Separation of Peanut Proteins by Capillary Electrophoresis

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Peanut proteins have been extensively characterized by employing conventional methods such as low-pressure column chromatography, high-performance liquid chromatography, and polyacrylamide gel electrophoresis. However, these methods are tedious and time-consuming and require large amounts of sample. In this study an attempt was made to test the suitability of capillary electrophoresis (CE) for resolving peanut proteins. Proteins and peptides were extracted from seeds, leaves, and cell cultures of peanut and resolved on a fused-silica capillary column using a UV detector. The CE separated peanut proteins into several major and minor components and required no major sample preparation steps. The data were consistent and reproducible. The results showed that CE is well suitable for obtaining excellent resolution of peanut proteins and peptides.

Keywords: *Callus; capillary electrophoresis; leaves; peanut; peptides; protein*

The emergence of biotechnology, which utilizes recombinant DNA techniques, and protein and polypeptide synthesis has increased the demand for sophisticated analytical instrumentation and methodologies. In biological samples, proteins, nucleic acids, and polysaccharides are present in very small quantities, requiring highly sensitive separation techniques. Since biological samples, especially of plant origin, are quite complex, more than one technique is often used to complement the assays. High-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) are two such complementary methods that provide reliability in the analytical results.

Peanut seed proteins have been extensively characterized using column chromatography (Basha and Pancholy, 1981a), HPLC (Basha, 1988), and polyacrylamide gel electrophoresis (PAGE) (Basha, 1979, 1990; Basha and Pancholy, 1981b, 1984; Tombs, 1965; Cherry et al., 1973, Cherry, 1974; Jones et al., 1994). However, these methods are laborious and time-consuming. Depending upon the plant part used, these methods often require extensive sample cleanup and concentration. This is especially true when in the analysis of peanut root, leaf, and tissue culture material.

In this study an attempt was made to test the suitability of CE technique for analyzing the proteins and peptides from peanut leaf, seed and tissue culture material.

MATERIALS AND METHODS

Plant Material. Peanut (*Arachis hypogaea* L. cv. Florunner) seeds were a gift from Dr. Daniel Gorbet of University of Florida, Marianna, FL. The leaves were obtained from peanut (*A. hypogaea* L. cv. Florunner) plants grown in the greenhouse in 6 ft × 4 ft plots filled with potting soil. The callus was induced from Florunner peanut by placing a surface-sterilized cotyledon onto a media containing Murashige and Skoog (1962) salts and vitamins, 3% sucrose, 0.5 mg/L picloram, and 0.8% agar (Ozias-Akins et al., 1992). Suspension cultures were established by transferring a portion of the callus material into the above media without agar. The cultures were routinely subcultured every 2 weeks by transferring a portion of the cultured cells into fresh media.

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Protein Extraction. *Peanut Seed.* Raw peanut seeds were ground into a meal using a Hamilton Beach laboratory grinder. The resulting full-fat meal was suspended in hexane to solubilize the fat (Basha et al., 1976) and centrifuged in a Beckman Model J2-21M centrifuge at 20000*g* for 20 min at 15 °C. The supernatant was discarded, the pellet was reextracted with hexane three times, and the final pellet was air-dried and used as a defatted meal for protein extraction.

Seed proteins were extracted from defatted peanut meal (6 mg) by homogenizing for 2 min (30 s pulses) with 0.5 mL of 0.3% (w/v) sodium borate buffer, pH 8.3, using a Polytron homogenizer. The homogenate was centrifuged using a Beckman J2-21M centrifuge at 20000*g* for 20 min at 15 °C, and the supernatant was used for protein analysis.

Peanut Leaves and Callus Tissue. Whole peanut leaves and callus tissue were freeze-dried, and a portion (100 mg) of the sample was ground to a powder (5−7 min) using a coffee grinder. The ground material was extracted with acetone (leaf material) or 80% ethanol (tissue culture material) using a Polytron homogenizer (3 min) and centrifuged at 20000*g* for 15 min. The supernatant was discarded and the pellet homogenized for 2 min (with 30 s pulses) on ice with 50 mM imidazole−HCl buffer, pH 6.4/0.5 mM EDTA using a Polytron homogenizer. The homogenate was centrifuged in a Beckman Model J2-21M centrifuge at 20000*g* for 20 min at 4 °C, and the supernatant was used for protein analysis. Acetone and ethanol extraction step is necessary to remove chlorophyll and other pigments from leaf and tissue culture material. These pigments interfere with protein resolution and result in artifacts.

Peptide and Acid-Soluble Protein Extraction. Defatted peanut meal (5 mg) was extracted with 0.5 mL of 0.1 M sodium phosphate buffer, pH 2.5, by homogenizing for 2 min (30 s pulses) on ice using a Polytron homogenizer. The homogenate was centrifuged in a Beckman Model J2-21M centrifuge at 20000*g* for 20 min at 4 °C and the supernatant used for CE analysis.

