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Biocatalytic Activity of Fungal Protease on Silver Nanoparticle-Loaded Zeolite X Microspheres

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Silver nanoparticles are excellent biocompatible surfaces for the immobilization of enzymes. However, separation of the silver nanoparticle–enzyme bioconjugate material from the reaction medium is often difficult. In this study, we investigate the assembly of the silver nanoparticles on the surface of the amine-functionalized zeolite microspheres or formation of zeolite–silver nanoparticle "core–shell" structure and thereafter, using obtained structure in immobilization of fungal protease. The assembly of silver nanoparticles on zeolite surface occurs through the amine groups present in 3-aminopropyltrimethoxysilane (3-APTS). The fungal proteases bound to the massive "core–shell" structures can be easily separated from the reaction medium by mild centrifugation and exhibited excellent reuse characteristics. The biocatalytic activity of fungal protease in the bioconjugate was marginally enhanced relative to the free enzyme in solution.

Keywords: Silver Nanoparticles, Zeolite Microspheres, Fungal Protease, Biocompatible Surfaces, Bioconjugate Materials.

1. INTRODUCTION

In the last decade, nanosized materials have been widely used as support for immobilization. Among these materials, gold and silver nanoparticles are very popular when used in conjunction with biological materials including proteins, peptides, enzymes antibodies and nucleic acids, because of their unique properties. Although we are still at the beginning of exploring the use of these materials for biocatalysis, various nanostructures have been examined as hosts for enzyme immobilization via approaches including enzyme adsorption, covalent attachment, enzyme encapsulation.

Biotechnology is undergoing impressive advances in the synthesis of biocompatible surfaces for the immobilization of a range of biomolecules with important applications in biosensing and medicine.[1–2] Insofar as enzymes are concerned, advantages of the immobilized enzymes over their counterparts in solution include enhanced temperature, temporal stability of the bioconjugate materials and ease of biocatalyst separation from the reaction medium, enabling their multiple reuses.[3–7]

A number of templates have been used for enzyme immobilization, such as silica nanotubes, phospholipid bilayers, self-assembled monolayers, Langmuir–Blodgett films, polymer matrices, galleries of α-zirconium phosphate, mesoporous silicates such as MCM-41, silica nanoparticles, and thermally evaporated lipid films, each with its characteristic advantages and disadvantages.[8–11]

In this investigation, we demonstrate that silver nanoparticles loaded on amine-functionalized X zeolite particles (pore diameter 2.3 Å, average diameter of the particles ~800 nm) provide a biocompatible surface for the immobilization of the enzyme fungal protease. The enzyme binds strongly to the silver nanoparticle surface and shows excellent biocatalytic activity, temperature stability in the immobilized form. The fungal protease nano silver–zeolite biocatalyst could be easily removed from the reaction medium by simple sedimentation and exhibited excellent reuse characteristics.

2. EXPERIMENTAL DETAILS

2.1. Silver Nanoparticle Synthesis

In a typical experiment, 100 mL of 1.5×10^{-4} M concentrated aqueous solution of silver nitrate ($AgNO_3$) was reduced by 0.01 g of sodium borohydride ($NaBH_4$) at room temperature to yield a solution containing silver nanoparticles of 3–5 nm in diameter.

2.2. Formation of Nanosilver–X-Zeolite Composites

15 milligrams of the amine-functionalized zeolite was dispersed in 50 mL of the nano silver solution under

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continuous stirring. After 14 h of stirring, the originally white color zeolite attained a gray hue. The zeolite particles capped with silver nanoparticles were separated by mild centrifugation, washed with double distilled water, and air-dried for further use.

2.3. Formation of Fungal Protease–Nanosilver–Zeolite Bioconjugate

Fifteen milligrams of the nanosilver–zeolite powder was dispersed in 2 mL of glycine–HCl buffer (0.05 M, pH 3). To this solution, 100 μL of a stock solution consisting of 50 mg/mL of fungal protease in glycine–HCl buffer (0.05 M, pH 3) was added under vigorous stirring. After 1 h of stirring, the fungal protease–nano silver–zeolite bioconjugate material was separated by centrifugation (Fig. 1). The loss in absorbance at 280 nm in the supernatant (arising from $\pi - \pi^*$ transitions in tryptophan and tyrosine residues in the enzyme)[2, 12] was used to quantify the amount of F-prot bound to the nanosilver–zeolite for specific activity determination. The powder thus obtained was rinsed several times with glycine–HCl buffer (0.05 M, pH 3) solution and re-suspended in the buffer solution (pH 3) and stored at 4 °C for further experimentation. Because it is possible that the zeolite particles suspended in the supernatant could lead to an error in the quantitative analysis of the protein by UV-Vis spectroscopy in the bioconjugate materials. Fluorescence measurements were carried out on the initial concentration of the F-prot in solution at pH 3 and supernatant of F-prot in solution after centrifugation of the bioconjugate using a luminescence spectrophotometer. The enzyme was excited at 280 nm and the emission band was monitored in the range 300 to 500 nm.[2, 11] The decrease in fluorescence intensity (arising from $\pi - \pi^*$ transitions in tryptophan and tyrosine residues in proteins) in the F-prot solution before and after reaction with silver-nanoparticle-loaded zeolite was used to quantify the amount of F-prot bound to the nanosilver–zeolite spheres.

