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Received June 13th, 1963

Biochim. Biophys. Acta, 77 (1963) 671–673

SC 11087

Purification of bovine plasminogen

Partial purifications of bovine plasminogen (EC 3.4.4.14) have been described[1–4] but few characterizing data given. When it was reported[5,6] that, among other ω-amino acids, ε-ACA and lysine markedly increased the solubility of sparingly soluble human plasminogen preparations at neutral pH, these amino acids were tried in the purification of human plasminogen[7,8]. Recently WALLÉN[9,10] published an improved method for the purification of human plasminogen in which the rather specific interaction between plasminogen and ε-ACA was utilized. This method was used for bovine material with suitable modifications. The plasminogen preparation obtained was almost homogeneous in starch-gel electrophoresis at different pH values. It contains aspartic acid as N-terminal amino acid.

The starting material used was fresh bovine citrated plasma (1 volume 0.14 M trisodium citrate and 9 volumes blood). The purity and yields throughout the different steps were followed by analyses of the freeze-dried fractions. The proteolytic activity obtained after activation with urokinase (human urokinase[11] containing about 500 Ploug and Kjeldgaard units/mg) was usually determined by a caseinolytic method[12], but the purer fractions were also assayed for fibrinolytic activity by a clot lysis technique using reagents free from plasminogen[13]. Homogeneity was tested by starch-gel electrophoresis[14]. The N-terminal analyses were carried out by the Edman technique as applied by ERIKSSON AND SJÖQUIST[15].

The procedure elaborated for the purification of bovine plasminogen is as follows: From 30 l of bovine citrated blood, 16 l of plasma are obtained and Fraction I (mainly fibrinogen) precipitated according to Method 6 of COHN *et al.*[16]. After centrifugation the supernatant is adjusted to pH 5.3 with acetic acid, whereupon most of the plasminogen precipitates. This precipitate (Fraction IP), which usually contains about 100 g of protein (dry weight), is used directly for further fractionation. It is suspended in 3.3 l distilled water at 0° by means of a vibrating mixer (Vibro-Mischer, Chemap A.G., Männedorf, Switzerland) and the slurry is acidified to pH 4.5–4.6 with acetic acid. To the opaque solution obtained, 74 ml of 4 M sodium acetate are added, giving an ionic strength of about 0.08 and a pH of 5.4 $\pm$ 0.1. The plasminogen activity again precipitates. It is then dissolved and precipitated once more as described above. The precipitate thus obtained is suspended in 1.2 l distilled water at 0° by using the Vibro-Mischer and the pH is adjusted to 4.5–4.6 with acetic acid

Abbreviation: ε-ACA, ε-aminocaproic acid.

in order to dissolve most of the protein. 300 ml 1 M ε-ACA solution at 0° was added. The pH rises to 5.3–5.5 and a heavy precipitate consisting of inert proteins is formed. The activity remains in the supernatant (Fraction B) which after centrifugation is clear. From this solution more impurities are precipitated by adding methanol and $(NH_4)_2SO_4$ to a final concentration of 20% and 0.016 M, respectively. The pH is adjusted to 4.0 with acetic acid and the temperature is lowered to −3°. To the supernatant more methanol and $(NH_4)_2SO_4$ are added to give a final concentration of 40% methanol and 0.04 M $(NH_4)_2SO_4$ at −15°. The plasminogen precipitate obtained under these conditions is centrifuged down and freeze-dried (Fraction B2,3). This fraction is further purified on CM-cellulose (from Serva Entwicklungslabor, Heidelberg, Germany). The plasminogen activity is adsorbed on a CM-cellulose column previously equilibrated with a 0.04 M sodium phosphate–0.02 M ε-ACA

TABLE I

PURIFICATION AND YIELDS OF BOVINE PLASMINOGEN

The figures from Fractions IP, B and B2,3 refer to mean and range from 11 preparations and the figures from Fraction CMC-II are means and ranges from 4 preparations. As spontaneous proteolytic activities in all preparations are less than 0.1% of the corresponding plasminogen value they are not included in the table. Purification factor = the specific activity of the fraction divided by the specific activity of Fraction IP.

