Abstract

Chemical and biosensors serve as analytical tools that use a recognition element as the primary sensing component coupled with a transducer to obtain analytical measurements in a convenient and user-friendly format. Synthetic molecularly imprinted polymer receptors, or MIPs, have emerged as cost-effective and stable alternatives to biological or chemical receptors. The research group of the Laboratory of Biofunctional Materials at TalTech has developed numerous MIP-based electrochemical sensors for the specific and selective detection of clinically relevant proteins, such as viral protein of SARS-CoV-2 and neurotrophic factor as BDNF. However, while most MIP technology studies focus on detecting large protein molecules, there is limited research on using short peptide sequences, which offer advantages such as lower cost, simpler structure, and greater availability compared to proteins. Therefore, the development and optimization of peptide MIP-based sensors are essential to enhance the versatility of MIP technology for detecting various targets, including proteins, and peptides.

The goal of this thesis was to study the molecular imprinting of the ACE2 peptide fragment - peptide QAKTFLDKFNHEAEDLFYQ (AM194) - to develop a peptide-selective MIP-based electrochemical sensor. This peptide, containing a sequence of 19 amino acids, was used as a model target template to prepare AM194-selective MIP (AM194 sensor). The length of AM194 was expected to be an appropriate model for synthesizing AM194-MIP film by the electrochemical surface imprinting approach. Although this imprinting approach has been used exclusively for proteins, this thesis tested its applicability for imprinting peptides of relevant lengths, highlighting the novelty of this research. It is also important to note that this study is the first to report on the molecular imprinting of the peptide sequence AM194. Initially, the appropriate functional monomer, dopamine, was selected using molecular docking modeling method. After subsequent optimization of the electrodeposition conditions, it was found that the electrodeposition of 5 mM dopamine in PBS solution by galvanostatic method at a current of 500 µA for 5.6 seconds corresponding to charge density of 5 mC/cm^2 allowed to obtain AM194-MIP with enhanced affinity to AM194. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) electroanalytical techniques were used to characterize all stages of AM194-MIP formation. The responses of the prepared AM194 sensor to the target oligopeptide at the concentration range of 1 to 100 pg/mL were determined by DPV measurements in the presence of redox probe solution. AM194 sensor demonstrated the capability to detect AM194 with a linear concentration range of 50-500 pg/mL, limit of detection of 24 fg/ml and a limit of quantitation 72 fg/ml in PBS. Additionally, it exhibited significantly higher selectivity towards AM194 compared to other peptide analogues. The obtained results lay the groundwork for future research aimed at improving peptide MIP-based sensors, which will significantly contribute to the development of cost-effective and rapid analytical tools for clinical diagnostics and environmental monitoring.

The topic is related to multiple disciplines, including electrochemistry, sensor technology, peptide detection, and molecular imprinting technology. The study was carried out in the Laboratory of Biofunctional Materials at the Department of Materials and Environmental Technology at TalTech.