Analysis of the Proteins in Crude Plant Extracts by Polyacrylamide Slab Gel Electrophoresis

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ABSTRACT

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A discontinuous system for polyacrylamide-SDS slab gel electrophoresis of plant proteins is described. High resolution of low-molecular-weight protein bands was achieved even when working with protein preparations obtained from highly pigmented, crude plant extracts.

Additional key words: subcellular fractionation, fluorography, plant proteins.

Since the first reports on polyacrylamide disc electrophoresis by Davis (3) and Ornstein (6) and the vertical polyacrylamide slab gel electrophoresis by Jordan and Raymond (5) and Ritchie et al. (7), a number of modifications have been described. They have been quite successful, especially when used in the analysis of simple mixtures of proteins. However, they fail to achieve clear resolution of the protein bands in crude extracts of plant materials. This is particularly true for the protein bands of lowest molecular weights associated with the running front, which normally are the most diffuse. The problem of polyphenols and other interfering pigments further complicates the results obtained. In studies of the effect of citrus exocortis viroid (CEV) on the protein of *Gynura aurantiaca* DC. by Conejero and Semancik (2), the following procedure was developed. It has applications not only for crude plant extracts, but also for the detection of proteins translated following injection of RNA species into *Xenopus laevis* oocytes (8) and nematode characterization (4).

This present paper describes a discontinuous system for vertical slab electrophoresis in polyacrylamide-SDS gels as a modification of that described by Studier (9,10), which through a balanced combination of stacking and separating gels, a wide range of protein bands are resolved.

MATERIALS AND METHODS

Subcellular fractions.—Subcellular fractionation of isolated protoplasts and homogenized leaves from *Gynura aurantiaca,* and the feeding with ^{14}C-labeled

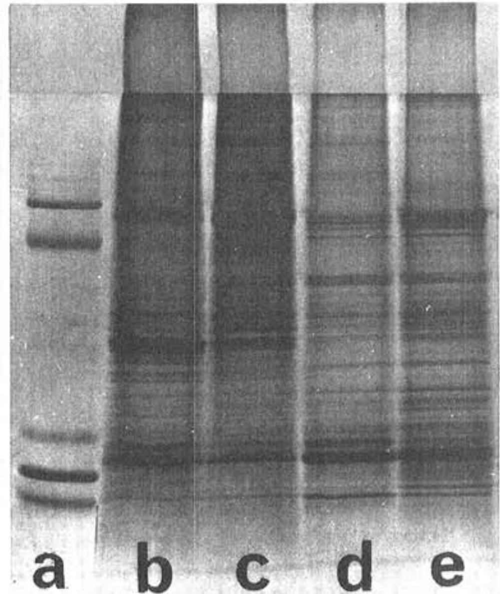

Fig. 1. Protein profiles in polyacrylamide-SDS slab gel electrophoresis of different subcellular fractions of homogenized protoplasts from *Gynura aurantiaca* leaves, stained with Coomassie brilliant blue. Profiles a to e represent proteins from: (a) a standard reference protein mixture: 1) cytochrome C (11,700 daltons), 2) ribonuclease A (13,700 daltons), 3) tobacco mosaic virus protein (17,500 daltons), 4) ovalbumin (43,000 daltons), and 5) catalase (60,000 daltons); (b) a 250 $g \times$ 10-min pellet (nuclei-rich fraction); (c) a 10,000 $g \times$ 10-min pellet (chloroplast- and mitochondria-rich fraction); (d) a 40,000 $g \times$ 20-min pellet (membrane-rich fraction); and (e) a 100,000 $g \times$ 2-hr pellet (ribosome-rich fraction).

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amino acids of cut leaves were carried out according to Conejero and Semancik (2). Protein preparations from *Xenopus laevis* oocytes were performed as previously described (8).

Preparation of samples of electrophoresis.—Before electrophoresis, the samples were boiled for 4 min in 77 mM Tris-HCl pH 8.0, 1.8% sodium dodecyl sulfate (SDS), 4.4% 2-mercaptoethanol (MCE) and 3.0% glycerol. Optimum resolution and detection of protein bands was obtained when 25-75 μg of protein was applied per sample.

Stock solutions.—The following stock solutions were used to prepare the gels:

Solution A: 30% (w/v) acrylamide, 0.8% (w/v) bisacrylamide.

