

**ENZYMATIC AND RADIOACTIVE TRACER
STUDIES ON THE BIOSYNTHESIS OF
THREONINE AND OTHER AMINO ACIDS
OF THE ASPARTIC ACID FAMILY**

**A THESIS SUBMITTED TO
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LIST OF ABBREVIATIONS

ATP	Adenosine-5'-Triphosphate
GTP	Cytidine-5'-Triphosphate
Cys or cys	Cysteine
DEAE-Cellulose	Diethylaminoethyl cellulose
EDTA	Ethylenediaminetetra-acetic acid
Ile or ile	Isoleucine
Lys or lys	Lysine
Met or met	Methionine
NAD ⁺	Nicotinamide adenine dinucleotide
NADH	Reduced Nicotinamide adenine dinucleotide
NADP ⁺	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced Nicotinamide adenine dinucleotide phosphate
pCMB	p-chloromercuribenzoate
SDS	Sodium dodecyl sulphate
Thr or thr	Threonine

SYNOPSIS

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The regulation of cell metabolism in biological systems and especially the regulation of the biosynthesis of compounds involving branched pathways has become an important field of study in Biochemistry in recent years. The biosynthesis of threonine, lysine and other amino acids of the aspartate family is one such highly branched pathway on which much attention has been focussed. A number of review articles have appeared in literature during the last six to eight years on the enormous amount of work concerning the various aspects of this pathway, including the regulatory properties of the enzymes involved therein. However, most of the work done so far has been with bacteria and very little information is available on plants.

During the course of our investigations, we studied the pattern of regulation for the biosynthesis of the amino acids of the aspartate family in bacteria, with special reference to Serratia marcescens. Our initial work on Micrococcus glutamicus and certain plant systems had to be discontinued for reasons explained in the thesis.

The thesis consists of four chapters:

In Chapter I, a general review of the known regulatory mechanisms in biological systems is presented. The various modes of control existing for the regulation of branched biosynthetic pathways in general, and for the regulation of the biosynthesis in bacteria and plants of the threonine, lysine and other amino acids of the aspartate family in particular, have been surveyed. An abstract of the work done during the course of the present investigations is included at the end of the chapter.

Chapter II contains a description of the materials used and the various experimental procedures (chemical, enzymatic, biological and others) employed in our studies.

Chapter III describes the experimental work done and the results obtained. The chapter has been divided into four sections. In the first section, results of our preliminary work with bacteria and plants are given. Both aspartokinase and homoserine dehydrogenase activities were detected in the two bacterial species - Serratia marcescens and Micrococcus glutinis. Of the plant species studied, fresh cotyledons of french beans (Phaseolus vulgaris) had both the enzyme activities, while seedlings of Phaseolus mungo and Phaseolus

radiating had only weak aspartokinase activity. The reasons for discontinuing our studies with some of the bacterial and plant systems and concentrating on the regulatory enzymes of Serratia marcescens are also mentioned.

The second section of Chapter III includes results of studies with labelled compounds. ^{14}C -U-aspartate is utilized by S. marcescens cells for the synthesis of lysine, threonine, methionine and isoleucine: these amino acids accounted for the majority of the radioactivity incorporated into the cell protein. Indications for the presence of an alternate pathway in the same bacterium for the production of isoleucine from uniformly labelled glutamate are described.

The third section of the same chapter gives an account of the work done on the pattern of repression of synthesis and inhibition of activity of the two enzymes aspartokinase and homoserine dehydrogenase of S. marcescens by the amino acid end products. These experiments have indicated the presence of two isoenzymes of homoserine dehydrogenase. The activity of one isoenzyme is inhibited specifically by L-threonine while the synthesis of the other is completely repressed by methionine. The activity and synthesis of aspartokinase are both affected by L-lysine.

Section 4 gives a detailed description of the experiments on purification and properties of the three enzymes - the threonine-sensitive and methionine-repressible isoenzymes of homoserine dehydrogenase and the lysine-sensitive aspartokinase of S. marcescens. The methionine-repressible homoserine dehydrogenase has been purified to homogeneity as determined by disc gel electrophoresis at two pH values. Properties of the enzyme studied indicated two identical subunits in the enzyme each of a molecular weight of 75,780 while the molecular weight of the native enzyme was obtained as 155,000 by gel filtration techniques. Properties of the other two enzymes such as pH optimum, substrate and cofactor specificity, temperature optimum etc. have been studied.

Chapter IV includes a discussion of the pattern of regulation of the biosynthesis of the aspartate family of amino acids in S. marcescens and the regulatory properties of the enzymes involved, based on the experimental results detailed in the previous chapter. A comparison of the properties of corresponding regulatory enzymes of different bacterial and plant systems has also been made.

The thesis concludes with a summary of the results obtained and conclusions drawn from them and a list of references cited in the text.