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Lipase-Catalysed Reactions of Prostaglandins

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Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for any degree or examination.

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II Vallikivi, I., Villo, L., Metsala, A., Pehk, T., Parve, O. The lipase-catalyzed alcoholysis of prostanoid derivatives in low-water media. *Manuscript,* **2004**.

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ABBREVIATIONS

Ac	Acetyl group
Asp	Aspartic acid
BCL	*Burkholderia cepacia* lipase
CALB	*Candida antarctica* lipase B
cAMP	Cyclic adenosine monophosphate
CRL	*Candida rugosa* lipase
COX	Cyclooxygenase, prostaglandin H_2 synthase (PGHS)
DAG	Diacylglycerol
DEF ABS	RMS deviation of a selected set of atoms from their centre of the mass mean value
e.e.	enantiomeric excess
Gln	Glutamine
Gly	Glycine
His	Histidine
HPLC	High-performance liquid chromatography
IP_3	Inositol 1,4,5-triphosphate
IP_4	Inositol 1,3,4,5-tetrakisphosphate
MD	Molecular dynamics
NMR	Nuclear magnetic resonance spectroscopy
NSAID	Nonsteroidal anti-inflammatory drug
PG	Prostaglandin
PGHS	Prostaglandin H_2 synthase, cyclooxygenase (COX)
PPAR	Peroxisome proliferator-activated receptor
RML	*Rhizomucor miehei* lipase
RT	Room temperature
Ser	Serine
THP	Tetrahydropyranyl
THPMA	Tetrahydropyranyl-protected mandelic acid
TI	Tetrahedral intermediate
TLL	*Thermomyces (Humicola) lanuginosa* lipase
Thr	Threonine
Trp	Tryptophan
TX	Thromboxane

INTRODUCTION

Prostaglandins and their derivatives are natural compounds which are of great interest for medicine. Although the compounds have been extensively studied for several decades, their research is still of high significance. Prostaglandins play an important role in living systems (mammals and corals, for example). The origin of some of their derivatives, which can be found in living systems, is still unknown. The lipase-catalysed reactions of prostaglandins may account for the appearance of these products.

The lipase-catalysed reactions of natural compounds usually occur with a high stereo- and regioselectivity. Therefore, one of the most important fields of application of lipases is a selective protection of the functional groups of complex natural compounds that are used as a starting material in the semi-synthesis of physiologically active substances. The lipases are also used in the total synthesis of natural products for both the kinetic resolution of racemates and the asymmetrisation of prochiral and meso compounds.

In this work, the lipase-catalysed reactions of prostaglandins in low-water media were investigated. Mainly, the transesterification and different alcoholytic reactions were explored. We found the velocity and regioselectivity of lipase-catalysed reactions to depend essentially on the lipase and the acyl donor used as well as on the relatively small differences in substrate structure. Also, the reaction medium has a great effect on the results of the lipase-catalysed reactions of prostaglandins. By changing the medium it is possible to steer these reactions in an appropriate direction. The lipase-catalysed derivatisation of prostaglandins occurs under mild conditions and can afford products with high stereo-, regio- and chemoselectivity.

The molecular modelling methods have successfully been used for the prediction of the occurrence and character of lipase-catalysed reactions. In the current work, molecular dynamics simulations were performed to predict the regio- and stereoselectivity of the acetylation reaction of the *Candida antarctica* lipase B towards prostaglandins of type F.

1. REVIEW OF THE LITERATURE

1.1. Introduction

The synthesis and modification of natural compounds is an important field of research, especially in medicine. The enantiomeric purity of the compounds used in medicine is of crucial importance. Therefore methods used for the asymmetric synthesis or separation of enantiomers are of great interest to researchers.

Several prostaglandins (PG) are currently used as medicines and many more are being subjected to extensive biological and medical research. In the last years attention has also been paid to the synthesis and research of prostaglandin-like compounds. Some pathways of prostaglandin biosynthesis are quite well known, but the origin of some of their derivatives, which can be found in living systems is still unknown. One possible explanation for the formation of the products is the lipase-catalysed reactions of prostaglandins.

The synthesis and semisynthesis of prostanoids is continuously topical. The extensive use of lipases may greatly contribute to the development of appropriate synthetic methods. Lipases are remarkable biocatalysts which are capable of accepting a wide array of complex natural and artificial molecules as substrates. They are often exquisitely selective, catalysing reactions with unparalleled enantio- and regioselectivities.

Depending on the reaction medium the lipase-catalysed reactions occurring in low-water media that can be applied in synthesis include either acylation, deacylation, esterification, hydrolysis, or even elimination.

The molecular modelling of enzyme-catalysed reactions can help us to understand the mechanism of these reactions as well as make predictions about the enantio- and stereoselectivity of enzymes towards specific substrates.

The current literature overview reflects the most important features in accordance with the above fields and problems studied in this work.

First, the definition of enantiomers is given and their properties are described. After that a short overview is given of prostaglandins, starting from their use in medicine. Emphasis is put on prostaglandin biosynthesis. This is done in order to help to understand the possible role of lipases in the formation of the prostanoids and their derivatives isolated from natural sources.

Further, methods of semisynthesis of prostaglandins as well as the most important features of the use of lipases in synthesis are shortly referred to. A detailed review of achievements in the molecular modelling of lipase-catalysed reactions is presented.

1.2. Enantiomers. The biological properties of enantiomers.

Enantiomer is defined as one of a pair of molecular entities which are mirror images of each other and nonsuperposable. This means that although two enantiomer molecules are built in the same way and have the same number of atoms, their properties may differ drastically since the molecules differ in three-

dimensional structure. Enantiomers can differ from each other in smell, taste or, more importantly, pharmacological action.

R-Thalidomide *Sedative* **S-Thalidomide** *Toxic* **R-Limonene** *Orange fragrance* **S-Limonene** *Lemon fragrance* **S-Aspargine** *Bitter taste* **R-Aspargine** *Sweet taste*

The separation of enantiomers is an important issue since in the case of many biologically active compounds only one enantiomer has a desired activity, while the other enantiomer may be inactive, or possess different, sometimes even an opposite or undesirable activity. A tragic example is thalidomide both enantiomers of which had the desired sedative and teratogenic effects, but the *S*-thalidomide had toxic side-effects which caused foetal malformations.

1.3. Preparation of enantiomers with a high enantiomeric excess

Several pairs of enantiomers have been reported to have not only an opposite sign of the optical rotation but also opposite biological effects. A good example is Japanese beetle pheromone: (*R*)-enantiomer attracts male beetles while (*S*)-enantiomer interferes, even as a 1% impurity[1]. The same effect is also known to occur in some active compounds contained in medicines, including prostanoids (for example, in cloprostenol, which is a highly potent luteolytic agent used in veterinary medicine).

Regulatory guidelines require the determination of a physiological effect of each enantiomer contained in a drug candidate. However, a problem of how to estimate precisely the level of an enantiomeric excess of novel compounds remains.

Highly pure single enantiomers of physiologically active compounds can be prepared by using a sequential kinetic resolution[2]. For this, enzymatic or chemical processes with a repeated chiral recognition (with a multiple enantioselection) are used. In the course of the enzymatic sequential resolution the substrate has to go through the active site of the enzyme at least twice which results in a high total apparent enantioselectivity.

A double enantioselection has been used for the demethoxycarbonylation of possible precursors for carbacyclin synthesis[3]. The highly efficient double enantioselection by lipase has been used for the transesterification of (*R,S*)-carboxylic acid vinyl esters with (*RS*)-1-phenylethanol[4]. In this process, the CALB showed an excellent enantioselectivity (E>100) toward (*RS*)-1-phenylethanol, and a moderate enantioselectivity (E~10) toward carboxylic acid vinyl esters. Also, the CALB-catalysed doubly enantioselective aminolysis reactions have been reported[5]. The preparation of (*S*)-2-ethylhexyl-*para*-methoxycinnamate by the lipase-catalysed sequential kinetic resolution has been described[6].

A double enantioselection by the *Thermomyces lanuginosa* lipase (TLL) has been shown to occur in the case of hydrolysis of a bicyclic bis-acetylated

bromodiol. The resulting bromodiol (**3**) was found to contain the minor enantiomer in a lower quantity than the detection level (Scheme 1)[7].

Scheme 1. The doubly TLL-catalysed enantioselective kinetic resolution; the lipase-triggered cascade reaction resulting in epoxide (**4**).

In the lipase-catalysed kinetic resolution of carboxylic acids and alcohols of simple structure the most common problem to solve by a sequential resolution is to improve a good, moderate and even fair enantioselectivity of the process in order to gain a product with an e.e. of at least 98%.

The determination of a very high enantiomeric excess is probably more complicated than the problem of preparation of samples of a super-high enantiomeric purity. The lipase-catalysed kinetic resolution has been used to aid the determination of high enantiomeric purity by concentrating the minor enantiomer to decrease the e.e. of the sample to 98% or lower, which is the detection level for most of the suitable methods[8].

The use of highly enantiopure compounds as medicines is sometimes considered to be arguable because of the occurrence of the racemisation of a number of chiral compounds in the living organism. This may be true only in the case of simple compounds bearing one stereogenic centre. For complex molecules like prostanoids, which bear up to 5 stereogenic centres, the racemisation of one (or more) stereogenic centre could be considered a normal metabolic inactivation.

1.4. Prostaglandins

Prostaglandins are fatty acid derivatives with a cyclopentane ring bearing two side chains (α-chain: C_1-C_7 and ω-chain: C_{13}-C_{20}), one of which holds the carboxyl terminal[9].

PGE$_2$ **5**   PGF$_{2\alpha}$ **6**

Prostaglandins are classified and named by a functional groups on the cyclopentane ring (A, B, C, D, E, F, etc) and the number (1, 2, or 3) of double bonds in the side-chains. The subscript lettering α or β after the number refers to whether the C_9-OH group is above or below the cyclopentane ring plane.

The chemical synthesis of prostaglandins such as $PGF_{2\alpha}$, PGE_2, PGI_2, and several structural analogues is at a highly developed stage, with the result that these substances can be efficiently produced synthetically[10].

1.4.1. The use of prostaglandins in medicine

Natural prostaglandins are biologically potent, short-lived local hormone metabolites which are derived, via cyclooxygenase (COX) enzymes, from a nutritionally essential precursor, polyunsaturated fatty acids. They are present in virtually all mammalian tissues. Prostaglandins participate in a variety of normal physiological processes such as maintaining the blood pressure and body temperature, protecting organs from the damage caused by a disease, a traumatic injury and stress, regulating the parturition and involvement in sleep-wake cycles. PGs can exert a host of pharmacological effects and have therefore been of interest to the pharmaceutical industry for a long period of time and continue so to be. There are now more than thirty prostaglandin drugs, including natural PGs and prostacyclin and its analogues, marketed throughout the world, with many more under development. Current preclinical and clinical research will for sure lead to the introduction of an increasingly diverse range of innovative prostaglandin products for use in many areas of medicine[11].

1.4.2. The biochemical and physiological action of prostaglandins

PGs possess very diverse functions depending on type and the tissue in which they are found. The minor differences in molecular structure may result in major differences in potencies and the type of response elicited. At the functional level it appears that many PGs are rapidly biosynthesised, exert their biological effect and are rapidly metabolised, thereby functioning as autocoids or local hormones[12].

