ELECTROPHORETIC ANALYSIS OF HYDROLASES FROM GROWN GALL TISSUES

P.B. GAHAN, K. SHEIKH, Viviane MAGGI
Faculty of Medicine, Memorial University of Newfoundland, Canada

M. STROUN
Institut de Botanique Générale, Université de Genève, Suisse

and

A.R.W. SMITH
Biological Sciences, Thames Polytechnic, London, England

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1. Introduction

Crown gall (plant tumour) arises when a virulent strain of *Agrobacterium tumefaciens* is introduced into a plant conditioned by wounding. In addition to the presence of the bacterial nucleic acids in the host cells at the time of tumour induction [1, 2], an increase in acid phosphatase and esterase activities has been recorded cytochemically in infected cells of *Lycopersicon esculentum* with a maximum activity observed 6–7 days after inoculation [3]. These experiments did not indicate if the increased enzyme activity was due to increased activity of the existing hydrolases or to the appearance of new enzymes in the system. This situation has been resolved by disc gel electrophoresis studies on the normal plants and induced tumour tissue.

2. Methods

Plants of *L. esculentum*, var. Money Maker, were grown at 19° until 3 nodes high, when the plants were transferred to a temperature between 25° and 28°. After one week, the apices of the plants were removed, and 24 hr later the cut regions were inoculated by needle puncture with *A. tumefaciens* strain B6. Twenty days later the terminal portions of the shoots were harvested. As controls, other plants were treated in a similar way, except for the omission of the bacteria at the time of inoculation.

Extracts of the plant enzymes were prepared by grinding 40 g of either the control plant tissues or the tumour tissue for 10 min in a pastle and mortar with white, acid-washed sand in 20 ml of 50 mM acetate buffer, pH 5.0. The slurry was filtered through muslin and was centrifuged at 10,000 *g* for 30 min at 4°. The supernatant was diluted in buffer to a final protein concentration of 8–10 mg/ml, as determined by the method of Lowry et al. [4], and solid sucrose was added to a final molarity of 0.5. The resultant preparation was used directly for polyacrylamide gel separation.

The bacterial enzymes could not be made by a similar procedure due to the resistance of their cell walls to rupturing by grinding. Instead, 10 g of packed cells, suspended in 10 ml of 50 mM acetate buffer at pH 5.0 were subjected to ultrasonic irradiation for 4 min at 4° using a 100 W ultrasonic disintegrator. The crude cell extract was centrifuged at 10,000 *g* for 30 min at 4°. The supernatant was diluted as described above for the plant extracts and used for gel separation.

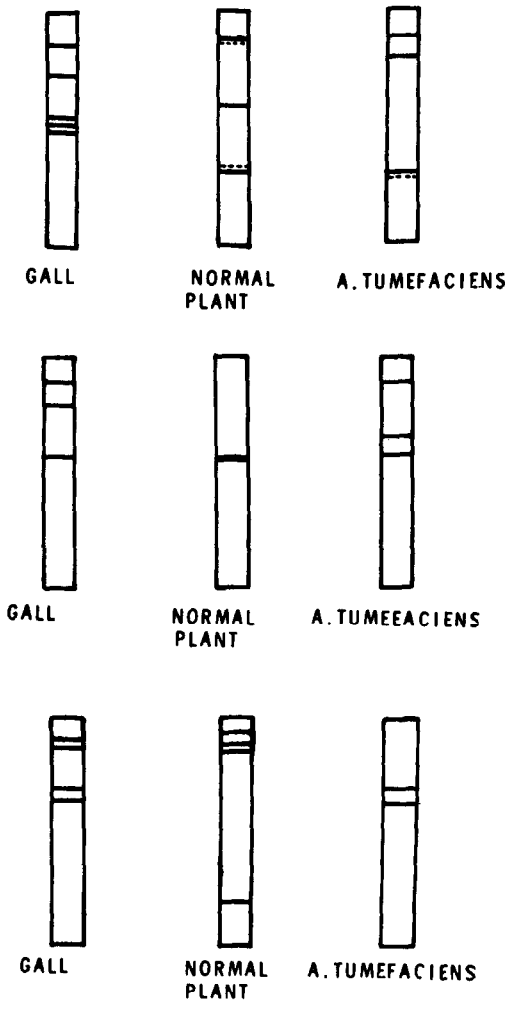

Fig. 1. Diagrams of gels showing the electrophoretic pattern of (A) esterases, (B) acid β-glycerophosphatases, (C) acid naphthol AS-BI-phosphatases from: normal plant i.e. wounded, non-infected shoots of *L. esculentum; A. tumefaciens*, gall i.e. wounded shoots of *L. esculentum* 21 days after infection with *A. tumefaciens.*

