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Note

Rapid method for the direct observation of aryl-β-glucosidases after separation on polyacrylamide gel

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The localization of proteins and most enzymes after polyacrylamide disc gel electrophoresis is generally carried out by non-specific staining with dyes such as amido black or coomassie blue, after fixation of the proteins. For the localization of enzymes more specific methods may be used[1]. Most of these techniques are time-consuming and require the extrusion of the gels[2,3]. More sophisticated techniques such as layering a second substrate-impregnated gel over the extruded gel have been used by some workers[4–9]. Padjak and Padjak[10] replaced a glass rod in the middle of the gels by substrate-impregnated agar gel after electrophoresis. The inclusion of a lipidic substrate in the agar gel for the detection of lipases has been reported[11], but more than 24 h were necessary for enzyme visualization.

In our investigations on the cellulolytic complex of a bacterium[12] we examined the possibility of impregnating the gels, before electrophoresis, with a substrate which would allow a very rapid detection of some enzymes after the electrophoretic run. The results obtained with aryl-β-glucosidases were very encouraging and the method can certainly be extended to other enzymes.

EXPERIMENTAL

Polyacrylamide gel electrophoresis was carried out with an Acrylophor 140 from Pleuger, Wijnegem, Belgium. Both compartments contained a Tris–glycine buffer of pH 8.3 (3 g of Tris and 14.4 g of glycine in 1 l of distilled water). The gels were prepared by mixing equal volumes of (1) 30% acrylamide and 0.8% N,N′-methylene-bis(acrylamide) in water, (2) 1% of N,N,N′,N′-tetramethylethylenediamine in the Tris–glycine buffer (pH 8.3) of 10× strength, (3) distilled water, (4) 0.48% of ammonium persulphate in water and (5) 5 m*M* *o*-nitrophenyl-β-D-glucopyranoside in water, in glass tubes (67 × 9.0 mm). The aryl-β-glucosidase-containing samples[12], crude bacterial extracts or extracts purified by DEAE–Sephadex chromatography (30–200 μg of protein) were layered on top of the gels after mixing with a few grains of saccharose.

The experiments were started by applying a constant current of 2 mA/gel to avoid convection of the sample before it had entered the gel[13]. The current was then increased to 5 mA/gel and the run was stopped when free *o*-nitrophenol had reached

the bottom of the gel cylinder (*ca.* 45 min). The temperature was kept at 15°. The tubes containing the gel were then removed from the apparatus and immediately incubated at 37° until sufficient *o*-nitrophenol had been liberated by the separated aryl-β-glucosidases to give visible yellow bands. Control gels were stained for proteins according to the method of Diezel *et al.*[14] using Coomassie Brilliant Blue G-250. Enzyme protein was located in the stained gels by comparing the R_F values, taking the solvent front as a reference point.

RESULTS AND DISCUSSION

Electrophoresis of the crude enzyme extract[12] resulted in the clear detection of at least nine protein bands (Fig. 1A). Three of the bands exhibited aryl-β-glucosidase activity (Fig. 1B). Purification of the extract by DEAE–Sephadex chromatography also gave three peaks with aryl-β-glucosidase activity. Electrophoresis of an aliquot of each peak resulted in the detection of one active band for each fraction (Fig. 1C). When coomassie blue was used more bands were evident. Using the same method we have been able to detect aryl-β-glucosidase activity in several other cellulolytic bacteria, and thus this method permits the rapid screening of strains.

