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PhD dissertation summary

Studies on the nature of nucleic acids – analyte interactions in recognition layers of biosensors

Nucleic acids (NA) play a fundamental role in the biological processes including cell division and apoptosis, as well as protein expression. However, currently they are not concerned just as the genetic information storage units, since they can exhibit catalytic or receptor features likewise. Nucleic acids bind primarily to the complementary nucleotide sequences, but can also interact with molecules such as metal cations, amino acids, proteins and bacteria cells. Moreover, nucleic acids constitute a material, which is sensitive to the degradation caused by specific enzymes and other chemical agents. Furthermore, hybridized DNA provides the platform for the efficient charge transfer along its double - strand. For all those reasons, nucleic acids are contemporary intensively exploited for the preparation of the recognition layers of biosensors.

This PhD thesis, presented as a coherent collection of publications, focuses on the studies on the modes of interactions between nucleic acids and analytes occurring in receptor layers of electrochemical sensors.

The first project referred to the development of an oligonucleotide-based sensor for determination of lead ions. The studies indicated that by the selection of a specific aptamer sequence – herein a thrombin binding aptamer probe - as a recognition layer, Pb^{2+} ions can be detected with high selectivity. The pronounced affinity of aptamer-based monolayer to lead ions was attributed to the formation of G-quadruplex structure stabilized by Pb^{2+} ions, which resulted in the rearrangement of the receptor layer at the gold electrode surface. The binding of aptamer to lead ions was analyzed by the use of voltammetric and impedimetric techniques with the application of methylene blue and ferri/ferrocyanide redox indicators. On the basis of electrochemical studies the working parameters of aptasensor were defined and the feasibility of its use for the analysis of Pb^{2+} cations in real sample was tested.

In the second project the influence of Cr(VI) on the stability of dsDNA deposited on Au electrode was examined. For that purpose the DNA immobilization technique along with the experimental conditions such as pH and incubation time were adjusted. It was shown that chromium(VI) induced a pronounced DNA damage and its overall effect was quantified in the presence redox indicator – methylene blue. Moreover, the degree of the DNA cleavage caused

by Cr(VI) was altered by the addition of ascorbic acid and hydrogen peroxide to the degrading solution.

Another project was dedicated to the elaboration of aptamer-based sensor for detection of potassium ions. As K^+ ions can also stabilize G-quadruplex structure, three DNA aptamer strands, which varied in length and number of guanine bases, were employed for the preparation of recognition layer. The electrochemical experiments enabled the selection of an electroactive indicator, which evidenced the specific interaction between recognition layer and potassium ions. The studies revealed that the thrombin binding aptamer exhibited the strongest binding to K^+ cations in comparison to other tested DNA sequences. Based on voltammetric data, the working parameters of the aptasensor were determined. The results also indicated that the sensor selectivity should be improved so that it could be considered as an alternative tool for the potassium detection in clinical diagnostics.

Next project was focused on the elaboration of DNA aptamer-based sensor for detection of dopamine. High affinity of aptamer probe towards dopamine led to the accumulation of this neurotransmitter in the close proximity to the electrode surface. Thus, a direct measurement of dopamine oxidation current was performed. To further enhance the working parameters of the proposed sensor, an intermediate layer between aptamer probe and glassy carbon electrode was introduced, which consisted of reduced graphene oxide and gold nanoparticles. The resulting aptamer-based platform allowed the selective detection of dopamine within the micromolar range of concentration.

The last project was dedicated to the development of an aptamer-based assay for cancer biomarker - urokinase plasminogen activator (uPA). For this purpose, an RNA aptamer was attached to gold surface *via* phosphorothioated deoxyadenine tail, which was bound to aptamer 3' end. The experiments revealed a significant influence of the type of the redox indicator used for the electrochemical studies, as the recognition layer was subjected to a potential-induced rearrangement. The high affinity of aptamer towards uPA was evidenced in the presence of methylene blue electroactive molecule and the degree of binding was enhanced upon addition of bovine serum albumin to the target protein solution.

The studies showed a broad range of possible applications of nucleic acid - based recognition layers. It was indicated that several issues should be considered while the design of the nucleic acid-based sensors, namely the choice a nucleic acid sequence, method of nucleic acid immobilization on the solid surface as well as the manner of generation of the analytical signal.

To conclude, the main advantages of the elaborated nucleic acid-based sensors include the simplicity of fabrication, ease of operation and distinctive working parameters. Hence NA - based biosensors could serve as alternative tools in areas such as environmental and clinical analysis.