[1] E. C. LOOMIS, C. GEORGE, JR. AND A. RYDER, *Arch. Biochem.*, 12 (1947) 1.
[2] A. DALBY, E. R. COLE AND E. T. MERTZ, *Can. J. Biochem. Physiol.*, 38 (1960) 1029.
[3] N. ALKJAERSIG, *Federation Proc.*, 19 (1960) 58.
[4] E. T. MERTZ AND J. Y. S. CHAN, *Federation Proc.*, 22 (1963) 561.
[5] N. ALKJAERSIG, A. P. FLETCHER AND S. SHERRY, *J. Biol. Chem.*, 234 (1959) 832.
[6] J. J. HAGAN, F. B. ABLONDI AND E. C. DE RENZO, *J. Biol. Chem.*, 235 (1960) 1005.
[7] P. WALLÉN AND K. BERGSTRÖM, *Acta Chem. Scand.*, 13 (1959) 1464.
[8] P. WALLÉN AND K. BERGSTRÖM, *Acta Chem. Scand.*, 14 (1960) 217.
[9] P. WALLÉN, *Arkiv Kemi*, 19 (1962) 451.
[10] P. WALLÉN, *Arkiv Kemi*, 19 (1962) 469.
[11] K. BERGSTRÖM, *Arkiv Kemi*, in the press.
[12] S. MÜLLERTZ, *Biochem. J.*, 61 (1955) 424.
[13] K. BERGSTRÖM AND P. WALLÉN, *Arkiv Kemi*, 17 (1961) 503.
[14] O. SMITHIES, *Biochem. J.*, 71 (1959) 585.
[15] S. ERIKSSON AND J. SJÖQUIST, *Biochim. Biophys. Acta*, 45 (1960) 290.
[16] E. J. COHN, L. E. STRONG, W. L. HUGHES, JR., D. J. MULFORD, J. N. ASHWORTH, M. MELIN AND H. L. TAYLOR, *J. Am. Chem. Soc.*, 68 (1946) 459.
[17] M. RYBÁK AND M. PETÁKOVÁ, *Clin. Chim. Acta*, 8 (1963) 133.

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.

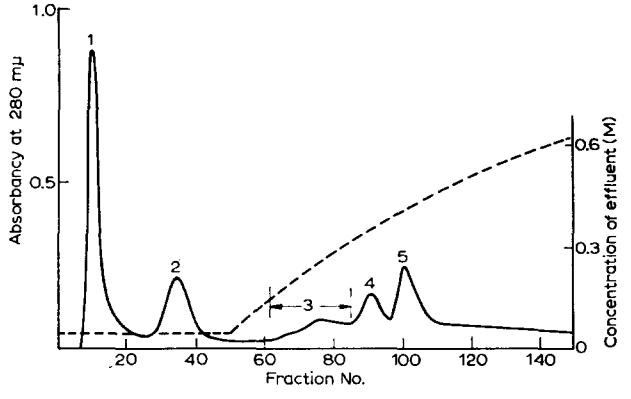

Fig. 1. Chromatography of "euglobulin precipitate" on a 2.5 × 16-cm column of DEAE-cellulose. 38.4 mg protein was added to the column. The pH throughout was 8.9. NaCl gradient was begun at Fraction 50. Mixing-chamber volume 558 ml; fraction volume 5 ml; flow rate 30–35 ml/h.

clear supernatant is then dialyzed for 24 h against 16 l of distilled water. The dialysis step in addition to removing undesirable salts prior to freeze-drying, also removes the 280-mμ absorbing non-protein compound responsible for Peak 2 (see Fig. 1).

The method of SACHAR *et al.*[6] using orcein-impregnated elastin (Worthington Biochemicals) was used in the assay of activity. Protein determinations were made using the method of LOWRY *et al.*[7].

In Fig. 1 are presented the results of chromatography of a sample of "euglobulin precipitate" on a column of DEAE-cellulose. The elution diagram obtained is similar to that presented by LEWIS AND THIELE[8] in that four major peaks and one other area of 280-mμ absorbing material (Peak 3) were obtained. The first peak, designated as Peak 1, is pancreatopeptidase E. Peaks 2, 3, 4 and 5 are elastolytically inactive. Approx. 90% of the protein added as the "euglobulin precipitate" is recovered in five peaks shown in Fig. 1. 72% of the activity is, consistently, the extent of the recovery of pancreatopeptidase E during column chromatography of the "euglobulin precipitate".

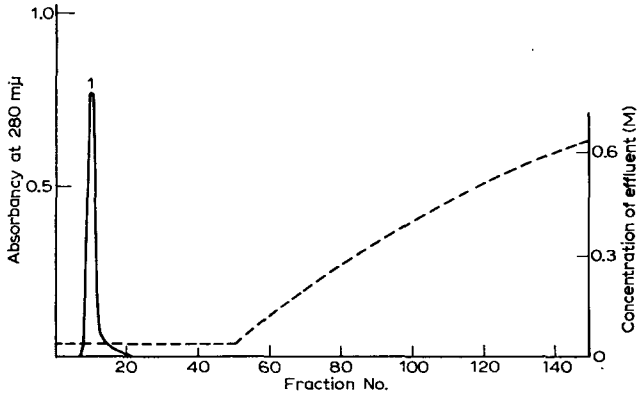

Fig. 2. Chromatography of batch-purified pancreatopeptidase E on DEAE-cellulose. 13.3 mg protein was added to the column. Conditions as in Fig. 1.

