Characterization of Disulfide-Linked Forms of Seed Proteins in Peanuts (*Arachis hypogaea* L.)

Esmail H. Shokraii* and Asim Esen

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

When the disulfide bonds in the peanut seed proteins arachin and conarachin were reduced by 2-mercaptoethanol (2-ME), a number of new bands appeared after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Analysis of the individual protein bands under reducing and nonreducing conditions by SDS–PAGE showed the extent of various disulfide bondings in different protein components. Most reduced protein subunits had lower molecular weight and greater electrophoretic mobilities than those unreduced, except three monomeric components whose mobilities decreased upon reduction. The hydrogen bond disrupting agent (6 M urea) had no effect on polypeptide profiles in the presence of SDS and 2-ME. When the effects of urea and 2-ME were studied individually in nondenaturing native gels, neither significantly affected the protein profiles. However, when urea and 2-ME were used together, they had a synergistic effect and drastically changed the polypeptide profiles. Immunological studies showed that antiserum raised against reduced proteins reacted with both the unreduced and reduced seed proteins. The results of this study contributed to an improved understanding of the kinds of disulfide bonding of peanut proteins and their physicochemical properties.

The storage proteins of peanut have been extensively studied by several investigators during the past 60 years (Jones and Horn, 1930; Tombs, 1965; Singh and Dieckert, 1973; Basha, 1979; Basha and Pancholy, 1981, 1982a,b; Cherry et al., 1973; Cherry, 1974, 1977; Yamada et al., 1979, 1980; Shokraii et al., 1985). These studies focused on methods of extraction and purification as well as chromatographic and electrophoretic separation of the various protein fractions and their subunits. These authors showed that the peanut storage protein was composed of two major fractions, arachin and conarachin and that these could be separated from each other by adding ammonium sulfate to 40–45% final concentration to the whole meal extract (Tombs, 1965). We investigated the polypeptide composition of the peanut protein in 22 cultivars and breeding lines, with electrophoretic and isoelectric focusing (IEF) techniques (Shokraii et al., 1985). The results showed no major differences among the polypeptide profiles of various peanut cultivars except for the presence or absence of a polypeptide of 36 kDa which is a component of the acidic subunit of arachin (Krishna et al., 1986). Krishna and Mitra (1987) studied the patterns of polypeptides among 90 genotypes and found four different forms of arachin resulting from charge and size variations in the arachin polypeptides.

The present study was undertaken to investigate the effects of the disulfide bond reducing agent, 2-ME, and the hydrogen bond disrupting agent, 6 M urea, on the polypeptide profiles of peanut protein obtained by electrophoresis under native and denatured states. Such studies would enable one to determine the extent of intra- and intermolecular disulfide bonds linking oligomeric units of these proteins. Earlier studies on this subject were described by Tombs (1965), Singh and Dieckert (1973), and Cherry and Ory (1973). Singh and Dieckert (1973) investigated the effects of varying concentrations of SDS and 2-ME on the profile of arachin subunits by SDS–PAGE and urea–PAGE. The study used tube gels for SDS–PAGE according to the procedure of Weber and Osborn (1969), which tends to have poorer resolution than slab gels used with the Laemmli system (Laemmli, 1970). Cherry and Ory (1973) studied the effect of thiol reducing agents, dithiothreitol (DTT) and 2-ME, and showed significant changes in molecular weight and structure of peanut proteins. Other studies with 2-ME and 6 M urea were limited to their use in the extraction and electrophoresis media (Yamada et al., 1979; Krishna and Mitra, 1987). However, the data on polypeptide composition of peanut proteins in various gel systems and the nature of disulfide bonds among different components are not conclusive, and more detailed studies need to be undertaken. Another objective of this study was to investigate the immunological reactivity of the various protein components before and after treatment with 2-ME. Combinations of the immunochemical and electrophoretic studies should help to improve understanding of the physicochemical properties of peanut proteins and the tertiary and quaternary structures of arachin and conarachin and their subunits.

* Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Plant Materials. Peanut (*Arachis hypogaea* L.) seeds were obtained from the Tidewater Research Center in Suffolk, VA. The meal was prepared by grinding 12 randomly selected and carefully deskinned seeds from each cultivar or breeding line to a fine powder in a prechilled mortar with a pestle. The meal was then defatted three times with ethyl ether using a solvent volume (milliliters) to meal weight (grams) ratio of 10:1 and stored in a freezer until use.