Solution B: 0.5 M Tris HCl, pH 8.0, 0.4% SDS.

Solution C: 1.5 M Tris HCl, pH 8.8, 0.4% SDS.

Solution D: 10% ammonium persulfate (freshly prepared).

Preparation of polyacrylamide-SDS gel slabs.—The gel slabs (1-mm thick) were set up forming a discontinuous system of three layers. To prepare the polymerizing mixture for each layer, the corresponding amounts of solutions A, B (or C), water, and solution D previously chilled were added to a flask set in ice. After stirring, the mixture was degassed under vacuum for about 5 min, TEMED (N,N,N′-tetramethylmethylenediamine) was added and the final mixture stirred and poured into the glass plate frame at room temperature. To obtain a flat interface between the resolving and the spacer gels and between the spacer gel and the stacking gel, very careful overlayering with water was carried out immediately after the corresponding layer was poured. The water was removed prior to addition of the next layer. To produce the sample slots in the stacking gel, a teflon comb was used. Compositions of the three layers were (i) *14% acrylamide resolving* gel: 8.6-cm layer of a mixture composed of 6.065 ml of solution A, 3.25 ml of solution C, 3.865 ml of water, 110 μliters of solution D and 6 μliters of TEMED, (ii) *6.3% acrylamide spacer gel:* 1.5-cm layer of a mixture composed of 1.375 ml of solution A, 1.625 ml of solution C, 3.5 ml of water, 30

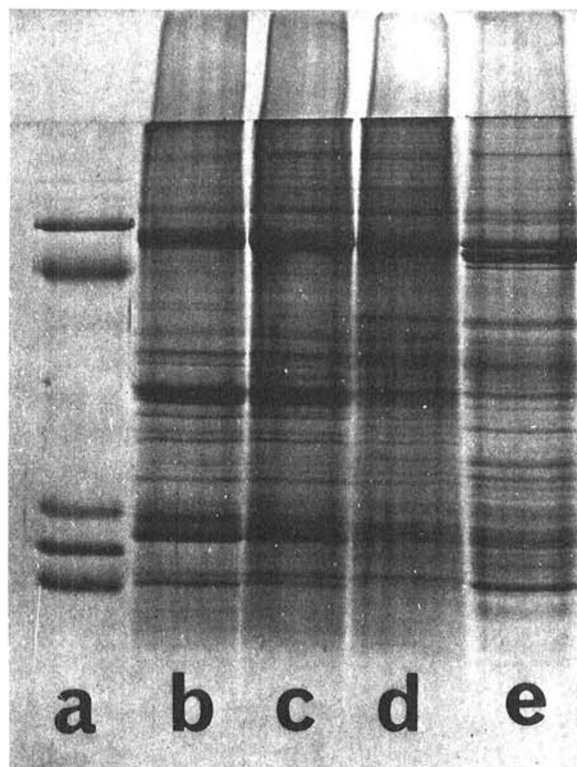

Fig. 2. Protein pattern in polyacrylamide-SDS slab gel electrophoresis of homogenized leaves from *Gynura aurantiaca* stained with Coomassie brilliant blue. Profiles a to e represent: (a) a standard reference protein mixture (see Fig. 1); (b) proteins from a 750 × *g* pellet (nuclei-rich fraction); and (c, d, and e) as described in Fig. 1.