Their very low concentrations (10^{-9} M) and synthesis in most tissues support the concept that PGs are local hormones. They act via the receptors analogous to the adrenergic receptors coupled to G proteins. Once induced, the concentration of second messengers (a cyclic adenosine monophosphate (cAMP), IP_3, IP_4, DAG, Ca^{2+}, etc.) varies by stimulating the adenylate cyclase and phospholipase C, opening/closing Ca^{2+} and K^+ channels, or promoting the Na^+/H^+ exchange[13].

PGE_1 and prostacyclin act as vasodilators and prevent platelet aggregation. PGI_2 strongly dilutes blood in coronary blood vessels, while TXA_2 induces platelet aggregation.

In some cases, PGs are diarrheic and also inhibit the gastric secretion to protect the epithelium of the gastrointestinal tract. PGs of type E inhibit the response to an adrenergic nerve stimulation and release of noradrenalin from nerve endings[14,15]. In addition, PGs play a certain role in control of the body temperature, the onset of rheumatoid arthritis, and some bone cancers[16].

Once a call responds to a prostaglandin by changing the intracellular concentration of some key substance, these changes then trigger a sequence of reactions that produce a physiological response (i.e. contraction, secretion, excitation, etc.).

Since the number of prostaglandins and the target cells is high, the effects exerted by these hormones are very different. In several cases, prostanoids can act as opposing pairs, for example PGE_1 relaxes the venous smooth muscle, while $PGF_{2\alpha}$ causes its contraction.

The opposing action of a pair of prostaglandins is also seen in the case of blood coagulation. PGI_2, which is produced by endothelial cells, acts on the local smooth muscle by relaxing them and decreasing the blood pressure. With a receptor it also acts on the platelet by inhibiting aggregation. It has been suggested that PGI_2 synthesis prevents platelets from aggregating on and/or sticking to vessel walls. When a vessel is injured, the platelets produce TXA_2 which blocks the PGI_2 binding, thus permitting platelet aggregation. Furthermore, TXA_2 either inhibits PGI_2 synthesis, or it has a direct action on the smooth muscle cell which is opposite to that of PGI_2, thus increasing the muscle contraction and the blood pressure.

8-*iso*-$PGF_{2\alpha}$ is a potent agonist of TXA_2/PGH_2 receptors in the rat vascular smooth muscle. It primarily acts as an antagonist of TXA_2/PGH_2 receptors in both rat and human platelets. Therefore, the vasoconstriction induced by 8-*iso*-$PGF_{2\alpha}$ would not be expected to be accompanied by platelet aggregation *in vivo*[17].

1.4.3. Synthetic prostanoids: natural prostaglandins and their analogues

The extraction of prostaglandins from natural sources is an expensive and tedious process. Currently it is more cost-effective to synthesise prostaglandins and their analogues using methods developed by several groups.

Synthetic prostaglandin analogues have been developed with the aim of obtaining compounds which are more stable, have a longer duration in action and a more specific effect.

The prostaglandins used as uterine stimulants in obstetrics and gynaecology include $PGF_{2\alpha}$ (dinoprost) and its analogue carboprost, PGE_2 (dinoprostone) and its analogues metenoprost and sulprostone, and gemeprost, an analogue of PGE_1. The synthetic analogues of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ are used as luteolytic agents in veterinary medicine.

(±)-Cloprostenol is a synthetic analogue of $PGF_{2\alpha}$. It is an FP receptor agonist and a potent luteolytic agent in rats and hamsters. (±)-Cloprostenol is 200 times more potent than $PGF_{2\alpha}$ in terminating pregnancy when administered subcutaneously at a daily dose of 0.125 μg/kg in rats and hamsters, without the side effects associated with $PGF_{2\alpha}$[18].

The prostaglandins used as vasodilators and inhibitors of platelet aggregation include PGE_1 (alprostadil) and its analogue limaprost, prostacyclin (epoprostenol) and its analogues beraprost, ciprostene, and iloprost, and viprostol, an analogue of PGE_2.

Table 1. Some examples of prostaglandins and their analogues used as medicines.

Name	Structure	Application
Alprostadil (Prostaglandin E_1)		Impotence, congenital heart disease, vasodilator
Dinoprostone (Prostaglandin E_2)		Labour induction
Dinoprost (Prostaglandin $F_{2\alpha}$ Tromethamine salt)		Termination of pregnancy, luteolytic agent
Misoprostol		NSAID induced duodenal/gastric ulcer
Limaprost		Peripheral vascular disorders
Cloprostenol Sodium Salt		Luteolytic agent, labour induction

The dissociation constants of interaction between some prostaglandin analogues and a PGF_2 receptor in bovine *corpora lutea* were determined. With regard to both the affinity for the receptor and the luteolytic potency the most active analogues were 17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ and 15-methyl-$PGF_{2\alpha}$. It was demonstrated that the alkyl side-chain of PGs could be modified considerably without altering the affinity for the receptor. In this way metabolism by 15-hydroxyprostaglandin dehydrogenase could be blocked. Some of these compounds had a significantly increased luteolytic effect. The substitution of a phenyl group for the 3 terminal carbon units of the alkyl side-chain of PGs increased both the affinity for the receptor and the luteolytic activity *in vivo*[19].

1.4.4. Enantiomerically pure prostaglandins vs. racemic prostaglandins

It is not always recommendable to use racemic compounds in medicine since enantiomers might have opposite or adverse activities.

An example of an inhibiting activity can be observed in case of cloprostenol. An effective dose of (±)-cloprostenol sodium salt is 500 µg compared to the 150 µg of (+)-cloprostenol sodium salt[20].

1.4.5. The use of prostaglandin derivatives and stereoisomers

The derivatives of different natural prostaglandins often have similar biochemical or physiological actions. In some cases, they have also been found to be less potent than natural PGs. However, the derivatisation of native as well as synthetic PGs aims at producing more effective, selective and potent active ingredients of medicines.

Investigation was conducted with a series of enantiomeric forms of PGF_2 and PGE_2 that were compared for potency in a hamster antifertility test. In the PGF_2 series, the enantiomeric isomers investigated had a lower potency than the corresponding natural structures. For the PGE_2 series, an opposite effect was observed – 11α-(15S)-*ent*-PGE_2 methyl ester was 10-fold more potent than PGE_2. Altering the C_9 hydroxy configuration in the PGF_2 series from natural α to β decreased the potency dramatically for compounds tested[21].

1.5. Biosynthesis and the primary activity of prostaglandins

Prostaglandins are formed by most cells in mammals and act as autocrine and paracine lipid mediators (i.e., they signal at or immediately adjacent to their site of synthesis). They are not stored but are synthesised *de novo* from the membrane-released arachidonic acid when the cells are activated by a physical (stretching, squeezing, vibration, electrical) stimulation or by a specific cytokine, growth factor, and other stimuli.

1.5.1. Prostaglandin metabolism

1.5.1.1. Arachidonic acid release in cells

Arachidonic acid, the most abundant precursor of PGs in mammals, is cleaved from cell membrane phospholipids by the action of phospholipase A_2 and phospholipase C. These phospholipases are activated by a variety of intercellular and intracellular mediators[22]. Small amounts of arachidonic acid are always present in our diets. Arachidonic acid is mainly synthesized from the dietary linoleic acid.

After synthesis, the arachidonic acid is incorporated into a phospholipid, phosphatidyl choline or phosphatidyl inositol, usually on the middle or second carbon of the glycerol. The phospholipid is incorporated and functions as part of the membrane.

Arachidonic acid is released directly from the phospholipid by a phospholipase A_2 reaction. When phosphatidic acid provides the arachidonic acid, a phospholipase A_2 mediates the release.

The release of arachidonic acid is the rate-limiting step of prostanoid synthesis.

1.5.1.2. Pathways of prostaglandin biosynthesis

The prostaglandin H_2 synthase (COX; EC 1.14.99.1) is a heme-containing protein and is located in microsomes[23]. There are two isoforms of the cyclooxygenase which have a high level of structural homology and catalyse the same reaction. COX-1 is regularly expressed in various tissues as a 'house-

keeping' enzyme which carries out a normal, physiological production of prostaglandins. COX-2 is an inducible enzyme believed to be responsible for the PG synthesis at the site of inflammation. The COX-2 expression is significantly upregulated in inflamed periodontal tissues. The COX-2 expression can be induced by a variety of factors – different oncogenes as well as polypeptide hormones (such as PDGF, EGF, tumour necrosis factor α (TNF-α), endothelin, FSH, LH, thrombin, interferon γ (INF-γ), TGF β, FGF, parathyroid hormone, IL-1α, IL-1β, kit ligand, BDNF and NF_3); pharmacological agents (e.g., phorbol esters and forskolin); prostaglandins $PGF_{2\alpha}$, PGE_1, PGE_2; LPS/endotoxin, neurotransmitters (norepinephrine and serotonin); depolarisation and aggregation IgE receptors. As other inducible proteins, COX-2 rapidly degrades. COX-2 can be found in epithelial, endothelial, smooth muscle, synovial microvascular, granulosa, uterine stromal, amnion, decidual and mast cells; calvariae, osteoblasts, mesangial cells, macrophages, monocytes and neurons. COX-2 plays an important role in a variety of cellular responses to many cellular stimuli[24,25].

Both inflammatory cytokines such as IL-1β and bacterial constituents may be responsible for the enhanced COX-2 expression and PGE_2 synthesis *in vivo*[26].

The mechanism of transformation of arachidonic acid to PGH_2 is shown in Scheme 2. First the cyclooxygenase enzyme converts arachidonic acid into PGG_2, after that PGG_2 will be transformed into PGH_2. Then PGH_2 rapidly transforms into tissue specific prostanoids by PGD or PGE isomerases and $PGF_{2\alpha}$, PGI_2, or thromboxane A_2 synthases (Scheme 3).

Scheme 2. The mechanism of transformation of arachidonic acid to PGH_2.

PGF_2-isoprostanes are PGF_2-like compounds that are produced *in vivo* in humans by a non-enzymatic free radical catalysed peroxidation of arachidonic acid. 8-*iso*-$PGF_{2\alpha}$ is one of the compounds that can be produced by this mechanism.

PHOSPHOLIPIDS

Phospholipase A_2

COOH

Arachidonic acid

2 O_2

COX - cyclooxygenase activity

NSAIDs

O
O
COOH
PGG_2 OOH

6-keto-$PGF_{1\alpha}$
6-keto-PGE_1
15-keto-PGI_2

2 e^-

COX - peroxidase activity

O
O
COOH
PGH_2 OH

COOH

Isomerases **Synthases**

O
HO OH
PGI_2

O
COOH
HO OH
PGE_2

HO
COOH
HO $PGF_{2\alpha}$ OH

OH
COOH
O OH
PGD_2

O
O
COOH
TxA_2 OH

15-keto-PGE_2
19-OH-PGE_2
$9\alpha,11\beta$-PGF_2
13,14-dihydro-15-keto-PGE_2
PGA_2
PGC_2
PGB_2
PGE-M

13,14-dihydro-15-keto-PGD_2
$9\alpha,11\beta$-PGF_2
PGJ_2
Δ^{12}–PGJ_2

15-keto-$PGF_{2\alpha}$
19-0H-$PGF_{2\alpha}$
13,14-dihydro-15-keto-$PGF_{2\alpha}$
PGF-M

TXB_2
11-dihydro-TXB_2

Scheme 3. The biosynthetic pathways of some prostanoids in mammals.

