Homoserine Dehydrogenase of *Serratia marcescens*

By M. S. SHAILAJA and M. R. RAGHAVENDRA RAO
(Discipline of Biochemistry and Applied Nutrition,
Central Food Technological Research Institute,
Mysore-2A, India)

Homoserine dehydrogenase is a key enzyme in the biosynthesis of methionine, threonine and isoleucine (Greenberg, 1969; Meister, 1965) from aspartate. So far there appear to be only two instances of homoserine dehydrogenase subject to complete repression by methionine, namely in *Escherichia coli* K12 (Patte *et al.*, 1967, 1969) and in *Salmonella typhimurium* (Cafferata & Freundlich, 1969, 1970). However, these organisms possess two homoserine dehydrogenases and the methionine-repressible enzyme is a minor one.

A strain of *Serratia marcescens* seems to possess two homoserine dehydrogenases, one NAD-dependent and the other NADP-dependent. The NAD-dependent enzyme is completely repressed by methionine, homoserine or cysteine (at 1 mM), but is not inhibited by any amino acid either singly or in combination. The NADP-dependent enzyme is partially repressed (70–80%) by the above amino acids. Unlike in *E. coli* K12 (Patte *et al.*, 1967, 1969), these activities are not associated with aspartokinase, as evidenced during purification and in mutants. Two threonineless mutants of this organism lack homoserine dehydrogenase activity but possess normal aspartokinase activity. Further details about the purification and properties of the enzymes will be discussed.

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Studies on Mammalian Hetero- β -galactosidases

By B. SEETHARAM and A. N. RADHAKRISHNAN
(Wellcome Research Unit, Christian Medical College
Hospital, Vellore-4, India)

Hetero- β -galactosidase was purified from the soluble fractions of monkey liver (550-fold) and small intestine (110-fold). Both enzymes are active over a wide range of pH, the liver enzyme with an optimum at pH 5.6 and the intestinal enzyme with an optimum

at pH 7.0. The enzymes are active towards a variety of synthetic hetero- β -galactosides and β -glucosides, but have no action on lactose or cellobiose. The K_m for *o*-nitrophenyl β -D-galactoside was 8.6 mM with the liver enzyme and 8.9 mM with the intestinal enzyme. Phlorrhizin was not a substrate for the enzymes. In both cases there was no evidence for heterogeneity by gel filtration or by ion-exchange chromatography, in contrast with the results obtained with ox liver (Chytil, 1965) and rat liver (Furth & Robinson, 1965) enzymes. On polyacrylamide-gel electrophoresis both the hetero- β -galactosidase and hetero- β -glucosidase activities were present in the same major protein band. Mixed-substrate inhibition revealed that the glucosidase and galactosidase activities are exerted by the same protein. The pig kidney (Patel & Tappel, 1969) and rat kidney (Robinson *et al.*, 1967) purified fractions appear to catalyse the hydrolysis of both hetero- β -galactosides and hetero- β -glucosides. However, a partial separation of β -galactosidase and β -glucosidase activities of rat brain has been reported (Gatt & Rapport, 1966). The purified enzymes from both tissues from the monkey are inhibited by heavy-metal ions such as Cu^{2+} , Hg^{2+} and Ag^+ and by tris, gluconolactone and galactonolactone. The liver enzyme is most probably lysosomal (Alpers, 1969; Sellinger *et al.*, 1960), whereas in the intestine there are two hetero- β -galactosidases, one with a neutral pH optimum in the soluble fraction and one with an acid optimum pH in the particulate fraction (Swaminathan & Radhakrishnan, 1969).

