

**Short Communication****Isolation and Isoelectric Focusing of Plasma Membrane Proteins from Potato Tubers**

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Philadelphia, Pennsylvania 19118 USAKey Term Index: Plasma membranes, protein pattern, isoelectric focusing; *Solanum tuberosum***Summary**

Proteins were successfully extracted from the plasma membranes of several potato tuber varieties by means of selected nonionic detergents. Protein patterns produced by analytical isoelectric focusing in polyacrylamide gels of total protein extracts exhibit a high degree of complexity.

The patterns of all varieties observed are different from each other. The electrofocusing patterns of the membrane proteins are also different from those of the soluble storage proteins of the same potato tuber varieties. It is therefore apparent that varietal differences are exhibited by the membrane proteins, as well as the storage proteins.

At least part of the difficulty generally associated with studying membrane proteins is related to their inherent insolubility in commonly used aqueous media coupled with the need to achieve separation without changing native structure. The availability of new nonionic, nondenaturing detergents has helped greatly in overcoming the solubility problems (HELENIUS and SIMONS 1975; TANFORD and REYNOLDS 1976), and enhanced the use of isoelectric focusing as a tool for separating and studying the complex protein populations characteristic of plasma membranes (BOOZ and TRAVIS 1981; HJELMELAND et al. 1979). CRIBBS and STELZIG (1978) have studied the plasma membrane proteins from protoplasts of the potato leaf. This report presents a comparison of polypeptide patterns from a membrane-rich fraction from tubers of a variety of potato cultivars.

*Plant materials*

The plant materials used in these experiments included cv. Kennebec, Katahdin, Cherokee, Superior, Catoosa, and Russet-Burbank potato tubers. They were stored at 10 °C until used.

*Isolation of membrane rich fraction*

The plasma membrane-rich fraction was isolated by means of previously published procedures and methods (LEONARD and HOTCHKISS 1978; BOOZ and TRAVIS 1980) modified to suit the tissues

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*Abbreviations:* TES (N-tris[Hydroxymethyl]methyl-2-aminoethyl) sulfonic acid; DIECA, diethyldithio-carbamate; DTT, dithiothreitol; BHA, butylated hydroxyanisole; Tris, tris (hydroxymethyl) aminomethane; EGTA, ethyleneglycol-bis( $\beta$ -aminoethyl ether)N,N'-tetraacetic acid; TEMED, N,N,N',N'-tetramethyl-ethylenediamine; TCA, trichloroacetic acid

of the potato tuber. All operations were carried out at 0 to 4 °C. Potato tubers were washed, chilled, cut into pieces, and then homogenized for 1 min in a Waring Blendor with 1 volume of grinding medium (GM) of 25 mM TES-Na buffer (pH 7.5), 0.25 M sucrose, 4 mM DIECA, 4 mM DTT, and 100 µg/ml BHA.

The homogenate was diluted with two more volumes GM and then filtered through two layers of Miracloth (VWR Scientific, Pittsburgh, PA). The filtrate was successively centrifuged at 3,400 · g for 5 min, 11,000 · g for 15 min, and 80,000 · g (Beckman SW 28 rotor) for 30 min. The clear supernatant containing the soluble proteins was dialyzed against 0.1 M Tris-HCl buffer (pH 7.5) and concentrated before use by surrounding the cellulose tubing dialysis sac with Carbowax 20,000 (Fisher Scientific, Phila., PA). The 80,000 · g pellet was resuspended in GM and recentrifuged at 80,000 · g. The pellet was then suspended in 2 mM TES-Na buffer, pH 7.5, containing 15 % sucrose, 1 mM EGTA, 1 mM DTT, and 100 µg/ml BHA. The suspension was layered upon 30 ml hand-formed discontinuous gradients of 20, 34, and 45 % sucrose in 2 mM TES-Na (pH 7.5), 1 mM EGTA, 1 mM DTT, and 10 µg/ml BHA, and centrifuged for 3 h at 80,000 · g. The fraction rich in plasma membrane vesicles was collected at the 34/35 % (w/w) aqueous sucrose interface with a Pasteur pipette. This fraction was diluted and washed once with 200 mM KCl 10 mM TES-Na (pH 7.5), 1 mM EGTA, 1 mM DTT, and 100 µg/ml BHA, and centrifuged at 80,000 · g for 30 min. The pellet obtained was used for subsequent experiments.