In regard to eicosanoid-protein recognition features recent interesting results should be referred to: in the research of cyclooxygenase mechanisms[27] the structure of prostaglandin H synthase 1 at 3 Å resolution with arachidonic acid bound in a chemically productive conformation has been determined[28].

1.5.1.3. Prostaglandin catabolism

Prostaglandins have a very short half-life. They are degraded rapidly and efficiently *in vivo* by different enzymes.

Normally PGE and PGF compounds do not survive a single pass through the circulatory system. Prostaglandins are rapidly catabolised to different extents in different tissues by several enzymes. PGE_2 is converted to an inactive PGA_2 which, in turn, is converted to PGC_2 and PGB_2. TXA is converted to an inactive TXB. PGI_2 undergoes a spontaneous hydrolytic transformation into 6-keto-$PGF_{1\alpha}$ before further catabolism. In the samples of tissues and fluids, 6-keto-$PGF_{1\alpha}$ is measured in lieu of PGI_2.

The decomposition pathways of various PGs are different but their metabolism has several common features:

1) oxidation of the C_{15}-OH group resulting in the corresponding keto derivatives;
2) β-oxidation of the carboxyl terminus leading to di-nor PGs; and
3) ω-oxidation of the methyl terminus affording dicarboxylic compounds[,24,29].

1.5.2. The inhibition of prostaglandin biosynthesis

A major mechanism to explain the anti-inflammatory action of certain steroids like corticosteroids has been traced to their inhibition of phospholipase A_2. This inhibition causes a decrease of arachidonic acid level and other polyenoic acids which are the precursors of the PG_1 and PG_3 series. Therefore, the inhibition of prostaglandin synthesis takes place.

A cyclooxygenase is inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, indomethacin and phenylbutazone[30]. In fact, the only known biochemical affect of aspirin is the prostaglandin synthesis suppression via the cyclooxygenase inhibition. Conventional nonsteroidal anti-inflammatory drugs inhibit both COX-1 and COX-2. The clinical efficacy of NSAIDs is primarily related to the inhibition of COX-2 activity, whereas much of the toxicity, particularly gastrointestinal, is related to the COX-1 inhibition.

1.5.3. Natural sources of prostaglandins

Prostaglandins are known to be of wide occurrence in mammal tissues where they are formed from polyunsaturated fatty acids and are rapidly metabolised. However, the most abundant source of prostaglandins in nature is corals viz. the horny coral *Plexaura homomalla*[31,32,33,34], the soft coral *Lobophyton depressum*[35], etc. For example, in *P. homomalla* the content of PGA_2 and PGE_2 esters amounts on average to 2-3% of its dry weight[,36]. It seems noteworthy that the 11-acetylated derivatives of F-type prostanoids as well as 15-acetylated A-type prostaglandins and their esters have been the most prevailing components in the prostanoid fractions isolated from the corals.

Shortly after the chemical structure of prostaglandin hormones was elucidated, a rich natural source of prostaglandins was discovered in the Caribbean gorgonian *P. homomalla*. Weinheimer and Spraggins analysed *P. homomalla*

collected in the Florida Keys. They found that the main constituents were (*15R*)-PGA_2 methyl ester acetate and (*15R*)-PGA_2 methyl ester, products that have an opposite configuration to mammalian prostaglandins at the 15-position. It was subsequently discovered that the *P. homomalla* colonies collected in other Caribbean locations contain the same prostaglandin derivatives but with the (*15S*) configuration[37,38]. These latter studies established that *P. homomalla* also contains PGE_2 methyl ester as well as minor constituents, including the methyl ester 15-acetate derivatives of 5,6-*trans*-PGA_2 and 13,14-*cis*-PGA_2.

From the soft coral *Lobophyton depressum* 11-acetyl-$PGF_{2\alpha}$ and its methyl ester as well as 18-acetoxy-11-acetyl-$PGF_{2\alpha}$ and its corresponding methyl ester has been isolated. The 18-acetoxy-derivatives of $PGF_{2\alpha}$ may have been formed upon 1-3 lipase-catalytic steps and enzyme-catalysed functionalisation of 17,18-unsaturation (characteristic of PGs of 3rd series), since the isolation of a single compound, and not of a diastereomeric mixture, has been reported.

1.6. Semisynthesis of prostaglandins

A number of prostanoids are continuously used in biological, pharmacological and medical research. The simplest approach to the synthesis of different prostaglandins is based on interconversions starting from $PGF_{2\alpha}$, PGE_2, etc.[39,40] Prostanoids are (poly)hydroxycarboxylic acids of complex structure. So, the further development of methodologies of the chemo- and regioselective protection of the functional groups of prostaglandins under mild conditions is of crucial importance for their semisynthesis. The choice of the methods of synthesis, as well as of protecting groups is determined by the stability of target compounds which can often be both acid- and base-sensitive.

1.6.1. Interconversions of naturally occurring prostaglandins

A key feature of the interconversions between the PGE, PGF, and PGD series has been an extensive use of silyl protecting groups to facilitate both selective oxidations[41,42,43] and reductions[44,45,46].

The reduction of PGE_2 is also carried out with $NaBH_4$ yielding $PGF_{2\alpha}$ and $PGF_{2\beta}$[47].

Pike *et al.* have described the acid-catalysed dehydration of PGE_1 and PGE_2 leading to the corresponding 10,11-unsaturated ketones[48], PGA_1 and PGA_2, respectively.

Bundy *et al.*[49] describe the semisynthesis of PGs from the coral *Plexaura homomalla*. The ester derivatives of both (*15S*)- and (*15R*)-PGA_2 were epoxidised, then reduced and separated by silica gel chromatography. However this procedure, being tedious and expensive, was replaced by a total synthesis of PGs (for example, by the Corey lactone route).

1.6.2. Synthesis of the stereoisomers of $PGF_{2\alpha}$

The esterification-inversion procedure is one possible way to convert $PGF_{2\alpha}$ to $PGF_{2\beta}$ derivatives[50]. The procedure starts from the 11,15-bis-tetrahydropyranyl ether of $PGF_{2\alpha}$ methyl ester and, using *p*-phenylbenzoic acid as an acy-

lating agent, the esterification-inversion sequence followed by the removal of THP-groups gave the $PGF_{2\beta}$ derivative in a 70–75% yield. After saponification the latter yielded $PGF_{2\beta}$.

The treatment of prostaglandins of type F with formic acid causes an inversion at C_{15} even at 0°C. This configurational change proceeds presumably through an allyl cation intermediate.

Taber and Kanai have developed a practical synthesis of the four enantiomerically pure isomers of 15-F_{2t}-isoprostane, including 8-*iso*-$PGF_{2\alpha}$, using an enzymatic resolution of the pseudo-meso diol as a key step[51].

1.7. Lipases

Lipases have been established as valuable asymmetric catalysts in organic synthesis and today there are already more than 30 lipases commercially available[52,53,54,55]. Due to their generally excellent enantioselectivity and the mild reactions conditions lipases have a widely recognised potential in the production of enantiomerically pure chiral compounds[56]. They are frequently used in both the kinetic resolution of racemates and the asymmetrisation of prochiral and meso compounds[57,58]. They allow us to perform a number of stereo- and regioselective transformations in the synthesis of different physiologically active compounds. Especially valuable is the contribution of lipases to the semisynthesis of complex natural products.

Several textbooks[53,59,60,61,62] and review papers[,-76] have been published to discuss various aspects of the use of lipases as tools in organic synthesis. The use of lipases in synthesis as well as in medicine, industrial chemical processes, etc., with emphasis on the structure of lipases and the mechanism of lipase-catalysed reactions has been discussed in a review by Schmid and Verger. The lipase-supported synthesis of biologically active compounds[63] and pharmaceuticals[64], use of preparative biotransformations in organic chemistry[65,66,67], lipase-catalysed ester synthesis[68], use of lipases in racemic resolutions[69] and polymer synthesis[70] have been dealt with in detail by several authors. Some papers interesting for synthetic chemists on more general problems in lipase catalysis have been published as well. These are focused on:

1. the activity of lipases in organic solvents vs. water[71],
2. the interfacial activation of lipases[72],
3. the possible multiplicity of catalytic sites and functions of an enzyme molecule[73],
4. the commercial scale biocatalysis: pros and cons[74].

The molecular recognition and mechanism of some reactions catalysed by different hydrolases have been discussed in a review by Holmquist[75]. A review of the molecular modelling of biocatalysis has been published by Kazlauskas[76].

Lipases have been defined as fat-splitting enzymes which are able to act on the interface between two phases. However, they usually retain some residual activity also in bulk aqueous phase hydrolysing esters in the molecular solutions of the latter. Lipases maintain their catalytic activity in low-water media

(organic phase, supercritical fluids, ionic liquids) and tolerate a wide variety of artificial substrates.

The conventional functions of lipases involve the acyl transfer resulting in:

1. hydrolysis of esters;
2. ester synthesis;
3. transesterification;
4. amidation, etc.

Recently, some unconventional functions of lipases, for example, the decyclisation of hemiacetals followed by the trapping of an open-chain hydroxyaldehyde upon acetylation have also been revealed[77].

Lipases usually recognise chiral substrates and nucleophiles in a stereo-selective manner. Thus, the tolerance to different media and artificial substrates, the ability to work as phase-transfer agent on the interfaces of a multiphase system, the high stereoselectivity of catalytic performance along with an intriguing versatility make lipases the most favourable biocatalysts as well as a challenge for synthetic chemists.

1.7.1. Features of a lipase-catalytic process important for synthesis

When using lipase catalysis we need a high reaction velocity and a high selectivity corresponding to the goal. Usually the following types of selectivity of a lipase-catalysed reaction have been acknowledged’’:

1) **substrate selectivity** – the ability of the enzyme to distinguish and act on a subset of compounds within a larger group of chemically related compounds;
2) **functional group selectivity** – the ability of the enzyme to act on one functional group selectively in the presence of other equally reactive or more reactive functional groups in the molecule;
3) **stereoselectivity** – the ability of the enzyme to act on a single enantiomer or diastereomer selectively – this is the most important feature of lipase-catalysis allowing kinetic resolution of enantiomers and diastereomers;
4) **regioselectivity** – the ability of the enzyme to act on one location in a molecule selectively allowing the regioselective protection and/or deprotection of complex molecules (polyols, polycarboxylic acids, etc.);
5) **chemoselectivity** – the ability of the enzyme to produce a certain product selectively upon acting on a functional group; in some cases, more than one chemically different alternative product can be formed upon acting on one certain functional group (for instance: the acylation of hemiacetal moiety).

