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Studies on Purified Bovine Spleen Adenosine Deaminase

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Adenosine deaminase has been purified from bovine spleen. All the purified spleen preparations had a typical protein extinction spectrum with a maximum at 278 m μ . A study of the substrate specificity showed that the enzyme deaminates deoxyadenosine at approximately the same rate as adenosine, and the results are comparable with those obtained for adenosine deaminase from bovine duodenal mucosa. 6-Chloropurine riboside was dechlorinated by the spleen enzyme at a slower rate than by the duodenal enzyme. There was a marked difference between the heat stability of the two enzymes. The pH optimum and stability of the enzyme were similar to that reported by Brady & O'Sullivan (1967) for the duodenal enzyme.

Ag⁺, Hg²⁺ and Cu²⁺ ions as well as iodosobenzoic acid and iodine inhibited the enzyme. *p*-Chloromercuribenzoic acid and *N*-ethylmaleimide completely inhibited the enzyme, and these results point to the importance of a thiol group for the activity of the enzyme. In this respect it was similar to the bovine duodenal enzyme. The stability of the enzyme to freeze-drying decreased as enzyme purity increased, which again was similar to the duodenal enzyme. By electrophoresis the number of isoenzymes present in spleen extracts was found to vary from one to five, and the original pattern persisted throughout the purification. The electrophoretic pattern of the duodenal enzyme varied from intestine to intestine, giving a pattern of five or six isoenzymes when the intestines from various animals were mixed. The Michaelis constants of both enzymes were very similar.

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Disulphide Bonds in Relation to the Activity and Structure of Adenosine Deaminase

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Amperometric-mercurimetric titration of adenosine deaminase, with both inorganic and organic mercurials, revealed the presence of two disulphide bonds in the protein molecule. Results obtained by using two different procedures of the above titration technique (Leach, 1966), were compared and assessed in relation to the total sulphur content of this enzyme.

Reduction of the disulphide bonds of adenosine deaminase with sodium sulphite resulted in an instantaneous and marked fall in the enzyme activity. The residual activity of the reduced enzyme when assayed at pH 9.3 and 7.0 was similar, showing that reformation of disulphide bridges, which might be favoured by the lower pH, was not accompanied by reactivation of the enzyme. Iodoacetamide, which was shown to have no effect on the activity of the native enzyme, accelerated the rate of inactivation by sulphite.

Finally, the effect of reducing the disulphide bonds on the overall shape of the enzyme molecule was studied by the Sephadex-gel filtration technique. Comparable studies were carried out on other proteins of known disulphide bond content, and results were compared with those obtained for adenosine deaminase.

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The Induction of Glutamate Dehydrogenase and Aspartate Aminotransferase in *Rhizobium*

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Enzymes involved in the fixation of atmospheric nitrogen in legumes have been located in the bacteroid fraction of the root nodules (Kennedy, Parker & Kidby, 1966; Koch, Evans & Russell, 1967; Bergersen & Turner, 1967). Several enzymes active in the assimilation of ammonia, the primary product of nitrogen fixation, were also detected in the bacteroid fraction of legume root nodules and in *Rhizobium* grown *in vitro* (Fottrell & Montgomery,

1968). The most important of these ammonia-assimilating enzymes appeared to be glutamate dehydrogenase and aspartate aminotransferase, and the present communication describes studies on the mechanism involved in the induction of these enzymes in *Rhizobium japonicum*.

When *R. japonicum* was transferred from a medium containing glutamate or aspartate to one containing 2-oxoglutarate plus ammonium chloride, the glutamate dehydrogenase and aspartate aminotransferase activity increased with time. Glutamate dehydrogenase activity in the latter medium was maximal after about 27 hr., after which the activity declined and reached its former value after about 90 hr. Aspartate aminotransferase, on the other hand, reached maximum activity after about 90 hr., after which it also began to decrease and reached its former value after about 120 hr.

