

Enzyme-Substrate Modifications And Their Industrial And Biomedical Significance

BY

K. ANJANEYULU

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

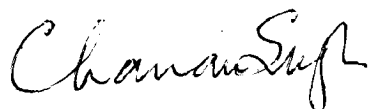
**DEPARTMENT OF CHEMISTRY
DECEMBER, 1978**

DEDICATED
TO
MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled "Enzyme-substrate modifications and their industrial and bio-medical significance" being submitted by K. Anjaneyulu 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. He 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.



(CHANAN SINGH)
Thesis Supervisor,
Assistant Director,
Biochemistry Division,
C.D.R.I., Lucknow, 226001.



(R. D. DUA)
Thesis Supervisor
Professor, Chemistry Department,
Indian Institute of Technology,
Delhi, New Delhi, 110029.

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

I would like to express special gratitude to my revered teachers, Dr. Chanan Singh and Prof. R. D. Dua, for their able guidance, advice, criticism and encouragement, at every step of this investigation,

Thanks are due to the authorities of the Indian Institute of Technology, Delhi and Central Drug Research Institute, Lucknow for providing me the facilities for carrying out and successfully completing this project.

I am also grateful to all the scientists and various organisations, who generously extended their co-operation and made available to me a large number of chemicals and biochemicals, but for which present work would have never reached fruition.

I especially appreciate the help and co-operation of my colleagues in the Biochemistry Laboratories of I.I.T., Delhi, and C.D.R.I., Lucknow, and value their comments and suggestions to improve this thesis.

A word of appreciation is also due to Shri A.N. Khurana, for his excellent typing of this thesis.

I shall be charged of dereliction, if I do not acknowledge the patience, co-operation and understanding from my wife, Dr. Renu Anjaneyulu, Laboratoire De Medicine

Experimentale, Universite' Libre de Bruxelles, Belgique.
She has been a source of inspiration and encouragement
to me during all this period.

Last but not least, I shall like to thank the
Council of Scientific and Industrial Research, New Delhi,
for providing me the financial assistance to complete
this investigation.



(K. ANJANEYULU)

December, 1978.

TABLE OF CONTENTS

		Page
Part I	STUDIES ON THE ACTIVATION OF α -AMYLASE (HOG PANCREAS)	
	Introduction	1
	Materials and Methods	19
	Reagents	19
	Assay of various enzymes	21
	Determination of kinetic parameters	26
	Results and Discussion	29
	Activation of α -amylase	29
	<u>In Vivo</u> studies	47
	Studies on trasylol	49
Part II	STUDIES ON IMMOBILIZATION OF α -AMYLASE (HOG PANCREAS)	
	Introduction	53
	Materials and Methods	66
	Reagents	
	Immobilization on carboxy-methyl cellulose	66
	Preparation of Sephadex bound α -amylase	68
	Dextran- α -amylase conjugate preparation	69
	Results and Discussion.	70

Part III	STUDIES ON THE INFLUENCE OF SUBSTRATE MODIFICATION (MODIFICA- TION OF RNA) ON ITS HYDROLYSIS BY THE ENZYME, RIBONUCLEASE	
	Introduction	75
	Materials and Methods	82
	Reagents	82
	Ribonuclease assay	82
	Complexation of RNA with various poly cationic compounds	82
	Results and Discussion	84
Part IV	SUMMARY AND CONCLUSIONS	89
	BIBLIOGRAPHY	92