#### *Solubilization of membrane proteins*

The washed membrane pellet was mixed thoroughly with twice its volume of solubilizing buffer: 20 mM TES-Na, pH 7.5, 1 mM EGTA, 1 mM DTT, 10 µg/ml BHA, 30 mM octyl-β-D-glucopyranoside (Calbiochem-Behring, La Jolla, CA). The mixture was incubated for 1 h at room temperature before centrifugation at 100,000 · g for 30 min. The supernatant was removed from the pelleted membrane residue and used for isoelectric focusing (CATSIMPOOLAS 1968; WRIGLEY 1968).

#### *Isoelectric focusing in polyacrylamide gels (IEF)*

IEF was carried out in cylindrical (5 mm D × 850 mm L) polyacrylamide gel rods (T = 5.1 %, C = 2.6%)<sup>1</sup> containing 1 % glycine and 8 M urea. The pH 4.5 to 7 ampholyte was prepared by mixing the commercially available (Bio-Rad, Richmond, CA) pH ranges 4 to 6 (80 %) and 3 to 10 (20 %); the pH 3 to 7 ampholyte was used as received. The final ampholyte concentration in each gel rod was 2 %. Conditions for polymerization and IEF have been described elsewhere (LEONARD and HOTCHKISS 1978; BOOZ and TRAVIS 1980); 0.05 % w/v (NH<sub>4</sub>)<sub>2</sub> S<sub>2</sub>O<sub>8</sub> and 0.05 % (v/v) TEMED were used as polymerization initiators. The catholyte (top reservoir) was 0.02 N Ca (OH)<sub>2</sub> and 0.04 N

Table 1. *Extraction of protein from potato tuber plasma membranes by various detergents*

Detergent concentration (%)	Solubilized protein (µg/mg vesicles)		
	Octyl-β-D-glucopyranoside	Zwittergent 3-08	TritonX-100
0.2	28.6	0.8	31.4
0.4	34.7	4.0	35.0
0.6	36.0	6.5	39.5
0.8	37.7	8.8	52.7
1.0	39.2	13.8	67.7

All samples were incubated for 30 min at 25 °C and then centrifuged at 100,000 · g for 2 h. Protein was measured in supernatant.

<sup>1</sup> T = total concentration of acrylamide + bisacrylamide, C = bisacrylamide percentage of total acrylamide present.

