ON THE PRESENCE OF TWO DISTINCT PROTEOLYTIC COMPONENTS IN PANCREATIC CRYSTALLINE ELASTASE

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The complex nature of pancreatic elastase has been described by many workers, Lewis et al. (1956), Banga (1952), Czerkawski and Hall (1958), Loeven (1963). Donovan (1964) working in Dr. Hanes' laboratory detected 5 different species of elastase.

However, the presence of more than one proteolytic component, active towards elastins in twice crystallized elastase (Lewis et al. 1956), has not been clearly established.

Our investigations show that crystalline porcine pancreatic elastase preparation (Lewis et al. (1956)) contains two distinct proteolytic components. One of these appears to be identical to the electrophoretically pure elastase (Lewis et al. (1956)) and the second is proteolytic but non-elastolytic in nature.

The present communication describes the detection and preliminary separation of this second proteolytic component from crystalline elastase.

METHODS AND MATERIALS

Twice crystallized elastase (CR elastase) (identical to the preparation commercially available from Worthington Biochemical Corporation) was prepared according to the method of Lewis et al. (1956). Electrophoretically

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pure (EP) elastase was purchased from Worthington Biochemical Corporation, Freehold, N.J.

Elastin was prepared from bovine ligamentum nuchae by repeated autoclaving in water according to the method of Partridge, Davis and Adair (1955).

High voltage paper electrophoresis was carried out on Whatman No. 3 HR paper, with the use of pyridine-acetic acid-water buffer pH 3.6 at 38 V/cm for 2 hrs.

Borate starch gel and formate-urea-starch gel electrophoresis were carried out according to the methods of O. Smithies (1959). The former was run in borate buffer pH 8.8, the latter in formate buffer pH 3.1 and both at 4 V/cm for 20 hrs.

Preliminary separation of the second proteolytic component was carried out by adsorbing 20 mg of CR elastase on DEAE sephadex (A 50) in borate buffer pH 8.7. The DEAE sephadex was washed with the borate buffer and the second proteolytic component eluted from the DEAE sephadex with 0.05 M acetate buffer, pH 3.0.

The borate buffer washings of the DEAE sephadex were combined, adjusted to pH 5.0 and loaded on a carboxymethyl cellulose (CMC) column, and elastase eluted with a sodium chloride gradient. A major peak containing purified elastase appeared at about 0.2 M NaCl and was collected. This peak and the DEAE sephadex component were separately lyophilized and dialysed against borate buffer pH 8.8.

To test the activity of different fractions, 5 mg samples of elastin were suspended in 2.0 ml of borate buffer pH 8.8 and digested at room temperature with different enzyme fractions or preparations. The digests were subjected to high voltage paper electrophoresis and the electrophoretograms were developed with ninhydrin to reveal the ninhydrin positive components.

RESULTS AND DISCUSSION

As seen in Fig. 1a a dark band due to the liberation of free amino

acids was present in the CR elastase digest which was absent from the digest obtained with EP elastase. Starch gel electrophoresis of CR elastase in borate buffer pH 8.8 revealed two major protein bands. One of these bands had the mobility identical to that of the major band shown by EP elastase. The unstained portion of this band from CR elastase was cut. The elastin digest prepared with it showed electrophoretic pattern similar to the one obtained with whole CR elastase, which suggested that either the CR elastase had different specificity from that of the EP elastase or it contained at least one additional proteolytic component, with the same mobility as elastase under the conditions applied. Urea-starch gel electrophoresis in formate buffer pH 3.1 indicated that the latter was the case.