There are several methods to improve the results of the lipase-catalytic process. These can be divided into:

1) **physicochemical methods**:
   – immobilisation of lipase (to improve stability),
   – solubilisation,
   – chemical derivatisation (for instance, nitration of lipase);

2) **engineering methods**:
   – the appropriate choice of reaction (for instance, hydrolysis vs. esterification, etc.),
   – substrate engineering (choosing the best fragments for a molecule of putative substrates from alternatives),
   – control of water activity,
   – the appropriate choice of acyl donor (vinyl acetate vs. acetic acid),
   – the appropriate choice of solvent (ionic liquids, supercritical fluids may be used to enhance the rate and selectivities),
   – process engineering (use of proper mixing conditions, microwave-promotion, etc.);
3) **molecular biology methods**:
   – rational protein design, (to alter enantioselectivity)
   – directed evolution (to alter substrate specificity, increase stability).

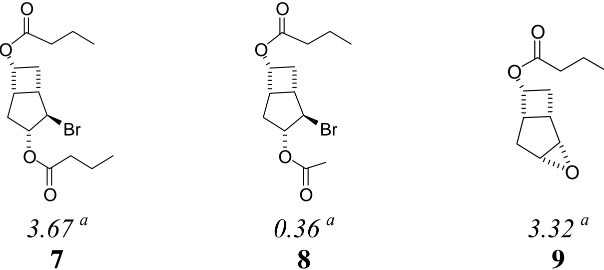
1.8. The inhibition of lipases

Usually the main concern is how to enhance the rate of lipase-catalysed reactions and how to do this as selectively as possible. For this reason several factors inhibiting the lipase-catalytic reactions have been identified and their influence has been tried to diminish.

The knowledge of inhibiting factors could allow control of the changes occurring in several biochemical systems, including those which occur *in vivo*. The digestive process could be an example in the case of which the lipase inhibitors are currently used as drugs for treating obesity.

Inhibitors can be used in the study of structural and mechanistic properties of lipases. Both the fields have been well reviewed by several groups[78,79].

The structural changes in the substrate molecule apart from the reaction centre may lead to a significant inhibition of hydrolysis (Scheme 4)[80].



Scheme 4. Cyclobutanol ester substrates. A nonsteric influence of hydrolysis by a remote structural change.
a: the initial rate of the TLL-catalysed hydrolysis (µmol/min per 1.0 ml of Lipolase).

The inhibitory effect illustrated in Scheme 4 has been assumed to arise from the possibility of two orientations of bipolar substrate molecules on the interface between water and the substrate.

Recent studies of lipase inhibitors mainly focus on the synthesis of 2-oxo amide triacylglyceride analogues[81] and the determination of their inhibitory effect on pancreatic[82] and gastric lipases[83,84]. To inhibit lipases, conventional

inhibitors of serine-hydrolases – alkylphosphonates, sulfonyl fluorides, etc., are also useful. For instance, the lipoprotein lipase has been efficiently inhibited by alkanesulfonyl fluorides[85]. An interesting inhibitory effect of quercetin on the *Candida rugosa* lipase (CRL) has also been reported[86].

1.9. The lipase-catalysed reactions of prostanoids and their derivatives

So far only few works can be found in the literature about the research of the lipase-catalysed reactions of natural prostanoids. In many cases, lipases have been described to allow the chemo-, regio-, and stereoselective protection and deprotection of functional groups of several synthetic prostanoids[87,88,89].

Taber and co-workers have successfully used the lipase for the acetylation of the C_9-OH or C_{11}-OH groups to gain monoacetates of several isoprostanes’. Taber and Jiang have also acetylated the C_{15}-OH group of isoprostane derivatives to separate two enantiomers of racemic alcohol. Although the acetylation was very slow, it however, proceeded a with very high enantioselectivity (the e.e. of the remaining alcohol was over 99%).

The active site structure of several lipases is known from X-ray difractometric studies. Thus, the modelling of certain binding modes for prostanoids in lipase active site could be resolvable by using computational methods supported by some guidelines of empiric origin.

1.10. The molecular modelling of lipase-catalysed reactions

The rapid advances in structural biology which began in the early 1990s have revealed three-dimensional structures of many biocatalysts. Molecular modelling can therefore be the tool that links these structures with experimental observations. As a qualitative tool, current modelling methods can be extremely useful. They can explain unusual features of enzymatic reactions on a molecular level. They can also predict how to increase the selectivity – by either the substrate modification or site-directed mutagenesis. Quantitative predictions, for example, the degree of enantioselectivity, are still not very reliable although they are constantly improving with the development of quantum chemical programs and algorithms.

1.10.1. Methods used in the molecular modelling of lipase-catalysed reactions

The prediction of enantiopreference of lipases has been currently realised most often by molecular dynamics simulations using different force fields (Amber, CHARMM, etc.) and tetrahedral intermediates as models of transition states in the active site of lipases[90,91]. The enantiopreference is then determined from the resulting ensembles on the basis of: 1) potential energy differences corresponding to the whole enzyme-substrate complexes[92,93,94] or 2) distance and angle geometry comparisons at the active site[95,96].

Lately, the energy of non-minimised complexes has been used for the study of the enantioselectivity of lipase towards alcohols and their esters[97]. Schultz *et al.* were able to distinguish substrates, for which the *Burkholderia cepacia*

lipase (BCL) shows high enantioselectivity, from those for which enantiomers are poorly distinguished on the basis of the HN_{ε}–$O_{alcohol}$ distance for the slow-reacting enantiomer. Raza *et al.* studied the enantioselectivity of the CALB considering the mean, function-based conformational energy of an unoptimised enzyme-substrate system representing the core structural elements of the modelled transition state. They obtained a reasonable agreement with the experimentally determined free energy of activation.

Usually, the fast-reacting enantiomer can be predicted with very good results, but a quantitative estimation of enantioselectivity is hard to achieve[98]. The results from molecular modelling and x-ray crystallography[99] show independently that the slow-reacting enantiomer has a problem of forming all of the hydrogen bonds needed for a functional transition state when docked into the active site in the same way as the fast-reacting enantiomer.

However, it is necessary to know the exact details of the molecular structure of substrates in order to get well-defined parameters for accurate force field calculations of lipase-substrate interactions. The parameters which describe the molecules are equilibrium bond-lengths, angles and torsion angles, and the forces to maintain each of these repulsive and attractive forces in the van der Waals interactions and repulsive and attractive forces in the hydrogen-bond interactions. The electrostatic interaction of the enzyme and the substrate is one of the most significant factors in enzymatic catalysis[100]. Therefore, a significant feature in the molecular mechanics calculation is the energy derived from electrostatic interactions. In this respect, the applications of *ab initio* methods to the molecular structure studies of these systems are very useful for finding parameters for force field calculations with high accuracy[101]. Independently the *ab initio* methods explore usually interactions between well-defined small parts of the enzyme (few atoms of each active site residue) and the substrate, and can explain in great detail the mechanism of reaction.

A new approach was suggested by Tomić and Kojić-Prodić for predicting the enantioselectivity of enzymes towards racemic compounds. It was based on a comparative binding energy (COMBINE) analysis[102]. This approach was used to rationalise the enantioselectivity of the BCL towards thirteen racemic 3-(aryloxy)-1,2-propanediols in the process of acylation. According to their molecular modelling study the two 3-(aryloxy)-1,2-propanediol enantiomers bind in the BCL active site in different orientations. To derive a quantitative structure-activity relationship (QSAR), the difference in interaction energy between two enantiomers with each amino acid residue was computed. These residue-based energy differences were then subjected to a chemometric analysis and 3D QSAR models were derived. Using these models the authors were able to unambiguously predict the fast-reacting enantiomer and the approximate magnitude of the enantioselectivity. The study enabled identification of interactions between the substrate and the lipase amino acid residues that play a key role in secondary alcohol enantiodifferentiation. On the basis of these results the authors were able to propose modifications to the substrate and the protein which could modify the enantioselectivity of the BCL towards secondary aryl-alcohols.

The molecular structure of several chiral compounds, substrates and nucleophiles in lipase-catalysed reactions (for example, 1-phenylethanol, 2-hexanol and 1-phenylethanol acetate), has been studied theoretically using *ab initio* methods[103]. Conformational analysis and electronic structure studies have been carried out with these molecules at STO-3G* and 6-31G* basis sets.

To study the interaction of lipases with substrates, a simplified model of the tetrahedral intermediate has been calculated at the 6-31G*//4-31G* level. Molecular mechanics simulations of interaction of these compounds with the CRL, BCL and RML lipases have been used to study the enantioselectivity of these lipases in the transesterification reaction of chiral alcohols. The theoretical results obtained using these methods were in good agreement with experimental.

Different methyl-branched chiral fatty acids have been used as model compounds for the CRL and in this case, modelling studies have predicted the existence of two different modes of binding the enantiomers of such a chiral acyl donor to the active site[104]. The fast-reacting enantiomer orients its hydrophobic acyl chain into the active-site tunnel, which is a unique feature of this kind of lipases[105], whereas the slow reacting enantiomer leaves the tunnel empty. The existence of these two substrate-binding modes has recently been experimentally confirmed[106]. Similarly, for the CALB, it has been shown that 4-methyl-branched acids can be resolved, and that the enantiomers orient their acyl chain differently in the active site of the enzyme[107]. Two different modes of binding the substrate enantiomers have also been described for homologous secondary alcohols with the CALB. In this case, longer alcohols have an inverted orientation in the enzyme active site.

2. THE OBJECTIVES OF THE STUDY

The objective of this work was to explore the lipase-catalysed reactions of prostanoids, viz. the transesterification as well as reactions occurring under alcoholytic conditions in low-water media.

In particular, the work was aimed at contributing to the synthesis and semi-synthesis of prostanoids upon developing methods for a 'mild' selective protection of functional groups. Additionally, the goal of the work was to contribute to a further understanding of the possible role of lipases in the formation of prostaglandins and their derivatives isolated from natural sources.

The molecular modelling was investigated with the aim to elaborating an approach to making the prognosis of accessibility of different hydroxyl groups of prostaglandins to the CALB-catalytic acetylation. The molecular dynamics simulations to be performed had to also provide further insight into the physical basis of selectivities of the CALB-catalysed acetylation of prostaglandins. In the modelling field, a specific objective of this work was to find criteria for testing the accessibility of prostaglandin hydroxyl groups allowing the prediction of the regio- and stereoselectivity of the CALB-catalysed acetylation of prostaglandins of type F.

3. RESULTS AND DISCUSSION

3.1. Resolution of the enantiomers of cloprostenol

3.1.1. Resolution of the enantiomers of cloprostenol using a chemical method

The derivatisation of racemic cloprostenol (**10**) with tetrahydropyranyl-protected mandelic acid (THPMA) was aimed at the semipreparative resolution of this highly potent luteolytic drug, as well as at the stereochemical studies of the derivatives.

A racemic cloprostenol (Scheme 5) was esterified under pTsOH catalysis in methanol, the corresponding methyl ester (**11**) was purified and THP-mandoylated with a diastereomeric mixture of THPMA. A diastereomeric mixture of CDA was used because, based on previous results we suggested a better chromatographic separability of free trismandelates (**13**)/(**15**) than of the THP-protected ones.

10: R=H
11: R=CH_3
12: X=THP
13: X=H
14: X=THP
15: X=H

a: the arc line indicates coplanar fragments of the molecule;

b: the atoms showing the greatest differential shielding effects in the NMR spectra caused by the ring-current influence of the phenyl group are indicated by the arrows.

Scheme 5. Synthesis of diastereomeric cloprostenol methyl ester trismandelates.

