PECTOLYTIC ENZYMES FROM BANANA*

O.Markovič, K.Heinrichová and B.Lenkey**

Institute of Chemistry,
Slovak Academy of Sciences, 809 33 Bratislava

Received February 2nd, 1974

The presence of six multiple forms of pectin esterase was detected in banana extracts by starch gel electrophoresis with simultaneous detection of proteins and enzymatic activity. The approximate molecular weight of some forms of pectin esterase was determined. The presence of endopolygalacturonase and exopolygalacturonase was also demonstrated. The endopolygalacturonase has been partially characterized.

Hultin and coworkers[1-3] have described the presence of at least three forms of pectin methyl esterase (pectin pectyl hydrolase, E.C. 3.1.1.11) in an extract of banana, prepared by extraction with distilled water and 0·15M solution of NaCl under various conditions. They have characterized the differences between the individual forms, designated according to the conditions of extraction as F I, F II, and F III, by the determination of their pH-dependence, activation by mono- and bivalent cations, differences in inhibition by final products, and also by the determination of differences in thermostability of the individual fractions. When they purified these fractions on DEAE- and CM-cellulose, they were able to increase the pectin esterase activity and to obtain one additional fraction, designated F I_A. These authors have considered the possibility of interconversion of the individual forms of pectin esterase and in view of the solubility of fraction F I in water and of fractions F II and F III in NaCl solutions – of differences in binding of these fractions to cell walls.

This paper reports on the use of starch gel electrophoresis with simultaneous detection of pectin esterase activity, proteins, and lipids for the characterization of multiple forms of pectin esterase in fractions obtained by extraction of banana under various conditions, as well as on the presence and characterization of endo- and exo-polygalacturonase (poly-α-1,4-galacturonide glycane hydrolase, E.C. 3.2.1.15).

EXPERIMENTAL

Material. Ripe bananas (*Musa sapientum*, var. *Gros Michel*) of Columbian origin were used to start with.

* Presented at Annual Meeting of Czechoslovak Biochemical Society, Plzeň, September 1973.

** Permanent address: Institute of Biochemistry, L. Kossuth University, Debrecen, Hungary.

The preparation of extracts and fractions was effected by the procedure described by Hultin and coworkers[1,3] except that the products after fractionation with ammonium sulfate (15–75% saturation) and dialysis were desalted on Sephadex G-25 and lyophilized. The following fractions were thus obtained: F I (after extraction with water), F II (after extraction with 0·15M-NaCl) and F III (after extraction with 0·15M-NaCl at pH 7·5) (ref.[1,3]).

Except for these three fractions, a total extract designated T was prepared as follows. Bananas were homogenized in Ultra-Turrax, Model TP 18/2 with 0·2M-NaCl at pH 7·8 (100 ml per 100 g of bananas, three-times), the extract was centrifuged at 3000 *g*, subjected to fractional precipitation with ammonium sulfate (15–75% saturation), dialysis, and lyophilization. The thus obtained product T was equilibrated with 0·05M phosphate buffer at pH 7·6, containing 0·05M-NaCl and passed over a column of Sephadex G-25, equilibrated with the same buffer. The fraction showing pectin esterase activity was fractionated on a column of DEAE-Sephadex A-50 and equilibrated again with the same phosphate buffer. A stepwise elution gradient of phosphate and NaCl, (concentration 0·10, 0·15, and 0·20M, pH 7·6) was used.

The pectin esterase activity was determined by a modified titration method[4] in an automatic titrator (Type SRB 2c/ABU 1c,/PHM 28/TTT 11, Radiometer, Copenhagen). Purified citrus pectin (degree of esterification 65·1%) was used as a substrate. One unit of pectin esterase activity is defined as 1 μequiv. of ester hydrolyzed in 1 min at pH 7·5 and 30°C.

