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A sensitive method to detect traces of peptidase activity

During the course of investigations on the possible presence of an ε-peptide bond in bovine growth hormone (BGH)[1,2] by means of ε-peptidase[3] and reduction of the disulfide bonds, there was evidence for the breakdown of the hormone both in the presence and absence of enzyme. The breakdown could be a result of digestion by α-peptidase activity present in the preparation of growth hormone. No peptidase activity was detected by the method of casein digestion[4] even when the incubation period was extended to 3 h. Two distinct protein peaks were obtained upon gel filtration of BGH (NIH-GH-8) from the Endocrinology Study Section, National Institutes of Health, Bethesda, Md., on a Sephadex G-100 column[5,6]; the second peak contained most of the biological activity[6]. A sensitive method for detection of the peptidase activity was developed which gave evidence for the presence of peptidase activity associated with the first peak[7]. This study has now been extended for assay of proteolytic enzymes at very low concentrations and to look for proteolytic contamination of commercial enzyme preparations.

Bovine serum albumin (BSA) (Pentex, Inc., Kankakee, Ill.) was acetylated with ^{14}C-acetic anhydride[8] (New England Nuclear Corp., Boston, Mass.). The unreacted ^{14}C-acetate was removed by extensive dialysis and gel filtration on Sephadex G-25. Peptidase activity was detected by using ^{14}C-acetyl BSA as substrate; the hydrolysis of peptide bonds was ascertained by following changes in the elution profile of the radioactivity during gel filtration on Sephadex G-75.

By casein digestion an increase of absorbance of 0.05 at 280 mμ was observed at a concentration of 1 μg of trypsin per ml of the incubation mixture[9]. Tryptic activity was better detected by using ^{14}C-acetyl BSA as the substrate as illustrated in Fig. 1. There were two peaks of radioactivity: the first one (unretarded) represented unhydrolyzed ^{14}C-acetyl BSA and the second one (retarded) was peptide from hydrolysis of labelled protein. The extent of hydrolysis is directly proportional to the total

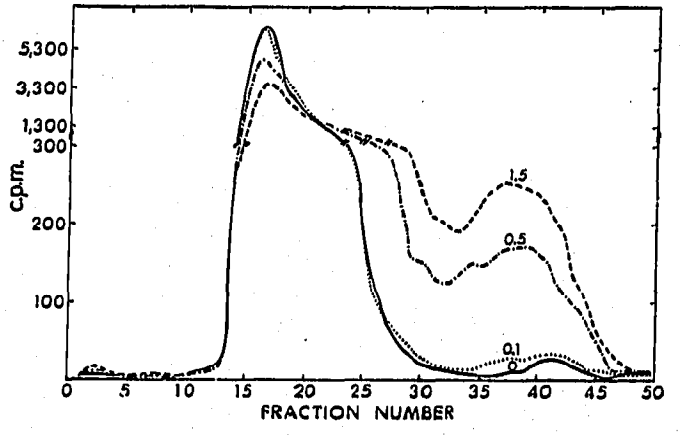

Fig. 1. Assay of trypsin using ^{14}C-acetyl bovine serum albumin. ^{14}C-Acetyl BSA (500 μg) and varying amounts of DFP treated trypsin (Worthington Biochemicals, Freehold, N.J.) in μg as indicated to a total volume of 0.2 ml in 1% NH_4HCO_3 were incubated at 37° for 1 h. The incubation mixture was subjected to gel filtration on a Sephadex G-75 column (31.5 × 0.8 cm) with 0.05 *M* NaCl as eluent. Fractions of 8 drops were collected and 5 ml of aqueous scintillation fluid[10] was added and counted in a Liquid Scintillation Spectrometer (Nuclear Chicago Mark I).

radioactivity of the second peak. The amount of radioactivity under the second peak was calculated by finding the area under the curve. There was a linear relationship between the amount of radioactivity of the second peak and the amount of trypsin up to 0.75 μg. Linearity was lost beyond 0.75 μg, probably because a significant portion of enzymatic hydrolysis involves further hydrolysis of peptides.

Using ^{14}C-acetyl BSA as the substrate and increments of chymotrypsin up to 1 μg, the enzyme was assayed. There were also two peaks of radioactivity, the second peak represented the extent of hydrolysis by chymotrypsin. Linearity was not observed between the amount of radioactivity of the retarded peak and concentration of enzyme. This may be due to the limited amounts of enzyme used or the broad specificity of hydrolysis of peptide bonds[11].

With ^{14}C-acetyl BSA as a substrate, low levels of trypsin and chymotrypsin which could not be detected by other methods were detected in a fast and easy way. In this method trypsin was not fully active on ^{14}C-acetyl BSA because many of the ε-amino groups were modified.

