THE DISC ELECTROPHORESIS OF PROTEINASES IN AN ACID MEDIUM

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The present paper gives the results of the application of the method of disc electrophoresis [1-3] to the investigation of acid proteinases.

For electrophoresis we selected a system of buffers giving a separation pH of 5.2 [4]. In 100 ml, solution A (pH 4.8) contained 1.13 g of creatinine (Chemapol, Czechoslovakia), 0.05 M HCl, and 1 ml of TMED* (Reanal, Hungary); solution B (pH 2.6) contained 0.57 g of creatinine, 0.05 M HCl, and 1 ml of TMED; solution C contained 30 g of Cyanogum-41 (Schuchardt, German Federal Republic); solution D contained 10 g of Cyanogum-41 and 1.5 g of m-b-acrylamide (Reanal, Hungary); and solution E contained 4 mg of riboflavin (Reanal, Hungary). The fine-pored gel contained equal volumes of solutions A and B diluted twofold with ammonium persulfate (2.8 mg/ml). The coarse-pored gel contained solutions B, D, and E in a ratio of 2:2:1. The electrode buffer consisted of 0.8 g of creatinine, 0.03 M glacial acetic acid, and water to 2 liters, pH 3.8. The polymerization of the 7.5% fine-pored gel was performed at 28°C, and that of the coarse-pored gel in the light of a fluorescent lamp. The length and diameter of the gels were 55 and 5 mm, respectively. Each tube was charged with from 30 to 100 μg of protein in 0.015-0.05 ml of a solution containing 20% of sucrose. Electrophoresis was performed for 2 h at a current strength of 4 mA in the tube (for the first 15 min, 2 mA in the tube). Before staining, the gel was kept for 30 min in 50% TCA, and it was then transferred for 30-60 min to a 1% solution of Coomassie Blue GL (Serva, German Federal Republic) in 20% TCA. The dye not bound to the protein was washed out with 7.5% acetic acid. The proteolytic activity was determined in parallel experiments, for which the gels were incubated in a mixture containing five parts of a 1% solution of hemoglobin in water and 1.25 part of 0.2 N HCl at 37°C for 30-60 min. The gels were washed free from the excess of hemoglobin (immersed for a few seconds in 0.2 N HCl), transferred to a closed tube, and incubated at 37°C in an atmosphere saturated with water vapor for 1 h. After incubation, the gels were washed repeatedly with 7.5% acetic acid and were then stained with a 0.5% solution of Amido Black 10B (Merck, German Federal Republic) and 7.5% acetic acid. After the excess of dye had been eliminated, the proteinase zones appeared in the form of light bands on a dark background of stained hemoglobin (Fig. 1.).

Fig. 1. Results of the electrophoresis of porcine pepsin in a medium with pH 5.2: a) coloration; b) proteolytic activity.

In resolving power, disc electrophoresis at a separation pH of 5.2 is not inferior to electrophoresis in an alkaline medium. The method described gave good results in a study of the acid proteinases of lower fungi, subtilisin, carboxypeptidase C, pepsinogen, and some pepsin derivatives and can be recommended for the study of various acid proteolytic enzymes.

*TMED – N,N,N',N'-tetramethylethylenediamine; m-b-acrylamide – N,N-methylene-bis-acrylamide; Cyanogum-41 contains 95% of acrylamide and 5% of m-b-acrylamide; TCA – trichloroacetic acid.

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LITERATURE CITED

1. B. J. Davis, Ann. N. Y. Acad. Sci. USA, 121, 404 (1964).
2. L. Ornstein, Ann. N. Y. Acad. Sci. USA, 121, 321 (1964).
3. S. Horiuchi, M. Honio, M. Yamasaki, and Y. Yamada, Sci. Pap. Coll. Gen. Educ. Univ., Tokyo, 19, 127 (1970).
4. F. I. Richards, J. A. Coll. Anal. Biochem., 3, 452 (1965).