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The Apparent Molecular Weights of Human Intestinal Aminopeptidase, Enterokinase and Maltase in Native Duodenal Fluid

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The apparent molecular weights of human intestinal aminopeptidase, enterokinase and maltase in native duodenal fluid were estimated by gel chromatography on Sephadex G-200 under different conditions of operational buffer and temperature. No evidence for environmentally induced changes in molecular form was found.

It has been shown in the pig (Baratti *et al.*, 1973a; Maroux & Louvard, 1976) that intestinal aminopeptidase and maltases are attached to the surface of the microvillus membrane of the enterocyte by a hydrophobic anchor, from which they may be cleaved and liberated into the luminal fluid by trypsin and other proteinases. Barns & Elmslie (1977) have demonstrated a similar membrane attachment for pig enterokinase. Pig aminopeptidase and enterokinase are multi-subunit glycoproteins; the apparent molecular weights of the purified enzymes have been estimated by ultracentrifugation to be 280000 for aminopeptidase (Maroux *et al.*, 1973) and 194000 for enterokinase (Baratti *et al.*, 1973a). The molecular weight of pig enterokinase has been estimated by gel filtration to be 212000 (Baratti *et al.*, 1973a) and 316000 (Grant & Hermon-Taylor, 1975), and highly purified human enterokinase has an apparent molecular weight of 296000 by gel filtration (Grant & Hermon-Taylor, 1976). The present study was designed to determine the apparent molecular weights of human intestinal aminopeptidase, enterokinase and maltase in native duodenal fluid, and whether these enzymes can exist in more than one active molecular form under different mildly dissociating conditions.

Materials and Methods

Sephadex G-200 and Sepharose 4B were obtained from Pharmacia (G.B.) Ltd., London W.5, U.K. Glycodeoxycholic acid was a product of Calbiochem, London W.1, U.K. α -N-Benzoylarginine ethyl ester, trypsinogen, leucine *p*-nitroanilide, peroxidase, glucose oxidase, bovine serum albumin, ovalbumin, alcohol dehydrogenase, xanthine oxidase, catalase and *o*-dianisidine were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Secretin/pancreozymin was obtained from Boots Pure Drug Co., Nottingham, Notts., U.K.

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Other chemicals were the purest grade obtainable. Maltase was assayed by the method of Dahlqvist (1967); enterokinase and aminopeptidase were assayed as previously described (Grant & Hermon-Taylor, 1976). Fractions after chromatography in 3M-NaCl were dialysed before assaying for enterokinase, because the enzyme activity is inhibited by high ionic strength (Baratti *et al.*, 1973b). Calibration of gel columns was performed as described by Andrews (1965). After re-equilibration in the different buffers the column calibration was checked with the standards.

Duodenal fluid was obtained, after secretin/pancreozymin stimulation (1 Clinical unit of secretin and 1 Ivy Dog unit of pancreozymin/kg body wt. intravenously), from eight patients undergoing routine clinical tests of pancreatic exocrine secretory function. A double-lumen tube was positioned in the second and third parts of the duodenum under fluoroscopic control; gastric secretion was continuously aspirated and discarded. One patient had a pancreatic carcinoma with decreased pancreatic enzyme output; pancreatic secretory function was normal in all other patients, although two had obstructive jaundice due to gall stones. Duodenal fluid was filtered (through Whatman no. 1 paper) and either chromatographed immediately or stored at -20°C . Chromatography was performed on Sephadex G-200 and Sepharose 4B at 4°C in 20mM-sodium phosphate buffer, pH7.4, containing one of the following: 0.2M-NaCl, 20% (v/v) ethylene glycol or 2.5mM-glycodeoxycholate. Chromatography on Sephadex G-200 at 37°C was performed in a water-jacketed column with 20mM-sodium phosphate buffer, pH7.4, containing 0.2M-NaCl as eluent.

