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SHORT COMMUNICATION

POLYACRYLAMIDE GEL ELECTROPHORESIS OF *SOLANUM TUBEROSUM* L.—I.

TOTAL SOLUBLE PROTEINS AND POLYPHENOL OXIDASE ACTIVITY

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Abstract—The total soluble protein complement of *Solanum tuberosum* L. (var. California Long White) was investigated. Seven consistent protein bands were resolved and several were inconsistently detected. The R_f values of consistent protein bands are reported and compared with the R_f values of sites of polyphenol oxidase activity on a number of phenol substrates.

INTRODUCTION

A NUMBER of plant tissues have been shown to contain several polyphenol oxidases (O-diphenol:O-quinone oxidoreductases).[1–4] The term isozyme has been proposed by Markert and Moller to describe the various molecular forms of these enzymes.[5] Previous investigations have shown the enzyme polyphenol oxidase to be associated with chloroplasts and mitochondria and to occur in a soluble form.[6–8]

Recent work by Stewart and Barber describes the separations of soluble plant proteins by the technique of polyacrylamide gel electrophoresis.[9] Similarly Steward, Lyndon and Barber have demonstrated the separation of soluble plant proteins in pea seedlings and determination of R_f values for individual components of the soluble protein complement.[10]

Patil, Evans and McMahill[11] have demonstrated that crude extracts of potato tubers contain electrophoretically different polyphenol oxidases. Similarly Patil and Zucker[12] have shown a phenolase preparation of potato peelings has been separated into two components on diethylaminoethyl cellulose.

The present investigation was undertaken to determine the total soluble protein complement of tuber tissue of *Solanum tuberosum* L. var. California Long White and to determine

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the R_f value of the individual components. In addition, the R_f values of proteins demonstrating polyphenol oxidase activity are compared to the R_f values of the total soluble protein complement.

RESULTS

Several soluble proteins were detected following polyacrylamide gel electrophoresis. Seven bands were found to be consistently present while the remaining components were detected only occasionally and only in trace amounts. Of the seven consistent bands, four appeared quite prominent and had R_f values of approximately 0·40, 0·45, 0·66 and 0·84, respectively. All seven bands appeared in five consecutive protein separations. The relative positions and R_f values of these soluble protein components are shown diagrammatically in Fig. 1.

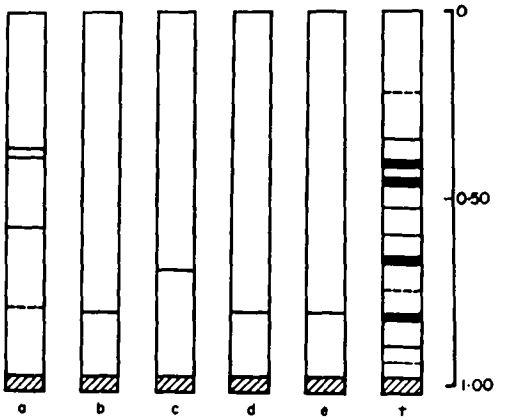

FIG. 1. RELATIVE POSITIONS AND R_f VALUES OF POLYPHENOL OXIDASE ACTIVITY COMPARED WITH TOTAL SOLUBLE PROTEINS.

Solid lines represent major reaction sites and dotted lines represent trace reactions. Shaded bands represent the dye front. Basal letters represent (a) dihydroxyphenylalanine (DOPA); (b) catechol; (c) caffeic acid; (d) chlorogenic acid, and (e) pyrogallol. Figure at right (t) represents total soluble protein bands.

It may be observed in Fig. 1 that the electrophoretic procedure was successful in separating several proteins which exhibited polyphenol oxidase activity and that the R_f values of these sites closely correlated with the R_f values of the soluble proteins. It was found that one protein band (R_f 0·82) appeared capable of oxidizing L-dihyroxyphenylalanine (DOPA), catechol, chorogenic acid, and pyrogallol. It was also observed that four isozymes were capable of oxidizing L-dihydroxyphenylalanine. Three of these isozymes were correlated with soluble protein bands.