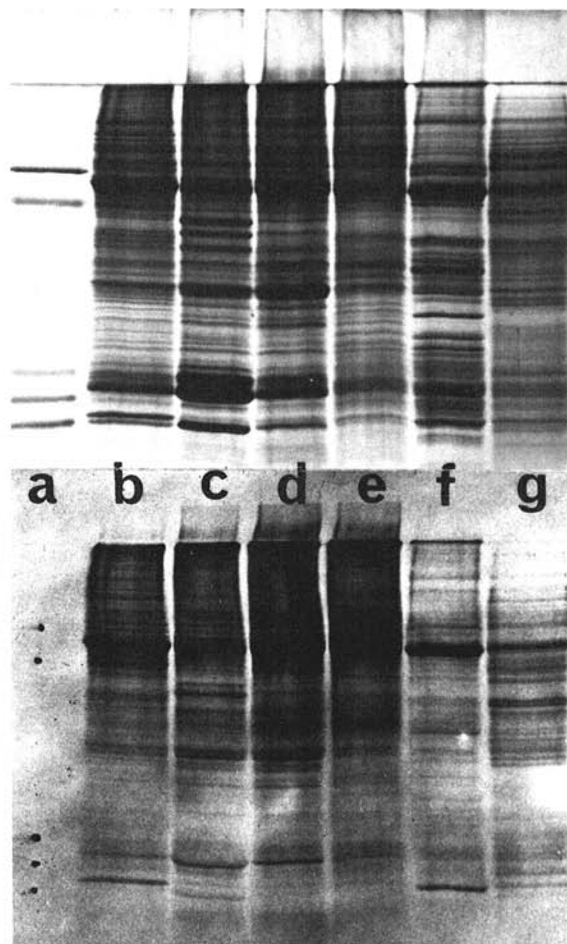

Fig. 3. Coomassie brilliant blue-stained (upper) and fluorographic (lower) protein patterns corresponding to different subcellular fractions from homogenized *Gynura aurantiaca* leaves after feeding with ^{14}C-Argenine, leucine, lysine, and valine. Profiles a to g represent proteins from: (a) a standard reference protein mixture (see Fig. 1); (b) the total extract; (c) a 750 *g* × 10-min pellet; (d) a 10,000 *g* × 10-min pellet; (e) a 40,000 *g* × 20-min pellet; (f) a 100,000 *g* × 2-hr pellet; and (g) the supernatant from f.

μliters of solution C and 3 μliters of TEMED, (iii) *4% acrylamide stacking gel:* 1.5-cm layer of a mixture composed of 0.665 ml of solution A, 1.250 ml of solution B, 3.085 ml of water, 30 μliters of solution D, and 5 μliters of TEMED.

Electrophoresis.—The electrophoresis was run in a 4-C air cooled chamber at constant current (20 mA) for about 2.5-3.0 hr. The apparatus and the electrode buffer (Tris, 20.16 gm; glycine, 4.3 gm; SDS, 0.7 gm in 700 ml H_2O) used were essentially those described by Studier (9,10). After electrophoresis, the gels were fixed overnight in an aqueous solution of 12.5% TCA (w/v), 25% isopropanol (v/v), stained 24 hr with a 0.1% aqueous solution of Coomassie brilliant blue G, and destained in water.

RESULTS AND DISCUSSION

The method described was applied to a number of protein preparations from different sources and generated in various ways, ranging from green plant *(Gynura aurantiaca)* protoplasts and tissue homogenate to *Xenopus laevis* oocyte and nematode proteins.

A comparative study of the proteins obtained from lysed protoplasts and homogenized tissue was carried out to detect the variation in the corresponding profiles. The patterns (Fig. 1 and 2), are quite similar in the number, relative intensity, and position of the bands along the profile. The quality of the patterns achieved also is quite similar, indicating that the proteins derived from tissue homogenization and those prepared from protoplast lysates reflect a common distribution of protein species.

Polypeptides with apparent molecular weights of 10^4 to 10^5 daltons were observed in the resolving gel after a relatively short time of electrophoresis, without leaving significant amounts of protein of the highest molecular weights at the gel interface or in the spacer gel. Despite the great number of bands of the patterns, they are finely separated, and sharp along the whole profile. Even though the number of distinct protein bands that can be detected per 50 μg of protein in the photographs taken after destaining the gel is about 60-90, the actual resolution by direct observation is greater. Furthermore, we have observed excellent reproducibility, from aliquots of preparations made before SDS treatment, common samples analyzed in different positions of the same gel, and comparable preparations from distinct experiments.

The electrophoretic system also was applied to study the ^{14}C-labeled proteins of subcellular fractions from *Gynura aurantiaca* leaves. After leaf pieces had been vacuum-infiltrated with the labeled amino acids, the proteins detected both by Coomassie staining and the fluorographic (1) patterns indicated the distribution of the newly-synthesized protein species (Fig. 3).

It should be noted that the preparations which have been used to obtain the protein profiles presented in this paper were very crude. This feature is especially notable in the case of *Gynura aurantiaca* extracts, in which the interference of the great amount of pigments present has been overcome, and emphasizes the general application of this procedure in analysis of plant proteins. The utilization of this technique in the comparative analysis of healthy and diseased systems already has been demonstrated in the detection of the proteins associated with viroid infection (2).

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