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Note

Detection of tannase in polyacrylamide gels

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The localization of enzymes after polyacrylamide gel electrophoresis is generally determined by specific detection for enzymatic activity and by non-specific staining for protein. Many techniques for each enzyme have been developed for detecting their activities in gels[1]. However, the use of tannase (tannin acylhydrolase, E.C. 3.1.1.20) in localization procedures for enzymatic activity has never been studied, although it has been reported to be isolated from various sources[2–7].

In our investigations on *Penicillium* tannase[4], we examined a localization procedure for tannase activity in polyacrylamide gels. The enzyme is highly specific for its natural substrate tannic acid, which interacts with quinine specifically, and forms white and water-insoluble complexes. These are favourable characteristics for localizing tannase activity in gels. When gels are incubated with tannic acid and subsequently with quinine, they will be covered with the complexes of tannic acid and quinine. If tannase activity is contained in the gels, the portion of gels containing the activity will be distinguishable from the other part, because the enzyme in the gels hydrolyses the substrate into glucose and gallic acid, which do not form complexes with quinine.

In this paper, we present a simple and rapid procedure for the detection of tannase activity in polyacrylamide gels.

EXPERIMENTAL

Materials

Tannic acid, quinine hydrochloride and Amido Black 10B were purchased from Wako Pure Chemicals (Osaka, Japan) and bovine serum albumin from Schwarz/Mann (New York, N.Y., U.S.A.). All reagents were of analytical-reagent grade.

Tannase. Tannase was prepared from a culture broth of *Penicillium* No. 80 B' by fractionation with ammonium sulphate and acrinol (ethacridine lactate monohydrate) and column chromatography on DEAE-Sephadex, CM-Sephadex and Sephadex G-200. The enzymatic activity was assayed spectrophotometrically by the method reported by Iibuchi *et al.*[8]. The enzyme in a gel was assayed after the gel had been cut into 2-mm slices and the enzyme eluted with 0.01 *M* phosphate buffer (pH 5.6) at 4° overnight.

Gel preparation

Gels were prepared according to the systems of Williams and Reisfeld[9]. All separating gels (7.5% gels) were subjected to pre-electrophoresis before preparation of stacking gels in the gel buffer[9] used for their polymerization at 3 mA per gel and room temperature for 2 h in order to remove residual ammonium persulphate. Stacking gels were prepared without sucrose, because sucrose, part of which migrated into separating gels during electrophoresis, deformed them in tannic acid solution.

Electrophoresis

The enzyme was applied to three individual gels, which were then subjected to electrophoresis at pH 8.0 (ref. 9), 2 mA per gel and 4° for 2 h and separately to localization of the enzymatic activity, staining with Amido Black 10B and enzyme assay.

Localization of tannase

After electrophoresis, the stacking gel was removed and the separating gel was incubated with 0.5% tannic acid in 30 ml of 0.5 *M* acetate buffer (pH 5.5) at 25° for 15 min. After the gel had been rinsed with 50 ml of 0.25 *M* acetate buffer (pH 5.5) with two changes of the buffer, it was transferred into 30 ml of 0.25 *M* acetate buffer (pH 5.5) containing 0.5% quinine hydrochloride and incubated with it at 25°. When white complexes appeared on the surface of gel except where the tannase activity was located, the gel was placed in a small test-tube (7 × 95 mm) which was filled with the quinine solution used for complex formation and photographed.

RESULTS AND DISCUSSION

When the gel was incubated with tannic acid after electrophoresis and subsequently with quinine, it was rapidly covered with white, insoluble complexes of tannic acid and quinine. The complexes became uniform on the gel after 20 min in the quinine solution, but the portion of 2–4 mm from the top of gel remained transparent as a single band without formation of the complexes, as shown in Fig. 1(A). As tannase would have hydrolysed tannic acid in the gel to glucose and gallic acid, which do not form the complexes with quinine, the enzyme seemed to exist in the transparent portion. It actually coincided with the main band of protein stained with Amido Black 10B [Fig. 1(B)] and tannase activity [Fig. 1(C)] assayed after the gel had been sliced and the enzyme eluted from it.

Bovine serum albumin was also tested as a control sample, because the transparent portion might appear in a location of the gel that contains both protein and tannase activity. However, as shown in Fig. 2, the whole surface of gel was covered with the complexes of tannic acid and quinine, although the protein was contained in part of the gel. These results show that only tannase activity brings about the transparent portion and can be localized in the gel by the proposed procedure.

As tannase has a high specificity for its substrate tannic acid, which also has a high specificity for quinine in the formation of complexes between them, the procedure is specific for the localization of tannase activity in polyacrylamide gels.