Pronase was assayed by casein digestion[12]. At a concentration of 2.75 μg of pronase per ml of the incubation mixture, an increase in absorbance of about 0.05 at 280 mμ was observed. Pronase (less than 2.75 μg) was assayed by using ^{14}C-acetyl BSA as the substrate. The second radioactive peak indicated the peptidase activity of the enzyme as in the case of trypsin and chymotrypsin.

Low concentrations of papain were also detected by using ^{14}C-acetyl BSA as substrate. Since ^{14}C-acetyl BSA was precipitated at low pH, it could not be used to detect pepsin, which acts best at about pH 2[13].

Peptidase activity was detected in samples of ribonuclease in 0.1 *M* tris-HCl, 0.05 *M* KCl, pH 7.4 (Fig. 2). It was absent in electrophoretically pure deoxyribonuclease in 0.1 *M* sodium acetate 0.015 *M* Mg^{2+}, pH 6.5 at which DNase was most active[15].

Peptidase activity at low concentrations of trypsin, chymotrypsin, pronase and papain was detected by using ^{14}C-acetyl BSA as substrate and observing changes in the radioactive profile upon gel filtration on a Sephadex G-75 column. Peptidase

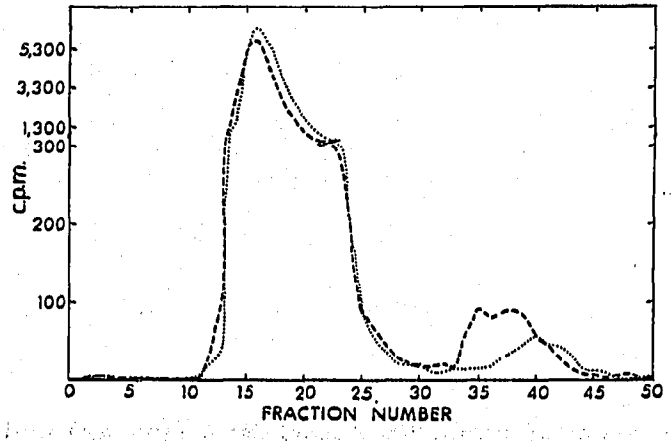

Fig. 2. Gel filtration of ^{14}C-acetyl bovine serum albumin treated with ribonuclease. ^{14}C-Acetyl BSA (500 μg) and ribonuclease (100 μg) to a total volume 0.2 ml in 0.1 *M* tris-HCl, 0.05 *M* KCl, pH 7.4[14] were incubated at 37° for 1 h with stirring and then subjected to gel filtration on Sephadex G-75 column (31.5 × 0.8 cm). Fractions of 8 drops were collected and counted for radioactivity. (- - -) ^{14}C-acetyl BSA treated with RNase; (· · · · ·) ^{14}C-acetyl BSA without RNase.

activity was found in samples of pancreatic ribonuclease. This method could be employed to detect traces of peptidase in biological preparations.

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1 C. H. LI, in M. L. ANSON, K. BAILEY AND J. T. EDSALL (Editors), *Advances in Protein Chemistry*, Vol. XI, Academic Press, New York, 1956, p. 101.
2 C. H. LI, *Cancer*, 10 (1957) 698.
3 J. D. PADAYATTY AND H. VAN KLEY, *Biochemistry*, 5 (1966) 1394.
4 M. KUNITZ, *J. Gen. Physiol.*, 30 (1947) 291.
5 J. M. DELLACHA AND M. SONENBERG, *J. Biol. Chem.* 239 (1964) 1515.
6 H. G. BURGER, H. EDELHOCH AND P. G. CONDLIFFE, *Endocrinology*, 78 (1966) 98.
7 J. D. PADAYATTY AND H. VAN KLEY, *Endocrinology*, 80 (1967) 782.
8 P. J. COLLIPP, S. A. KAPLAN, D. C. BOYLE AND C. S. N. SHIMIZU, *J. Biol. Chem.*, 240 (1965) 143.
9 M. LASKOWSKI, in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. II, Academic Press, New York, 1955, p. 26.
10 F. E. KINARD, *Rev. Sci. Instr.*, 28 (1957) 293.
11 A. M. KATZ, W. J. DREYER AND C. B. ANFINSEN, *J. Biol. Chem.*, 284 (1959) 2897.
12 M. NOMOTO AND Y. NARAHASHI, *J. Biochem.*, 48 (1960) 906.
13 R. M. HERRIOTT, in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. II, Academic Press, New York, 1955, p. 3.
14 S. R. DICKMAN, J. P. AROSKAR AND R. B. KROPE, *Biochim. Biophys. Acta*, 21 (1956) 539.
15 M. KUNITZ, *J. Gen. Physiol.*, 33 (1950) 363.

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