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Note

Separation and detection of lipases using polyacrylamide gel electrophoresis

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In recent years, various methods have been used for the separation and identification of lipases by electrophoresis. Lipases have been separated by paper electrophoresis and the enzyme activity determined directly from the strips of paper using titrimetry[1]. Separation of lipases has also been achieved by electrophoresis on cellulose acetate strips and polyacrylamide gels, detection being effected by incubating the inert support on agar gel impregnated with tributyrin. The lipase activity appeared as clear zones in the emulsion[2]. A modification of this method has been reported[3] in which the released fatty acids were deposited at the site of lipolysis as insoluble calcium soaps. In another method[4], Tween 60 was impregnated in the polyacrylamide gel on which the lipase-containing sample was subjected to electrophoresis. Once again the liberated fatty acids were detected as white bands of calcium soaps.

This paper describes a sensitive technique for the separation of lipases on substrate-impregnated polyacrylamide gels and detection by a staining technique.

EXPERIMENTAL AND RESULTS

Materials

Nile Blue A (0.1 g) was dissolved in 100 ml of 0.4 *N* sulphuric acid by heating. The solution obtained was stable for at least 2 months.

A 48-ml volume of 1 *N* hydrochloric acid, 36.6 g of Tris and 0.46 ml of N,N,N′,N′-tetramethylethylenediamine (TEMED) were dissolved in distilled water and the volume made up to 100 ml.

An acrylamide solution was prepared by dissolving 30 g of acrylamide and 0.9 g of N,N-methylenebisacrylamide in distilled water to give a final volume of 100 ml.

An ammonium persulphate solution containing 0.3 g of ammonium persulphate in 100 ml of distilled water was prepared.

Pure substrates of triolein, diolein and monoolein were obtained from Centron Laboratories (Bombay, India) and tributyrin from E. Merck (Darmstadt, G.F.R.). The purity of the lipid samples was ascertained by thin-layer chromatography.

Preparation of gels

The gels were prepared[5] by mixing the solutions of TEMED, acrylamide, water and lipid substrate in the ratio 1:2:1:0.1 with the aid of a vortex mixer for 5

min, until an uniform emulsion formed. This emulsion was mixed with an equal volume of ammonium persulphate solution and 2-ml aliquots of the mixture obtained were transferred into glass tubes (7 × 0.6 cm). Another set of gels were prepared in a similar manner but without addition of the substrate. The top surface of the gel was layered with distilled water, the tubes were left at room temperature for gelation to occur and then the gels were cooled at 4° for 2 h.

Gel electrophoresis

IV instar larvae were homogenized to give a 15% homogenate in 0.05 *M* Tris–hydrochloric acid buffer of pH 7.4 and centrifuged at 1000 *g* for 10 min. Then 0.05–0.1 ml of the supernatant (35–70 μg of protein), with a lipolytic activity of 0.1–0.2 μmole of free fatty acids released per hour, was applied to the gel.

Electrophoresis was carried out at 4° using 0.05 *M* veronal–hydrochloric acid buffer of pH 8.6 at a voltage of 50 V per four gels. Tracking dye was used to detect completion of electrophoresis, which took about 2 h.

Detection of lipase activity on the gel

The gels were removed and one of them was immediately immersed in a boiling water-bath for 2 min; this gel served as a control. The other impregnated gels were incubated at 37° in 0.05 *M* Tris–hydrochloric acid buffer of pH 7.4, 10 m*M* in calcium chloride, for a few hours. The tributyrin-impregnated gels were incubated in a humid atmosphere. At the end of incubation, the gels were immersed in boiling water for 2 min and, after cooling, they were stained in Nile Blue A solution for 2 min[6]. The gels were washed free of the stain until a pale pink background was obtained. As the butyric acid is soluble in water, care was taken to minimize the washing of gels impregnated with tributyrin. The enzyme activity appeared as a blue band against the pink background at the site where fatty acids were liberated. The colour was stable for several weeks when kept in a cold, humid atmosphere. The gels that had no substrate were cut into 3-mm sections and each section was assayed for lipase activity.

Lipase assay

The substrate was prepared by mixing 0.05 *M* Tris–hydrochloric acid buffer, pH 7.4, containing 1% of bovine serum albumin, with 50 μmole of lipid substrate per millilitre. The mixture was sonicated for 15 min at 20 kHz, the container being cooled in crushed ice. The assay system consisted of 0.2 ml of enzyme and 0.2 ml of sonicated substrate in a final volume of 1 ml, made up with the Tris–hydrochloric acid buffer. The final concentration of bovine serum albumin was adjusted to 25 mg/ml. The incubation was carried out at 37° as described above. The lipids were extracted by the method of Bligh and Dyer[7] and the fatty acids were determined according to the method of Verudin *et al.*[8].

On electrophoresis and staining of the tributyrin-impregnated gels, *Culex* larval homogenate exhibited five enzyme bands. The lipase activity could also be detected when the gel sections from the corresponding areas of unimpregnated and unstained gels were assayed. Triolein-, diolein- and monoolein-impregnated gels showed one enzyme band each (Fig. 1). The bands obtained with triolein and diolein were similar to each other and distinctly different from that of monoolein-containing

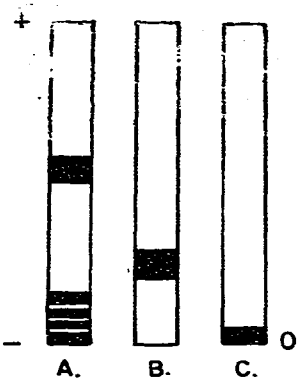

Fig. 1. Lipase activity on gels impregnated with (A) tributyrin, (B) triolein or diolein and (C) monoolein. O = origin.

gel. This result was confirmed by assaying the enzyme in the gel section. The gel section which hydrolyzed triolein also hydrolyzed diolein, but not monoolein, suggesting that a single enzyme hydrolyzed both triglyceride and diglyceride while the enzyme acting on monoglyceride was different. Our recent studies on the characterization of different lipases of the insect confirmed these observations; the details will be published elsewhere.

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