

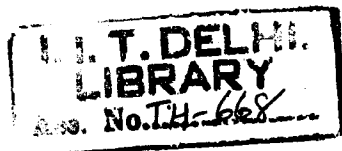
TH-668

MECHANISTIC STUDIES ON GOAT PANCREAT CARBOXYPEPTIDASE B (EC 3. 4. 12. 3)

BY
S. K. SRIVASTAVA

THESIS SUBMITTED IN FULFILMENT OF THE
REQUIREMENT FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
TO THE
INDIAN INSTITUTE OF TECHNOLOGY, DELHI

DEPARTMENT OF CHEMISTRY
MARCH, 1979



TH
547.29
SR1-M

C E R T I F I C A T E

This is to certify that the thesis entitled "Mechanistic Studies on Goat Pancreatic Carboxypeptidase B (EC 3.4.12.3)" being submitted by S.K. Srivastava 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 him. S.K. Srivastava 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 full to any other university or institute for the award of any degree or diploma.


(R.D. Dua)
Thesis Supervisor
Professor, Chemistry Department
I.I.T, Delhi
New Delhi 110029

ACKNOWLEDGEMENTS

I express my profound gratitude to Prof. R.D. Dua for his valuable guidance and constant encouragement throughout the course of these investigations.

I wish to express my sincere thanks to my colleagues in the Biochemistry Laboratory for their help and cooperation during the period of my stay here.

Mr. N.K. Nautiyal needs a special mention here who maintained the instruments in the Biochemistry Laboratory in good shape which helped me to finish the project satisfactorily.

A word of appreciation is due to Mr. S.L. Aneja for his neat typing of the manuscript and to Mr. N.L. Arora for the skill and care he took in making the diagrams.

I gratefully acknowledge the financial assistance and research facilities provided by the authorities of Indian Institute of Technology, Delhi.

New Delhi 110 029
March, 1979.


(S.K. Srivastava)
Department of Chemistry,
Indian Institute of Technology, Delhi

CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	29
Collection of Pancreatic Juice	29
Activation of Pancreatic Juice	29
List of Chemicals	30
Preparation of Substrate Solutions	33
Assay for Carboxypeptidase B Activity	34
Assay for Carboxypeptidase A Activity	34
Definition of Activity Unit	35
Assay of Esterolytic Activity	35
Amino Acid Standards	35
Purification of Methylcellosolve	36
Ninhydrin Reagent	36
Assay of Chymotrypsin Activity	36
Protein Determination	36
Preparation of DEAE-cellulose Column	38
Preparation of CM-Sephadex C-50 Column	38
Preparation of Sephadex G-25, Sephadex G-50 and Sephadex G-100 Columns	39
Disc Gel Electrophoresis	40
Molecular Weight Determination	44
Determination of Isoelectric pH	49
Determination of NH ₂ -terminal Amino Acid Residue	52

	Page
Determination of Amino Acid Composition	53
Preparation of Reagents used in Chemical Modification Studies	58
RESULTS AND DISCUSSION	62
Purification and Characterization of Goat Carboxypeptidase B	62
Purification Procedure	62
Purity of Enzyme Preparation	65
Optimum pH	70
Optimum Temperature	71
Effect of Ionic Strength	72
Effect of Buffers and Buffer Concentrations	73
Michaelis-Menten Constant	76
Molecular Weight	76
NH ₂ -Terminal Amino Acid Residue	77
Isoelectric pH	78
Optical Properties	79
Amino Acid Composition	80
Inhibition Studies with Goat Carboxypeptidase B	85
Chemical Modification of Goat Carboxypeptidase B	90
Preliminary Approach for the Detection of Active Site Amino Acid Residues	90
Arginine Modification by Phenylglyoxal	99
Tyrosine Modification by N-Acetylimidazole	110
Tyrosine Modification by Iodine	115
Histidine Modification by Photooxidation	121

	Page
Carboxylic Group Modification by 1-cyclohexyl-3(2-Morpholinoethyl)-Carbodiimide Metho-p-Toluene Sulfonate	125
Carboxylic Group Modification by N-Ethyl-5-Phenylisoxazolium-3'-Sulfonate	132
Studies on Thermal Stability of Goat Carboxypeptidase B	136
SUMMARY	149
BIBLIOGRAPHY	151