

Enhanced Enzyme Activity with Ferritin Nanocages

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ABSTRACT

In this study, a novel enzyme system based on ferritin nanocages was designed. α -Amylase was covalently crosslinked on surface of ferritin nanocages by EDC/NHS. The activity of immobilized enzyme was monitored by using UV-vis spectrophotometer. The optimum temperature of immobilized enzyme shifted from 50 °C to 70 °C due to the ferritin nanocages. In addition, The K_m and V_{max} values of immobilized enzymes were 5.19 mg mL⁻¹ and 3.3x10⁻⁵ U mg⁻¹, respectively. This novel enzyme system displayed higher catalytic activity and enhanced stability.

Keywords:

Ferritin nanocages; α -amylase; Enzyme immobilization; EDC/NHS; Covalently crosslinked

INTRODUCTION

In recent years, the combined use of chemistry, biology and nanotechnology has made significant contributions to the field of medical diagnosis and treatment. For example, quantum dots or fluorochrome-linked macromolecules have been developed as cell and tissue imaging materials. Iron oxide nanoparticles have also been developed for use as biomedical applications, such as hyperthermia, cancer diagnosis and therapy, and as a contrast agent in magnetic resonance imaging (MRI). However, these nanostructures, which have been subjected so much research on them, they have toxic effects. For this reason, the search for biocompatible nanomaterials in diagnosis and treatment of diseases has become intense. Protein lattices such as ferritin and virus capsules (protein layer outside the virus) are versatile nanoscale perfect architectures that allow both genetic and chemical modification. Nanoscale protein architects with multiple functionalities have a good potential, especially in medical imaging and treatment fields. Protein lattice structures have different subunits that can be used as the interface between the inner and outer and subunits. The subunits can be easily modified genetically and chemically to demonstrate versatile functionality. Ferritin is a very large iron storage and detoxification protein found in many organisms [1,2]. In nature, ferritins are filled with iron. Ferritin is a natural nanocage and also ready to be combined with biomolecules for preparing nanobiosensors. Thus,

biomedical applications have a promising potential for sensitive and robust bioassays. Based on these properties, ferritin is not only due to its inherent nano-size and nanostructure, but also by its high stability and specific structures [3-9]. In addition to these superior properties, nanotechnology researchers have found that nanosized biomaterials such as ferritin, apoferritin in biomedical field, such as diagnosis and treatment of diseases because they are biodegradable, biocompatible and non-toxic properties [10-12].

In this study, it is aimed to developed ferritin nanocage-based platform to construct an enzyme system. To prepare this system, α -amylase enzyme was covalently crosslinked on the outer surface of the ferritin nanocages. The activity of this novel enzyme system was investigated for different temperatures and substrate concentrations using UV-vis spectrophotometer.

EXPERIMENTAL

Materials

All chemicals were used for the preparation enzyme immobilization ferritin nanocages. Ferritin nanocages Type I: From Horse Spleen and α -amylase (E.C.3.2.1.1, 47 kDa) were obtained from Sigma.

Enzyme immobilization

To immobilize α -amylase onto the ferritin nanocages, firstly, ferritin nanocages were suspended in phosphate buffer solution (pH 7.4) by ultrasonic bath

