Colloids and Surfaces A: Physicochem. Eng. Aspects 414 (2012) 190–197

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Improved preparation of immobilized trypsin on superparamagnetic nanoparticles decorated with metal ions

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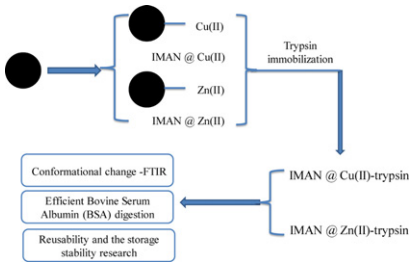
HIGHLIGHTS

- Immobilized metal affinity magnetic nanoparticles (short as IMANs).
- The presented synthetic route allows preparing high-quality IMANs in a large scale.
- Newly IMANs (Cu(II) and Zn(II)) enhanced trypsin conformational stability.
- Both immobilized trypsin could be used for efficient BSA digestion.
- The application of the IMANs may find much potential in trypsin immobilization.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 February 2012
Received in revised form 28 May 2012
Accepted 7 August 2012
Available online 19 August 2012

Keywords:
Enzyme immobilization
Catalytic properties
Trypsin
Immobilized metal affinity chromatography

ABSTRACT

The superparamagnetic carboxymethyl chitosan nanoparticles (Fe_3O_4 (PEG + CM-CTS) NPs) which prepared by chemical coprecipitating were treated with Cu(II) and Zn(II) ions solutions to obtain the novel immobilized metal affinity magnetic nanoparticles (IMANs), short as IMAN @ Cu(II) NPs and IMAN @ Zn(II) NPs. Immobilization of trypsin onto the obtained IMANs, as a result of metal affinity interaction was confirmed by Fourier Transform Infrared (FTIR) spectroscopy. FTIR data demonstrated that the IMAN @ Cu(II) NPs and IMAN @ Zn(II) NPs were capable of preventing the trypsin unfolding. Due to the large specific surface area and excellent dispersibility, the adsorption equilibrium of trypsin onto the nanoparticles was achieved quickly within 30 min, and adsorption equilibrium of trypsin onto the IMANs fitted well with the Langmuir model. The results of kinetic parameters (Michaelis constant, K_m) showed that, both immobilized trypsin, compared with free trypsin, have higher affinity to the substrate, furthermore, they could be used for fast and efficient Bovine Serum Albumin (BSA) digestion under very facile processes, as well as the greatly reduced digestion time (from 12 h to 15 min) which confirmed by MALDI-TOF MS analysis. Both immobilized trypsin exhibited a sound stability. Considering that the studied IMANs possess the advantages of high efficiency, cost-effectiveness and lack of negative effect on trypsin bioactivity, such IMANs may hold potential applications in immobilization and stabilization of trypsin.

1. Introduction

Proteases are widely used in industrial and biomedical applications, among which trypsin was most extensively concerned. However, their applications are limited due to their instability and rapid losing of catalytic activity during the operational and storage periods. The immobilized enzyme can overcome these limitations

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http://dx.doi.org/10.1016/j.colsurfa.2012.08.016