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Demonstration of two forms of human pancreatic carboxypeptidase A

Carboxypeptidase A (EC 3.4.2.1), an exopeptidase from the pancreas, and different forms of its zymogen, procarboxypeptidase A, have been extensively studied in bovine and porcine pancreas[1–15]. Four forms of the bovine enzyme[10–12], carboxypeptidases A_α, A_β, A_γ and A_δ, and three forms of the porcine enzyme[7], carboxypeptidases A_1, A_2 and A_3, have been described, with differences occurring in their N-terminal sequence. Furthermore, allelomorphs of bovine carboxypeptidase A_α have been shown to contain either leucine or valine in their terminal hexapeptide[14,15]; either or both forms of the enzyme may be present in any one animal and the differences are believed to be the result of a generic trait. The presence of procarboxypeptidase A has also been demonstrated in human pancreatic juice[16], and in human duodenal juice, carboxypeptidase A activity has been determined as a measure of exocrine pancreatic function[17–20].

In the present investigation, it is demonstrated that carboxypeptidase A from human duodenal juice and homogenates of activated human pancreatic tissue also occurs in two forms with different electrophoretic mobility. Evidence is also presented which suggests that during the activation of procarboxypeptidase A by trypsin (EC 3.4.4.4), the slower migrating form of the enzyme appears first and is converted into the faster migrating form in the presence of endogenous endopeptidases.

Human duodenal juice was collected from healthy adults using a three-lumened duodenal tube[20] in order to avoid contamination of duodenal fluid with gastric contents. Carboxypeptidase A was measured by its activity towards *N*-carbo-β-naphthoxy-DL-phenylalanine[21]. Homogenates of human pancreatic tissue (7.5%, w/v) were prepared in 0.15 M NaCl containing 0.1% Triton X-100 (ref. 22). Activation of the zymogens was carried out at 37° by the addition of bovine crystalline trypsin. One ml of activation mixture contained 230 μg trypsin (Sigma), 2 μM $CaCl_2$, 125 μM NaCl, 0.9 mg Triton X-100, 4.5 mg protein[23] and 5 μM Tris buffer (pH 8.0). Trypsin and chymotrypsin (EC 3.4.4.5) activities in duodenal juice and in homogenates of pancreatic tissue were measured by the procedure of LUNDH[24]. Electrophoresis was carried out on cellulose polyacetate membranes (Sepraphore III, Gelman). The conditions of electrophoresis are described in the legend to Fig. 1. After electrophoresis,

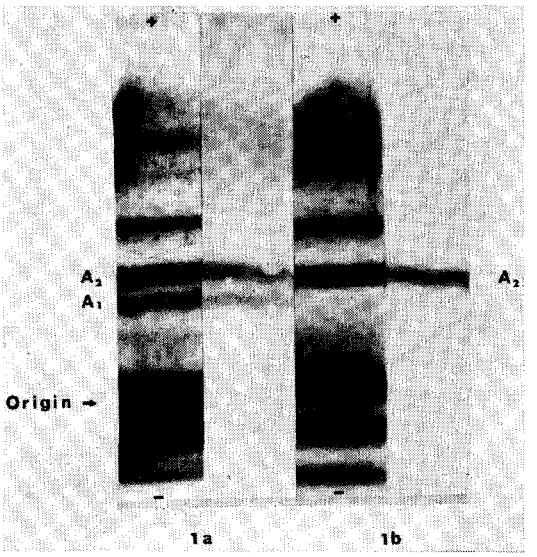


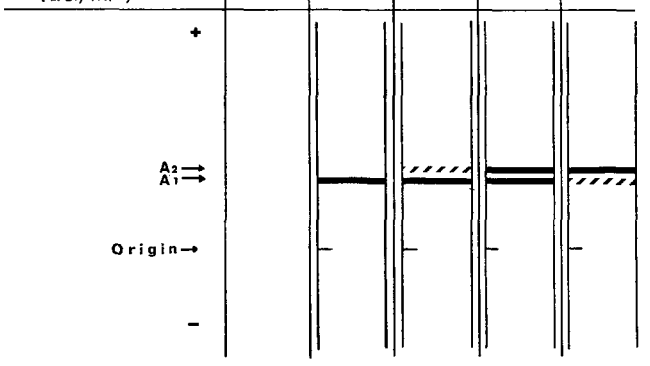
Fig. 1. Electrophoresis of 10 μl human duodenal juice on cellulose polyacetate (width 2.5 cm) in 0.025 M Tris–barbital–sodium barbital buffer, pH 8.8. 1.5 mA per strip for 40 min at room temperature. (a) Represents electrophoresis of fresh duodenal juice. (b) Juice kept at 4° for 18 h. A_1 and A_2 represent carboxypeptidases A_1 and A_2, respectively. In each case, the left strip shows protein bands and the right strip the specific stain for carboxypeptidase A.

