IMMOBILIZATION OF GOAT PANCREATIC CARBOXYPEPTIDASES AND ITS APPLICATIONS

By

SANJAY KUMAR

Thesis submitted
in fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

INDIAN INSTITUTE OF TECHNOLOGY, DELHI

1985

Dedicated

To My

AUNT

(Sharda Bhatnagar)

Who Has Untimely Left Us Forever

C E R T I F I C A T E

This is to certify that the thesis entitled, "Immobilization of Goat Pancreatic Carboxypeptidases and Its Applications" being submitted by Mr. Sanjay Kumar to the Indian Institute of Technology, Delhi for the award of degree of 'Doctor of Philosophy', is a record of bonafide research work carried out by him. He has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our 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.

Padma Vasudevan

(Padma Vasudevan)
Thesis Supervisor
Professor and Head,
Centre for R.D. & A.T.,
I.I.T., Delhi
New Delhi - 110 016.

(R.D. Dua)
Thesis Supervisor
Professor,
Department of Chemistry,
I.I.T., Delhi
New Delhi - 110 016.

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

It is my profound pleasure and privilege to put on record my deep sense of gratitude to Professor R.D. Dua, Department of Chemistry and Professor (Mrs.) Padma Vasudevan, Head, Centre for R.D. & A.T., for their invaluable suggestions, inspiring guidance and critical supervision, at every stage of this investigation.

My sincere thanks are due to Professor B.L. Khandelwal, Head, Department of Chemistry and Professor R.P. Gandhi, for their kind care.

I wish to express my indebtedness to Professor M.S. Sodha, Department of Physics, for his constant and enduring help and encouragement throughout my research career.

I appreciate the cooperation of my friends and staff in the Biochemistry Laboratories and Chemistry Department, especially Drs. Alok Misra, F. Hussain and Messers Rishi Srivastava, Atul Gupta, Narendra Bhandari and Mr. N.K. Nautiyal.

Finally, I would like to thank Mr. Jagdish Kumar for his competent and dependable typing.

Sanjay Kumar

SANJAY KUMAR)

C O N T E N T S

		Page
(1)	INTRODUCTION	1
2)	PURIFICATION OF CARBOXYPEPTIDASE A AND B FROM GOAT PANCREAS	
	Collection of Pancreatic Juice	
	Activation of Pancreatic Juice	29
	Isolation of the Carboxypeptidase A Activity	30
	Preparation of Acetone Powder	30
	Preparation of Acetone Powder Extract	30
	Ammonium Sulfate Fractionation	31
	Column Chromatography on CM-Sephadex C-50	32
	List of Chemicals	32
	Preparation of Substrate Solutions	34
	Assay for Carboxypeptidase A Activity	
	Colorimetric Method	34
	Amino Acid Standards	35
	Spectrophotometric Method	35
	Assay for Carboxypeptidase B Activity	35
	Definition of Activity Unit	36
	Purification of Methylcellosolve	36
	Ninhydrin Reagent	36
	Protein Determination	36
	Preparation of CM-Sephadex C-50 Column	38
	Disc Gel Electrophoresis	38
	Michaelis-Menten Constant	43

Page

(3) STUDIES ON IMMOBILIZATION OF GOAT CARBOXY-PEPTIDASE A ON CELLULOSIC MATRICES

(i) Oxidized Cellulose 45

Materials and Methods 46

Reagents 46

Preparation of Oxidized Cellulose 46

Determination of Oxygen Consumption 48

Immobilization of Carboxypeptidase A 48

Formation of Reduced Carrier-Enzyme Complex 49

Results and Discussion 49

(ii) Acid Chloride of Oxidized Cellulose 53

Materials and Methods 54

Reagents 54

Preparation of Acid Chloride of Oxidized Cellulose 54

Immobilization of Carboxypeptidase A 56

Results and Discussion 56

(4) STUDIES ON IMMOBILIZATION OF GOAT CARBOXY-PEPTIDASE A ON SYNTHETIC POLYMERIC CARRIERS

(i) Copolymer Styrene Maleic Anhydride 63

Materials and Methods 63

Reagents 63

....(iii)

Page

Crosslinking and the Activation of Carrier 64

Immobilization of Carboxypeptidase A 66

Results and Discussion 66

(ii) Copolymer Ethylene Maleic Anhydride 73

Materials and Methods 74

Reagents 74

EMA-hydrazide Resins 75

Coupling of Anionic and Cationic EMA-hydrazide Resins to Carboxypeptidase A 77

EMA - MDA Resins 78

Coupling of Anionic and Cationic EMA-MDA Resins to Carboxypeptidase A 79

Results and Discussion 80

(5) APPLICATIONS OF GOAT CARBOXYPEPTIDASES IN SEQUENTIAL ANALYSIS

Introduction 90

Materials and Methods 94

Sequential Analysis 94

Tetrapeptide (L-Leu-L-Leu-L-Val-L-Tyr) 94

Preparation of Dowex 50X4 Resin Column 95

Ninhydrin Test 97

Biuret Assay 98

....(iv)

Page

Experimental 98

Column Chromatography on Dowex 50X4 Resin 100

Conclusions 102

Tetrapeptide (L-Leu-L-Leu-L-Val-L-Phe) 108

α-Chymotrypsin 109

Immobilization of Carboxypeptidase B 110

(6) SUMMARY AND CONCLUSIONS 114

BIBLIOGRAPHY 118