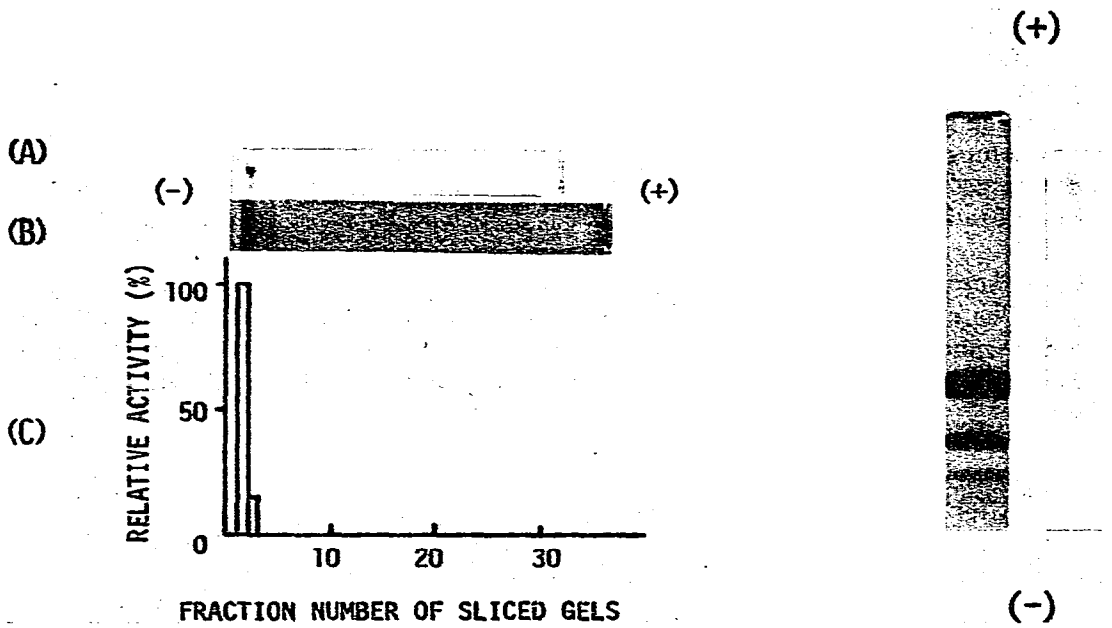

Fig. 1. Detection of tannase activity (A), Amido Black 10B pattern of the enzyme (B) and its enzymatic activity (C) in polyacrylamide gels. Amounts of 54, 24 and 36 μg of tannase were run on gels A, B and C, respectively. Gel B was stained with 1% Amido Black 10B in 7% acetic acid for 2 h and swollen about 10%. Enzymatic activity was assayed after the gel had been cut into slices of 2 mm and the enzyme eluted from them.

Fig. 2. The left-hand gel, containing bovine serum albumin, was covered with white complexes over the whole surface when it was incubated with tannic acid and subsequently with quinine after electrophoresis. The right-hand gel was stained with 1% Amido Black 10B in 7% acetic acid for 2 h and swollen about 10%. The amount of the protein applied to each gel was 44 μg.

In addition, the patterns of the enzymatic activity in gels can be obtained within 1 h after electrophoresis with a simple operation.

Part of the complexes on the gel was gradually released from it 24 h after complex formation, so that it is necessary to photograph the patterns before this alteration of the complexes occurs.

REFERENCES

1 O. Gabriel, *Methods Enzymol.*, 22 (1971) 578.
2 W. Madhavakrishna and S. M. Bose, *Bull. Cent. Leather Res. Inst. Madras*, 8 (1961) 153.
3 E. Haslam, R. D. Haworth, K. Jones and H. J. Rogers, *J. Chem. Soc.*, (1961) 1829.
4 H. Nishira and N. Mugibayashi, *Leather Chem.*, 11 (1965) 93.
5 S. Iibuchi, Y. Minoda and K. Yamada, *Agr. Biol. Chem.*, 32 (1968) 803.
6 H. Yamada, O. Adachi, M. Watanabe and N. Sato, *Agr. Biol. Chem.*, 32 (1968) 1070.
7 K. Aoki, R. Shinke and H. Nishira, *Agr. Biol. Chem.*, 40 (1976) 79.
8 S. Iibuchi, Y. Minoda and K. Yamada, *Agr. Biol. Chem.*, 31 (1967) 513.
9 D. E. Williams and R. A. Reisfeld, *Ann. N.Y. Acad. Sci.*, 121 (1964) 373.