The results clearly showed that chlorogenic acid and caffeic acid are oxidized by different isozymes with R_f values of approximately 0·80 and 0·69, respectively. There was no observable oxidation of quinic acid.

DISCUSSION

The data presented in this paper again demonstrate the ability of polyacrylamide gel electrophoresis to resolve significant, reproducible results in the separation of total soluble proteins from plant tissues.

While the total number of soluble proteins separated appears to be rather low, it is highly probable that many enzymes present in the cell are in such small quantities that they are not detected by the present technique. Moreover, it appears quite feasible, as suggested by Steward, Lyndon and Barber,[10] that single bands may contain several proteins of similar mobility under the electrophoretic conditions used. In this instance, those bands with R_f values of 0·40, 0·45, 0·66 and 0·84, on the basis of quantity, appear most likely to be a group of proteins. It was previously observed that one soluble protein band (R_f 0·82) appeared to be capable of oxidizing L-dihydroxyphenylalanine (DOPA), catechol, chlorogenic acid and pyrogallol. These results would tend to indicate the presence of either a group of isozymes, or of one isozyme capable of oxidizing several different phenols. It cannot be concluded from the data presently available which of these cases actually occurs.

The comparison of chlorogenic acid and caffeic acid as substrates for polyphenol oxidase activity showed that, although both are oxidized, they are oxidized by different isozymes with R_f values of approximately 0·80 and 0·69, respectively. Apparently the isozyme which oxidized chlorogenic acid is dependent on the specific ester combination of caffeic acid and quinic acid as it does not oxidize either of them separately.

An observation was made that one of the isozymes which was responsible for the oxidation of L-dihydroxyphenylalanine (DOPA) was not correlated with a soluble protein band. This observation illustrates that minor proteins may well be present in the polyacrylamide gel, but in concentrations too low to be detected by our procedure.

EXPERIMENTAL

Potatoes (*Solanum tuberosum* L. var. California Long White) were purchased at local supermarkets and stored at 2–4°. The tuber tissue was homogenized in cold 0·05 M tris (hydroxymethyl) aminomethane-HCl buffer at pH 7·5 (1·5 ml/g fresh wt.) in a pre-chilled mortar. The resulting slurry was strained through a double layer of cheese-cloth and the residue discarded. The remaining liquid was centrifuged at 1500 rev/min (520 × *g*) for 2 min. The supernatant was decanted, mixed with an equal volume of saturated ammonium sulfate, and allowed to stand at room temperature for 25–30 min. Other than the precipitation, all steps were carried out at 0–3°. Following the precipitation with ammonium sulfate, the liquid was centrifuged at 2800 rev/min (1900 × *g*) and the pellet was resuspended in 12–15 ml of the extracting buffer. The precipitation was repeated to thoroughly wash the protein. The protein was finally resuspended in 3–5 ml of the buffer solution.

The polyacrylamide gel was prepared according to the procedure recommended by Davis.[13] All electrophoretic separations were carried out at 5 mA per tube. Following electrophoresis the gels were immersed in 7 per cent acetic acid containing a 1 per cent amido Schwartz solution for staining soluble protein. The gels were stained for 1 hr and subsequently destained in fresh 7 per cent acetic acid with gentle agitation for approximately 42 hr. The destaining solution was changed every 12 hr.

The isolation and electrophoretic separation of soluble proteins for studies of polyphenol oxidase activity was identical to that used in studying the total protein complement. Following protein separation, the gels were immersed in various phenols. The phenols utilized in this study were L-dihydroxyphenylalanine (DOPA), chlorogenic acid, catechol, pyrogallol, and quinic acid. All substrates were 0·02 M in concentration. Following oxidation the R_f values of visible quinone bands were recorded for comparison with the R_f values of the soluble protein bands.

All R_f value determinations were by visual measurements. All measurements were made from the upper end of the small pore gel to the center of the indicator dye front.

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