Starch gel electrophoresis was carried out in the horizontal arrangement[5] in Tris-HCl buffer at pH 7·5 and 4°C, 18 h at a potential gradient of 3 V/cm. After the electrophoretic run had been completed, the gel was cut horizontally. One layer was used for the detection of pectin esterase activity by the printing technique: a paper sheet impregnated with the substrate (1% pectin in 0·1M-NaCl adjusted to pH 7·5) and with an alkaline solution of bromothymol blue[6] was employed. Yellow spots on a blue background appeared after a few minutes at sites where pectin esterase activity was located. After the print had been taken, the same part of the gel was used for protein staining with amido black 10 B or nigrosine. The other part of the gel was used for staining of lipids[5] with sudan black B.

Endopolygalacturonase activity was established by determination of the increase of reducing groups according to Somogyi[7]; polygalacturonic acid of mean molecular weight 27000, purity 95·7%, and esterification degree 0, prepared from citrus pectin[8] was used as substrate. The reaction mixture contained 0·5 ml of 0·5% polygalacturonic acid and 0·5 ml of enzyme solution in 0·1M acetate buffer at pH 4·5. Incubation was carried out at 30°C.

Exopolygalacturonase activity was determined by the same method; digalacturonic acid, prepared according to Rexová - Benková[9], was used as a substrate (2 μmol in 1 ml of reaction mixture, pH 4·5, 30°C).

Molecular weights were determined by chromatography on thin layers of Sephadex G-150 Superfine equilibrated with 0·9% NaCl. Ribonuclease A, chymotrypsinogen, ovalbumin, and human serum albumin were used as standards.

Proteins were determined by the method of Lowry and coworkers[10].

RESULTS AND DISCUSSION

In fractions of banana extracts, prepared according to Hultin[1,3] the following percentage of pectin esterase activity was found: 62% in fraction F I, 7% in fraction F II, and 31% in fraction F III.

These values represent the mean of three extractions; the activity was determined at pH 7·5 and 30°C in individual extracts.

Products suitable for electrophoresis were obtained by fractional precipitation with ammonium sulfate, dialysis, lyophilization, and desalting on Sephadex G-25. The large quantity of contaminants in the original extracts did not allow us to obtain satisfactory electropherograms. Fractions F I and F III were stable even after the above described purification process, fraction F II lost a substantial part of its pectin esterase activity.

The fractions of banana extracts on electropherograms after the detection of pectin esterase activity showed a behavior different from that of the multiple forms of tomato pectin esterase, which were separated to advantage by this method[5]. Whereas only basic forms had been found in tomato extracts, three forms moving to the anode and three forms moving to the cathode were found in banana extracts. Four forms of pectin exterase were found in aqueous extract F I, two anodic (No 2 and No 3) and two cathodic (No 5 and No 6). Fractions F II and F III afforded identical forms of pectin esterase, namely one basic (No 4) and two acidic (No 1 and No 2) (Fig. 1). The results of electrophoresis suggest the probable correctness of the theory of Hultin and coworkers[3] postulating conversion of the forms of pectin esterase in fraction F II into forms of fraction F III, since we find electrophoretically identical forms in these fractions.

All six forms of pectin esterase, detected in fraction F I through F III (Fig. 1) could be detected on the electropherogram of total banana extract T.

When the pectin esterase activity was being detected, the rate of appearance of individual spots was not identical. After the gel had been covered by the paper impregnated with pectin and bromothymol blue, the individual forms appeared at pH 7·5

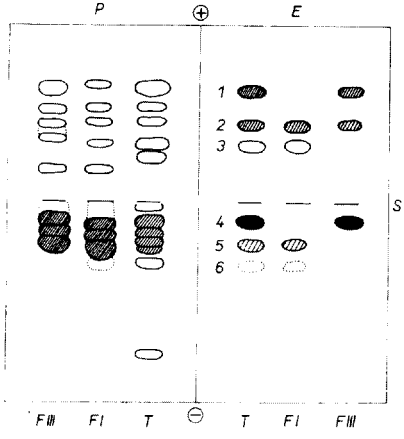

FIG. 1

Starch Gel Electrophoresis of Banana Extracts

P Detection of proteins by nigrosin, *E* detection of pectin esterase activity by the printing technique using a paper impregnated with pectin and bromothymol blue. *S* origin. T total extract of banana (extraction with 0·2M-NaCl at pH 7·8, fractional precipitation with ammonium sulfate and desalting). F I extracted with distilled water (treated as sample T). F III extracted with 0·15M-NaCl at pH 7·5 (treated as sample T). 1—6 multiple forms of pectin esterase.