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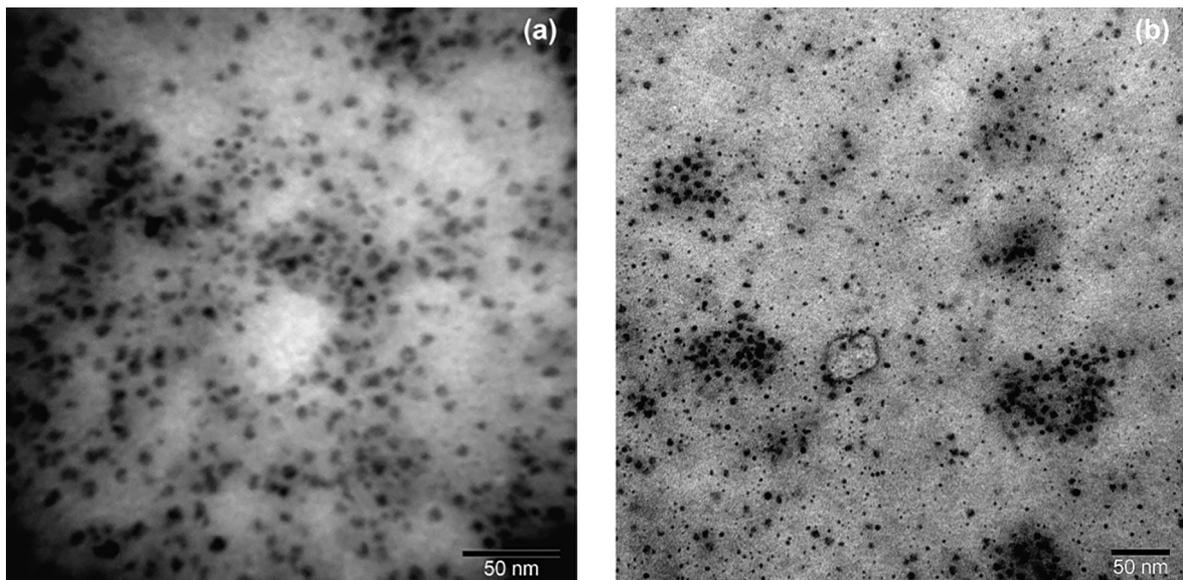


Figure 1. TEM images of a) ferritin nanocages b) enzyme immobilized ferritin nanocages

during 45 min. Then, α -amylase was also preactivated with EDC:NHS (1:1, w:w) and in phosphate buffer solution for 30 min, and added to the ferritin nanocage suspension, and stirred at room temperature for 4 h with constant shaking. The enzyme immobilized ferritin nanocages were washed with the buffer solution. Finally re-dispersed in phosphate buffer solution (pH 7.4). After redispersed in PBS (pH 7.4) enzyme immobilized ferritin nanocages were kept at +4 °C until further use.

Immobilization experiments

The activity of α -amylase was determined via examining the reduction of starch in different conditions. The concentration of substrate was determined via UV-vis spectrophotometer at 560 nm.

Immobilization efficiency was determined by using the Eq.1:

$$\text{Immobilization efficiency} = 1 - \frac{[\text{enzyme}]_{\text{upper phase}}}{[\text{enzyme}]_{\text{initial}}} \quad (1)$$

Where $[\text{enzyme}]_{\text{supernatant}}$ is the enzyme concentration in the upper phase and $[\text{enzyme}]_{\text{initial}}$ is the initial concentration of the enzyme.

Characterization

TEM investigation was performed using a JEOL 100CX instrument working at 120 kV. UV-vis absorbances were determined by a Shimadzu UV-2450 spectrophotometer at room temperature.

RESULTS AND DISCUSSION

Preparation of novel enzyme system

Firstly, α -amylase enzyme was covalently crosslinked

onto the ferritin nanocages via EDC/NHS. The TEM characterization results were summarized Fig. 1a.

Fig. 1a demonstrated the TEM images of bare ferritin nanocages. The bare ferritin nanocages were uniform size and no exhibited agglomeration in Fig. 1. Their average diameters 13.7 ± 0.54 nm. With the enzyme immobilized, the ferritin nanocages well dispersed with an average diameter 17.3 ± 0.82 nm was determined in Fig. 1b.

Enzyme immobilization was accomplished via reacting a constant concentration of ferritin nanocages with a fixed concentration of enzyme solution. α -amylase enzyme was preactivated by EDC/NHS in solution before it was linked on the ferritin nanocage surfaces. Finally, α -amylase enzyme was immobilized on the surface of the ferritin nanocages by reacting between the amino groups of nanocages and carboxyl groups of enzyme. The efficiency of immobilization was determined to be 97.3%. This result indicated that, binding process was successful.

