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

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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.

E. R. thanks the Department of Education, Dublin, for a grant and F. B. is grateful to the Irish Committee for International Biological Programme for support. The financial support of An Foras Talúntais is gratefully acknowledged.

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

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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

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coeliac patients by using Leu-Pro, Phe-Pro and Glu-Pro, the zymograms were almost identical. These present results and the findings of others (Lindberg, Norden & Joseffson, 1968; Douglas & Booth, 1969) strongly suggest that the decreased activities of some peptidases in untreated coeliac intestine may be due to secondary phenomena attributable to the abnormal morphology of the mucosa.

To obtain more specific information about the intestinal hydrolysis of gluten, ^{125}I -labelled gliadin peptides were used to study intestinal peptidases. Results will be presented on the suitability of these peptides as substrates for intestinal peptidases.

We thank the Medical Research Council of Ireland for a grant.

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Multiple Forms of Pig Intestinal β -Galactosidase and Related Activities

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Studies on humans (Hsia, Makler, Semenza & Prader, 1966; Gray & Santiago, 1969), rats (Asp & Dahlqvist, 1968) and a number of other species indicate that intestinal β -galactosidases fall into two main groups. 'Acid' β -galactosidases have low pH optima, are readily solubilized, work best on hetero galactosides and are inhibited by *p*-chloromercuribenzoate. 'Neutral' β -galactosidases are essentially disaccharidases with neutral pH optimum that are associated with brush-border particles. They are not readily inhibited by *p*-chloromercuribenzoate. Multiple forms may occur within these groups and the substrate specificity may include glycosides with a β -glucosidic configuration.

We have examined pig intestinal mucosa and compared its enzymes with those described in pig kidney by Abrahams & Robinson (1969). Four enzymes with activity towards 4-methylumbelliferyl β -D-galactoside can be separated on DEAE-cellulose at pH 6.5 in 10mm-phosphate buffer by using a NaCl gradient. Form I is unretained by the columns; forms II, III and IV are eluted successively with increasing salt concentration. Form I has narrow specificity in requiring the galactose configuration at C-4, but hydrolyses hetero glyco-

sides (β -galactosides and α -arabinosides) as well as lactose. Forms II and III have identical specificities, hydrolysing β -glucosides and β -galactosides and the corresponding pentosides, but have no effect on lactose. Form IV has hexosidase but no pentosidase activity, and it is most effective on lactose. Unlike forms I, II and III it is not readily inhibited by *p*-chloromercuribenzoate and closely resembles the 'neutral' lactases previously reported. On starch gel electrophoresis at pH 6.8, form I remained at the origin, forms III and IV migrated rapidly towards the anode at the same rate, and form II showed intermediate mobility. Forms III and IV could be resolved electrophoretically on thin agarose gels. At pH 5.0 form I is dissociated into two bands migrating in opposite directions but retaining β -galactosidase activity. The two forms of soluble β -glucosidase reported by Abrahams & Robinson (1969) in pig kidney have the same substrate specificities and electrophoretic mobility as forms II and III reported here.

This study was supported by a grant from the Medical Research Council.

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Isoenzymes in the Study of Wild Populations

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The superior resolution of starch-gel electrophoresis in the separation of proteins has added a new dimension to studies in genetics. This technique has enabled us to examine variation at the molecular level. It has been shown that normal body proteins are polymorphic and that the actual molecular variants present are genetically determined. Thus with the aid of this biochemical technique it is now possible to detect and analyse the action of both directed and stabilizing selection in natural populations at the molecular level.

In the present study samples from a wild population of the house mouse, *Mus musculus*, living on the small island Skokholm off the coast of Pembrokeshire in South Wales (Berry, 1968) were surveyed at six loci. Gene frequencies were computed at the beginning and end of the breeding