in the following order: No 4, No 1, No 2, No 5, at finally No 3 and No 6. This indicates either quantitative differences in the distribution of the individual forms in banana extracts or differences in their pH-optimums; a similar observation has been made with fractions F I and F III by Hultin earlier[1,3].

We characterized earlier tomato pectin esterase and eliminated the possibility that this enzyme is of lipoprotein character[5]. On the basis of the results obtained in the earlier study, we used the method of starch gel electrophoresis to examine the content of lipid components in banana extracts. The staining of the electropherograms by Sudan black revealed in total extract T the presence of four zones of lipid character which were not identical with any of the forms of pectin esterase.

The separation of total extract T by chromatography on the DEAE-Sephadex A-50 column afforded four peaks; the protein concentration (A_{280}) in these peaks corresponded to the pectin esterase content determined in the individual effluent fractions by the titration technique (Fig. 2). The electropherogram of the material contained in the first two peaks, eluted by 0·1M phosphate-NaCl, showed the presence of three basic forms of pectin esterase (No 4, 5 and 6). Anodic forms No 2 and 3 were present in the peak eluted by 0·15M phosphate-NaCl, and forms No 1 and 2 were detected in the peak eluted by 0·2 M buffer.

We made use of the possibility of direct detection of pectin esterase activity by the printing technique for the determination of molecular weights of pectin esterase in the individual fractions. In fraction F I we were able to detect by thin-layer chromatography on Sephadex G-150 Superfine only one spot showing pectin esterase activity of a molecular weight of c. 35000. Their spots, corresponding to material of molecular

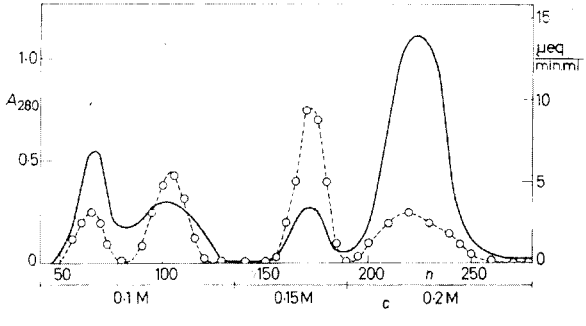

FIG. 2

Fractionation of Total Extract T on Column of DEAE-Sephadex A-50

Full line protein concentration as recorded by Uvicord, dashed line pectin esterase activity of individual fractions, n number of fractions, c concentration of phosphate and NaCl, pectin esterase activity μequiv./δ min/δ ml.

weight close to 46000, 35000, and 10000 (Fig. 3), were present in fractions F II and F III. In view of the presence of glycide or glycoprotein components respectively in partly purified banana extracts, we must take into account the fact that these values of molecular weights are affected by these contaminants[6].

The presence of endo- and exopolygalacturonase activity was examined in desalted, lyophilized banana extracts. The presence of endopolygalacturonase was found in fraction F I and in fraction F III. When these fractions were tested for activity and equal quantities of lyophilized material in terms of protein content were used, differences in specific activity were found. Fractions F I and F III showed the same pH-optimum curves with a maximum at pH close to 4.6. The specific activity of fraction F I at this pH was 1·3 μequiv. of red. groups/min/mg prot. and fraction F III gave a value of 2·1 μequiv. of red. groups/min/mg prot.

Since tomato endopolygalacturonase is activated by monovalent cations[11,12], we examined also the effect of various NaCl concentrations on the rate of the reaction catalyzed by banana endopolygalacturonase (examined with total desalted extract T). A 0·15M concentration of NaCl increased the activity of the enzyme by 49%, whereas a 25% increase of activity compared to controls was observed when 0·315M-NaCl was used.