	Fraction IP	*Fraction B*	*Fraction B2,3*	*Fraction CMC-II*
Specific caseinolytic activity (ACU/mg)*	2.9 (2.3–3.9)	29 (23–39)	58 (46–78)	139 (120–163)
Purification factor	(1)	10.3 (8.0–14.4)	20.6 (17.2–26.9)	47.9 (41.4–56.2)
Yield of plasminogen (%)	(100)	29 (20–42)	31 (18–41)	6 (4–10)

* Caseinolytic activity per mg freeze-dried material after activation with urokinase. ACU = Arbitrary Caseinolytic Units as used by WALLÉN[9].

buffer (pH 6.0). Most of the inert protein passes through the column and material not adsorbed is washed out from the column with equilibration buffer (Fraction CMC-I). Plasminogen activity can then be eluted by a 0.02 M sodium phosphate–0.02 M ε-ACA buffer (pH 7.0) (Fraction CMC-II). More plasminogen activity is eluted by changing to 0.08 M sodium phosphate–0.02 M ε-ACA (pH 7.0) (fraction CMC-III). After acidifying the effluents to pH 4.5–5.0 with acetic acid the protein in the different fractions is precipitated at −15° by adding ethanol (about −60°) and $(NH_4)_2SO_4$ to a final concentration in the mixture of 30% and 0.035 M, respectively. The precipitates are collected by centrifugation and freeze-dried. The fractions contain small amounts of $(NH_4)_2SO_4$ and ε-ACA which can be removed by gel filtration through Sephadex G 75 (AB Pharmacia, Uppsala, Sweden).

The purification and yields of plasminogen have been compiled in Table I. The Fractions CMC-II and CMC-III show about the same specific caseinolytic activity. Fraction CMC-II appears to be almost homogeneous, however, while Fraction CMC-

III contains at least two components as shown by the starch-gel electrophoretic pattern (Fig. 1). The nitrogen content of the gel-filtered Fraction CMC-II varies between 15 and 16% as determined by a micro-Kjeldahl technique. The only N-terminal amino acid found is aspartic acid, amounting to one mole per 75 000–85 000 g of protein when correlated to the yield of aspartic acid from a crystalline bovine serum albumin preparation, run simultaneously.

In contrast to human material, bovine plasminogen preparations were found to contain little or no spontaneous proteolytic activity. It was therefore unnecessary