Thus, the THP groups of (**12**) and (**14**) were cleaved on the acidic hydrolysis. The resulting cloprostenol methyl ester trismandelates (**13**) and (**15**) were separated by short-column chromatography over silica and further purified by semipreparative HPLC in order to isolate the minute amounts of impurities detected. HPLC as well as NMR analysis established the resulting trismandelates (**13**) and (**15**) to be highly pure compounds.

The alkaline hydrolysis of esters (**13**) and (**15**) afforded (+)-cloprostenol and (-)-cloprostenol, respectively, in satisfactory yield. This final assignment of the absolute configuration of these earlier described products by polarimetric results was in accordance with our results of the absolute configurational assignment by the NMR spectra of trismandelates (**13**) and (**15**). Regions of the molecules affected by the ring-current influence of the phenyl groups of the mandoyloxy moieties were identified (Scheme 5).

The process suggested for the resolution of cloprostenol enantiomers affords both of the enantiomers with a very high enantiomeric purity: the enantiomeric purity of the chiral derivatising agent (e.e.) was ≥98% (three molecules of mandelic acid were introduced) and all detectable (possibly diastereomeric) impurities were separated by semipreparative HPLC; consequently, the e.e. of cloprostenols could be even as high as 99.9998%.

3.1.2. The lipase-catalysed acetylation of cloprostenol

One way for the resolution of enantiomers is to use an enantioselective enzyme-catalysed reaction and afterwards separate the product and the unreacted starting material. This was clearly demonstrated for prostaglandins using both cloprostenol enantiomers as model substrates. The CALB-catalysed acetylation of the 11-hydroxyl group of (+)-cloprostenol ((+)-**10**) with vinyl acetate was found to occur highly chemo- and regioselectively, while (-)-cloprostenol ((-)-**10**) did not give any detectable access to 11-acetyl-derivative upon treatment under the same conditions.

The very high enantioselectivity of the CALB-catalysed acetylation of prostanoids of type F has been proven by this trial.

Scheme 6. The lipase-catalysed acetylation of cloprostenol.

3.2. The lipase-catalysed acylation of prostaglandins

3.2.1. The lipase-catalysed acylation of PGE_2 and its derivatives

Both the C_{11}- and C_{15}-OH groups of PGE_2 can be acetylated using vinyl acetate by the CALB. The acetylation occurs in strict order: the C_{11}-OH group is acetylated first and at a rate more than ten times higher than the C_{15}-OH group. In the case of PGE_2 methyl (as well as ethyl) ester, the C_{15}-OH group was not acetylated by the CALB: the corresponding diacetate was undetectable after 257 hours of incubation. During the same period and under the same conditions PGE_2 as a free carboxylic acid afforded the corresponding diacetate (**19**) with a yield higher than 50%. The conclusion is that the accessibility of the C_{15}-OH group to the enzyme depends crucially on the interactions between the carboxyl group of PG and the active site region of the CALB.

When vinyl laurate was used for acylation, instead of vinyl acetate, the C_{15}-OH group of PGE_2 was not acylated by the CALB (Scheme 7). In this case also

the velocity of the lipase-catalysed acylation is much lower than that of acetylation with vinyl acetate.

The C_{11}-OH group of PGE_2 can be acetylated by the CALB as well as by the *Thermomyces lanuginosa* lipase (TLL) and *Rhizomucor miehei* lipase (RML). However, neither the TLL nor RML catalyse the acetylation of the C_{15}-OH group of PGE_2.

Scheme 7. The lipase-catalysed reactions of PGE_2 and its derivatives.

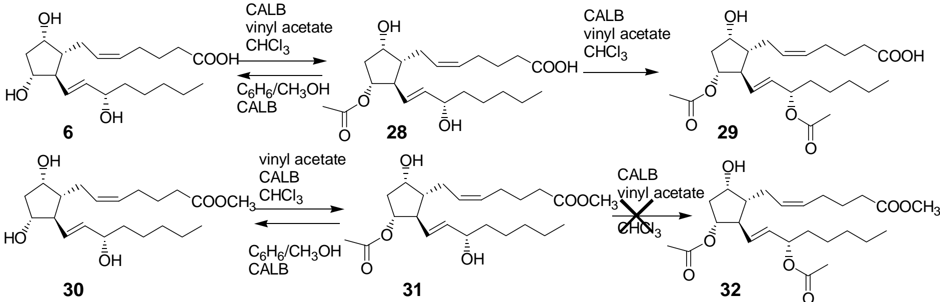
3.2.2. The lipase-catalysed acylation of PGE_1

PGE_1 (**25**) is acetylated CALB-catalytically easily to 11-acetyl-PGE_1 (**26**). The CALB-catalysed acetylation of PGE_1 did not give 11,15-diacetyl-PGE_1 (**27**) when the same conditions as for the acetylation of PGE_2 were used.

Scheme 8. The lipase-catalysed acetylation of PGE_1.

3.2.3. The lipase-catalysed acylation of $PGF_{2\alpha}$ and its derivatives

The CALB-catalysed acetylation of $PGF_{2\alpha}$ (**6**) in a $CHCl_3$/vinyl acetate solution proceeds smoothly and is highly chemo- and regioselective. The crude product contained the target 11-acetyl-$PGF_{2\alpha}$ (**28**) of over 99% purity by NMR spectroscopic analysis (reaction time 18 hrs; the yield of 11-acetate was >95% after chromatography). However, negligible amounts of several by-products, mainly 11,15-diacetyl-$PGF_{2\alpha}$ (~1% after 48 h; ~3% after 96 h), were detectable by NMR (and TLC) beginning from the 6th hour of incubation. The 11-acetyl-$PGF_{2\alpha}$ (**28**) was also purified using an alternative way, recrystallisation, from $CHCl_3$/n-hexane (2/3) at -15°C. As noted, the main by-product of this reaction was 11,15-diacetyl-$PGF_{2\alpha}$ (**29**). The difference in acetylation velocity between the C_{11}-OH and C_{15}-OH groups of $PGF_{2\alpha}$ was more than 100 times.



Scheme 9. The lipase-catalysed acetylation of $PGF_{2\alpha}$ and its derivatives.

3.2.4. Comparison of the kinetics of the lipase-catalysed acylation of PGE_2 and $PGF_{2\alpha}$ and their methyl esters

The acetylation of prostaglandins $PGF_{2\alpha}$ and PGE_2 and the corresponding methyl esters catalysed by the CALB was monitored by using ^{1}H NMR spectroscopy in order to get a quantitative estimation of the velocities of the reactions occurring in the system. Apparent second-order rate constants were calculated using the kinetic curves obtained from experimental results.

The apparent second-order rate constants of acetylation of the C_{11}-OH group of PGE_2, $PGF_{2\alpha}$ and their methyl esters differed by up to two orders of magnitude (Table 2).

Table 2. Investigation of the kinetics of the lipase-catalysed acetylation of PGs.

Substrate	PG [mg; µmol]	Novozym 435 [mg]	$CDCl_3$ [ml]	Vinyl acetate [µl, mmol]	Time of monitoring [h]	Conversion of PG [%]	$k_{II} \pm$ SE [min^{-1} $(g/ml)^{-1}$][a]
$PGF_{2\alpha}$	24; 67.7	50	1	60; 0.65	17	>99	0.092±0.004
$PGF_{2\alpha}$ Me-ester	25; 67.8	50	1	60; 0.65	36	84	0.0266±0.002
PGE_2	24; 68.1	50	1	60; 0.65	32	36	0.00596±0.0001
PGE_2 Me-ester	26; 70.9	50	1	60; 0.65	36	10	0.00116±0.0003

a: apparent second-order rate constants (per g/ml of Novozym 435) calculated for the acetylation of the C_{11}-OH group of the prostanoids

The contribution of the carboxyl group of prostaglandins versus the ester group to the enhancing of the acetylation velocity was 4/1. The effect of the C_9-OH group of $PGF_{2\alpha}$ and its methyl ester vs. the C_9-oxo group of PGE_2 and its methyl ester on acetylation velocity of the C_{11}-OH group was determined to be as high as 20/1. From this we can conclude that the carboxyl group as well as the C_9-OH group participate in the interactions important for the molecular

recognition and binding of prostaglandins by the acetyl-CALB involving the stabilisation of the corresponding tetrahedral intermediate.

3.2.5. The modelling of the CALB-catalysed acetylation of $PGF_{2\alpha}$ and its stereoisomers; NMR spectroscopic investigation of modelled reactions

Five stereoisomeric prostaglandins of type F were investigated by means of the molecular dynamics simulation methods to describe the different behaviour of these compounds in the active site of the CALB. On the basis of the results obtained conclusions were made regarding the regio- and stereoselectivity of acetylation. However, the modelling results obtained by the investigation of the tetrahedral intermediates created by the docking of compounds in the active site of lipases should be evaluated by comparing the empiric characteristics of the reactions. In this work, we investigated the acetylation kinetics of PGF stereoisomers by the NMR, in addition to the modelling research.

Scheme 10. The lipase-catalysed acetylation of $PGF_{2\alpha}$ and its stereoisomers.

The molecular dynamics simulation results obtained from the CALB-catalysed acetylation of the above stereoisomers were adjusted by the kinetic results to give the criteria that could allow prognostication of the stereo- and regioselectivity of the lipase-catalysed acetylation of PG isomers, derivatives, etc.

The molecular dynamics runs were performed with PG stereoisomers which were covalently bound to the active site Ser 105 over the acetyl moiety and via

the C_9-, C_{11}- or C_{15}-OH group of PGs. Altogether 13 different 100 ps molecular dynamics runs were performed. Three runs with the abovementioned hydroxyl groups for each of the four PGs (**6, 35**, **39**, **41**) and one run on the 8-*iso*-$PGF_{2\alpha}$ (**33**) (docked via C_{11}-OH group) were performed.

The results of molecular dynamics calculations (potential energies for the whole system and the function-based subset, the structures taken every 0.2 ps of a MD run, hydrogen bonding, etc.) and experimental were analysed, the criteria were found. The corresponding characteristics are given in Table 4.

The CALB-catalysed acetylation of $PGF_{2\alpha}$ and its stereoisomers (Scheme 10) was monitored by using the ^1H NMR spectroscopy. The consumption of PGs followed the pseudo first-order kinetics suggesting that there is no extensive complex formation of PG with the lipase before its nucleophilic attack to the acyl-enzyme. Apparent second-order rate constants (k_{II}) for the acetylation reactions were calculated (Table 3) using the least squares method.

Table 3. The CALB-catalysed acetylation of $PGF_{2\alpha}$ and its stereoisomers.