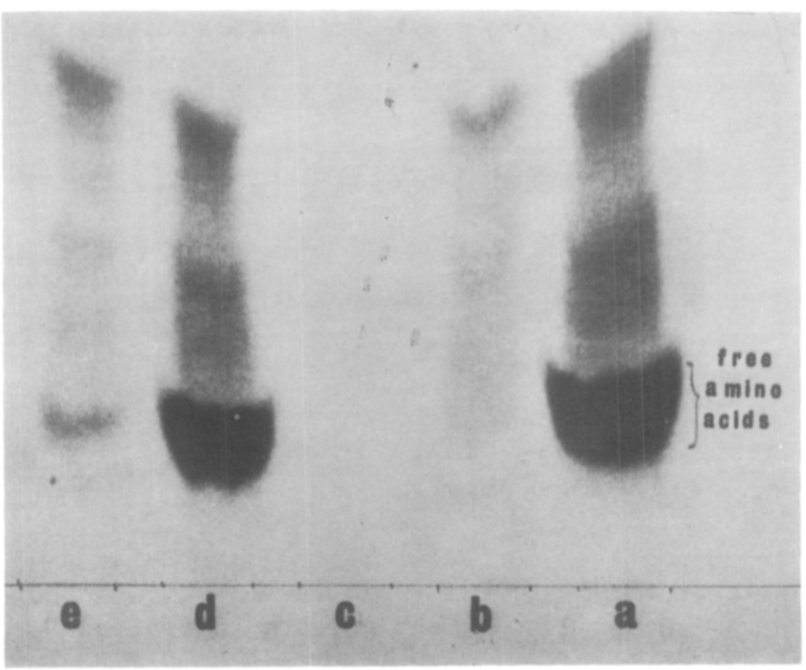

Fig. 1. Paper electrophoretic patterns of elastin digests with (a) CR elastase, (b) elastase purified from CR elastase, (d) purified elastase plus the second proteolytic component, (e) EP elastase.

Fig. 2a shows the urea-starch gel electrophoresis of CR elastase and 2c is that of EP elastase. As can be seen, CR elastase yielded 3 major protein bands. One of these bands had the same mobility as the major band of EP elastase and another slightly slower mobility. The elastin digest prepared with the band having the same mobility as the major band from EP elastase, after dialysing out the urea, gave electrophoretic pattern identical to the one obtained with the elastin digest of EP elastase. These observations suggested that the differences in electrophoretic patterns of elastin digests prepared with EP elastase and CR elastase were probably due to the presence of a second proteolytic component in CR elastase. This was confirmed by separating the two activities from CR elastase on DEAE sephadex (A 50) as mentioned under Methods.

Fig. 2b and 2c are urea-starch gel electrophoresis of elastase purified

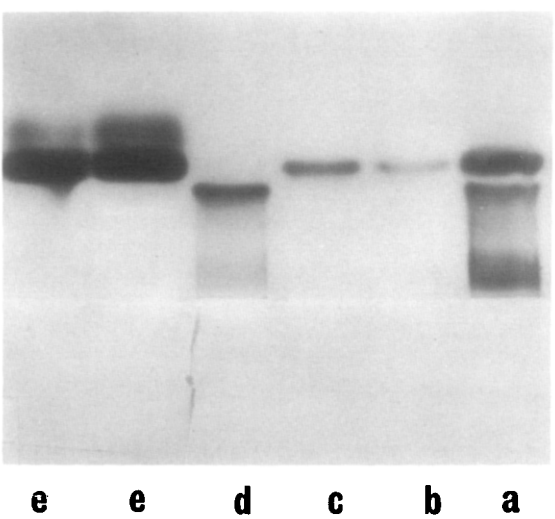

Fig. 2. Urea-starch-gel electrophoresis in formate buffer, pH 3.1. (a) CR elastase, (b and c) elastase purified from CR elastase, (d) the second proteolytic component separated from CR elastase, (e) EP elastase.

from CR elastase and separated from the second component. They show single bands with the same mobility as that of the major band from EP elastase (Fig. 2e). The elastin digest prepared with this purified elastase gave an electrophoretic pattern (Fig. 1b) which was identical with the one obtained with the elastin digest of EP elastase (Fig. 1e).

Fig. 2d shows urea-starch gel electrophoresis of the second proteolytic component isolated from CR elastase. This component is now free of elastase band present in both the CR elastase and EP elastase. The elastin digest prepared by mixing this second proteolytic component with the purified elastase gave electrophoretic pattern (Fig. 1d) identical to the one obtained from elastin digest of CR elastase. Similar results were obtained by adding this second component to the elastin digest of EP elastase. This then clearly establishes the presence of a second proteolytic component in CR elastase in addition to elastase. Preliminary experiments suggest that this component is non-elastolytic, acts on insulin and is different from carboxypeptidases, chymotrypsins and trypsin.

Recently Gjessing and Hartnett (1962) and McConnell and Gjessing (1966) have reported the isolation of estero-proteolytic enzyme and estero-proteolytic zymogen from porcine pancreas. Further work is in progress to completely characterize this second proteolytic component and to establish its relationship with the other pancreatic proteases.

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