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Binding of trypsin and chymotrypsin by human intestinal mucosa

Although dog intestinal juice contains proteolytic enzymes associated with cellular material[1,2], no proteolytic activity was detected when pancreatic secretions were excluded[3]. Trypsin and chymotrypsin are partly present in an insoluble form in rat intestinal contents[4] and in human faeces[5,6]. Evidence is now presented that these enzymes are adsorbed by intact human intestinal epithelium without loss of activity.

Ileostomy material from human subjects was collected within 1 h of voiding and homogenized in 4 vol. 0.15 M NaCl at 4° with the 'Ato-Mix Blender' (Measuring and Scientific Equipment, London) for 3 min at 12 000 rev./min. Segments of fresh normal human small intestine were obtained during resection of colonic lesions, opened longitudinally, and thoroughly washed with cold 0.25 M sucrose. The mucosa was lightly scraped off with a blunt scalpel and homogenized in cold 0.25 M sucrose with a glass-coated teflon pestle (Sireica, New York) at 6000 rev./min until the preparation was judged by microscopic examination to consist predominantly of discrete unbroken epithelial cells. Trypsin and chymotrypsin activities were measured as the initial rates of hydrolysis of *p*-toluene-L-arginine methyl ester and *N*-acetyl-L-tyrosine ethyl ester, respectively, using a pH-stat automatic recording titrator of the Radiometer Corporation (Copenhagen, Denmark) as previously described[6]. Twice-crystallized trypsin (salt-free) and thrice-crystallized α-chymotrypsin (salt-free) from bovine pancreas were obtained from Koch-Light Laboratories, Colnbrook, England, for use in recovery experiments. Nitrogen estimations were made, where appropriate, by a microkjeldahl procedure[7].

When pancreatic enzymes were added to human ileal contents, they became bound to particulate material sedimentable at 3000 × *g* for 15 min without loss of activity (Table I). Repeated freezing and thawing (up to 15 times) did not release bound enzymes into the supernatant, nor did incubation at 37° for periods up to 4 h. Addition of H_2SO_4 in final concentration ranging from 0.005 to 0.1 M 15 min before centrifugation did not liberate bound enzymes; the pH range spanned was 1.6–4.5.

TABLE I

RECOVERY OF TRYPSIN AND CHYMOTRYPSIN FROM HUMAN ILEAL CONTENTS

10 mg of each enzyme were added to 100 ml of ileal contents homogenized in 0.15 M NaCl and adjusted to 10 mg nitrogen per ml. Endogenous enzyme content of homogenates ranged from 6.8 to 13.8 mg/100 ml of each enzyme. After equilibrating for 15 min at room temperature, the recovery of the enzymes in the homogenates and their distribution between pellet and supernatant separated at 3000 × *g* for 15 min was determined, allowance being made for the activity originally present in homogenates, pellets and supernatants. All assays in duplicate. Mean ± S.E. of 10 experiments.

Source	*Trypsin (mg)*	*Chymotrypsin (mg)*
Homogenate	9.98 ± 0.09	10.09 ± 0.24
Supernatant (S)	6.41 ± 0.08	5.40 ± 0.28
Pellet (P)	3.87 ± 0.12	5.42 ± 0.30
S + P	10.28 ± 0.18	10.82 ± 0.36

p-Chloromercuribenzoate in amounts sufficient to give final concentrations ranging from 10^{-9} M to 10^{-4} M failed to release enzymes already bound, or to prevent the binding of enzymes subsequently added. Neither NaCl nor KCl in final concentrations up to 2.0 M exercised any notable effect.

The following were tested for ability to block the binding of added enzymes, final concentration being given in parentheses: Calf thymus DNA (10 mg/ml), yeast RNA (10 mg/ml), bovine serum albumin (25 mg/ml), whole human plasma proteins (25 mg/ml), cytochrome *c* (10 mg/ml), polylysine (10 mg/ml), rat liver glycogen (10 mg/ml), and heparin (10 mg/ml). All results were negative. The non-ionic detergent Nonidet P 40 (100% polyethylene oxide condensate, British Drug Houses, Poole, England) released in 4 experiments a mean of 42.0% of trypsin and 23.8% of chymotrypsin from particulate ileal material. Sodium deoxycholate in a final concentration of 0.25% liberated 30.1% of trypsin and 17.2% of chymotrypsin (mean of 2 experiments). Pancreatic enzymes were not adsorbed to suspensions of heat-denatured serum albumin, whole human plasma proteins and cytochrome *c*. Negative results were also obtained when slurries of potato starch, dextran Grade C and 'Chromedia' CF 1 Cellulose (all from British Drug Houses) were prepared in 0.15 M NaCl and tested for adsorptive capacity.

Both trypsin and chymotrypsin, when added to suspensions of intact intestinal epithelial cells, were partly adsorbed, and readily sedimented at 3000 × *g* for 15 min. The recovery in 8 experiments ranged from 93 to 102%, there being no evidence of enzyme inactivations or inhibition. The pH dependance of this binding was demon-

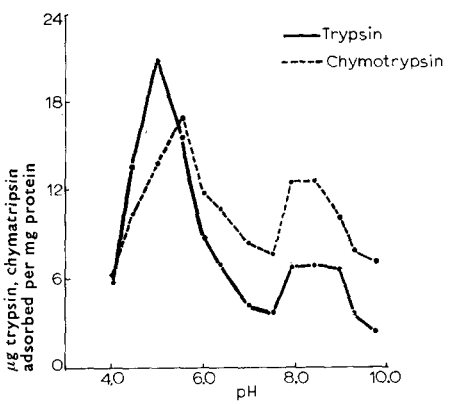

Fig. 1. Effect of pH on binding of trypsin and chymotrypsin by homogenates of human small intestinal mucosa in buffer system of DAVIES[8]. All assays in duplicate, reaction mixture being 4.0 ml mucosal preparation (nitrogen content, 3–4 mg/ml); 1.0 ml enzyme solution (1 mg/ml); 5.0 ml buffer.

strated in the buffer system of DAVIES[8]. Adsorption for both enzymes was maximal at pH 5.0 with a subsidiary peak at pH 8.5. The same profile was obtained when enzyme distribution was measured by casein digestion[9] in place of esterase activity. When 3 samples of intestinal contents were tested in this buffer system all demonstrated maximal adsorption at pH 5.0, and 2 showed the subsidiary peak at pH 8.5.

We conclude that the capacity of human ileal contents to adsorb trypsin and chymotrypsin is largely due to their content of exfoliated epithelial cells, and have

noted that a higher percentage of the chymotrypsin found in these contents is usually associated with insoluble material. The binding does not seem to involve covalent or disulfide bonds, and no evidence could be obtained for the participation of protein, nucleic acid or polysaccharide, although adsorption of trypsin to cellulose membranes has been reported at very low ionic strength[10]. In all aspects so far studied, binding of both enzymes to intestinal debris manifests the same chemical properties as binding to human intestinal epithelial cells. Since the latter were mainly intact, and no 'lag' period was observed in the kinetic assay of cell-bound enzymes, adsorption probably occurred at the cell surface. This is important direct evidence in support of UGOLEV's[11] theory of membrane digestion. The effect of detergent and deoxycholate may tentatively be regarded as favouring binding of the enzymes to lipoprotein.

It is of interest that adsorption of the enzymes is not accompanied by loss of activity since water-insoluble derivatives prepared chemically lose 70–85% activity[12,13]. Finally, previous work[14] describing inactivation of trypsin and chymotrypsin in the human small intestine will require reconsideration, since estimations were made on soluble enzymes and did not take account of adsorption of active enzymes to insoluble intestinal material.

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