

Summary

The main aim of the study presented in the thesis was to develop new, label-free and rapid analytical methods to measure the concentration of the selected proteases in natural samples, which can be used as competitive methods for ELISA test.

Three new SPRI biosensors were constructed for quantification of proteases: MMP-1, MMP-2, cathepsin L. The development of new biosensors sensitive to these proteases consisted of the following stages:

1. choosing the appropriate tiol,
2. selecting the appropriate receptor, which could bind the tested enzyme from the sample and determining its optimum concentration on the biosensor surface,
3. preparation of the calibration curve by which the concentration of this enzyme was determined in the samples,
4. for a cathepsin L- sensitive biosensor, determining the pH of the solution in which it exhibits optimal activity against the inhibitor and most effectively it binds on the biosensor surface,
5. determination of selectivity,
6. determination of precision and accuracy of the method,
7. determination of detection and quantification limit,
8. measurement of concentration of each enzyme in a variety of natural samples.

During the construction of the individual biosensors, the formation of subsequent layers of compounds on its surface was monitored by atomic force microscope (AFM, for MMP-1 and MMP-2) or by scanning electron microscope (SEM, for cathepsin L).

A very important element in the validation process of the newly developed methods/ biosensors was the comparison of the determined concentrations of each of the enzymes in various natural samples by using SPRI biosensors with results obtained by using ELISA test. The comparison confirmed the good correlation between these methods, what makes it possible to use these SPRI biosensors in laboratory diagnostics as a competitive method.

The measurements of cathepsin L enzymatic activity were also shown in the study and confirmed the tendency of changes in cathepsin L amount in natural samples, which was also determined by the SPRI biosensor.

In order to confirm the diagnostic utility of SPRI biosensors sensitive to such proteases as cathepsins B, D and cathepsin B inhibitor- cystatin C, a number of measurements of the concentration of these enzymes in various natural samples (plasma, serum, urine) were carried out.

The results of these studies have confirmed the possibility of measuring the concentration of these enzymes in a disease control and monitoring of patients having had inflammation, treatments or injuries.