Electrophoresis was performed using a continuous buffer system of 10 mM tris-glycine, pH 8.4–8.6, in a Vokam disc electrophoresis apparatus. Gels were prepared [5, 6] with a starting concentration of 7% (w/v of Cyamogun 41. 0.05 ml of cell-free preparation was applied to the top of each gel, and the continuous buffer system was run for 60–90 min at 2° (1.5–2.5 mA/gel).

The gels were reacted for general proteins with amido black, or for naphthyl esterases [7] with α-naphthyl acetate as substrate, or for acid phosphates [7, 8] with either naphthol AS BI phosphate or sodium β-glycerophosphate as substrates. After reaction, all gels were cleared [9].

3. Results

Seventeen protein bands were present in the extract of the tumour, as opposed to only 9 from the extract of the normal plant tissue and 14 bands from the bacterial extract.

The normal tissue extract yielded 4 bands and the bacterial extract 3 bands which reacted with α-naphthyl acetate as substrate. The gall tissue extract yielded 3 strongly positive bands which were not present in either the normal plant extract or the bacterial extract (fig. 1A). In addition, in the tumour extract there were two weakly reacting bands, one of which may have corresponded with a similar band in the bacterial extract.

Acid β-glycerophosphatase activity was revealed as 3 bands in the tumour extract, 1 band in the normal tissue extract, and 3 bands in the bacterial extract (fig. 1B). Of these, the second band from the tumour did not correspond with any band from either the extract of the bacteria or the normal plant.

The acid naphthol AS BI phosphatase reaction indicated that 2 of the 4 tumour bands correspond to 2 of the bacterial bands and 2 correspond to ones similar in extracts of the normal tissue. Two of the bands present in the normal tissue extract are absent from extract of the tumour tissue (fig. 1C).

Discussion

Electrophoretic studies indicated that proteins of tumour tissue extract were more heterogenous than were extracts of normal plant. Reaction of the gels with the enzyme substrates showed an increase in the number of esterase and β-glycerophosphatase bands of comparable intensity in the tumour preparation over the number from normal tissue and a change in the number of acid naphthol phosphatase bands (fig. 1A–C). Thus the originally reported increment

of hydrolytic activity identified cytochemically [3] is associated with a change in the types of phosphatase and esterase involved.

The appearance of the new bands in the tumour tissue has at least three possible explanations which relate to three observations upon the gels. The first concerns the appearance of new bands in the tumour extract which correspond in R_f to those present in the bacterial extract (fig. 1C). It is unlikely that enzymes were released directly from the bacteria by the grinding process used since bacterial cells are not normally ruptured in this way. It is possible that (a) these enzymes are secreted by the bacteria (which are normally extracellular) and diffuse into the affected plant cells, or (b) as indicated by Stroun et al. [1, 2], on infection of the plant cells with *A. tumefaciens*, the bacterial DNA is released into the host cells and transcribed. It is conceivable that messenger RNAs thereby formed are translated into bacterial enzymes in the plant cells.

After infection, there was a diminution in the transcription of host DNA [1, 2] which could account for the absence of enzymes of the normal plant from the tumour tissue extracts (fig. 1A and C) due to the suppression of transcription of gene sites for acid phosphatases and esterases.

A third explanation is required to account for the appearance of new bands which correspond to those neither from the bacteria nor from the normal plant tissue (fig. 1A and B). It is possible that these enzymes arose by derepression of existing plant or bacterial genes, or both, as a result of biochemical host-parasite interaction.

The above possibilities are being investigated further.

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