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A Rapid Method for the Detection of Trypsinogen and Chymotrypsinogen After Electrophoretic Separation of Pancreas Extract on Polyacrylamide Gel

Several methods have been described for the visualization of proteolytic activity on electropherograms obtained with starch (1,2), agar and agarose (3–6), paper (7), and cellulose acetate (8–11) as supporting media. In most of these reports casein was used as a (nonspecific) substrate. In only one case (11), the authors used substrates specific for trypsin and for chymotrypsin.

The proteins in a pancreas extract could be satisfactorily separated by electrophoresis on polyacrylamide gel and we looked for the possibility of localizing proteolytic activities in this gel.

Recently (12,13) methods for the direct localization of proteolytic activity in polyacrylamide gels were published, but no attempt was made in these articles to distinguish between trypsin and chymotrypsin. In this paper we will describe a method for the detection of Tg[1] and ChTg after their activation in the polyacrylamide gel. The method allows a rapid and reliable localization of the two proenzymes.

MATERIALS

DCC-treated bovine trypsin type XI, 1 × crystallized, bovine α-chymotrypsin type II, 3 × crystallized, and BAPNA were purchased from Sigma Chemical Co. (St. Louis, Missouri); purified enterokinase from Miles Laboratories Inc. (Kankakee, Illinois); tris-(hydroxymethyl)-methylamine and urea from BDH Chemicals Ltd. (Poole, England); *N,N'*-methylene-bisacrylamide from Fluka A.G. (Buchs, Switzerland); *N,N,N',N'*-tetramethylenediamine from Koch-Light Laboratories Ltd. (Colnbrook, England); ATPNA and all other reagents (of analytical grade) from E. Merck Co. (Darmstadt, Germany).

METHODS AND RESULTS

Rat Pancreas Extract

Rat pancreas extract was prepared by homogenization of the rat pancreas in 0.2 M $NaHCO_3$–NaOH pH 8.4 at 0°C by means of a Potter-

[1] Abbreviations: Tg, trypsinogen; ChTg, chymotrypsinogen; BAPNA, α-*N*-benzoyl-*dl*-arginine-*p*-nitroanilide HCl; ATPNA, *N*-acetyl-l-tyrosine-*p*-nitroanilide.

Elvehjem homogenizer. After a first centrifugation of 30 min at 5000*g* the supernatant was centrifuged for 15 hr in a Spinco ultracentrifuge L-50 at 105,000*g*. The pancreas extract was stored at −20°C and thawed just before use.

Detection of Trypsinogen and Chymotrypsinogen

After completion of the electrophoresis of the pancreas extract the gels were split longitudinally and the two halves were incubated for 2 min in cold incubation medium, consisting of 0.05 M Tris–HCl pH 8.2 + 0.02 M $CaCl_2$. Activation of Tg was achieved by incubating one-half for an additional 30 min at 37°C in the incubation medium to which was added enterokinase (1 mg/ml). In the other half ChTg was activated by the addition of trypsin to the incubation medium to a final concentration of 50 μg/ml also for 30 min at 37°C. For Tg localization, the gel has to be completely freed from adhering enterokinase since this enzyme reacts with the (trypsin specific) BAPNA substrate. It was sufficiently achieved by washing this half of the gel during 2 min in incubation medium at 37°C. Subsequently the gels were gently blotted and placed on 3 MM Whatman paper strips moistened with incubation medium containing either BAPNA or ATPNA (specific for chymotrypsin) in a concentration of 5 mM. Addition of dimethylsulfoxide in a final concentration of 10% was needed to obtain a clear solution of the substrate. After 5–15 min of incubation at 40°C in a moistened chamber bright yellow bands could be seen, beautifully sharpened by the gel acting as a lens. With this method we found two BAPNA and two ATPNA hydrolyzing bands after electrophoresis, as is illustrated in Fig. 1. Both Tg

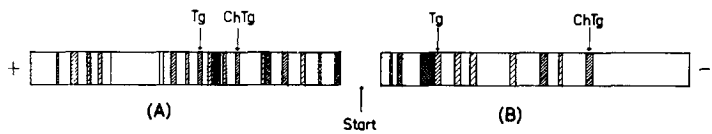

FIG. 1. The electrophoresis was performed in the cold in tubes (i.d. 9 mm) using 0.025 M Tris–0.19 M glycine buffer pH = 8.5 to which was added 1.6 M urea. The gels were formed using 7.5% (w/v) acrylamide, 0.375% (w/v) *N,N'*-methylene-bis-acryl-amide, 0.03% (v/v) *N,N,N',N'*-tetramethylenediamine and 0.02% (w/v) ammoniumpersulphate. 100 μl of the rat pancreas extract (1 mg protein), made 30% in sucrose, was applied onto the gel. A constant current of $6.3 \cdot 10^{-2}$ mA/mm² was maintained during the electrophoretic run (20–30 V/cm). The gels were stained for 1 hr with 0.5% Amido Black 10B solution containing 5% mercurichloride and destained with 5% acetic acid using the leaching method. The measure of shading reflects the intensity of the stained protein bands.
(A)—anodic electrophoresis for 2–3 hr.
(B)—cathodic electrophoresis for 4–6 hr.

and ChTg occur in the rat pancreas in a cationic and anionic form. Without activation of the proenzymes no proteolytic activity could be detected.

The qualitative method reported here appeared to be rapid (~50 min), simple and cheap.

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