Thermal stability of immobilized α -amylase was

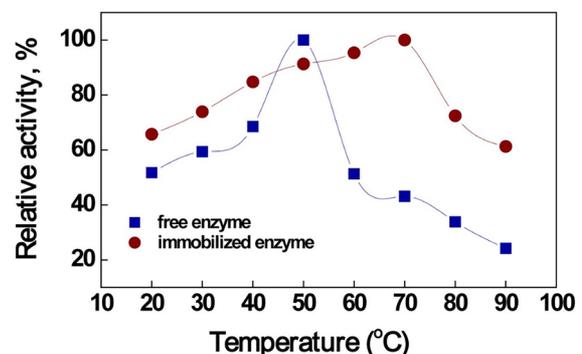


Figure 2. Effect of temperature on free and immobilized enzyme activity

Table 1. Kinetic parameters of free and immobilized enzymes

	K_m mg mL ⁻¹	V_{max} U mg ⁻¹
free enzyme	2.63	10.6x10 ⁻⁵
immobilized enzyme	5.19	3.3x10 ⁻⁵

compared with the free enzyme at different temperatures (20-90 °C). The results were showed in Fig 2.

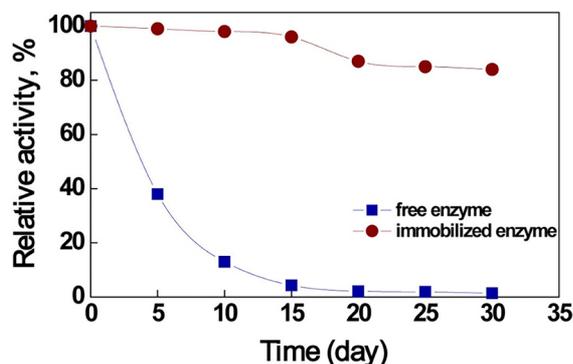
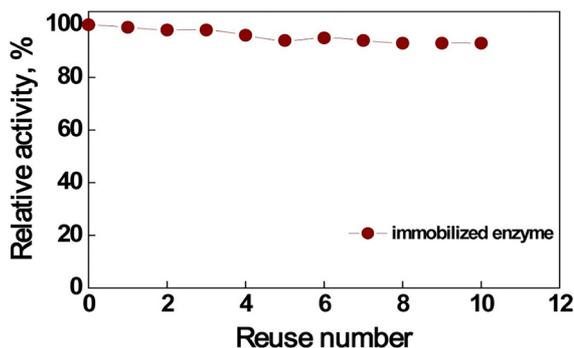
Free enzyme very stable and can be incubated at 50 °C. Optimum temperature of immobilized enzyme was changed from 50 °C to 70 °C. As a result, immobilized enzyme more resistant to heat than the free enzyme. It can be probably ferritin nanocages were enhanced enzyme activity at higher temperatures. Moreover, it can be the hydration layer around the ferritin nanocages prevented the thermal denaturation of the enzyme.

Kinetic parameters of free and immobilized enzymes were determined by using the Michaelis-Menten equation. The results were summarized in Table 1.

The K_m values of free enzyme and immobilized enzymes were 2.63 and 5.19 mg mL⁻¹, respectively. The V_{max} values of free enzyme and immobilized enzymes were found to be 10.6x10⁻⁵ and 3.3x10⁻⁵ U mg⁻¹, respectively. The K_m value of immobilized enzyme higher then the free enzyme. It can be due to the changes in the conformation of the enzyme molecules after the immobilization and it may be increased enzyme activity [13].

Generally, enzymes may lose their activities due to environmental conditions. So that it is important their storage stability for industrial usage. In order to determine the storage stabilities of free and immobilized α -amylase enzyme, which were kept in phosphate buffer solution (pH 7.4) at +4 °C. Their activities were investigated in every 5 days during 30 days. (Fig. 3)

Free enzyme was lost its about all initial activity within 15 days but immobilized enzyme preserved about 96 % of the initial activity. After 30 days, the immobilized enzyme retained about 84 % of the initial activity. According to

**Figure 3.** Storage stability of free and immobilized enzyme activity**Figure 4.** The reusability of immobilized enzyme

the results, the immobilization process and the ferritin nanocages increased the storage stability and durability of immobilized α -amylase.

