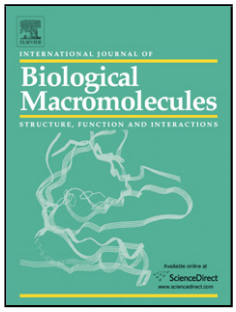
Accepted Manuscript



Title: Covalent Immobilization of Trypsin onto Modified Magnetite Nanoparticles and its Application for Casein Digestion

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PII: S0141-8130(16)31976-6
DOI: http://dx.doi.org/doi:10.1016/j.ijbiomac.2017.01.023
Reference: BIOMAC 6929

To appear in: *International Journal of Biological Macromolecules*

Received date: 13-10-2016
Revised date: 20-12-2016
Accepted date: 4-1-2017

Please cite this article as: Keziban Atacan, Bekir Cakiroglu, Mahmut Ozacar, Covalent Immobilization of Trypsin onto Modified Magnetite Nanoparticles and its Application for Casein Digestion, International Journal of Biological Macromolecules http://dx.doi.org/10.1016/j.ijbiomac.2017.01.023

<AT>Covalent Immobilization of Trypsin onto Modified Magnetite Nanoparticles and its Application for Casein Digestion

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<ABS-Head><ABS-HEAD>Graphical abstract
<ABS-P>► The illustration of modification and immobilization process on magnetic iron oxide nanoparticles for casein digestion.

<ABS-HEAD>Highlights► Fe_3O_4 MNPs was developed to immobilize the enzyme as reusable carriers. ► Trypsin was immobilized onto tannic acid modified MNPs via covalent bonding. ► A possible mechanism of trypsin immobilization onto Fe_3O_4 NPs was proposed. ► The casein protein was efficiently and fast digested by Fe_3O_4 -TA-TR. ► The digested protein was characterized by LC-MS/MS and SDS-PAGE.

<ABS-HEAD>Abstract

<ABS-P>The immobilization method consists of the production magnetite nanoparticles (Fe_3O_4) by solvothermal treatment of $FeCl_3$ and sodium acetate (NaAc) in the presence of ethylene glycol. Subsequently, the surface of magnetite nanoparticles was modified with a well-known polyphenol tannic acid. Trypsin was covalently immobilized on the tannic acid modified magnetite nanoparticles after exposing the modified nanoparticles to pH 9.4. Then, tryptic digestion of casein by free and immobilized trypsin was carried out for 13 h and 1 h, respectively. TGA curves, FTIR spectra, and magnetization curves demonstrated the decent amount of trypsin immobilization without compromising the enzyme activity. Digestion efficiency of casein was investigated using liquid chromatography–mass spectrometry (LC-MS/MS) technique. LC-MS chromatograms confirmed the efficient digestion of casein by immobilized trypsin compared to free trypsin owing to prevention of autohydrolysis. Also, the sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis confirmed the satisfactory digestion of casein by immobilized trypsin.

<KWD>Keywords: Fe_3O_4 nanoparticles; trypsin immobilization; covalent attachment; casein digestion.

<H1>1. Introduction

Magnetic iron oxide nanoparticles (Fe_3O_4 MNPs) have been used in various fields owing to their unique properties including large specific surface area and simple separation with magnetic field [1]. Because of the high surface energy, the pristine Fe_3O_4 (magnetite) nanoparticles are generally unstable and tend to aggregate easily, which strongly affects their dispersion in aqueous medium. In addition, Fe_3O_4 nanoparticles are highly susceptible to oxidation to γ-Fe_2O_3 nanoparticles in the presence of oxygen [2]. To overcome such limitations, various surface modification methods have been developed to modify the surface of pristine Fe_3O_4 nanoparticles via loading of other chemicals or biological materials during or after the synthesis process to improve the dispersibility, stability, biocompatibility and