BBA 66391

FOUR FORMS OF HUMAN PANCREATIC PROCARBOXYPEPTIDASE A DEMONSTRATED BY ISOELECTRIC FOCUSING

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(Received May 7th, 1971)

SUMMARY

Four forms of procarboxypeptidase A (peptidyl-L-amino acid hydrolase, EC 3.4.2.1) were found in human pancreatic juice obtained from 22 specimens from 9 patients by isoelectric focusing using Ampholine carrier ampholyte pH 5–8 formed in a thin layer of polyacrylamide gel. The isoelectric points of these procarboxypeptidases A were A_1, 6.72; A_2, 6.56; A_3, 6.37; and A_4, 6.22. A_1, A_2 and A_4 were converted to A_3 after 18 h incubation at 4° with bovine trypsin.

INTRODUCTION

Four forms of carboxypeptidase A (peptidyl-L-amino acid hydrolase, EC 3.4.2.1) have been found in the bovine enzyme[1–3], and three forms of the porcine enzyme[4]. Only two forms of human carboxypeptidase A have been previously demonstrated by ourselves[5] in pancreatic juice obtained from the duct and in duodenal secretions by HADORN AND SILBERBERG[6]. Cellulose acetate strip electrophoresis was used in both of these studies. Since the most effective way of separating enzymes is through the use of isoelectric focusing, we have studied pancreatic juice obtained from the duct by this method[7–9].

MATERIAL

Pancreatic juice was obtained from a tube left in the duct after a pancreatic resection for carcinoma in one case; from a transpapillary tube left in the duct after an operation for recurrent acute pancreatitis in four cases; chronic pancreatitis in two cases; and for no pancreatic disease in one case; and from external drainage of a long standing pancreatic cyst in one case. The juice was collected every 8 h in a collecting bag at room temperature, then stored as frozen juice at −20°.

METHODS

Isoelectric focusing of pancreatic juice was carried out on the Ampholine

carrier ampholytes (LKB production, Stockholm, Sweden) formed in a thin layer of polyacrylamide gel. We mixed 0.4 ml of human pancreatic juice with 1.2 ml of 3% Ampholine carrier ampholytes (either pH 3–10 or pH 5–8) and 6.6 ml of distilled water, 4.1 ml of freshly prepared gel mixture[9] was added. The mixture was quickly transferred between two glass plates (15 cm × 7.5 cm) which were 1.5 mm apart. The gel was polymerized at room temperature by placing it under fluorescent lighting for 75 min and then under normal lighting for 75 min. One glass plate was carefully removed leaving the thin layer of gel adhering to the other plate. The plate was then inverted so that the gel made good contact with the graphite electrodes in a humidified chamber. Before each run the negative electrode was soaked in 0.4% ethylenediamine solution and the positive in 0.2% sulfuric acid. The electrodes were placed 12 cm apart and a voltage of 200 V (12 mA) was maintained until the current dropped to 0.5 mA then it was raised to 300 V. The total time for electrofocusing was 24 h at 4°.

On completion of the run, the pH gradient was determined by cutting a linear sequence from negative to positive of 5-mm discs of gel with a cork borer. Each piece was suspended in 1 ml of distilled water at room temperature for 2 h and the pH was then measured with a Radiometer pH-stat, type TTT1C.

The gel was split longitudinally, half of the gel was placed in 5% trichloroacetic acid overnight to wash out the ampholytes and then stained with Coomassi Brilliant blue. The other half of the gel was used for identification of the procarboxypeptidase A. Procarboxypeptidase A was identified by incubating the gel strip in the solution containing the chromatogenic substrate for carboxypeptidase A, *n*-carbo-*β*-naphthoxy-L-phenylalanine and coupling reagent. The solution was prepared by dissolving 10 mg *n*-carbo-*β*-naphthoxy-L-phenylalanine in a 0.5 ml dimethylformamide and diluting with 10 ml Tris–Ca^{2+} buffer (pH 7.9) containing 100 mg Diazo blue B and 5 mg bovine trypsin (2 times crystalline salt-free trypsin, Worthington Biochemical Corporation, Freehold, N.J.) to activate the procarboxypeptidase A.