Active protein and RNA synthesis was necessary for the induction of both enzymes. When actinomycin D (1.0 $\mu\text{g./ml.}$) was included in the growth medium, no increase in the activity of either glutamate dehydrogenase or aspartate aminotransferase was detected. Likewise, puromycin (2.5 $\mu\text{g./ml.}$) completely inhibited the induction of both enzymes. When cycloheximide was included in the growth medium at concentrations of 2 $\mu\text{g./ml.}$, 3 $\mu\text{g./ml.}$ and 4 $\mu\text{g./ml.}$, the induction of aspartate aminotransferase was inhibited by 60%, 88% and 90% respectively.

The induction of both the aforementioned enzymes was completely reversible. For example, when the cells were transferred to a medium containing glutamate or aspartate, the glutamate dehydrogenase activity was completely repressed. Likewise, the activity of aspartate aminotransferase was completely repressed by aspartate in the growth medium. Considerable though not complete repression of glutamate dehydrogenase took place when the bacteria were transferred to a medium containing glutamine, ornithine or alanine.

These results show that enzymes active in ammonia assimilation in *R. japonicum* are induced or repressed by different nitrogen sources in the growth medium.

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Dissociation of the Prosthetic Group of Aspartate Aminotransferase

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Aspartate aminotransferase (EC 2.6.1.1) contains pyridoxal 5-phosphate (PLP), whose removal from the apoprotein is facilitated by conversion into pyridoxamine 5-phosphate (PMP), by acid pH, and by either phosphate or precipitation with ammonium sulphate (Banks & Vernon, 1961; Wada & Snell, 1962; Scardi, Scotto, Iaccarino & Scarano, 1963). Jenkins & D'Ari (1966) converted the enzyme into its PMP form, overcoming the unfavourable equilibrium constant, by gel filtration through a band of amino acid, so that the enzyme constantly encountered fresh reagent. We present a similar method for obtaining the apoenzyme.

Pig heart enzyme was obtained by extraction, heat treatment and ammonium sulphate fractionation modified from the method of Jenkins, Yphantis & Sizer (1959), followed by chromatography on microgranular CM-cellulose in 64 mM-sodium acetate-30 mM-acetic acid. The enzyme was then gel-filtered into water and freeze-dried.

For removal of the prosthetic group a column (70 cm. $\times$ 1 cm.) of dextran particles (Sephadex G-25, fine grade) was washed with water. Buffer (2 ml. of 50 mM-tris-25 mM-HCl) was added, then 1 ml. of water, and then 10 ml. of m- NaH_2PO_4 -50 mM-glutamic acid-0.2 M-sodium glutamate. The enzyme (up to 50 mg.) dissolved in 1 ml. of 0.2 M-sodium glutamate was added, and the column was eluted with water at 40 ml.cm.⁻² hr.⁻¹. The protein peak was detected by its extinction at 280 m μ . It proved to have only about 2% of its potential activity until PLP was added, when 60% of the initial activity appeared. Activity was measured by rate of increase of extinction at 265 m μ in 10 mM-aspartic acid-4 mM- α -oxoglutaric acid-0.1 M-tris-50 mM-HCl. The apoenzyme was freeze-dried.

The glutamate added did not merely convert the PLP prosthetic group into PMP, but assisted the dissociation of the PMP. This was shown by gel-filtering samples (5 mg.) first through 2 ml. of 0.1 M-glutamic acid in 0.2 M-tris, which completely converted the enzyme into the PMP form, and then through 1 ml. of water followed by 5 ml. of a reaction mixture. Loss of coenzyme was complete in m- NaH_2PO_4 -50 mM-sodium glutamate-50 mM-glutamic acid, but was only partial when the glutamate was replaced by acetate, glutarate or aspartate. The phosphate, however, could be replaced by sulphate.

Thus the method of Scardi *et al.* (1963) for removing the prosthetic group by treating the PMP form

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