Synthesis, characterization and application of the magnetic nanobiocatalyst Fe_3O_4@silica-thermolysin in peptide chemistry

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Thermolysin was bound to magnetic nanoparticles. Fe_3O_4@silica-TLN was characterized and used in dipeptide synthesis.

Introduction

Peptides are synthesized by all live organisms. These compounds can act as hormones, antimicrobial agents, cell-cycle regulators, analgesics, surfactants or many other vital functions. This importance has motivated the search for methods applicable for their isolation, purification, analysis, identification, quantification and synthesis.

Chemical peptide synthesis (in solution or on a solid phase) is well established and applicable for any peptide, but it requires full amino acid protection, employs drastic conditions, generates not eco-friendly wastes and may lead to amino acid enantiomerization. Thus, the enzymatic method has been considered as a greener alternative to synthesize important biologically active dipeptides, such as Ala-Gln, for parental nutrition, or Tyr-Arg, a neurotransmitter. In fact, the use of enzymes in peptide synthesis is advantageous owing to their specificity and stereo selectivity, ability to work under mild and clean conditions, reduction of costs and wastes. Yet, purified enzymes are expensive, can denature, can suffer autolysis, are difficult to recover from reaction media and, often, are not reusable.

Thermolysin (TLN) is a protease produced by *Bacillus thermoproteolyticus*. It has been used in the production of aspartame, the artificial dipeptide sweetener[1]. Immobilization on solid supports, including nanoparticles (NPs), minimizes these disadvantages. Superparamagnetic NPs are especially useful for this purpose since magnetic nanobiocatalysts can be easily recovered from reaction media[2].

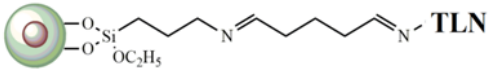
Results and Discussion

The procedures used to synthesize Fe_3O_4@silica-NH_2 and react it with TLN followed our previous studies[2,3]. Amidase activity assays were based on digestion of bovine casein and on the hydrolysis of the dipeptide FAGLA (N-(3-[2-Furyl]Acryloyl)-Glycine-Leucine Amide). Protein contents were obtained by thermogravimetric analysis (TGA), the Bradford method and full acidic hydrolysis/amino acid analysis of the hydrolyzate. Autolysis was detected by SDS-PAGE under reducing conditions. Preliminary attempts of Fe_3O_4@silica-TLN to catalyze dipeptide synthesis employed mild green conditions. Monitoring of reactions was done by RP-HPLC. Product identification employed LC-MS and amino acid analysis. The nanobiocatalyst was recovered from the reaction media by a magnet.

Specific activities of free TLN and Fe_3O_4@silica-TLN were: (i) practically identical (0.34 U.mg$^-$1) against FAGLA; (ii) 117,800 PU.mg^{-1} (1 PU is 1 µg of tyrosine per min.) and 60,250 PU.mg^{-1}, respectively, against bovine casein. Activity was kept at 5 °C for almost 50 days.

Preliminary synthesis catalyzed by Fe_3O_4@silica-TLN yielded 23 % of Z-Asp-Phe-OMe, 29 % of Z-Ala-Phe-OMe, 27 % of FAGLA and 44 % of Z-Phe-Phe-OMe, precursors of sweetener, bitter or gel-forming dipeptides of high commercial values. Enzyme recovery from reaction media was easy and efficient. Enzyme reuse was confirmed.

Figura 1. The nanobiocatalyst studied.



Conclusions

Immobilization of TLN on Fe_3O_4@silica was achieved without of loss enzyme amidase activity. The Fe_3O_4@silica-TLN obtained can be stored in suspension at pH 7.5 at 5°C for 50 days without significant loss of activity. It can be efficiently recovered from reaction media using a magnet and reused at least twice. Despite synthetic conditions used so far require optimization, our results showed that Fe_3O_4@silica-TLN catalyzed peptide bond formation furnishing precursors of four dipeptides biologically and commercially important. They corroborate our previous studies[2,3].

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