RESULTS

When ampholyte pH 3–10 was used in the gel we were able to identify just two areas of procarboxypeptidase A activity in the juice (Fig. 1). When ampholyte pH 5–8 was used on identical samples of juice, four bands (A_1, A_2, A_3 and A_4) of procarboxypeptidase A activity appeared (Fig. 2a). Even after the gel was allowed to stand for 18, 24 or 48 h at 4°, four zones of activity remained. Only after 0.5 mg of trypsin was added to 1 ml pancreatic juice and allowed to stand 18 h at 4° prior to isoelectric focusing did this pattern change. All of the activity in specimens treated in this manner was at the zone previously identified as A_3 (Fig. 2b).

A total of 22 specimens from the nine patients mentioned above were studied in this manner (Table I). Procarboxypeptidase A_1 had a mean pI of 6.72 ± 0.043 and occurred in 40.9% of specimens. Procarboxypeptidase A_2 had a pI of 6.56 ± 0.028 and occurred in 72.7% of specimens. Procarboxypeptidase A_3 had a pI of 6.37 ± 0.037 and occurred in 54.4% of specimens. Finally procarboxypeptidase A_4 had a pI of 6.22 ± 0.033 and occurred in 40.9% of the specimens. The data, together with the

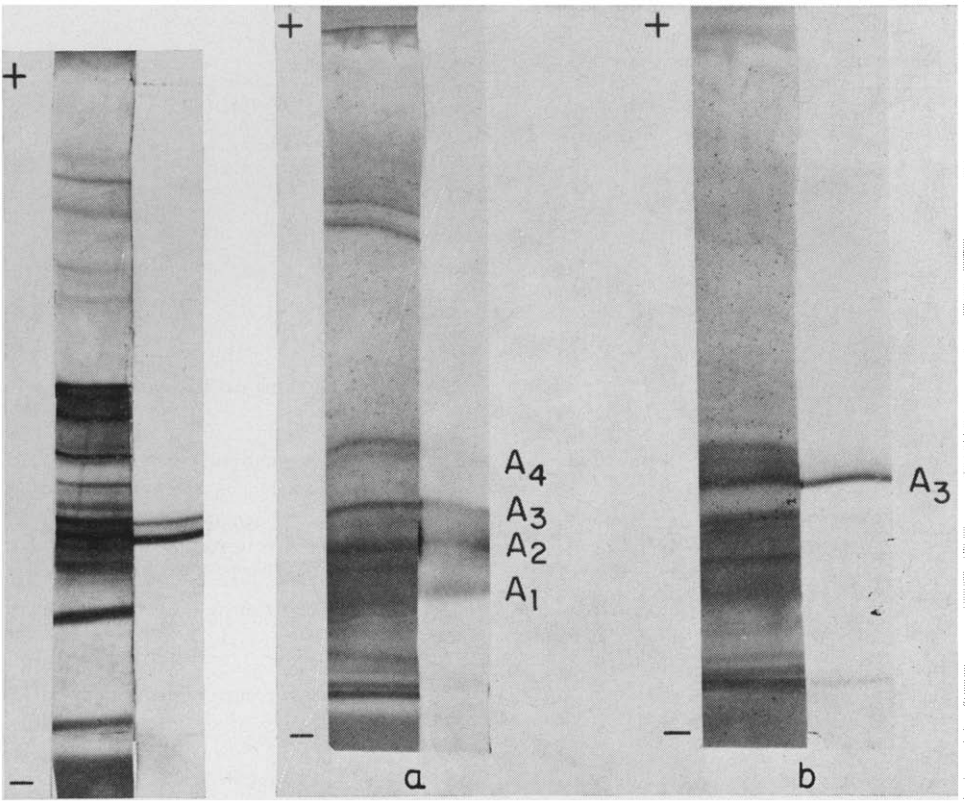

Fig. 1. Ampholine carrier ampholyte pH 3–10 isolelectric focusing of human pancreatic juice shows only two areas of procarboxypeptidase A activity as identified by a chromatogenic substrate *n*-carbo-β-naphthoxy-L-phenylalanine in the right hand strip. All of the proteins present can be seen to the left.

