STUDIES ON THE REACTIONS OF TRYPSIN WITH CROSSLINKING REAGENTS

by

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DEDICATED

TO

MY MOTHER

'The research work in biochemistry in the 20th century is probably like building of cathedrals in the middle ages. It is the work of many, and the identity of those who participated in their creation is a matter of little consequence and will soon be forgotton'.

taken from 'A new look
at mechanisms in bioenergetics'
by E. Racker

CERTIFICATE

This is to certify that the thesis entitled, **"STUDIES ON THE REACTIONS OF TRYPSIN WITH CROSSLINKING REAGENTS"** being submitted by Mr. Y.S. Rajput to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy, is a record of bona fide research work carried out by him. Mr. Y.S. Rajput 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 University or Institute for the award of any degree or diploma.

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(Y. S. RAJPUT)

ABSTRACT

The thesis has been divided into six chapters.

The Chapter. I is an introductory chapter wherein a review of literature on structure and function of trypsin and various attempts to stabilize it has been given. A brief introduction to crosslinking methods is also included in this chapter.

The Chapter II summarizes the optimization of reaction conditions for crosslinking of trypsin with three bifunctional reagents viz. glutaraldeyde, dimethyl suberimidate (DMS) and dimethyladipimidate (DMA). This optimization was aimed at obtaining a trypsin derivative which undergoes minimum amount of autolysis.

The Chapter III deals with the trypsin derivative obtained with DMA. This derivative showed more stability vis-a-vis autolysis as compared to the derivatives obtained with other crosslinking reagents. The routine data like pH optimum, Km value, temperature optimum, Ca^{++} activation, extent of amino groups modification, PAGE and SDS-PAGE patterns about this derivative are given. Inactivation by dithiothreitol and sodium metaperiodate has been studied. Data are also given for periodate oxidation of partially inactivated trypsin derivative by dithiothreitol. Activities towards small and large molecular weight substrates are also reported in this chapter.

Chapter IV describes the characterization of trypsin derivative obtained with DMS. Here also the autolysis of trypsin derivative was markedly lower than native trypsin though not as much less as in the case of DMA derivative. The pH optimum, Km value, extent of amino group modification, PAGE and SDS-PAGE patterns of this derivative are also given in this chapter.

Lately, there has been considerable interest in linking different enzymes together by crosslinking. In the present work, attempts have been made to link trypsin with alkaline phosphatase and chymotrypsin. These studies have been reported in Chapter V. Trypsin-chymotrypsin conjugate was prepared by most commonly used crosslinking reagent glutaraldehyde and also by more recently available N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP). The conjugate contained both enzymes in the ratio of 1:1 on mole basis. Comparison of the conjugate activities with small and large molecular weight substrates have been reported. The autolysis of conjugate was found to decrease still further after its reaction with DMA.

Enzyme aggregation is yet another approach for enzyme stabilization. Chapter VI deals with preparation of heteroenzyme insoluble aggregates from trypsin and chymotrypsin. The aggregate preparation has been analyzed for enzymatic activities and stability.

After Chapter VI, a summary of the work described in this thesis has been given. The list of references has been provided in the end.

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