Improvement of enzyme stability by synthesis of enzyme nanoparticles

Ph.D. thesis booklet

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1 Motivations and objectives

In order to make the industrial application of enzymes more attractive, as biocatalysts, by its increased stability, single enzyme nanoparticles were synthesized using α-chymotrypsin enzyme, cellulose enzyme complex and hemicellulose enzymes. These enzyme nanoparticles, which involve the enzyme species, are covered with thin, porous polymer layer in a few nanometers scale. The covered enzymes are more stable than the natural ones.

In general, the main disadvantage of enzymes for industrial application is their rather short lifetime. Elongation of enzymes’ lifetimes is very important for its effective industrial applications. Conservative immobilization techniques could significantly reduce the activity of enzymes. The relatively large size of the carrier particles can cause diffusive hindrance in transfer of the substrate molecules to the active center of the enzyme.

Three main methods exist for the synthesis of nanobioconjugates with size less than 50 nm. The so called “grafting onto” synthesis contains at least two main steps. In the first step the carrier nanoparticles are synthesized and the immobilization of the enzymes into or onto these nanocarriers is realized by a second step. During the “grafting from” synthesis the preparation of the carrier nanoparticle and the immobilization of the enzymes are carried out in a single step by polymerizing a covering layer initiated from the surface of the enzyme molecule. I have used the “grafting from” method to produce enzyme nanoparticles.

Enzyme nanoparticles are enzyme-polymer biocomposites where each enzyme molecule is covered individually with an approximately 3-5 nm thick cross-linked polymer layer. This thin polymer layer do not hinder the large polymer molecules from connecting to the cellulase enzyme enabling its catalytic action.

The main goal of this study was to investigate, how the above-mentioned methods can stabilize the industrially important enzymes, e. g. cellulase enzyme complex and hemicellulase enzymes, which are used on a large scale. Therefore, after successful experiments carried out with α-chymotrypsin enzyme, cellulase enzyme complex and hemicellulase enzymes were stabilized.

2 Synthesis methods

Two methods were studied in the literature to synthesize single enzyme nanoparticles (Kim et al., 2003; Yan et al., 2006). The method by Kim et al., (2003) contains three main steps. The first step is the enzyme modification on its surface by acryloylation of primer amino groups on side chains of amino acids of the enzyme molecule. After it, modified
enzyme molecules are solved in n-hexane by hydrophobic ion pairing. The second step is the polymerization of [3-(methacryloyloxy)propyl]trimethoxysilane initiated from the acryloyl groups on the surface of the enzyme molecule. Finally, cross-linkage between polymer chains was created around enzyme molecule.

Unfortunately, numerous enzymes are very sensitive to their microenvironment and do not dissolve in n-hexane and they can easily precipitate. Yan et al. (2006) worked out a new synthesis method, which does not involve organic solvents. A new preparation method was developed by me from the experiences of these two methods to avoid the dissolution of reagents in organic solutions. The surface modification was carried out by an acryloyl chloride reagent in aqueous solution and the polymerization step was realized also in aqueous solution using acrylamide-bisacrylamide polymerization. The unreacted reagents were separated by dialysis instead of expensive gel chromatography. Synthesis of single enzyme nanoparticles was successfully prepared by this new method.

3 Theses

Thesis 1:
I have developed a new two-step method modifying the literature one of Yan et al., (2006) to prepare single enzyme nanoparticles for stabilization of enzyme activity. This new method is simpler and more efficient than methods used in the literature.

A few nanometer thick spatial polymer layer was synthesized around individual enzyme molecule on the surface of the enzyme molecules. In contradiction to the previous methods in the literature, the significant advantage of this new method is that every step of the preparation is realized in aqueous solution and the modification of the surface of the enzyme molecules is proceeded with acryloyl chloride that is broken down easily in water and does not need to use n-acryloxsuccimide reagent dissolved in dimethylsulphoxyde and 4-aminopyridine as stabilizer. The unreacted reagents are separated by dialysis membrane instead of the more complex gel chromatography.

Thesis 2:
I have proved that
- the stability of single enzyme nanoparticles prepared by the two-step method, developed by me, has got a much longer lifetime, than that published in the literature,
larger substrate molecules, e.g. polysaccharides, can also be reacted by the pretreated enzyme, proving the biocatalytic activity of the covered enzyme.

**Thesis 3:**
I have first prepared and stabilized single enzyme nanoparticles using multiple enzyme complex i.e. industrial cellulase enzyme complex ‘Celluclast BG’ isolated from *T. reesei* organism.

First, I synthesized single enzyme nanoparticles using sensitive enzymes (*e.g.* endocellulase, *β*-D-mannosidase, *β*-D-xylosidase, endoxylanase and endomannanase enzymes) that could not or could hardly be stabilized. It was proved that the stability of these enzymes is at least fifty times higher than that of native ones.

**Thesis 4:**
I have proved that cellulase and hemicellulase enzymes stabilized by the presented two-step method are even stable at such a high temperatures as well, where native enzymes have not got any activity any more.

One example for hemicellulase enzymes is *β*-D-xilozidase. Single enzyme nanoparticles prepared by this enzyme have retained about 20% of its original activity for 24-hour incubation time at 80 °C and 150 rpm. In contrary to it, native *β*-D-xilozidase enzyme loses its activity after a half an hour incubation time.

**Thesis 5:**
I have proved, that single enzyme nanoparticles synthesized from *α*-chymotrypsin enzyme and cellulase enzyme complex keep their pH-stability at extreme acidic and basic pH values, as well.

Single *α*-chymotrypsin enzyme nanoparticles retain about a half of their activity obtained at pH = 7.8, under extremely acidic conditions (pH = 1.5), as well. Contrary to it, native *α*-chymotrypsin enzyme has not got any activity at pH = 1.5. In the case of cellulase enzyme complex, the activity of enzyme nanoparticles does not decrease significantly at extremely acidic (pH = 1.5) or at extremely basic (pH = 12) pH values. But the activity of native cellulase enzymes at the above-mentioned pH values (pH = 1.5 and pH = 12) lower below 10% and 30% of their activity respectively, comparing it to their original activity measured at pH = 6.0.
4 This PhD work based on the following publications

4.1 Scientific periodicals


4.2 Book chapter


4.3 Oral and poster presentations

6. Hegedüs I., Nagy E., Kukolya J., Barna T., Fekete Cs. A., Akrilamid réteggel stabilizált egyedi enzim nanorészecskék aktivitásának vizsgálata (Activity measurement of single


5 Publications not related to the theses

5.1 Scientific periodicals


5.3 Oral and poster presentations