CE. CE was performed on a Beckman P/ACE 2100 system (Beckman Instruments, Inc., Palo Alto, CA) controlled by a computer equipped with System Gold software. The protein and peptide separations were performed in uncoated fused-silica capillaries (75 μm i.d. × 57 cm). Electrophoresis was conducted at 25 °C and voltages of 10 kV (protein) and 20 kV (peptide). The detector was set at 214 nm, and 6 nL (30 μg) of sample was injected (10 s). The capillaries were rinsed sequentially between successive electrophoretic runs with 1% (w/v) sodium hydroxide (5 min), deionized water (5 min), 0.3% (w/v) sodium borate buffer, pH 8.3 (for protein analysis), or 0.1 M sodium phosphate, pH 2.5 (5 min; for peptide analysis). Separations were performed in either 0.3% sodium borate

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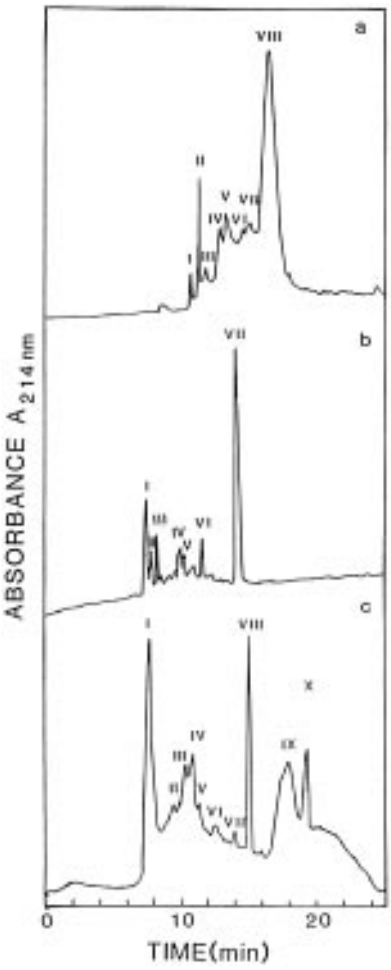

Figure 1. Electropherograms of protein extracts from peanut seed (a), cell culture tissue (b), and leaf (c). Protein was extracted with 0.3% sodium borate buffer, pH 8.3, and an aliquot (6 nL) of the extract was pressure injected (10 s) into the capillary. Electrophoresis was performed at 20 kV toward cathode.

buffer, pH 8.3 (for protein), or 0.1 M sodium phosphate buffer, pH 2.5 (for peptides).

RESULTS AND DISCUSSION

Seed Proteins. Figure 1 shows the electropherograms of peanut seed (a), cell culture (b), and leaf (c) tissue. Following CE the seed proteins were resolved into one major (peak VIII) and several minor (peaks I−VII) peaks (Figure 1a). The major peak represents the arachin protein (the predominant storage protein of peanut seed), while the minor peaks represent the non-arachin proteins. The CE protein pattern is consistent with the PAGE (nondenaturing) pattern of peanut seed protein in which arachin resolves as a major band (not shown) and the non-arachin proteins resolve into several minor bands (Basha, 1979). These results show that CE profiles of peanut proteins are similar to those obtained using gel electrophoresis and, hence, CE can be substituted for PAGE to obtain seed protein profiles of peanuts.

Cell Culture Proteins. Proteins form peanut cell cultures resolved into one large (peak VII) and several smaller peaks (Figure 1b). The protein profile of cell culture (derived from seed) showed a different electrophoretic profile than that of the seed proteins, indicating that they differ in their composition. The CE successfully resolved proteins from cell culture tissue into at least seven components, while loading similar amounts of protein on a conventional PAGE gel failed to show (data not shown) protein bands. This indicated that CE was successful where PAGE failed. These data thus suggest that unlike gel electrophoresis, which requires large amounts of sample material and extensive sample and gel preparation steps, the CE is simple, sensitive, and effective.

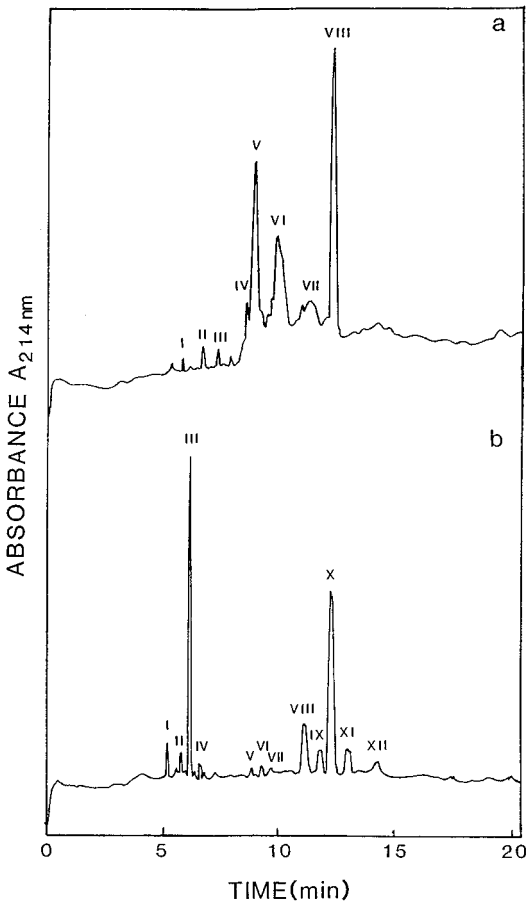

Figure 2. Electropherograms of peptide and protein extracts from peanut seed (a) and cell culture tissue (b). The proteins were extracted with 0.1 M sodium phosphate buffer, pH 2.5, and an aliquot (6 nL) of the sample was pressure injected (10 s) and electrophoresed at 20 kV toward cathode.

