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Improvement of Enzymological Properties of Pepsin by Chemical Modification with Chitooligosaccharides

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Abstract

In order to improve the enzymological properties and to enhance the stability of pepsin (PP, EC 3.4.23.1), chitooligosaccharide (COS) was used to chemically modify enzyme molecules of PP. The results showed that one protein band appeared on the PAGE diagram, indicating that the enzyme samples reached electrophoresis purity after purification. The molecular weights of the enzymes before (PP) and after (COS-PP) modification were 38.68 KD and 49.55KD, respectively. The K_m and V_{max} of PP were 2.40 mg/mL and 1.1×10^6 mg /（mg Pro·min）. The K_m and V_{max} of COS-PP were 4.44 mg/mL and 8.3×10^5 mg /（mg Pro·min）, respectively. The optimal pH values of PP and COS-PP were 1.5-2.0. The optimal temperatures of PP and COS-PP were 57℃ and 59℃, respectively. After being incubated at 60 ℃ for 1 hr, the residual activities of PP and COS-PP solutions were 26.56% and 74.72%, respectively. After being incubated at 65 ℃ for 1 hr, the residual activities of PP and COS-PP solutions were 14.74% and 46.40%, respectively. After being kept at room temperature (25℃) or 4 ℃ refrigerator for 4 days, PP solution had basically no activity, whereas the residual activities of COS-PP solutions were 5.40% and 33.94%, respectively.

Key words: Pepsin, Chemical modification, Chitooligosaccharide, Enzymological properties, Stability.

1 Introduction

Pepsin (PP)(EC 3.4.23.1) is a monomeric enzyme with 2 structural domains. Its secondary structures are mainly β - sheets. Because its peptide chain contains more acidic amino acid residues, PP has a very low pI value [1]. PP digests protein molecules under very acidic environment in stomach and converse protein macromolecules into smaller polypeptides. When PP hydrolyzes protein or polypeptide molecules, it has certain specificity for the sequence of amino acid residues. PP tends to hydrolyze the peptide bonds in which the carboxyl terminals are aromatic amino acids, including phenylalanine, tryptophan and tyrosine or leucine residues. If the third amino acid residue at the amino terminal of a peptide bond is an alkaline amino acid residue (e.g. lysine, arginine and histidine), or, if the amino terminal of a peptide bond is arginine residue, the