The reusability of immobilized enzyme is very important feature for their large-scale applications. The reusability of immobilized enzyme was measured for 10 reuse in 1 day. (Fig.4)

In Fig. 4, after 10 reuses immobilized enzyme protected about 93% of the initial activity. This result showed that there was no significant loss after the 10 reuses. Therefore the ferritin nanocages can be reused and they could be an economic platform for enzyme immobilization.

CONCLUSION

A novel enzyme system was developed by using natural protein cages. Model enzyme α -amylase was covalently immobilized onto the nanocages and optimum immobilization conditions were determined. The immobilized enzyme showed better temperature resistance than the free enzyme. In addition, storage stability of immobilized enzyme was excellent when compared the free enzyme. These results can be attributed to the ferritin nanocages. As a result, ferritin nanocages are very important platforms for enzyme immobilization because of their enzyme mimic activity.

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REFERENCES

- Bou-Abdallah F, Zhao G, Biasiotto G, Poli M, Arosio P, Chasteen ND, Facilitated diffusion of iron(II) and dioxygen substrates into human H-chain ferritin. A fluorescence and absorbance study employing the ferroxidase center substitution Y34W. *JACS*, 130 (2008) 17801-17811.
- Bulvik BE, Berenshtein E, Meyron-Holtz EG, Konijn

- AM, Chevion M, Cardiac protection by preconditioning is generated via an iron-signal created by proteasomal degradation of iron proteins. *PLoS ONE*, 7 (2012), 1-9.
3. Ullrich A, Horn S, Structural investigations on differently sized monodisperse iron oxide nanoparticles synthesized by remineralization of apoferritin molecules. *J. Nanopart. Res.* 15 (2013), 1-9.
 4. Zhen ZP, Tang W, Guo CL, Ferritin nanocages to encapsulate and deliver photosensitizers for efficient photodynamic therapy against cancer. *ACS Nano*, 7 (2013) 6988-6996.
 5. Tang Z, Wu H, Zhang Y, Li Z, Lin Y, Enzyme-Mimic Activity of Ferric Nano-Core Residing in Ferritin and Its Biosensing Applications. *Anal. Chem.* 83 (2011) 8611-8616.
 6. Tedesco AC, Oliveira DM, Lacava ZGM, Azevedo RB, Lima ECD, Morais PC, Investigation of the binding constant and stoichiometry of biocompatible cobalt ferrite-based magnetic fluids to serum albumin. *Journal of Magnetism and Magnetic Materials.* 272-276 (2004) 2404-2405.
 7. Powell JJ, Bruggraber SFA, Faria N, Poots LK, Hondow N, Pennycook TJ, Latunde-Dada GO, Simpson RJ, Brown AP, Pereira DIA, A nano-disperse ferritin-core mimetic that efficiently corrects anemia without luminal iron redox activity. *Nanomedicine: Nanotechnology, Biology, and Medicine* 10 (2014) 1529-1538.
 8. Rakshit T, Mukhopadhyay R, Tuning band gap of holoferritin by metal core reconstitution with Cu, Co and Mn. *Langmuir* 27 (2011) 9681-9686.
 9. Zhao J, Liu ML, Zhang YY, Li HT, Lin YH, Yao SZ, Apoferritin protein nanoparticles dually labeled with aptamer and horseradish peroxidase as a sensing probe for thrombin detection. *Anal. Chim. Acta* 759 (2013) 53-60.
 10. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE, Biodegradable polymeric nanoparticles as drug delivery devices. *J. Control. Release* 70 (2001) 1-20.
 11. Mundargi RC, Babu VR, Rangaswamy V, Patel P, Aminabhavi TM, Nano/micro Technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. *J. Control. Release* 125 (2008) 193-209.
 12. Chomoucka J, Drbohlavova J, Huska D, Adam V, Kizek R, Hubalek J, Magnetic nanoparticles and targeted drug delivering. *Pharmacol. Res.*, 62 (2010) 144-149.
 13. Tümtürk H, Yüksekdağ H, Acetylcholinesterase immobilized onto PEI-coated silica nanoparticles. *Artificial Cells, Nanomedicine, and Biotechnology* 44 (2016) 443-447.