Immobilization of enzymes on the biogenic magnetite for biological applications

Michaela Pecova[1], Marek Sebela[1], Katerina Polakova[2], Zdenka Markova[2], Fabio Vianello[3], Klara Safarova[2] and Radek Zboril[2]

[1]Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Protein Biochemistry and Proteomics, Faculty of Science, Palacky University, Slechtitelu 11, CZ-783 71 Olomouc, Czech Republic; [2]Regional Centre of Advanced Technologies and Materials, [Department of Physical Chemistry and Department of Experimental Physics], Faculty of Science, Palacky University, Slechtitelu 11, 783 71 Olomouc, Czech Republic; [3]Department of Biological Chemistry, University of Padua, Viale G. Colombo 3, 351 21 Padua, Italy
Contact: pecovamichaela@seznam.cz

Superparamagnetic nanoparticles of iron oxides with a suitable surface modification have proven useful in various biological applications including immobilization of biocomponents, magnetic resonance imaging, anticancer therapy using hyperthermia, magnetically guided site-specific drug delivery, cell labeling, immunoassays, bioseparation, purification of nucleic acids and so on. In order to become an appropriate biomaterial, magnetic nanoparticles need to be stabilized to avoid agglomeration[1]. Such a stabilization may reside in coating with polymeric substances (chitosan and its derivates, PEG, PVA, dextran, alginate etc.) resulting in biocompatibility, biodegradability and nontoxicity. These coating materials contain active groups which can bind bioparticles or biomolecules such as whole cells systems, proteins (including enzymes), hormones or drugs. Magnetotactic bacteria are interesting microorganisms that are able to produce by biomineralization membrane-enveloped crystals of magnetite called magnetosomes. Magnetic nanoparticles isolated from *Magnetospirillum gryphiswaldense* can be used as an excellent solid support for carrying immobilized enzymes[2]. After immobilization, the enzymes display improved properties like e.g. increased resistance to temperature, denaturants or organic solvents. Their stability is enhanced, which results in the possibility of repeated use. Whereas immobilized oxidoreductases are advantageous for constructing biosensors, immobilized proteases are attractive from the point of view of proteomics.

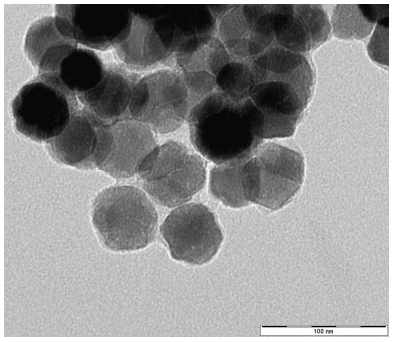
In this work, the biogenic magnetite nanoparticles from *M. gryphiswaldense* were used as a carrier for the immobilization of oxidoreductases (amine oxidase, peroxidase, sulfite oxidase) and proteases (trypsin, prolyl endoprotease). First, the material was covered by chitosan which provides appropriate hydroxy and amino groups for enzyme immobilization. Then the enzymes were attached to the surface using glutaraldehyde as a coupling agent. The immobilized amine oxidase and peroxidase were used for the preparation of a modified carbon paste electrode. This modified bioelectrode was then characterized by cyclic voltammetry and chronoamperometry, which indicated the possible applicability for the determination of amines in biological samples. A platinum electrode was modified by the immobilized sulfite oxidase and utilized for the determination of sulfite in food and beverages. Magnetic carriers containing the immobilized trypsin and prolyl endoprotease were applied for a rapid protein digestion. After immobilization, trypsin was more thermostable than its soluble form and this system was used repeatedly. Both free and immobilized enzymes were characterized by their kinetic parameters. The morphological properties and size of the biogenic magnetite before and after linking enzymes were measured by transmission electron microscopy.

References

[1] Kluchova K., Zboril R., Tucek J., Pecova M., Zajoncova L., Safarik I., Mashlan M., Markova I., Jancik D., Sebela M., Bartonkova H., Bellesi V., Novak P., Petridids D. (2009) Biomaterials **30** (15), 2855.

[2] Schüller, D. J. (1999) J. Molec. Microbiol. Biotechnol. 1(1), 79.

Figures



Magnetite nanoparticles isolated from magnetotactic bacteria and covered by chitosan