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Distribution of Sodium-plus-Potassium Ion-Stimulated Adenosine Triphosphatase along the Length of Rat Small Intestine and its Subcellular Localization

By M. S. GNANAPRAKASAM and L. M. SRIVASTAVA
(Department of Biochemistry, All India Institute of
Medical Sciences, New Delhi-16, India)

Ever since the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase* was first reported by Skou (1957), considerable sup-

*Abbreviation: ATPase, adenosine triphosphatase.

port has been forthcoming for the involvement of this enzyme in active transport. The epithelial cells of the intestine actively transport not only Na^+ and K^+ but also sugars and amino acids. It is now clear that this process is mediated by a common carrier and is linked to the Na^+ + K^+ -stimulated ATPase. This communication describes the distribution of Na^+ + K^+ -stimulated ATPase in various regions of the small intestine and its subcellular localization. It was found that different regions of the small intestine contained different amounts of the enzyme activity: the enzyme activity was highest in the jejunum and lowest in the terminal ileum. These results can be correlated with the reported observations of a higher rate of absorption by the upper part of the small intestine than by the lower part (Wilson & Vincent, 1955). Fractionation of homogenates of rat intestinal mucosa showed that about 90% of the Na^+ + K^+ -stimulated ATPase activity was localized in the brush-border and mitochondrial fractions. The enzyme activity required the combined presence of Na^+ and K^+ and was inhibited by ouabain, a specific inhibitor of active transport.

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Purification and Properties of Phenylalanine Ammonia-lyase from Plant Tissue Cultures

By M. DHANDAPANI, A. ANTONY and P. V. SUBBA RAO (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

Callus cultures were initiated from the hypocotyls and root meristem of germinating seedlings of *Trigonella foenum-graecum* L. and maintained in culture with Murashige & Skoog medium supplemented with casein hydrolysate and coconut milk. Cell-free preparations were found to deaminate L-phenylalanine and L-tyrosine to *trans*-cinnamate and *p*-coumarate respectively. These enzyme activities were followed during the growth of the hypocotyl callus cultures and maximal activity was found between day 20 and day 22.

Phenylalanine ammonia-lyase was purified from these cultures to near homogeneity and its properties were studied. The available literature on phenylalanine ammonia-lyase shows that this is the first report of its purification from plant tissue cultures.

Localization of Isocitrate Lyase in Germinating Seeds of Cotton (*Gossypium herbaceum* L.)

By G. PRATHAPASENAN (*Department of Botany, M.S. University of Baroda, Baroda-2, India*) and G. RAMANANDA RAO (*Microbiology and Pharmacology Laboratory, Indian Institute of Science, Bangalore-12, India*)

Previous studies on cotton have shown that there is a steady depletion of fat with a parallel increase in isocitrate lyase activity during germination (Prathapasenan *et al.*, 1969). The presence of isocitrate lyase in particles designated as 'glyoxysomes' was reported in castor bean (Briedenbach & Beevers, 1967), peanut and maize (Longo & Longo, 1970) and pine seed (Ching, 1970). In lettuce seeds the enzyme was present only in the soluble fraction (Mayer *et al.*, 1968). The present work involves the localization of isocitrate lyase in germinating cotton seeds.

Cotyledons from seeds germinated for 72h were homogenized for 15 min (Ching, 1970). The homogenate was centrifuged at 500g to remove the debris and then at 10000g for 20 min. Both the pellet and the supernatant were assayed for enzyme activity. The 10000g pellet was suspended in 1 ml of 32.5% (w/v) sucrose, layered over a discontinuous sucrose density gradient (Ching, 1970) and centrifuged at 90000g for 6h. The different bands were collected, dialysed and used for enzyme assays.

Isocitrate lyase was found both in the 10000g pellet and in the supernatant. The activity in the supernatant was at least twice that in the pellet. To exclude the possible release of enzyme from the particles during prolonged grinding, shorter durations (30s, 1 min, 2 min and 5 min) were tried. Irrespective of the duration of grinding, activity was found in both the fractions.

On sucrose-density-gradient centrifugation the 10000g pellet was further separated into six distinct bands. Isocitrate lyase activity was detected in a band with a peak sucrose density of 1.16. The same band showed catalase activity as well. Other bands are being characterized for their enzyme activities. These results indicate the presence of a soluble and particulate ('glyoxysomal') isocitrate lyase in cotton.

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