University of Bialystok

Faculty of Biology and Chrmistry

mgr Anna Sankiewicz

A summary of doctoral thesis

Development and analytical application of Surface Plasmon Resonance Imaging biosensors.

Recently the surface plasmon resonance (SPR) operating jointly with specific biosensors has been a widely applied technique for detection of molecular species of biological interest.

The first stage of the work was to develop the details of the construction of the biosensor basis, so that it would be useful for detection of biomolecules in many samples at the same time. To prevent mixing the samples, a special mask was used and an array with different number of measuring points for samples was prepared.

Two different immobilization methods of receptor were applied (covalent bonds, hydrophobic interaction). Depending on the strategy of immobilization, the various types of thiol to modify the gold on the chip were used.

Antibodies, natural and/or synthetic inhibitors as a bio-recognition element of biosensor were used. Thereby, various chemical interactions were used to capture the substance to be determined (antibody-antigen, inhibitor-enzyme).

During the construction of the biosensors, the creation of subsequent layers (gold, thiol and receptor layer) on the its surface was monitored by atomic force microscope (AFM) or by scanning electron microscope (SEM).

The important part of this work was the development of a method for regeneration of biosensors, which allowed reducing the cost of the analysis as well as omitting certain steps in the preparation of the sensor and saving time.

In general, seven analytical procedures for the diagnosis of useful molecules were developed using newly developed biosensors. Biosensors for determination of selected elements of ubiquitin-proteasome pathway (immunoproteasom, ubiquitin carboxy-terminal hydrolase L1-UCH-L1) and for detecting some substances of extracellular matrix (laminin-5, collagen IV and fibronectin) were developed.

The development of new analytical methods with application of constructed biosensors was proceeded in the following stages:

- selecting the appropriate receptor that could bind the tested biomolecules from the sample selectively
- choosing the immobilization strategy of receptor and appropriate thiol
- determination of optimum concentration of receptor on the biosensor surface,
- preparing the calibration curve to quantification of analyte in the samples,
- determination of selectivity,
- determination of precision and accuracy of the method,
- determination of detection and quantification limits,
- application of developed methods for the determination of selected biomolecules in various natural samples.
- validation of the newly developed methods by the comparison of the measured concentrations of each of the substances in the same samples by using SPRI biosensors with the results obtained by using ELISA test.

Based on the results obtained, each developed biosensor co-operating with SPR imaging may be a suitable tool for determination of concentration of biomolecules having potential diagnostic significance in different samples (blood plasma, serum, cerebrospinal fluid). The developed methods can be used in laboratory diagnostics as the competitive methods to existing techniques .

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Sawicwin Anna