Purification of Aspartate Aminotransferases from Soya-Bean Root Nodules and *Rhizobium japonicum*

By E. RYAN, F. BODLEY and P. F. FOTTRELL.
(Department of Biochemistry, University College,
Galway, Irish Republic)

High amounts of aspartate aminotransferase are present in soya-bean (*Glycine max*) root nodules, the sites of nitrogen fixation in these plants. The enzyme probably has an important role in the assimilation of 'fixed' nitrogen. At least two forms of aspartate aminotransferase are present in these nodules: one in the bacterial part and the other in the extra-bacterial part of the nodule (Fottrell & Mooney, 1969). To determine the functions of the two aspartate aminotransferases in nodules, the enzymes were purified from *Rhizobium japonicum* (symbiont of soya beans) and from the extrabacterial part of nodules.

Aspartate aminotransferase from nodules was purified by ammonium sulphate fractionation, acid precipitation and by passage through columns of DEAE-cellulose and DEAE-Sephadex. The procedures used gave an overall purification of 120-fold with a 45% yield. The enzyme was remarkably stable to a wide variety of inhibitors. The coenzyme pyridoxal 5-phosphate was resolved from the apoprotein only with difficulty by conversion into pyridoxamine 5-phosphate followed by precipitation with ammonium sulphate and dialysis to remove pyridoxamine 5-phosphate. The optimum pH of the enzyme was 7.5 and the molecular weight determined with Sephadex G-200 was about 100 000.

Lineweaver-Burk plots of initial reaction velocities of the enzyme against aspartate concentration at two fixed concentrations of 2-oxoglutarate suggested a binary mechanism of action for the enzyme.

The bacterial enzyme was purified from heat-treated extracts by passage through a column of Sephadex G-100. In contrast with the nodule enzyme described above the bacterial enzyme was unstable but could be stabilized by aspartate plus pyridoxal 5-phosphate. The molecular weight of the bacterial enzyme was about 100 000, and unlike the extrabacterial enzyme the coenzyme pyridoxal 5-phosphate was easily resolved from the apoprotein. The coenzyme could be separated from the apoprotein by passage of a crude bacterial extract, prepared with 66mM-phosphate buffer, pH 7.4, through Sephadex G-100 and eluting with 0.1M-NaCl. The enzyme was reconstituted by adding pyridoxal 5-phosphate to the apoprotein, and the apparent K_m for this reaction was 0.5×10^{-6} M.

A third aspartate aminotransferase has also been detected in mitochondria from soya-bean nodules,

and the possible roles of these three enzymes in nodule metabolism are under investigation.

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Molecular Heterogeneity of Human Intestinal Peptidases from Control Subjects and Children with Treated and Untreated Coeliac Disease

By ANN DILLON, J. DUFFY, J. O. DOLLY* and P. F. FOTTRELL. (Department of Biochemistry, University College, Galway, Irish Republic)

Previous studies showed that peptidase zymograms prepared from normal and coeliac intestinal mucosa were similar, although decreased amounts of some peptidases were detected in extracts from coeliac mucosa (Dolly & Fottrell, 1969). In view of the relevance of studies of this nature to the 'missing-peptidase' hypothesis for coeliac disease, this work has now been extended to study the hydrolysis of further peptides by intestinal extracts from control subjects (histologically normal intestinal mucosa) and untreated and treated (i.e. on gluten-free diets) coeliac patients.

The peptidases were separated by starch-gel electrophoresis from extracts of peroral intestinal biopsies as described by Dolly & Fottrell (1969).

Only slight decreases in the intensities of some peptidase bands were noticed when zymograms from control subjects and patients with untreated coeliac disease were compared by using the following substrates: Glu-Val, Pro-Leu, γ -Glu-Leu, Gly-Glu and Glu-Tyr-Glu. All the latter substrates were hydrolysed by more than one peptidase, e.g. four areas of peptidase activity were detected when Glu-Tyr-Glu was used as substrate. However, when intestinal peptidase zymograms were compared from control and untreated coeliac patients by using the substrates Leu-Pro, Phe-Pro and Glu-Pro, greatly decreased activities were noticed on zymograms prepared from the coeliac patients. Only one area of peptidase activity was detected on zymograms when these three substrates were used. These three substrates appeared to be hydrolysed by the same peptidase band on the zymograms.

When, however, intestinal peptidase zymograms were prepared from control subjects and treated

* Present address: Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.