PURIFICATION AND CHARACTERISATION OF VARIOUS FORMS OF CARBOXYPEPTIDASE-A FROM BUFFALO PANCREAS

BY

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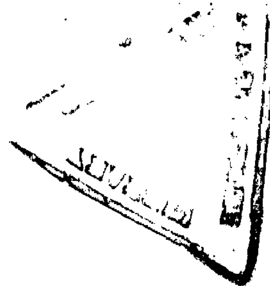

To My Parents

CERTIFICATE

This is to certify that the thesis entitled "PURIFICATION AND CHARACTERISATION OF VARIOUS FORMS OF CARBOXYPEPTIDASE A FROM BUFFALO PANCREAS" being submitted by Mira Sud to the Indian Institute of Technology, Delhi, for the award of the degree of DOCTOR OF PHILOSOPHY, is a record of bonafide research work carried out by her. Mira Sud has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which, to my knowledge, has reached the requisite standard.

The results contained in this thesis have not been submitted in part or in full, to any other University or Institute for the award of any degree or diploma.

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Abstract

Carboxypeptidase activities in buffalo pancreatic extract have been studied, using a wide range of substrates over a wide range of pH, so as not to miss any activity.

Two forms of buffalo carboxypeptidase A, designated as $CPAb_1$ and $CPAb_2$, have been purified. The various steps of purification include ammonium sulphate fractionation and ion exchange chromatography on DEAE-cellulose and on CM-Sephadex. The homogeneity of the two forms is established by (i) polyacrylamide disc gel electrophoresis, (ii) SDS-polyacrylamide gel electrophoresis and (iii) isoelectric focussing.

The two forms have been characterised for their optimum pH, temperature, K_m value and substrate specificities. The physical properties of the two forms, such as molar extinction coefficient, isoelectric pH and activation energy have been reported. Their molecular weights have been determined by gel filtration on Sephadex G-100 and by SDS-polyacrylamide gel electrophoresis. N-terminal amino acid and the amino acid composition of the two forms have also been determined.

The zinc content of the two forms has been determined chemically and by atomic absorption spectroscopy. Effect of metal chelating agents like EDTA and 1,10-orthophenanthroline on the two forms has been studied. 1,10-orthophenanthroline stabilises the enzyme in low concentration, while at higher concentration, it inhibits the enzyme by chelating zinc. Studies with CD spectra have shown that 1,10-orthophenanthroline in low concentrations changes the enzyme conformation such that it is resistant to thermal denaturation.

Product stabilization of the two forms has also been studied. During heat denaturation studies, it was found that carboxypeptidase Ab_1 and carboxypeptidase Ab_2 lost about 20% and 35% of the activity respectively, when incubated at their optimal temperature for 10 minutes, suggesting that the enzyme is being protected against thermal denaturation by the presence of substrate and/or either one or both of the products. Stabilization studies showed that both the forms are protected from heat inactivation by one of the reaction products,viz. L-phenylalanine,while the other reaction product hippuric acid provides only limited protection. It has also been

concluded that during buffalo carboxypeptidase A catalysed hydrolysis of hippuryl-L-phenylalanine, the slowest step is the release of L-phenylalanine. On the basis of this, a new tentative mechanism of carboxypeptidase A catalysed hydrolysis of acyl dipeptides has been proposed.

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