Results and Discussion

A typical elution pattern of human duodenal fluid at 4°C on Sephadex G-200 in 20mM-sodium phosphate, pH7.4, containing 0.2M-NaCl is shown

Fig. 1. Chromatography of native human duodenal fluid on Sephadex G-200

Native human duodenal fluid was chromatographed on a column (2.54 × 33.5 cm; volume 170 ml) of Sephadex G-200. Fractions (4 ml) were collected every 20 min. The column was eluted with 20 mM-sodium phosphate, pH 7.4, containing 0.2 M-NaCl. Standards were: 1, xanthine oxidase (mol.wt. 275 000); 2, catalase (mol.wt. 232 000); 3, yeast alcohol dehydrogenase (mol.wt. 150 000); 4, bovine serum albumin (mol.wt. 66 000); 5, ovalbumin (mol.wt. 43 000) ●, A_{280} ; □, aminopeptidase activity (nmol of nitroaniline formed/min per ml); △, enterokinase activity (pmol of trypsin formed/min per ml); ○, maltase activity (nmol of disaccharide hydrolysed/min per ml).

in Fig. 1. Duodenal fluid from all eight patients chromatographed in the same way in this buffer, with enterokinase activity being eluted just after the void volume (V_0) of the column and before aminopeptidase, which emerged in the same position as xanthine oxidase (mol.wt. 275 000). These results are consistent with an apparent mol.wt. of 296 000 previously described for highly purified human enterokinase (Grant & Hermon-Taylor, 1976).

Maltase activity was found in high-molecular-weight (approx. 300 000) and low-molecular-weight (<40 000) fractions. Controls showed that maltase activity in the low-molecular-weight fractions was not due to free glucose. This separation of maltase activity may have been due to differences in molecular size or retardation of the enzyme as a result of affinity for the polyglucan matrix of the Sephadex gel (Semenza & Kolinska, 1967). This possibility was examined by chromatography of two duodenal fluids under the same conditions on Sepharose 4B, for whose polygalactan matrix maltase should have no affinity. The maltase activity again separated into one major high- and one major low-molecular-weight species, showing that the separation was due to different molecular size.

Aminopeptidase and enterokinase also chromatographed in a position consistent with their apparent

molecular weights as estimated on Sephadex G-200. No active forms of the enzymes were found in higher-molecular-weight fractions. The possible existence of active subunits of the enzymes held in association by ionic or hydrophobic interactions was investigated by chromatography in 20 mM-sodium phosphate buffer, pH 7.4, containing 3 M-NaCl or 20% (v/v) ethylene glycol respectively. Chromatography of two duodenal fluids under each of these conditions showed no change in the elution positions of the enzymes.

Glycodeoxycholate is known to enhance enterokinase activity towards trypsinogen (Hadorn *et al.*, 1974). We tested the effect of this bile acid on the apparent molecular weights of the enzymes by chromatographing two duodenal fluids on Sephadex G-200, equilibrated with 20 mM-sodium phosphate buffer, pH 7.4, containing 2.5 mM-glycodeoxycholate. The enzyme activities chromatographed in their characteristic positions.

The effect of body temperature on the apparent molecular weights of the enzymes was studied by chromatography of two duodenal fluids on Sephadex G-200 in 20 mM-sodium phosphate buffer, pH 7.4, containing 0.2 M-NaCl at 37°C. Again no change in elution position of the enzymes was observed.

These findings suggest that aminopeptidase and enterokinase in native human duodenal fluid exist

in single stable physiological forms with apparent mol.wts. of 275000 and 300000 respectively. Maltase is present in two major chromatographically distinct forms, in agreement with the findings of Dahlqvist & Telenius (1969) and Semenza and co-workers (Semenza & Aurricchio, 1962; Semenza & Kolinska, 1967; Semenza *et al.*, 1975). The high-molecular-weight forms of these enzymes isolated by detergent extraction of duodenal mucosa (Maroux & Louvard, 1976; Barns & Elmslie, 1977) do not appear to be significant species in duodenal fluid, where the major degradative activity during the early stages of digestion occurs.

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