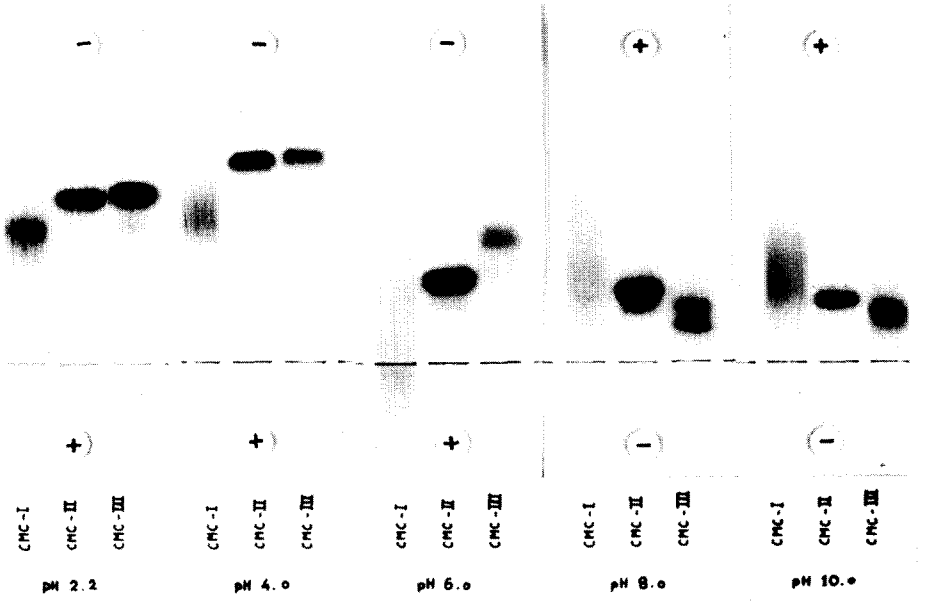

Fig. 1. Starch-gel electrophoreses at different pH values of a CM-cellulose (CMC) preparation. The electrophoreses are run vertically and descending at 0° for 24 h (pH 4 and 10) or 48 h (pH 2.2, 6 and 8) with a current density of about 3.5 mA/cm². The buffers used are: pH 2.2: glycine–HCl, I 0.1; pH 4.0: 0.03 M sodium acetate–0.02 M ε-ACA, acetic acid is added to pH 4.0; pH 6.0: 0.03 M NaH_2PO_4–0.02 M ε-ACA, NaOH is added to pH 6.0; pH 8.0: 0.02 M NaH_2PO_4–0.02 M ε-ACA, NaOH is added to pH 8.0; pH 10.0: 0.06 M glycine–0.02 M ε-ACA, NaOH is added to pH 10.0.

to include a step designed to remove this. ε-ACA is known to interfere with the activation of plasminogen[5]. In the solutions used for determination of proteolytic activity, however, the concentration of ε-ACA is too low to interfere. Yields and purification figures have been calculated from the euglobulin precipitate (Fraction IP) because of the difficulties in measuring plasminogen activity in plasma. As is the case with human plasminogen preparations[9,17], plasminogen activity could be found in at least two separate fractions. Although differing in starch-gel electrophoretic pattern (Fig. 1) the Fractions CMC-II and CMC-III were found to have about the same specific plasminogen activity with respect to caseinolytic and fibrinolytic properties.

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Received June 4th, 1963

SC 11093

Purification of pancreatopeptidase E by batch separation on DEAE-cellulose

Since the discovery of the enzyme pancreatopeptidase E (EC 3.4.4.7, formerly known as elastase) by BALO AND BANGA[1] many reports on the purification of the enzyme have appeared in the literature[2–5]. It is the purpose of the present communication to outline a rapid method for obtaining preparative amounts of purified pancreatopeptidase E by batch separation on DEAE-cellulose.

The source of the crude enzyme used in these studies was Trypsin 1-300 (Nutritional Biochemicals, Inc.) a commercial preparation of porcine pancreas. The preparation, usually 10 g, was extracted with sodium acetate buffer followed by fractionation with $(NH_4)_2SO_4$ according to LEWIS *et al.*[4]. The resulting "euglobulin precipitate" was used as the source of crude pancreatopeptidase E in the experiments to be described.

For the batch separation, 10 g of DEAE-cellulose (Whatman DE 50) are placed in a 250-ml centrifuge bottle. The adsorbent is then washed with three 50-ml aliquots of Na_2CO_3–HCl buffer (pH 8.9, *I* 0.04). The third wash is decanted and replaced with 20–50 ml of the crude pancreatopeptidase E preparation (about 200 mg protein), previously described. The preparation is thoroughly mixed and then placed in the cold for 1.5 h. The suspension is then centrifuged at 3000 rev./min at 4° for 10 min. The supernatant, containing the pancreatopeptidase E, is then decanted and saved. The DEAE-cellulose is then washed with three 20-ml aliquots of carbonate buffer (pH 8.9, *I* 0.04). Due to the low bulk density of the DEAE-cellulose the adsorbent was compressed with the aid of a tamper (constructed of borosilicate glass) and the resulting liquid was decanted. All supernatants were then pooled and filtered through Whatman No. 1 filter paper to remove extraneous DEAE-cellulose. The water-

* Formerly known as elastase.