The presence of exopolygalacturonase was detected in total extract T which cleaved digalacturonic acid. We examined the increase of reducing groups as a function of time and by paper chromatography. The use of digalacturonic acid as substrate permits us to determine the presence of exopolygalacturonase in parallel with endopoly-

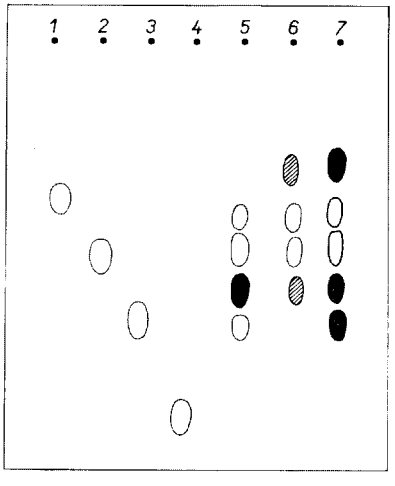

FIG. 3

Determination of Molecular Weights of Multiple Forms of Pectin Esterase from Banana by Thin-Layer Chromatography

1 Ribonuclease A; *2* chymotrypsinogen; *3* ovalbumin; *4* human serum albumin; *5* F I; *6* F II; *7* F III. Determined on Sephadex G-150 Superfine, equilibrated with 0·9% NaCl. The TLG Apparatus of Pharmacia (Uppsala, Sweden) was used. Developed 5 h at 22°C. The proteins were detected by the printing technique using Whatman 3 MM paper stained with 0·1% bromophenol blue. Pectin esterase was detected by the printing technique using paper impregnated with 1% pectin in 0·1M-NaCl and bromothymol blue at pH 7·5. Spots showing pectin esterase activity are marked as dashed and full spots.

galacturonase[13-15]. The changes of pectin products during the growth, ripeneng, and softening of banana have been studied by many authors[16] yet little attention has been devoted to the action of pectolytic enzymes in this respect, with the exception of pectin esterase[1-3]. The presence of endo- and exopolygalacturonase found in banana points to the importance of additional studies from this aspect as well as to the importance of more deteiled characterization of other polygalacturonases from higher plants.

REFERENCES

1. Hultin H. O., Levine A. S.: Arch. Biochem. Biophys. *101*, 396 (1963).
2. Hultin H. O., Levine A. S.: J. Food Sci. *30*, 917 (1965).
3. Hultin H. O., Sun B., Bulger J.: J. Food Sci. *31*, 320 (1966).
4. Chang L. W. S., Morita L. L., Yamamoto H. Y.: J. Food Sci. *30*, 218 (1965).
5. Markovič O.: This Journal *39*, 908 (1974).
6. Delincée H., Radola B. J.: Biochem. Biophys. Acta *214*, 178 (1970).
7. Somogyi M.: J. Biol. Chem. *195*, 19 (1952).
8. Rexová - Benková Ľ., Tibenský V.: Biochim. Biophys. Acta *268*, 187 (1972).
9. Rexová - Benková Ľ.: Chem. zvesti *24*, 59 (1970).
10. Lowry D. H., Rosenbrough N. J., Farr A. L., Randall R. J.: J. Biol. Chem. *193*, 265 (1951).
11. Pressey R., Avants J. K.: J. Food Sci. *36*, 486 (1971).
12. Pressey R., Avants J. K.: Biochim. Biophys. Acta *309*, 363 (1973).
13. Ayres A., Dingle J., Phipps A., Reid W. W., Solomons G. L.: Nature *170*, 834 (1952).
14. Saito H.: J. Gen. Appl. Microbiol. *1*, 38 (1955).
15. Demain A. L., Phaff H. J.: Wallerstein Lab. Commun. *20*, 119 (1957).
16. Von Loesecke H. W.: *Bananas*, 2nd Ed., Interscience, New York 1950.

Translated by V. Kostka.