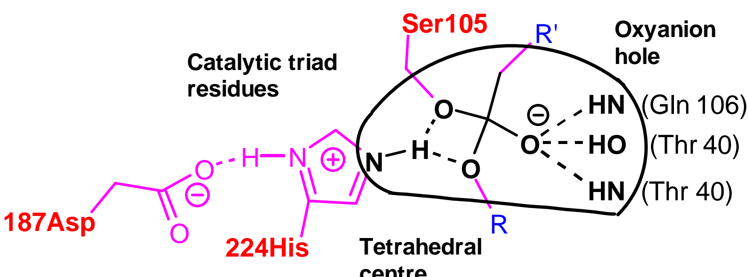
Substrate (amount [mg])	**Enzyme Novozym 435 [mg]**	**Medium CD_3COCD_3 [ml]**	**Acyl donor Vinyl acetate [µl]**	**Time [h]**	**Degree of conversion, %**	**k_{II} ± SE [min^{-1} (g /ml)$^{-1}$]**	**Products (ratio)**
$PGF_{2\alpha}$ (**6**), 10	20	1.0	50	25	99	0.31 ± 0.03	(**28**)
8-*iso*-$PGF_{2\alpha}$ (**33**), 9	18	0.9	46	120	85	0.022 ± 0.005	(**34**)
$PGF_{2\beta}$ (**35**), 9	18	0.9	46	11	95	0.285 ± 0.054	(**36**)/(**37**)/(**38**) (1/2/0.75)
(*15R*)-$PGF_{2\alpha}$ (**39**), 7	14	0.7	36	22	95	0.090 ± 0.008	(**40**)
(*15R*)-$PGF_{2\beta}$ (**41**), 8	16	0.8	42	21	95	0.22 ± 0.07	(**42**)/(**43**)/(**44**) (1/1/0.75)

From the experimental results some conclusions can be drawn:

1. the C_{11}-OH group was acetylated at a significant rate in the case of all isomers;
2. the rate of acetylation of the C_{15}-OH group for all of the compounds investigated was more than two orders of magnitude lower (if observed) than that of the C_{11}-OH group;
3. the C_9-OH group was acetylated in the case of (*9R*)-stereoisomers only;
4. the C_9-OH and C_{11}-OH groups of $PGF_{2\beta}$ and (*15R*)-$PGF_{2\beta}$ were all acetylated at a significant rate (Table 3). Somewhat unexpectedly, these hydroxyl groups were acetylated without a strict order of the reaction: both of the monoacetate products were simultaneously detected in the reaction mixture. This was important for the evaluation of the modelling

results described in this work because it demonstrates that there is a competition between the C_9-OH and C_{11}-OH groups as nucleophiles in the acetylation.

5. The absolute configuration of the stereogenic centre at C_{15} influences the regioselectivity of the CALB-catalysed acetylation: for $PGF_{2\beta}$ (*15S*) the rate of acetylation of the C_9-OH group was twice higher than that of the C_{11}-OH group, while in the case of (*15R*)-$PGF_{2\beta}$ no preference towards a certain OH group was observed.
6. In the case of $PGF_{2\alpha}$ and (*15R*)-$PGF_{2\alpha}$ (Table 3) the absolute configuration at C_{15} influences the reaction velocity as well: the C_{11}-OH group of $PGF_{2\alpha}$ was acetylated at a rate thrice the rate of acetylation of its (*15R*) counterpart.
7. The C_{11}-OH group of 8-*iso*-$PGF_{2\alpha}$, which in comparison with $PGF_{2\alpha}$, has an opposite configuration at C_8, was acetylated at a rate approximately tenfold lower than $PGF_{2\alpha}$. Our previous results have shown the decisive role of interactions between the PG carboxyl group and the functional groups of the active site region of the CALB for the acetylation of the C_{15}-OH group of PGs. Consequently, both the steric and non-steric interactions may cause a lower rate of 8-*iso*-$PGF_{2\alpha}$ acetylation.



Scheme 11. The function-based subset (dark) used by Raza *et al.* which describes the core structural elements of the modelled transition state in the CALB. The dotted lines mark the essential H-bonds needed for the catalysis of the reaction.

The following modelling and experimental results are given in Table 4:

1) The total potential energy of the whole system.
2) The function-based subset energy.
Raza *et al*. defined a function-based subset to be the one that represents the core structural elements of the modelled transition state. This particular function-based subset was chosen for our work because it was a good predictor of the enantioselectivity of several secondary alcohols. Also, it uses non-minimised structures, which means that the computation time is significantly reduced. We used the system Raza *et al.* studied where they took the function-based subset to be that illustrated in Scheme 11.

3) An essential hydrogen bonds percentage. This number shows the percentage of the structures generated during the molecular dynamics simulation (last 50 ps) that have at least four hydrogen bonds between the enzyme and the substrate which are considered essential for the catalytic process (see Scheme 11; 2 for His 224, 1 for Gln 106 and 2 for Thr 40).
4) The *DEF ABS* value defines the deformation of the specified group of atoms by calculating the root mean square (RMS) deviation from the group's centre of mass during a molecular dynamics run.
5) Apparent experimental second-order rate constants (k_{II}) according to the kinetic curves of acetylation of the hydroxyl groups.

The MD results obtained for $PGF_{2\alpha}$ (**6**) presented in Table 4 show that the value of the essential hydrogen bond percentage is high for the tetrahedral intermediates constructed by docking PG to the enzyme over the C_{11}-OH as well as the C_{15}-OH group. However, in the case of docking via the C_{15}-OH group the *DEF ABS* value of 5.26 is indicative of the problems of fitting PG into the active site. This was also visually confirmed since the structures at the end of the molecular dynamics run have deformities (unravelling of α-helix near the active site) in comparison with the starting structure. Our experimental results[III] showed the acetylation of the C_{11}-OH group to be possible for all the PGs tested under proper conditions (vinyl acetate, $CHCl_3$, RT), while the C_{15}-OH group of 11-acetyl-$PGF_{2\alpha}$ was acetylated at a rate more than two orders of magnitude lower and the C_9-OH group (*S* configuration) was not acetylated to any detectable extent (according to ^{1}H NMR).

The high *DEF ABS* value and high essential hydrogen bond percentage obtained in the combination can be explained by the "fitting" of a relatively bulky PG into the active site during the molecular dynamics run. This caused the enzyme atoms (α-helix) to expand away from the active site while the tetrahedral intermediate in the active site kept the essential hydrogen bonds mostly intact. When the unfolding (a high *DEF ABS* value) took place, we also observed the "evaporation" of water molecules (water molecules near the unfolding site were leaving the system). In the case of other tetrahedral intermediates whose *DEF ABS* value was between 1.1 and 2.1, almost no "evaporation" of water molecules took place.

MD runs of $PGF_{2\beta}$ (**35**) show that while the C_{11}-OH group could be accessible for acetylation, the acetylation of the C_9-OH group, which has an *R* configuration, is evidently even more favourable because of the higher essential bond percentage value. The C_{15}-OH group of $PGF_{2\beta}$ acts in the same way as in the case of $PGF_{2\alpha}$ (**6**), having high essential hydrogen bond values, but, at the same time, too high a *DEF ABS* value.

Table 4. The characteristics found by means of the MD calculations for $PGF_{2\alpha}$ and its stereoisomers (the factors that limit the accessibility of the hydroxyl group are in bold).

Compound	Position of (docked) OH-group	Potential energy [a] [kJ/mol]	Energy of function-based subset [b] [kJ/mol]	Essential hydrogen bond % [b,c]	*DEF ABS*[d]	k_{II} experimental [min^{-1} $(g/ml)^{-1}$]
$PGF_{2\alpha}$ (6)	9	-22426	**-93.3**	**25.1**	1.35	n.o.[e]
	11	-22468	-100.8	72.9	1.41	0.31
	15	-22635	-102.5	74.2	**5.26**	< 0.003
8-*iso*-$PGF_{2\alpha}$ (33)	11	-22397	-103.4	63.8	1.58	0.022
$PGF_{2\beta}$ (35)	9	-22510	-102.9	81.4	1.81	0.19
	11	-22468	-98.3	56.3	1.37	0.095
	15	-22552	-105.0	90.1	**5.59**	n.o.[e]
(*15R*)-$PGF_{2\alpha}$ (39)	9	-22343	**-91.6**	**23.9**	1.18	n.o.[e]
	11	-22301	-100.4	78.6	1.14	0.09
	15	-22635	**-93.7**	76.5	1.63	n.o.[e]
(*15R*)-$PGF_{2\beta}$ (41)	9	-22669	-97.1	70.1	1.34	0.11
	11	-22259	-101.7	87.3	2.07	0.11
	15	-22887	-100.8	83.4	**2.57**	< 0.0011

a: a potential energy of the whole system, the mean value given for the last 50 ps of a molecular dynamics run;
b: see Scheme 11;
c: the percentage of the bonds between the substrate and the hydrogen bond donor groups, including the corresponding hydrogen atoms associated with the tetrahedral centre, namely, His 224:N_{ε}, Gln 106:N, Thr 40:N, and Thr 40:O_{γ};
d: RMS deviation of the system backbone atoms from their centre of mass, the mean value from the last 50 ps of molecular dynamics run;
e: the formation of the product was not observed

Comparing the results of molecular dynamics results obtained for the (*15R*)-$PGF_{2\alpha}$ (**39**) docked into the active site of CALB via the OH groups of different positions similarities to the acetylation of $PGF_{2\alpha}$ (**6**), regarding the behaviour of the C_9- and C_{11}-OH groups, can be observed. The tetrahedral intermediate obtained by docking PG (**39**) into the CALB active site via the C_{15}-OH group, however, differs from the tetrahedral intermediate corresponding to $PGF_{2\alpha}$ (**6**) that had a relatively high value of the essential hydrogen bonding percentage combined with the low *DEF ABS* value. The value of the function-based subset energy of the tetrahedral intermediate obtained by docking PG (**39**) via the C_{15}-OH group differs from that of the other energies, being higher, thus showing that the acetylation of the hydroxyl in this position is more complicated.

Both the C_9- and C_{11}-OH groups should be accessible for the acetylation in the case of (*15R*)-$PGF_{2\beta}$ (**41**). This is also analogous with experimental as well as molecular dynamics results obtained for $PGF_{2\beta}$. The results of molecular dynamics for the tetrahedral intermediate constructed by the docking of PG (**41**) into the active site via the C_{15}-OH group show a higher *DEF ABS* value that does not meet the criterion for the accessible OH group. This was consistent with experimental.

From the results presented in Table 4 it can be concluded that the C_{11}-OH groups of the PG isomers investigated should be accessible for the acetylation in all cases. Indeed, this is consistent with experimental results.

In the case of 8-*iso*-$PGF_{2\alpha}$ (**33**), which has a different configuration at C_8, the percentage of the essential hydrogen bond is somewhat lower than that of other compounds docked via the C_{11}-OH group, probably due to the more difficult fit of this isomer into the active site region.

From the results presented it can also be concluded that the C_9-OH group with the *R* configuration should be accessible to or even preferred by the CALB (in comparison with the C_{11}-OH group).

The accessibility of the C_9-OH group of PG stereoisomers to the CALB can be well predicted by the results of molecular dynamics. This is clearly seen by comparing the essential hydrogen bond percentage values which differ almost three times for the substrates bearing the C_9-OH group in the *R* and *S* configurations, respectively. Also, the function-based subset energies show the preference of the CALB to the *R* configured C_9-OH group.

Comparing the tetrahedral intermediates constructed by docking the PG stereoisomers into the active site via the C_{15}-OH group, we can see that all of them do not meet at least one of the criteria characterising the OH group accessible to the CALB. This is in good agreement with experimental: all of the C_{15}-OH groups were inaccessible or weakly accessible to the CALB.