When the influent buffer (pH 8.9, I 0.04) was continued 35 fractions following the emergence of Peak 2 no additional protein was eluted. It was apparent from these results that at pH 8.9 and at I 0.04 the proteins responsible for Peaks 3, 4 and 5 in Fig. 1 are not eluted. This finding made it possible to obtain purified pancreatopeptidase E from the 5-component "euglobulin precipitate" by batch separation (Fig. 1). Pancreatopeptidase E purified by the batch method (dialyzed and freeze-dryed) when subjected to chromatography on DEAE-cellulose produced the results shown in Fig. 2. As can be seen in Fig. 2, Peak 2 is not present nor are the proteins that are responsible for Peaks 3, 4 and 5 (Fig. 1), when the same salt gradient which was used in the elution of the latter peaks in the "euglobulin precipitate" was employed (Fig. 1). This experiment provides ample evidence that the proteins responsible for Peaks 3, 4 and 5 are strongly adsorbed at the pH and I used.

When the sedimentation behavior of the column-purified product was compared to that of the batch-purified preparation, it was found that no difference was obtained. Both products gave single peaks. Comparison of the two products as to sedimentation coefficient, yield from the starting material and specific activity is presented in Table I. It can be seen in Table I that there is no significant difference

TABLE I

PURIFICATION AND SOME PROPERTIES OF PANCREATOPEPTIDASE E FROM TRYPSIN 1-300

Preparation	*Specific activity***	*Purification (fold)*	*Yield (%)****	$s_{20,w} \times 10^{-13}$
Trypsin 1-300*	1.8	—	—	—
"Euglobulin precipitate"	33.8	19	—	—
Batch purified	66.4	37	1.0%	2.9
Column purified	62.6	35	0.9%	2.9

* 1% suspension in carbonate buffer.
** Units per mg protein.
*** From Trypsin 1-300.

in the specific activity of pancreatopeptidase E purified by either method. The yield from the starting material is also approximately the same. Also both products produced single peaks when chromatographed on carboxymethyl-cellulose using the method outlined by NAUGHTEN AND SANGER[5]. Both products were negative for trypsin (EC 3.4.4.4) and chymotrypsin (EC 3.4.4.5) when assayed respectively on *N*-benzoyl-L-arginine ethyl ester[9] and *N*-benzoyl-L-tyrosine ethyl ester[10].

This work was supported by research grant NB 01962-05 from the National Institutes of Health, Bethesda, Md. (U.S.A.). Thanks are also due to Mr. G. DAYNES for the photographic work and to Miss D. COTE for typing the manuscript.

Department of Pathology and Medical Research,
St. Margaret's Hospital and
Department of Obstetrics and Gynecology,
Tufts University Medical School,
Boston, Mass. (U.S.A.)

J. S. BAUMSTARK
W. A. BARDAWIL
A. J. SBARRA
N. HAYES

[1] J. BALO AND I. BANGA, *Nature*, 164 (1949) 491.
[2] W. A. LOEVEN, *Acta Physiol. Pharmacol. Neerl.*, 9 (1960) 44.
[3] H. HERMANN AND T. FUJII, *Z. Physiol. Chem.*, 328 (1962) 65.
[4] U. J. LEWIS, D. E. WILLIAMS AND N. G. BRINK, *J. Biol. Chem.*, 222 (1956) 705.
[5] M. A. NAUGHTON AND F. SANGER, *Biochem. J.*, 78 (1961) 156.
[6] L. SACHAR, K. K. WINTER, N. SICHER AND S. FRANKEL, *Proc. Soc. Exptl. Biol. Med.*, 90 (1955) 323.
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
[8] U. J. LEWIS AND E. H. THIELE, *J. Am. Chem. Soc.*, 79 (1957) 755.
[9] E. W. SCHWERT AND Y. TANAKA, *Biochem. Biophys. Acta*, 26 (1955) 570.
[10] E. C. W. HUMMEL, *Can. J. Biochem. Physiol.*, 37 (1959) 1393.

Received May 21st, 1963
Revised manuscript received July 23rd, 1963

Biochim. Biophys. Acta, 77 (1963) 676–679

SC 11090

Apparent differences in enzymic rates of hydrolysis of commercial adenosinetriphosphate preparations

We wish to draw attention to the finding that commercial crystalline disodium ATP preparations differ greatly in their susceptibilities to hydrolysis by myofibrils or actomyosin and that the differences are likely to be attributable to slight contamination of ATP with Ca^{2+}.

Table I shows the differences in the rates of P_i production between two lots of ATP's purchased from Sigma Chemical Co., St. Louis, using myofibrillar ATPase

TABLE I

VARIATIONS IN MYOFIBRILLAR ATPASE ACTIVITY USING DIFFERENT LOTS OF ATP

The 2.5-ml reaction mixtures at 25° contained 40 mM Tris–acetate buffer (pH 7), 4 mM magnesium acetate, 4 mM ATP (as shown) and 1 mg of myofibrillar protein[1,2]. The ionic strength was adjusted to 0.15 by a mixture of 0.13 M KCl and 0.02 M potassium acetate solution. After 5-min reactions, 2.5 ml of 8% trichloroacetic acid was added, and P_i was measured by the method of FISKE AMD SUBBAROW[10] after removal of precipitate by centrifugation.

Myofibrillar preparation	*ATP*	*ΔP_i (μmole/min per mg protein)*
A	Sigma 52B728	0.381
A	Sigma 52B728	0.381
A	Sigma 22B721	0.126
A	Sigma 22B721	0.139
B	Sigma 52B728	0.196
B	Sigma 22B721	0.070

(EC 3.6.1.3). Even though the specific activities varied from one myofibrillar preparation to another, the percentage differences between the two ATP's remained the same. Further, as seen in Table II, the differences among various ATP's persisted even when the myofibrillar ATPase was coupled to the creatine kinase (EC 2.7.3.2) system.