Leaf Proteins. CE of leaf protein extracts separated proteins into six major and several minor components (Figure 1c). The large number of protein peaks revealed in leaf protein extract indicate the efficiency of CE procedure in resolving a complex protein mixture. It should be noted that fractionation of leaf proteins by PAGE usually fails to resolve peanut leaf proteins. This is attributed to the low levels of protein in the leaves and interference from other leaf components. Interestingly, CE resolved leaf proteins effectively and showed the presence of at least 10 components. This would suggest that CE improved peanut leaf protein resolution significantly and that it is less prone to interference from other leaf contaminants.

Peptide Analysis. Low molecular weight (<5000−7000) peptides and acid-soluble proteins were resolved by CE using a low pH (2.5) buffer. Under these conditions the seed proteins resolved into three major (peaks V−VIII) and eight smaller peaks. The peptide profile of the seed (Figure 2a) was quite different from the protein profile of the seed (Figure 1a), indicating that the protein compositions of the sodium borate, pH 8.3, and sodium phosphate, pH 2.5, extracts were

different and may contain different groups of proteins and peptides. Likewise, the composition of sodium phosphate, pH 2.5, extracts of cell cultures (Figure 2b) was different from that of sodium borate, pH 8.3, extracts of the cell cultures (Figure 1b). The sodium phosphate, pH 2.5, extracts of the cell culture showed the presence of two major (peaks III and X) and several minor peaks.

The overall results showed that CE effectively resolved peanut proteins and peptides into several discrete peaks and can be employed for determining protein and peptide composition of peanut seed, leaf, and tissue culture materials. Because of its sensitivity and small sample requirement, the CE may serve as a primary choice for analytical screening of different experimental peanut tissues, especially when small amounts of material (one cotyledon, one leaf, one small piece of callus) are available. In addition, unlike PAGE and HPLC, the CE minimizes exposure of researchers to polyacrylamide, reduces solvent consumption and sample preparation time, requires no staining and destaining steps, shows real time separation, and provides sample quantitation data immediately.

LITERATURE CITED

Basha, S. M. M. Identification of cultivar differences in seed polypeptide composition of peanut (*Arachis hypogaea* L.) by two-dimensional polyacrylamide gel electrophoresis. *Plant Physiol.* **1979**, *63*, 301−306.

Basha, S. M. Resolution of peanut seed proteins by high performance liquid chromatography. *J. Agric. Food Chem.* **1988**, *36*, 778−781.

Basha, S. M. Accumulation pattern of arachin in maturing peanut seed. *Peanut Sci.* **1990**, *16*, 7−73.

Basha, S. M. M.; Pancholy, S. K. Polypeptide composition of arachin and non-arachin proteins from Early Bunch Peanut (*Arachis hypogaea* L.) seeds. *Peanut Sci.* **1981a**, *8*, 6−10.

Basha, S. M. M.; Pancholy, S. K. Changes in the polypeptide composition of maturing seed from four peanut cultivars. *Peanut Sci.* **1981b**, *8*, 82−88.

Basha, S. M.; Pancholy, S. K. Variation in the methionine-rich protein composition of the genus *Arachis*. *Peanut Sci.* **1984**, *11*, 1−3

Basha, S. M. M.; Cherry, J. P.; Young, C. T. Changes in free amino acids, carbohydrates and proteins of maturing seeds from peanut (*Arachis hypogaea* L.) cultivars. *Cereal Chem.* **1976**, *53*, 586−597.

Cherry, J. P. Electrophoretic observations on protein changes and variability during development of the peanut. *J. Agric. Food Chem.* **1974**, *22*, 723−724.

Cherry, J. P.; Dechary, J. M.; Ory, R. L. Gel electrophoretic analysis of peanut proteins and enzymes. I. Characterization of DEAE-cellulose separated fractions. *J. Agric. Food Chem.* **1973**, *21*, 652−655.

Jones, K. G.; Basha, S. M.; Pancholy, S. K. Differences in skin and cotyledon composition of maturing peanut. *Oleagineux* **1994**, *49*, 109−113.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **1992**, *15*, 4473−497.

Ozias-Akins, P.; Anderson, W. F.; Holbrook, C. C. Somatic embryogenesis in *Arachis hypogaea*.: genotype comparison. *Plant Sci.* **1992**, *83*, 103−111.

Tombs, M. P. An electrophoretic investigation of groundnut proteins: the structure of arachin A and B. *Biochem. J.* **1965**, *105*, 181−187.

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