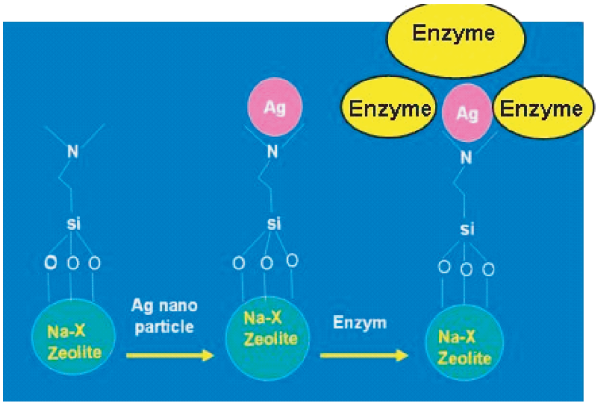

Fig. 1. Schematic representation of direct assembly of silver nanoparticles on amine-functionalized zeolite particles and, thereafter, immobilization of the enzyme F-prot on the nanosilver–zeolite template.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Spectroscopy Studies

Figure 2 shows UV-Vis spectrum recorded from the as prepared colloidal silver solution (curve 1) and the supernatant silver solution obtained after stirring with amine-functionalized zeolite for 12 h and filtration (curve 2). The surface plasmon resonance in the as prepared colloidal silver can be seen clearly at ca. 410 nm. After stirring the colloidal silver solution with the zeolite for 12 h, there was loss in intensity of surface plasmon resonance due to the decrease in concentration of silver nanoparticles in the aqueous solution (curve 2). This clearly indicates binding of the silver nanoparticles to the amine-functionalized zeolite through free amine groups of APTS.[9–12] The mass loading of the silver nanoparticles on the zeolite was estimated as 7 wt%. Because aggregation of the silver nanoparticles in solution can lead to the errors in estimation of silver nanoparticles loaded on the amine-functionalized zeolite material estimated from the UV-Vis spectrum. The silver nanoparticles bound to the silver nanoparticles in the amine-functionalized Na-X zeolite were dissolved in aqua regia and solutions were analyzed by atomic absorption spectroscopy (AAS).

3.2. TEM Measurements

Figure 3(a) shows TEM micrograph of the as prepared amine-functionalized zeolite and silver nanoparticle capped zeolite particles. The particles are fairly spherical, with slightly irregular edges. The silver nanoparticles (dark spots) bound to the surface of the amine-functionalized zeolite particles at fairly high density can be seen clearly. As mentioned previously, the silver nanoparticles bind to the zeolite template via the primary amine groups.

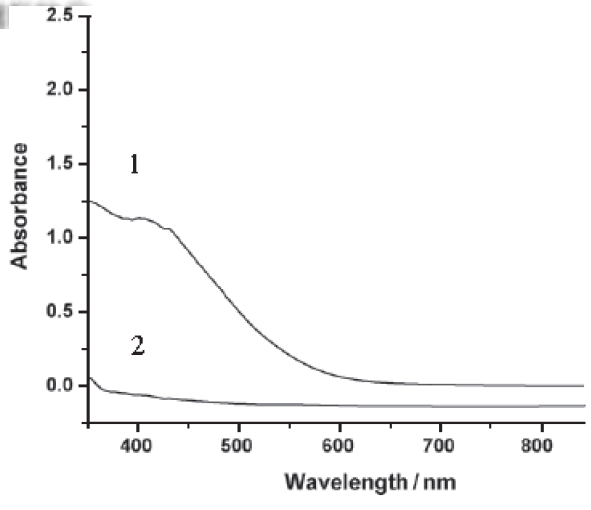

Fig. 2. UV-Vis spectrum recorded from the as-prepared colloidal silver solution (curve 1) and silver solution after stirring with amine-functionalized zeolite for 12 h and centrifugation (curve 2, see text for details).

