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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 mμ. 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^+, Hg^{2+} and Cu^{2+} 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,