

STUDIES ON THE CARBOXYPEPTIDASE B (EC. 3.4.12.3)  
FROM GOAT PANCREAS

(Purification and Characterisation)

A THESIS SUBMITTED TO THE  
INDIAN INSTITUTE OF TECHNOLOGY, NEW DELHI  
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY  
IN CHEMISTRY

M. PRADHAKARA RAO, M.Sc. (Ag.)

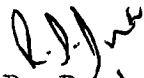
CHEMISTRY DEPARTMENT

OCTOBER, 1975

C E R T I F I C A T E

This is to certify that the thesis entitled "Studies on the Carboxypeptidase B (EC. 3.4.12.3) from Goat Pancreas (Purification and Characterisation)" being submitted by Mr. M. Prabhakara Rao to the Indian Institute of Technology, New Delhi for the award of the degree of Doctor of Philosophy in Chemistry, is a record of bonafide research work carried out by him. Mr. M.P. Rao 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.

  
(R. D. Dua)  
Thesis Supervisor  
Prof. of Chemistry  
I. I. T. Hauz Khas,  
New Delhi-110029.

## A C K N O W L E D G E M E N T

It is my immense pleasure to express my deep sense of gratitude to my revered **professor Dr. R.D. Dua** Head of the Department of Chemistry, for his guidance during the course of this project.

It is my duty also to thank Dr. R.C. Anand who helped to synthesise some of acyl peptide substrates commercially not available.

A word of appreciation to my dear colleagues in the Biochemistry laboratory especially to Mr. A. Dixit and Miss Rita Mitra for their constant encouragement during the period of my stay.

Mr. N.K. Nautiyal, senior laboratory assistant needs a special mention here. In spite of numerous difficulties, he maintained the instruments in Biochemistry laboratory in good shape which permitted me to finish the project satisfactorily.

May I also acknowledge here that completion of this project would have not been possible but for the authorities of the Andhra Pradesh Agricultural University, Hyderabad for agreeing to my study leave and the **lien**.

(ii)

I also wish to thank the authorities of the Indian Institute of Technology, New Delhi and the Council of Scientific & Industrial Research for the facilities and the financial assistance respectively.

At the end the credit for the neat execution of typing goes to Miss Sukhwarsha Devi and for tracings to Mr. N.L. Arora.

*M. Prabhakara Rao.*

M. PRABHAKARA RAO

## C O N T E N T S

	page
INTRODUCTION	1
MATERIALS AND METHODS	18
Collection of pancreatic juice	18
Activation of the pancreatic juice	19
Chemicals and sources	19
Synthesis of acyl peptides	21
Preparation of buffer solutions	31
Preparation of substrates	32
Assay of carboxypeptidase B & A	32
Definition of unit and activity	33
Amino acid standards	33
Ninhydrin reagent	33
Assay for esterolytic activity	34
Protein determinations	34
Chromatography of amino acids	36
Preparation of calcium phosphate gel	37
Removal of dissociable metal ions	37
Identification of metal ions	38
Zinc determination	38
Gel electrophoresis	41
RESULTS AND DISCUSSION	46
Evidence for carboxypeptidase B	46
Purification of carboxypeptidase B	56
Ammonium sulphate precipitation	58
DEAE cellulose batch adsorption and elution	61

(ii)

	page
Chromatography on DEAE cellulose	63
Stability	65
Rates of hydrolysis	68
pH Optimum	68
Temperature optimum	69
Effect of various buffers and buffer concentrations	69
Effect of anions	71
Michaelis-Menten constant	74
Homogeneity	75
Substrate specificity	82
Effect of C-terminal amino acid	90
Effect of penultimate amino acid	94
Stereospecificity	97
Metal studies	99
pH dependence of Zn removal	101
Zn removal by chelating agents	102
Zn substitution by other metal ions	106
SUMMARY	111
REFERENCES	114