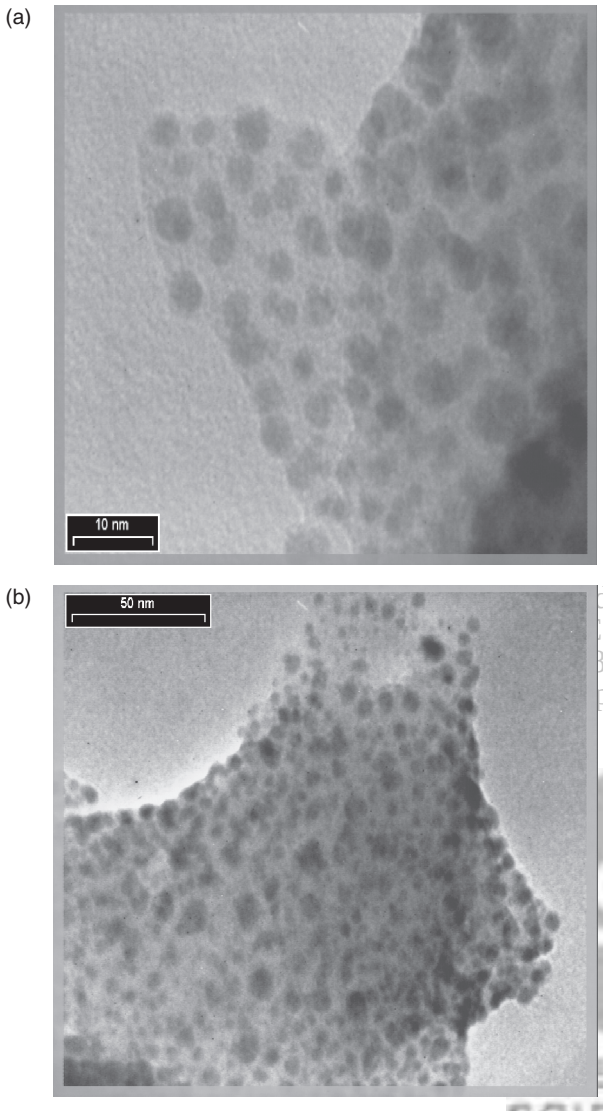

Fig. 3. (a) Representative TEM images of the nanosilver-amine-functionalized zeolite on a carbon-coated TEM grid. (b) TEM micrograph of the silver nanoparticles (dark spots) bound to the surface of the amine-functionalized zeolite particles-F-prot.

Figure 3(b) shows that TEM micrograph of the silver nanoparticles (dark spots) bound to the surface of the amine-functionalized zeolite particles-F-prot. The size of nano particle that loaded on X-zeolite was 3–5 nm.

3.3. Biocatalytic Activity Measurements

The most important aspect of this study concerns retention of the biocatalytic activity of F-prot after adsorption onto the nanosilver–zeolite surface. The biocatalytic activity of free F-prot in solution and of F-prot–nanosilver–zeolite bioconjugate in glycine–HCl buffer (0.05 M, pH 3) was determined by reaction with 0.5% Hb at 37 °C for 30 min. Control experiments on the biocatalytic activity of F-prot immobilized directly onto amine-functionalized zeolite were also performed under identical conditions.

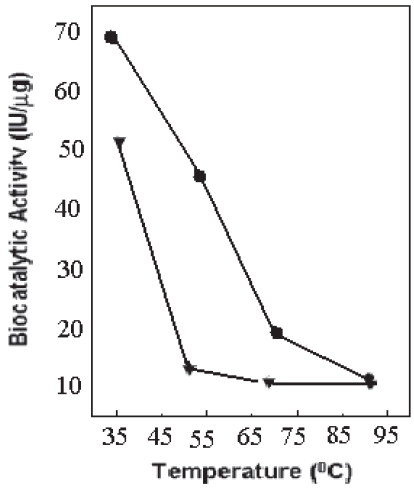

Fig. 4. Temperature dependent of biocatalytic activity of the free F-prot in solution (triangles) and F-prot in the nanosilver–X zeolite bioconjugate material (circles) pre-incubating for 1 h at different temperature.

The F-prot–nanosilver–zeolite and F-prot–zeolite bioconjugate materials were separated from the reaction medium by centrifugation (2000 rpm) for recycling studies. In typical experiments to estimate the biocatalytic activity of the bioconjugate, a carefully measured amount of the F-prot–nanosilver–zeolite/F-prot–zeolite bioconjugate in buffer was incubated with 1 mL of 0.5% HB solution at 37 °C for 30 min. Because the amount of F-prot bound to the bioconjugate could be estimated quite accurately from independent UV-Vis or fluorescence spectroscopy measurements, it is possible to compare the biocatalytic activity of the enzyme in the bioconjugate material and the free enzyme in solution under identical assay conditions (Fig. 4). The new biocatalyst material can be easily separated from the reaction medium by sedimentation or mild centrifugation and exhibits excellent reuse characteristics over four successive cycles. The variation in biocatalytic activity of free F-prot molecules in solution (triangles) and F-prot immobilized on the nanosilver–zeolite template (circles) as a function of temperature stability showed in Figure 4. As noted early this reaction was carried out after preincubation for 1 h at the different temperatures and measuring the biocatalytic activity at pH 3, 37 °C.[9–12]

4. CONCLUSIONS

In this study, we demonstrated the assembly of silver nanoparticles on amine-functionalized X-zeolite particles, with the binding of the nanoparticles occurring via complexation with the free amine groups present in the zeolite. The silver nanoparticles bound to X-zeolite particles act as excellent templates for the immobilization of the enzyme F-prot. F-prot in the bioconjugate system shows enhanced stability toward temperature conditions. The new biocatalyst material separated from the reaction medium by sedimentation or mild centrifugation and exhibits excellent reuse characteristics over four successive cycles.

The optimum temperature of the F-prot in the bioconjugate system was observed to be higher than that of the free enzyme in solution.

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