Fig. 2a. Ampholine carrier ampholyte pH 5–8 isoelectric focusing of human pancreatic juice shows the protein bands on the left and the four procarboxypeptidase A (A_1, A_2, A_3 and A_4) bands on the right.
Fig. 2b. Again the proteins are stained on the left, this time after incubation with trypsin for 18 h at 4°, and only carboxypeptidase A_3 can be seen as stained by *n*-carbo-β-naphthoxy-L-phenylalanine. This band is more concentrated than before addition of trypsin suggesting conversion of A_1, A_2 and A_4 to A_3.

diseases present in the patients from which the specimens were obtained is shown in Table I.

DISCUSSION

We feel that the two bands which can be seen when ampholyte pH 3–10 isoelectric focusing is used probably correspond to the A_1 and A_2 bands seen by HADORN AND SILBERBERG[6] in duodenal secretion and in our previous studies[5] on pancreatic juice. The more positive of the two bands on isoelectric focusing is probably the A_2 activity seen in both previous studies. It seems that this band separates into A_3

TABLE I

ISOELECTRIC POINTS OF PROCARBOXYPEPTIDASE A

Specimen	*Procarboxypeptidase A*				*Patient and illness*
	A_1	A_2	A_3	A_4	
1		6.59	6.42	6.25	No. 1/carcinoma of pancreas
2			6.33		
3	6.72		6.42		
4		6.55	6.35	6.18	
5			6.33		
6		6.56	6.38	6.20	
7			6.33		
8		6.54			No. 2/pancreatic cyst
9		6.54			
10		6.60			No. 3/chronic pancreatitis
11		6.57		6.25	
12		6.56		6.16	
13	6.68				No. 4/chronic pancreatitis
14	6.68	6.51	6.35		No. 5/recurrent acute pancreatitis
15		6.56		6.25	No. 6/No pancreatic disease
16	6.68				No. 7/recurrent acute pancreatitis
17	6.73	6.56	6.33		
18	6.73	6.61	6.40		No. 8/recurrent acute pancreatitis
19	6.73	6.51			
20	6.73	6.57	6.40	6.23	
21	6.82	6.60	6.40	6.20	
22		6.57		6.23	No. 9/recurrent acute pancreatitis
Mean ±S.D.	6.72± 0.043	6.56± 0.028	6.37± 0.037	6.22± 0.033	
Incidence (%)	40.9	72.7	54.4	40.9	

and A_4 in ampholyte pH 5–8 electrofocusing. The negative band seems to correspond to the A_1 found on electrophoresis[5,6] and may be a combination of A_1 and A_2 seen on ampholyte pH 5–8 electrofocusing.

Our human juice was not spontaneously autoactivated when allowed to stand for up to 48 h at 4°, probably because of the relatively high trypsin inhibitor content of human juice[10], and because we were dealing with juice obtained from the duct rather than pancreatic homogenate or duodenal secretions containing trypsin[6].

When trypsin was added to our juice specimens and incubated all four forms of procarboxypeptidase A were converted to A_3 (Fig. 2b). Our data correspond to that obtained by FOLK[11] on porcine pancreas extracts where carboxypeptidase A_1 was converted to A_2 after incubation with trypsin for 18 h at 0°. Also the aggregate forms of bovine procarboxypeptidase A (S6 and S5) are converted to one form by trypsin[12].

The isoelectric points of procarboxypeptidase A_1 through A_4 varied from 6.22 to 6.72 as opposed to 4.5 previously found by KELLER *et al.*[13] in bovine juice. We have not been able to find other data than our own on the isoelectric points of human procarboxypeptidase A.

ACKNOWLEDGMENT

This work was supported in part by U.S. Public Health Service Grant AM 11358.

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