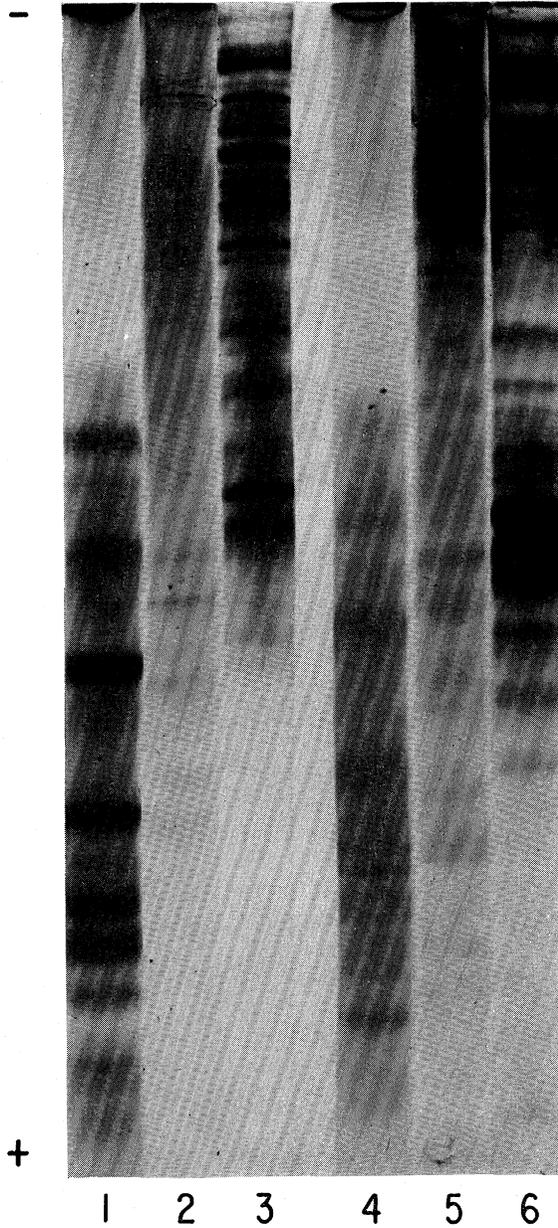
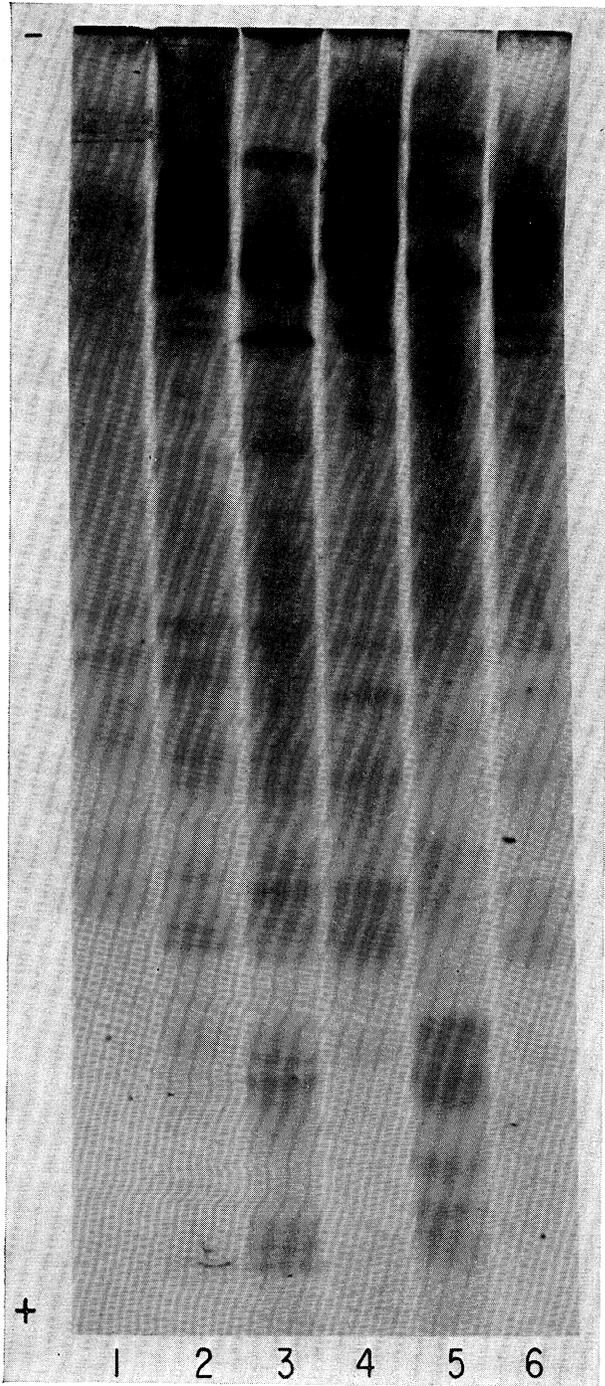
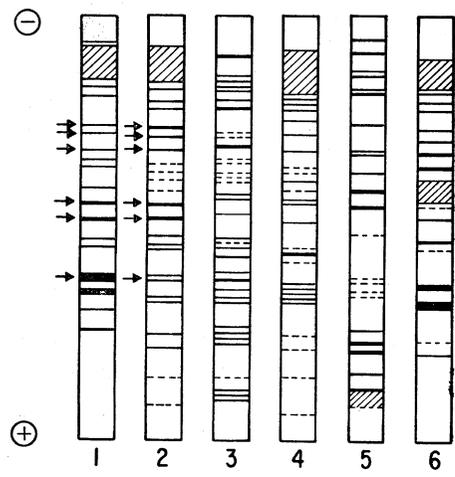


Fig. 1. Soluble proteins of varieties *Kennebec* (1) and *Katahdin* (4) and membrane proteins of *Kennebec* (2) and *Katahdin* (5) after electrofocusing in pH 4.5 to 7 ampholytes. Membrane proteins of *Kennebec* (3) and *Katahdin* (6) after electrofocusing in pH 3 to 7 ampholyte.