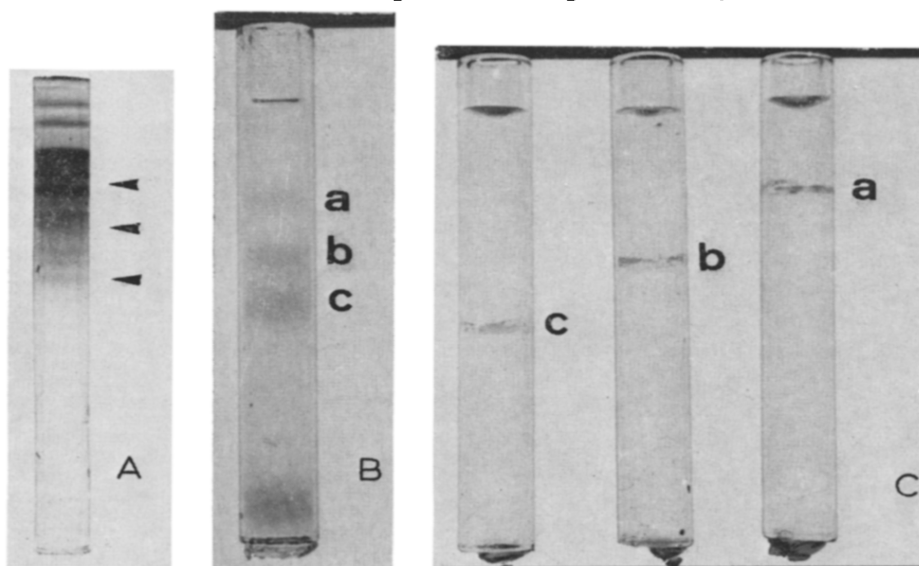

Fig. 1. (A) Polyacrylamide gel electrophoretic separation of proteins from a crude extract of a cellulolytic bacterium. The arrows indicate the approximate position of the enzymes with aryl-β-glucosidase activity. The proteins were stained with coomassie blue. (B) Direct localization of the aryl-β-glucosidases present in the same crude extract, using the method described in the text. Three different enzymes are detected: a, b and c. The lowest band is due to *o*-nitrophenol. (C) Direct localization of aryl-β-glucosidase activity found in fractions obtained by DEAE–Sephadex chromatography of the crude extract. Each fraction obtained corresponds to one enzyme. Enzyme c eluted first from the column, then b and finally a.

The method also provides a fast system for detecting the aryl-β-glucosidase activity during purification steps. During the electrophoretic run the substrate was always partly hydrolyzed by the enzymes and the liberated *o*-nitrophenol then acted

as the tracking dye. This hydrolysis occurred during the first phase of the run, when the enzyme penetrated the gel, in the presence of the low current. The method can be applied only when the enzymatic activity is sufficiently low to avoid the colouring of the whole gel during the run. This effect may be avoided by lowering the amount of enzyme applied to the gel. Another requirement is the use of non-migrating substrates, but it is expected that the method can be used whenever coloured products are formed from non-ionizable substrates. Thus, for the general detection of glucosidases acting on R-glucosides, where R is the coloured product, the method is certainly simple and rapid. Under these conditions the most striking adventages are: (1) there is no need for a tracking dye; (2) there is no need for staining and destaining; (3) the gels must not be extruded from the tubes; (4) the enzyme band is completely stained and (5) labile enzymes may be detected more accurately. The *o*-nitrophenol formed diffuses rapidly so that broad bands are obtained, as during the time necessary to take photographs (Fig. 1B).

REFERENCES

1 O. Gabriel, *Methods Enzymol.*, 22 (1971) 578.
2 J. Attias, J. L. Bonnet and J. L. Sauvagnargues, *Biochim. Biophys. Acta*, 212 (1970) 315.
3 B. Dahlmann and Kl.-D. Jany, *J. Chromatogr.*, 110 (1975) 174.
4 M. Ceska, E. Hultman and B. G. A. Ingelman, *Experientia*, 25 (1969) 555.
5 T. J. Davies, *J. Clin. Pathol.*, 25 (1972) 266.
6 O. Vesterberg and R. Eriksson, *Biochim. Biophys. Acya*, 285 (1972) 393.
7 O. Vesterberg, *Acta Chem. Scand.*, 27 (1973) 2415.
8 T. Wadström, *Ann. N.Y. Acad. Sci.*, 209 (1973) 405.
9 P. G. Righetti and J. W. Drysdale, *J. Chromatogr.*, 98 (1974) 271.
10 E. Padjak and W. Padjak, *Anal. Biochem.*, 50 (1972) 317.
11 A. E. Finkelstein, E. S. Strawich and S. Sonnino, *Biochim. Biophys. Acta*, 206 (1970) 380.
12 J. Bevers, *Doctoral Dissertation*, University of Louvain, 1975.
13 K. E. Cooksey, in J. R. Norris and D. W. Ribbons (Editors), *Methods in Microbiol.*, Vol. 5 B, Academic Press, London, New York, 1973, p. 573.
14 W. Diezel, G. Kopperschlager and E. Hofmann, *Anal. Chem.*, 48 (1972) 617.