TABLE I

ACTIVATION OF HUMAN PANCREATIC HOMOGENATE WITH CRYSTALLINE BOVINE TRYPSIN

Diagram represents positions of carboxypeptidases A_1 and A_2 after electrophoresis.

Time (min)	0 (Trypsin added)	5	30	60	240
Trypsin (μg/ml)	0	191	128	85	25
Chymotrypsin (μg/ml)	0	156	100	41	0
Carboxypeptidase A (I.U./ml*)	0.03	0.19	0.19	0.22	0.18



* I.U., international units.

the cellulose polyacetate strips were cut longitudinally and one-half of the strip was stained for protein with a solution of 0.1% nigrosine in 2% acetic acid containing 10% glycerol. The site of the enzymatic activity was located on the other half of the strip by covering it with two reagent strips. The first strip was soaked in the specific chromogenic substrate and the colour was developed with a second strip which had been soaked in the coupling reagent (fast blue salt, B. Merck). The colour developed within a few minutes and photomicrographs were taken immediately since the colour tended to fade as the strips dried.

If electrophoresis was carried out on fresh samples of duodenal juice, carboxypeptidase A activity was detected in two distinct bands which corresponded to two protein bands (carboxypeptidases A_1 and A_2 in Fig. 1a). However, when electrophoresis was performed on samples of the same duodenal juice which had been kept at 4° for 18 h only the faster migrating enzyme (carboxypeptidase A_2) was present (Fig. 1b). Measurement of the total carboxypeptidase A activity of the juice showed no loss of enzyme activity during the incubation. When samples of duodenal juice were initially treated with diisopropylphosphorofluoridate (DFP) (final concn. 10^{-3} M), both forms of the enzyme were present after incubation at 4° for 18 h. Since DFP inactivates trypsin and chymotrypsin-like endopeptidases[25] it was concluded that the conversion of the A_1-form into the A_2-form was due to the presence of endogenous endopeptidases in the duodenal juice.

It was of interest to investigate whether the two forms of human carboxypeptidase A represent discrete enzymatic entities arising from different zymogens or different activation products of a single zymogen. The following experiment was therefore undertaken. A homogenate of fresh human pancreatic tissue was prepared and zymogen solubilisation and activation were achieved with the methods described. At different time intervals during the activation process, aliquots of the activation mixture were treated with DFP and subjected to electrophoresis and staining for carboxypeptidase A activity. Simultaneously trypsin and chymotrypsin activities were measured in aliquots which were not treated with DFP. The result of one of these experiments is represented in Table I. Electrophoresis revealed that after incubation for 5 min, only the slower migrating enzyme (carboxypeptidase A_1) was present. By 30 min it was possible to detect the presence of carboxypeptidase A_2 and its concentration increased, until, at 4 h, virtually all the carboxypeptidase A activity was present in the A_2-form. It is also seen from this table that complete activation of carboxypeptidase A was reached within 5 min and that no significant change in activity was observed for the subsequent 4 h. In contrast the added trypsin activity showed a continuous decrease during the activation process. The appearance of a chymotrypsin-like activity was also noted.

From these results it was concluded that during the activation of human procarboxypeptidase A by trypsin, carboxypeptidase A_1 is produced first and then converted into the A_2-form. Since there is no change in the total carboxypeptidase A activity, it is thought that carboxypeptidase A_2 is the result of the direct action of an endopeptidase (trypsin or chymotrypsin) on the A_1-form of the enzyme.

A similarity is apparent between the above phenomenon and the porcine enzyme system where the conversion of carboxypeptidase A_1 into A_2 is the result of tryptic activity[6,7]. For both the human and porcine enzymes, the conversion of carboxypeptidase A_1 into A_2 resulted in similar changes of electrophoretic mobility.

For the bovine enzyme system, SAMPATH KUMAR *et al.*[12] believe that the different forms of carboxypeptidase A result from endopeptidase activity at different sites of a common precursor. Since the initial attack on the bovine zymogen by trypsin would result in the formation of carboxypeptidase A_α (ref. 12), it is possible that this enzyme resembles human carboxypeptidase A_1. If this is so, carboxypeptidase A_1, like the bovine enzyme, would then be subject to further attack in the presence of endopeptidase activity, thus accounting for the formation of a second enzyme, carboxypeptidase A_2.

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