The results of molecular modelling show that $PGF_{2\alpha}$ and its stereoisomers can fit into the active site of lipases (CALB) and undergo acetylation of the 11-OH and, in some cases, of the C_9-OH group as well. The influence of the stereochemistry of the C_{15}-OH group of PG on the selectivity of acetylation of the C_9- and C_{11}-OH groups is clearly evident from the experimental results.

The use of non-minimised structures and the function-based subset energy will provide us modelling results faster (there is no additional minimisation or selection of generated structures). A comparison of the values of the function-based subset energy with those of the *DEF ABS* and the essential hydrogen bonding percentage of different tetrahedral intermediates gave us the criteria necessary to describe the possibility of acetylation of PGs by the CALB.

The evaluation of the possibility of acetylation of an OH group of prostaglandin should be started by using the *DEF ABS* values. The OH groups accessible to the CALB were characterised by the *DEF ABS* value of up to 2.07 calculated for the corresponding tetrahedral intermediate. The evaluation has to be continued by comparing the function-based subset energy levels. The OH groups accessible to the CALB were characterised by the levels of not higher

than -97.1 kJ/mol calculated for the corresponding tetrahedral intermediates. An additional criterion for the evaluation of the possibility of acetylation is “the essential hydrogen bond percentage” value. The OH-groups accessible to the CALB were characterised by the values of not lower than 56.3% calculated for the corresponding tetrahedral intermediates. If any of the above three criteria was not met, then the acetylation of the corresponding hydroxyl group did not take place or proceeded at a very low rate.

When comparing the results of the modelling with experimental data the following conclusions can be made:

1. the C_{11}-OH group of prostaglandins in a native *R* configuration is always accessible for acetylation;
2. both the experiments and molecular modelling demonstrated well that the C_9-OH group is acetylated only when the stereogenic centre at C_9 has the *R* configuration;
3. the stereochemistry of the C_{15}-OH group influences the ratio of the acetylation products in the case of $PGF_{2\beta}$ (*15S*) and (*15R*)-$PGF_{2\beta}$. The acetylation of $PGF_{2\beta}$ gives 9- and 11-monoacetates at a ratio of 2:1, respectively, while (*15R*)-$PGF_{2\beta}$ shows no preference towards a certain OH group; in both cases the 9,11-diacetate is also formed;
4. experimental results showed that the C_{15}-OH groups are either inaccessible or weakly accessible. Modelling results were consistent with this finding. In all cases the tetrahedral intermediate of the compound docked via the C_{15}-OH group had either too high a value of the *DEF ABS* or of the function-based subset energy.

3.2.6. The modelling of the CALB-catalysed acetylation of PGE_1 and PGE_2

The characteristics calculated upon the molecular dynamics simulations for the OH-groups of PGE_1 and PGE_2 (Table 5) were in satisfactory accordance with the above criteria as well as with the experimental results[III,IV]. Only very slight differences in the values of function-based subset energies were observed. This proves that the method for prognosticating the accessibility of PG OH groups presented by us (3.2.5.) works quite well also in the case of other prostaglandins.

Table 5. The characteristics of a PGE_1 and PGE_2 found by means of MD calculations.

SUBSTANCE	Position of (docked) OH-group	Potential energy,[a] [kJ/mol]	Energy of function-based subset[b], [kJ/mol]	Essential hydrogen bond %[b, c]	*DEF ABS*[d]	Possibility of acetylation reaction
PGE_1	11	-22 298	-96.0	84.9	1.74	yes[f]
	15	-22 246	-99.3	92.1	**3.57**	no[f]
PGE_2	11	-22 656	-95.6	65.1	1.79	yes[g]
	15	-22 431	**-83.3**	**24.6**	1.23	no[g]

a-d: see Table 4

f: the acetylation of the C_{11}-OH group of PGE_1 is possible, the C_{15}-OH group was not acetylated[IV];

g: the acetylation of the C_{11}-OH group of PGE_2 is possible and proceeds at a lower rate than that of $PGF_{2\alpha}$, the C_{15}-OH group of PGE_2 was acetylated only after the acetylation of the C_{11}-OH group and therefore the results of the calculations are not relevant to the experimentally observed 15-acetylation that occurred at a rate more than ten times slower than that of the C_{11}-OH group[III].

3.3. The lipase-catalysed alcoholysis of prostaglandins

3.3.1. The lipase-catalysed esterification of prostaglandins

The esterification of prostanoids of type F has been usually performed by using a conventional protocol involving the acid-catalytic treatment of PG in alcohol, while acid-sensitive prostanoids of type E have been methylated preferably by using treatment with a harmful diazomethane[108]. Consequently, the lipase-catalytic, simple to handle, clean and high-yielding methodology for esterification of prostanoids proposed herein offers an attractive alternative.

Under the proper conditions PGs undergo the CALB-catalysed esterification of the carboxyl group (Scheme 12 ($PGF_{2\alpha}$) and Scheme 13 (PGE_2)).

OH COOH HO OH **6** → CALB CH_3OH C_6H_6 → OH $COOCH_3$ HO OH **30**

Scheme 12. The lipase-catalysed esterification of $PGF_{2\alpha}$ (**6**)(50 mg of PG; C_6H_6 (3 ml)/CH_3OH (0.5 ml), 500 mg Novozym 435, 24 hrs, 99% yield of (**30**)).

Scheme 13 structures: 5 → 22 (CALB, CH_3OH, C_6H_6); labels: O, COOH, HO, OH, COOCH3

Scheme 13. The lipase-catalysed esterification of PGE_2 (**5**)(50 mg of PG; C_6H_6 (3 ml)/CH_3OH (0.5 ml), 500 mg Novozym 435, 24 hrs, 99% yield of (**22**)).

Table 6. Investigation of the kinetics of the lipase-catalysed esterification of PGE_2 and $PGF_{2\alpha}$.

Compound	PG [mg; µmol]	Novozym 435 [mg]	$CDCl_3$ [ml]	CH_3OH [µl; mmol]	Time [h]	Conversion of PG [%]	k_{II} ± SE [min^{-1} (g /ml)$^{-1}$][a]
PGE_2	6; 17.0	100	1.25	30; 0.74	13.3	80	0.02275±0.001
$PGF_{2\alpha}$	6; 16.9	100	1.25	30; 0.74	14.2	95	0.06375±0.003

a: the apparent second-order rate constants (per g/ml of Novozym 435) calculated for the esterification

The synthesis of methyl esters $PGF_{2\alpha}$ and PGE_2 catalysed by the CALB was monitored by using ^1H NMR spectroscopy in order to get a quantitative estimation of the velocities of the reactions occurring in the system. The apparent second-order rate constants describing this reaction were calculated using kinetic curves obtained from experimental results.

The values of the apparent second-order rate constants of the CALB-catalysed methylation of $PGF_{2\alpha}$ and PGE_2 differed 2.5 times (Table 6).

3.3.2. The lipase-catalysed alcoholysis of 11-acetyl-PGF$_{2\alpha}$

Usually, the lipase-catalysed reactions are reversible: a certain OH-group of a molecule, if acylated by a lipase, becomes a constituent of an ester which should be a substrate for the same lipase. However, the change in the molecular structure obtained by the acylation that leads to the change in the mode of the molecular recognition of complex molecules bearing more than one functional group by a lipase, may lead to the formation of unexpected products upon alcoholysis instead of simple reversibility.

In the case of the lipase-catalysed methanolysis of the 11-acetyl-$PGF_{2\alpha}$ (**28**) (Scheme 14), only trace amounts of deacetylated products ($PGF_{2\alpha}$ (**6**) and $PGF_{2\alpha}$ methyl ester (**30**)) were detected at a low (2%) and high (100%) concentrations of MeOH in the reaction medium, respectively. The reaction occurring CALB-catalytically with acetate (**28**) at any concentration of MeOH in benzene was the methyl ester synthesis affording the product (**31**). The TLL was able to catalyse the same reaction only at a low concentration of MeOH (2%). The

difference between the results obtained by using the CALB and TLL in organic solvent media is in accordance with those published earlier[109].

Lipase

28 R_1=R_3= H, R_2= Ac, R_4=H
45 R_1=R_2=R_3= Ac, R_4=CH_3

31 R_1=R_3= H, R_2= Ac, R_4=CH_3
30 R_1=R_2=R_3= H, R_4=CH_3
6 R_1=R_2=R_3= H, R_4=H
46 R_1=R_3= Ac, R_2=H, R_4=CH_3
47 R_1=R_3= Ac, R_2=H, R_4=H

Scheme 14. The lipase-catalysed methanolysis of $PGF_{2\alpha}$ derivatives.

The deacetylation of 9,11,15-triacetyl-$PGF_{2\alpha}$ methyl ester (**45**) catalysed by the CALB afforded 9,15-diacetyl-$PGF_{2\alpha}$ methyl ester (**46**) at any concentration of the methanol tested. The CALB-catalysed hydrolysis of triacetate methyl ester (**45**) gave 9,15-diacetyl-$PGF_{2\alpha}$ (**47**) as an almost pure compound. The 11-acetyl group of triacetate (**45**) was not cleaved by the TLL at any concentration of MeOH in benzene.

3.3.3. The lipase-catalysed alcoholysis of 11-acetyl-PGE_2

The deacetylation of 11-acetyl-PGE_2 and its esters (Scheme 15) was performed using the CALB in a 2% solution (in benzene) of a proper nucleophile which does not cause any change in the status of the PG carboxyl group. The reaction rate was low and, depending on the nucleophile used, different. The degree of formation of PGA_2 (and esters, respectively) is practically the same in these systems confirming the spontaneous nature of the elimination reaction occurring in these cases.

CALB (Novozym 435)
1. C_6H_6/ 2 % H_2O
2. C_6H_6/ 2 % MeOH
3. C_6H_6/ 2 % EtOH

18 R=H
23 R=Me
48 R=Et

	R=H	R=Me	R=Et
5 R=H, **22** R=Me, **49** R=Et	64%	55%	48%
50 R=H, **51** R=Me, **52** R=Et	23%	23%	21%

Scheme 15. The lipase-catalysed deacetylation of PGE_2 derivatives (the reaction volume was 6 ml; the amount of the substrate ~15 mg; the reaction time 257 hrs; the amount of the enzyme 750 mg; the content of the compound (%) in the crude product is indicated).

Our preliminary results suggested that there is a dramatic relationship between the MeOH concentration in the reaction medium and the character of the TLL-catalysed methanolysis of 11-acetyl-PGE_2 (**18**). We proved that in the TLL-catalysed methanolysis carried out at a 2% MeOH concentration in benzene acetate (**18**) leads to the corresponding ester (**23**). Performing the same process in a neat MeOH gave rapid access to PGA_2 (**50**)(Scheme 16).

Scheme 16. The lipase-catalysed methanolysis of 11-acetyl-PGE_2 (**18**)(the reaction volume 4 ml; the amount of the substrate 15 mg; the reaction time 36 hrs).

The relationship between the catalytic properties of Lipolase 100T (TLL) and the concentration of methanol in benzene used as a reaction medium was investigated (Scheme 16). Our preliminary results showed that the esterification occurs at a 2%, the TLL was inactive at a 25 and 50% concentration of methanol in benzene and the formation of PGA_2 takes place at a MeOH concentration of 75 and 100%. As a next step of investigation, several synthetic trials were performed at different MeOH concentrations with a 2% step on the MeOH concentration scale between 2 and 22%. The critical concentration range was found to be between 18 and 22%. Between these concentrations a dramatic decrease in the catalytic activity of TLL was observed.