2a



2b

NaOH. The anolyte was 0.06 N H<sub>2</sub>SO<sub>4</sub>. The samples (100 to 150 μg membrane or "soluble" protein in 8 M urea) were layered on top of the gels and overlaid with 4 M urea, then the tubes were carefully filled with the catholyte. A DC potential of 20 v/cm gel length was applied overnight, then raised to 40 v/cm for an additional 2 h. The cylindrical gel rods were removed from the glass tubes and immediately transferred to a fixing bath of 12.5% TCA and 3% sulfosalicylic acid for 0.5 to 1 h. The gels were stained overnight in 0.02% Coomassie Blue R-250 dissolved in ethanol, water, and acetic acid (7:11:2 v/v), and the excess dye removed by soaking in a mixture of ethanol, acetic acid, water, and glycerol (5:2:13:5 v/v). Determination of pH gradients was accomplished after sectioning the gel rods with a razor blade and soaking each 1-cm gel slice in 1 ml 10 mM KCl overnight. The pH of each 1-ml extract was measured with a Beckman Zeromatic II pH meter equipped with a Thomas small diameter combination pH electrode (AWDEH et al. 1968; RIGHETTI and DRYSDALE 1971).

Efforts to solubilize and extract the proteins from the plasma membranes required the examination of nondenaturing detergents such as Triton X-100, octyl-β-D-glucopyranoside, and Zwittergent 3-08 (Calbiochem-Behring, La Jolla, CA). Increasing concentrations of detergent were added to a constant amount of membrane vesicles (Table 1), and the solubilized protein determined in the presence of the detergents by a modified Lowry procedure (MARKWELL et al. 1978). Under the experimental conditions used, Zwittergent 3-08 was the least effective at all detergent concentrations. Triton X-100 was the most effective. However, when samples containing this detergent were electrofocused, distorted bands and heavy streaking occurred. Although not as effective a solubilizer as Triton X-100, octyl-β-D-glucopyranoside-extracted samples gave reasonably clear patterns when electrofocused on polyacrylamide gels. Therefore, it became the detergent of choice for the work.

Fig. 1 compares electrofocusing patterns of soluble proteins and "solubilized" membrane proteins of two different varieties in pH gradients formed using pH 4.5 to 7 and pI 3 to 7 ampholytes. The patterns of the membrane proteins are different from the soluble ones, as well as from each other. Fig. 2a shows the electrofocusing patterns of the membranes proteins of several other varieties. To our knowledge, this is the first time potato tuber plasma membrane proteins have been separated and examined using the isoelectrofocusing technique. While it is apparent that there are varietal differences in the plasma membrane proteins, within these patterns several polypeptides having the same isoelectric point are present. Fig. 2b is a reconstruction of 2a and attempts to show consistent, as well as unique polypeptides. The significance of the differences and similarities in staining intensity are unclear at this time. The patterns shown are too complex to determine protein or proteins possibly associated with genetic traits for specific disease resistance against, for example, potato late blight. Commercial varieties Kennebec and Cherokee are known to carry the R<sub>1</sub> gene for resistance to

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Fig. 2. Membrane proteins of varieties Kennebec (1), Katahdin (2), Cherokee (3), Superior (4), Catoosa (5), and Russet-Burbank (6) after electrofocusing in pH 4.57 to 7 ampholytes.

a) Original photographs of gels. b) Drawings of the gels shown in Fig. 2a. The arrows indicate focused polypeptides of similar isoelectric value.

*Phytophthora infestans*, the cause of late blight disease. Perhaps comparison of the membrane proteins from samples of selfed USDA X96-56 (an experimental potato clone carrying the original gene for *P. infestans* resistance) that have been segregated according to resistance would be more informative since these samples would be derived, presumably, from near isogenic material.

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