ABSTRACT

Phosphorylation is one of the most common of all post-translational modifications of proteins and has been found in nearly all cellular processes. Abnormal phosphorylation is associated with many serious human diseases. One of the approaches used for the identification of protein phosphorylation sites is based on the application phosphatases and the comparison of MS analysis of samples before and after the sample treatment with the enzyme. The use of phosphatase immobilized to magnetic carriers is advantageous in comparison with the application of soluble enzyme: e.g. easy manipulation of samples, an increase of enzyme stability and a possibility of repeated use of immobilized enzyme.

Investigation of properties of enzyme reactor – bovine alkaline phosphatase from intestinal mucosa immobilized to magnetic particles is a subject of this Bachelor Thesis. The enzyme was coupled to cellulose magnetic particles after activation with divinyl sulfone via the protein free amino groups. p-Nitrophenylphosphate was used as a substrate for the phosphatase activity determination.

The effect of different conditions on the activity of soluble and immobilized forms of alkaline phosphatase was compared: the effect of pH and Mg^{2+} ions, storage stability and thermostability and possibility of repeated use of the immobilized enzyme.

Keywords:

- enzyme reactor
- properties of alkaline phosphatase
- magnetic particles
- immobilization of enzymes
- dephosphorylation