The RML (SP524) known to be similar to the TLL also exposed rather a high eliminating activity when used with acetate (**18**) in a neat MeOH. This lipase was a lyophilised powder, thus providing an additional confirmation of the absence of a crucial influence of the resin (used for immobilising the TLL in the Lipolase 100T) on the elimination.

In conclusion, in the case of the TLL-catalysed methanolysis of 11-acetyl-PGE_2 (**18**) a dramatic relationship between the catalytic performance of TLL and the MeOH concentration in the reaction medium was observed. The switch

of the reaction centre as well as the type was observed with varying MeOH concentration in the reaction medium.

3.3.4. The lipase-catalysed alcoholysis of 11-acetyl-PGE$_1$

The investigation of the TLL-catalysed methanolysis of 11-acetyl-PGE$_1$ (**18**) was undertaken (Scheme 17). Somewhat unexpectedly, the incubation of the acetate (**18**) with Lipolase 100T (TLL) either in a neat MeOH or in the mixture of MeOH (75%) in benzene did not give any remarkable access to PGA$_1$. Only an almost constant trace amount of PGA$_1$ was detected in the incubation mixtures.

TLL (Lipolase 100T), MeOH (2-50%) C_6H_6 → **53** (COOCH$_3$, OH) conv. >90%

26 11-acetyl-PGE$_1$ (COOH, OH)

TLL (Lipolase 100T), MeOH (75%) C_6H_6 → TLL is almost inactive (Trace amounts of unidentified products were formed)

TLL (Lipolase 100T), MeOH (100%) → TLL is almost inactive (Trace amounts of 11-acetyl PGE$_1$ methyl ester was formed)

Scheme 17. Methanolysis of 11-acetyl-PGE$_1$ (**18**)(the reaction conditions were the same as for 11-acetyl-PGE$_2$ (**10**) in Scheme 16).

In order to compare the esterifying activity of the TLL to 11-acetyl-PGE$_2$ (**18**) and 11-acetyl-PGE$_1$ (**26**), the latter compound was also incubated with the TLL in solvent mixtures containing 10-22% (by 2% steps) of MeOH in benzene. In this case no decrease in the catalytic activity of TLL was observed at these concentrations. The synthesis of 11-acetyl-PGE$_1$ methyl ester (**53**) was also observed at a 50% concentration of MeOH in C_6H_6. As noted, at MeOH concentrations of 75 and 100% the TLL appeared to be almost inactive towards the substrate (**26**).

4. SUMMARY

The lipase-catalysed transesterification and alcoholytic reactions of prostaglandins and their derivatives occurring in low-water media were explored.

Methods for a highly regioselective acylation of the C_{11}-OH group of $PGF_{2\alpha}$, PGE$_2$ and PGE$_1$ have been described. These methods have already made a significant contribution to the improvement of the preparative semisynthesis of PGD$_2$, PGA$_2$ and PGA$_1$, respectively.

A method of NMR spectroscopic monitoring of lipase-catalysed reactions in order to estimate the reaction velocities was elaborated.

An important task of the work was to elaborate an approach for testing the reactivity of the hydroxyl groups of prostaglandins in the lipase-catalysed acetylation. This investigation was performed on isomeric prostaglandins of

type F and the *Candida antarctica* lipase B. In parallel, the kinetics (using NMR) and molecular dynamics simulation were investigated. This work resulted in the criteria for a theoretical evaluation of the reactivity of a certain hydroxyl group.

With regard to the contribution to a better understanding of prostaglandin metabolism in organisms some features have been drawn out.

The best example of the versatility of lipase-catalysed reactions is illustrated in Scheme 18, where 11-acetyl-PGE_2 (**18**) is shown to undergo either:

1) the hydrolysis to PGE_2 (**5**);
2) further acetylation of the C_{15}-OH to afford 11,15-diacetyl-PGE_2 (**19**);
3) the esterification of the carboxyl group to afford 11-acetyl-PGE_2 methyl ester (**23**);
4) the elimination to afford PGA_2 (**50**).

Scheme 18. The lipase-catalysed reactions of 11-acetyl-PGE_2 (**18**).

It should be especially emphasised that the change of the methanol concentration in the reaction medium can steer the TLL-catalysed reaction either to the esterification or elimination of acetic acid from the acetyl-β-ketol moiety of 11-acetyl-PGE_2 (**18**).

The 11-acetylated derivatives of prostanoids of type F as well as 15-acetylated prostaglandins of type A and their esters have been the most prevailing components of the prostanoid fractions isolated from the corals (*Plexaura homomalla*, *Lobophyton depressum*)[31,35,37,38]. These natural derivatives of prostanoids have been shown above to be easily gained upon one to three lipase-catalytic reactions starting from $PGF_{2\alpha}$ and PGE_2, respectively.

5. CONCLUSIONS

The lipase-catalysed reactions of prostaglandins were investigated. Structurally similar PGs were found to behave very differently in lipase-catalysed reactions. The choice of the lipase and the acyl-donor can change the stereo- and regioselectivity of the acetylation of prostanoids. In regard of lipase-catalysed alcoholytic reactions, the effects of alcohol concentration on the catalytic performance of lipases in organic media were found to be dramatic, involving in some cases even a switch of the centre and the type of reaction. However, the latter results should be treated as preliminary and need further confirmation using other methods.

The following conclusions are drawn from the work:

1. The acylation of the 11-hydroxyl group of $PGF_{2\alpha}$ and PGE_1 as well as that of (+)-cloprostenol with vinyl acetate catalysed by the CALB (Novozym 435) were found to occur highly chemo- and regioselectively.
2. (-)-Cloprostenol did not give any detectable access to the 11-acetyl-derivative upon treatment with vinyl acetate catalysed by the CALB in chloroform showing the enantiospecificity of the process.
3. The rate of acylation of the C_{15}-OH group of PG was found to depend drastically on negligible differences in prostanoid structure, the structure of the acyl group to be transferred, and the enzyme used.
4. The apparent second-order rate constants of acetylation of the C_{11}-OH group of prostaglandins investigated differed by up to two orders of magnitude.
5. The contribution of the carboxyl group of prostaglandins vs. that of the ester group to acetylation velocity was 4/1. The effect of the C_9-OH group vs. that of the C_9-oxo group on the velocity of the acetylation of the C_{11}-OH group was determined to be as high as 20/1. Consequently, the carboxyl group as well as the $C_9\alpha$-hydroxyl group participate in the interactions important for the molecular recognition and binding of prostaglandins by the acetyl-CALB.
6. The C_{11}-OH group of $PGF_{2\alpha}$ stereoisomers in a native configuration is always accessible for acetylation.
7. Both the experiments and molecular modelling demonstrated that the C_9-OH group is acetylated only when the stereogenic centre at C_9 has the *R* configuration.
8. The absolute configuration of the stereogenic centre at C_{15} influences the regioselectivity of the CALB-catalysed acetylation: for $PGF_{2\beta}$ (*15S*) the rate of acetylation of the C_9-OH group was twice higher than that of the C_{11}-OH group, while in the case of (*15R*)-$PGF_{2\beta}$ no preference towards a certain OH group was observed.
9. Experimental results showed that the C_{15}-OH groups are either inaccessible or weakly accessible. Modelling results were consistent with this

finding. In all cases the tetrahedral intermediate of the compound docked via the C_{15}-OH group had either too high a value of the *DEF ABS* or of the function-based subset energy.

10. The CALB-catalysed synthesis of methyl esters of PGE_2 and $PGF_{2\alpha}$ occurred at comparable rates, showing a similar accessibility of the carboxyl groups of these prostaglandins to the active site of the CALB.
11. The lipase-catalysed methanolysis of 11-acetyl-PGE_2 using TLL afforded 11-acetyl-PGE_2 methyl ester at a low concentration of methanol (up to c=22%) in benzene. At higher methanol concentrations (75 and 100%) the same system produced exclusively PGA_2, while no reaction took place at intermediate concentrations of methanol (25 and 50%). In the case of 11-acetyl-PGE_1, no remarkable elimination affording PGA_1 was observed under the same conditions. The switch of the reaction type as well as the reaction centre was observed with varying alcohol concentration in the low-water reaction medium.

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// ABSTRACT

In this thesis, the lipase-catalysed reactions of several prostanoids in low-water media were investigated.

Synthetic, NMR spectroscopic kinetic and molecular dynamics simulation investigations were performed.

The lipase-catalysed reactions of prostanoids in low-water media often occur with high stereo-, regio- and chemoselectivity under mild conditions.

The velocity and regioselectivity of lipase-catalysed reactions were found to depend on the relatively small changes in the prostanoid structure, and on the choice of the lipase and the acyl donor. Also, the reaction medium had a great effect on the results of the lipase-catalysed reactions of prostaglandins. By changing the medium it is possible to steer these reactions in an appropriate direction.

The modelling investigation of tetrahedral intermediates led to characteristics that were further adjusted by the experimental data to give quantitative criteria which allowed classification of the hydroxyl groups of prostaglandin stereoisomers as those which are accessible or inaccessible to the *Candida antarctica* lipase B.

KOKKUVÕTE

Antud töös uuriti prostaglandiinide lipaas-katalüütilisi reaktsioone, mis kulgevad vähese veesisaldusega keskkonnas.

Viidi läbi sünteetilised, TMR spektroskoopilised ja molekulaar-modelleerimisuuringud.

Prostaglandiinide lipaas-katalüütilised reaktsioonid kulgevad vähese veesisaldusega keskkondades ’pehmetes’ tingimustes sageli kõrge stereo-, regio- ja kemoselektiivsusega.

Leiti, et prostanoidide lipaas-katalüütiliste reaktsioonide kiirust ning regioselektiivsust mõjutavad suhteliselt väikesed muutused prostanoidide struktuuris, kasutatav lipaas ning atsüüli doonor.

Otsustavat rolli prostaglandiinide lipaas-katalüütiliste reaktsioonide kiiruste ning selektiivsuste määrajana mängib reaktsioonikeskkond. Reaktsiooni-keskkonna koostist muutes on reeglina võimalik määrata prostaglandiinide lipaas-katalüütiliste reaktsioonide iseloom ning juhtida neid seega soovitud produktide tekke suunas.

Uuriti F-tüüpi prostaglandiinide hüdroksüülrühmade CALB-katalüütilist atsetüleerimist molekulaardünaamika meetodite abil. Paralleelselt teostati samade ühendite CALB-katalüütilise atsetüleerimisreaktsiooni kiiruse ja regio-selektiivsuse määramine tuumamagnetresonants-spektroskoopilisel meetodil. Tetraeedriliste vaheühendite modelleerimisuuringuga saadud karakteristikute hindamisel katseandmete alusel leiti oletatavad kvantitatiivsed kriteeriumid, mis loodetavasti võimaldavad testida erinevate prostaglandiinide hüdroksüül-rühmade reaktiivsust *Candida antarctica* lipaas B poolt katalüüsitavates atsetüleerimisreaktsioonides.

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Biokatalüütiliste enantioselektiivsete ja stereoselektiivsete sünteesimeetodite ning neil baseeruvate kemoensümaatiliste protsesside väljatöötamine.

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List of publications

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