ACID PHOSPHATASES OF THE TEA LEAF

A. S. L. Tirimanna

Phosphatases are a group of enzymes which catalyse the hydrolysis of phosphoric acid esters. This paper describes the separation and detection of acid phosphatases in the tea leaf. The enzymes were separated by starch-gel electrophoresis and were localized on the gel by a technique of staining dependent on enzyme activity. Six bands having phosphatase activity at pH 5. 0 were localized. These are probably isozymes of acid phosphatase and could play an important role in the biosynthesis of terpenoid compounds of the tea plant. Some of these terpenoid compounds may be partly responsible for tea aroma and flavour.

Introduction

The phosphatases are a group of enzymes which catalyse the hydrolysis of phosphoric acid esters. A great variety of phosphatases are found in living organisms (*eg* phosphomonoesterases, phosphodiesterases, phosphorylases, pyrophosphatases and nucleotidases). Phosphatases which show optimum activity under acid conditions are referred to as ' acid phosphatases '. Extensive studies on acid phosphatases in many plant tissues have already been carried out by various workers, *eg* in wheat germ (Joyce & Grisolia 1960 ; Brouillard 1965), in lupine seedlings (Newmark 1960), in bean seedlings (Roussos 1962), in pea leaves (Forti 1962) and in tobacco leaves (Shaw 1966). Phosphatases are usually associated with energy transfer mechanisms but in some cases (*eg* lower plants) it has been shown that these enzymes carry out certain digestive functions (Weinberg & Orton 1964).

A study of the acid phosphatases of the tea leaf was undertaken, because these enzymes may play an important role in the final stages of the biosynthesis of terpenoid compounds. A few of these terpenoid compounds may make some contribution to tea aroma and flavour.

This paper describes the separation and detection of the acid phosphatases in the tea leaf.

Methods

Preparation and partial purification of enzyme extract

A modification of the method of Roussos (1962) was used. Twenty g of flush (clone TRI 777), 10 g of Polyclar (General Aniline and Film Corporation, New York), and 1 g of sand were homogenized in a Waring Blender for 15 min, with 100 ml of 0.2 M KCl in 0.05 M phosphate buffer (pH 7.4). A few drops of 0.005 M EDTA were also added. The suspension was centrifuged. All subsequent operations were carried out at 4°C. A solution of 1 M acetate buffer (pH 4.2) was added to the cell-free extract in the proportion of 5 : 1 (v/v). After six hr, the precipitated inactive protein was removed by centrifugation. Using 30% ethanol the active fraction was precipitated, dissolved in phosphate buffer and subjected to horizontal starch gel electrophoresis.

Starch gel electrophoresis

Connaught-hydrolysed starch and tris-succinic acid buffer (pH 6.0) were used for the preparation of the gel. The gel was chilled for two to three hr, before the insertion of pieces of Whatman 3 MM chromatography paper which were soaked

in the enzyme extract. Acetate buffer (pH 4.0) was used for the bridge. Horizontal electrophoresis was carried out using Buchler equipment for 16 hr against a potential gradient of two volt per cm.

Localization of enzyme

The isozymes of acid phosphatases were localized on the starch gel by the azo-dye technique of Goldberg & Barka (1962). The incubation solution was freshly prepared from the following stock solutions :

A—Tris-succinic acid buffer (pH 6.0).

B—Substrate stock solutions, 'Naphthol AS-MX', 'Naphthol AS-BI' and 'p-nitrophenyl phosphate' (obtained from the Sigma Chemical Co.). Each substrate solution was dissolved in N, N-dimethyl-formamide in 10, g per ml concentration.

C—4 per cent sodium nitrite in distilled water.

D—2 g p-rosaniline hydrochloride (BDH) were added to 50 ml 2 N hydrochloric acid and gently heated. After cooling, the solution was filtered. Solutions A, B and C were stored at 4°C.

The incubation mixture was prepared as follows : Five ml of solution A and one ml of solution B were mixed with 13 ml of distilled water in a beaker. A 0.8 ml aliquot of solution C was added to 0.8 ml of solution D in a test tube. This mixture was added to the solution in the beaker. After mixing, the pH was adjusted to 5.0 with N sodium hydroxide.

Each sliced gel (14 × 8 cm^2) was incubated in this mixture at 37°C for 30 min. The sites of enzyme activity were localized by the formation of characteristic red azo-dyes (*eg* Naphthol AS-BI, Matador colour, Naphthol AS-MX, Rose Madder Lake colour).

Results

Six bands having acid phosphatase activity (hydrolyses mono phosphatases) were localized on the gel as shown in Figure 1.

Two of these bands carried a positive charge and it was observed that in this case azo-dye formation was slow. This probably indicated that the optimum pH for these two isozymes was not 5.0. It is probable that the isozymes of acid phosphatase in the tea leaf have different substrate specificities. Work is now in progress on the substrate specificities, inhibitors and other characteristics of these enzymes.

There is no evidence at present to show that the enzyme is bound to any particular fraction of the leaf extract, but it is interesting to note that Matile *et al* (1965) have presented considerable evidence to show the localization of some acid phosphatase activity in the spherosomes and dictyosomes of higher plant tissues.

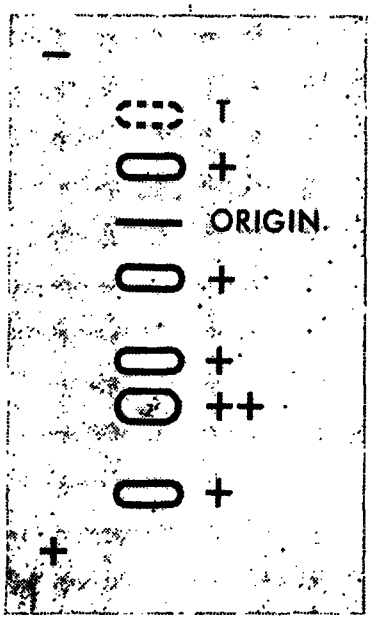

FIGURE 1—*Starch-gel electrophoresis of the isozymes of acid phosphatase using a discontinuous buffer system — The following abbreviations are used to denote enzyme activity at pH 5.0 T-little ; + moderate ; ++ appreciable*

Summary

1—A starch gel electrophoretic technique was used for the separation of the acid phosphatases of the tea leaf.

2—Six bands having acid phosphatase activity were detected by a technique of staining dependent on enzyme activity. Two of these bands carried a positive charge.

3—These acid phosphatases could play an important role in the biosynthesis of terpenoid compounds in the tea plant.

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