Sample Preparation. The defatted meal was extracted with 0.1 M Tris-HCl (pH 7.5) containing 1.5 M NaCl, 2 mM phenylmethanesulfonyl fluoride (PMSF), and 0.2% NaN_3. The meal weight (grams) to buffer volume (milliliters) ratio was 1:20, and the extraction lasted several hours with occasional shaking. The slurry was then centrifuged (14000*g*, 5 min) and the supernatant (total storage protein extract) saved. An aliquot of the extract was mixed with 4× concentrated SDS–sample buffer (0.125 M Tris-HCl, pH 6.8, containing 10% SDS, 5% 2-ME, 40% glycerol, and 0.05% bromophenol blue) at a ratio of 3:1 (Laemmli, 1970), heated for 2 min in a boiling water bath, centrifuged in a microfuge, and used for analysis by SDS–PAGE. In some studies the meal was directly extracted with a 1× sample buffer using the same weight to volume ratio (i.e., 1:20). The sample buffers used for electrophoresis under nondenaturing conditions were

0021-8561/92/1440-1491$03.00/0 © 1992 American Chemical Society

devoid of SDS but included 2-ME or 6 M urea when the effects of these two additives were being investigated.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). Electrophoresis was performed in a vertical slab gel apparatus (Bio-Rad Protean I or II) according to the method of Laemmli (1970) with either 8–15% gradient or 12% standard gels. The protein bands were visualized by staining the gel in Coomassie Brilliant Blue R-250 dye solution. SDS–PAGE was performed under both nonreducing and reducing conditions, in the absence or presence of 5% 2-ME, to identify the protein bands containing polypeptides held by interchain disulfide bonds. To establish relationships between the unreduced bands and those reduced, gel strips containing the bands of interest were cut from preparative gels run under nonreducing but denaturing conditions (SDS–PAGE without 2-ME in sample buffer). These strips were applied to the "sample wells" in the presence of 2-ME, urea, or both for a second electrophoretic separation.

Nondenaturing Gel Electrophoresis. These studies were performed at 4 °C under the same conditions and with the same apparatus but in the absence of SDS in the extraction, stacking, and resolving buffers. The native gels (7% T, 3% C) were prepared and run as described by Ornstein (1964) and Davis (1964).

Antiserum Preparation. Whole peanut protein from a selected cultivar (Florigiant) was used to immunize a New Zealand White rabbit. The antigen was prepared by extracting 20 mg of the defatted meal with 1 mL of 0.01 M Tris-HCl (pH 7.5) containing 1.5 M NaCl and mixing the extract with 0.32 mL of 4× concentrated SDS–sample buffer which contained 2-ME. For the first injection, an equivalent of 2 mg of protein in the extract (260 μL) was diluted with 490 μL of 1× sample buffer. This was mixed with an equal volume (0.75 mL) of Freund's complete adjuvant and injected subcutaneously into the rabbit. Injections were repeated three times at 2-week intervals, mixing 1 mg of protein solution with an equal volume of the incomplete adjuvant. A bleeding was taken before the first immunization (preimmune serum) and prior to each subsequent injection (immune sera).

Immunological Assays. An enzyme-linked immunosorbent assay (ELISA) was performed as described by Conroy and Esen (1984). The appropriate antigen concentration and antiserum dilutions were determined after the antigen was applied to the wells of a 96-well microtiter plate at varying dilutions in a solution of 6 M urea and it was reacted with the varying dilutions of the antiserum. The secondary antibody was protein A conjugated to the enzyme peroxidase. The reactivity was measured by reading the absorbance of the peroxidase reaction product in an automated microplate reader.

Western Blotting. The protein of the whole peanut meal extract was prepared like the one used for antiserum preparation. It was fractionated preparatively in a 12% SDS–PAGE gel and electrophoretically transferred to nitrocellulose at 4 °C at 60 V for 45 min and for 1 h at 100 V as described by Towbin et al. (1979). The gels were stained after electroblotting to check for the completeness of the transfer. The blots were rinsed in PBST (phosphate-buffered saline containing 0.05% Tween 20), dried at room temperature, and cut into 5 mm wide strips. The strips were rewetted with PBST in a reaction trough (S&S Accutran) and were reacted with the antiserum diluted with PBST (1/1000). Protein A–peroxidase conjugate was used as the second antibody, and immunoblots were developed with the peroxidase substrate 4-chloro-1-naphthol (Towbin et al., 1979).

RESULTS AND DISCUSSION

SDS–PAGE Analysis. The comparisons of the profile in the gradient vs homogeneous gels indicated no marked differences between them, except for the improved resolution of the low molecular weight polypeptides, components of the basic subunit of arachin (Krishna et al., 1986) and the 67 000 polypeptide (conarachin) which resolves into two distinct bands in the gradient gel (Figure 1). This finding confirms that conarachin is composed of two forms (α_1 and α_2) as was shown by Daussant et al. (1969) and Neucere (1972) using immunoelectrophoresis.

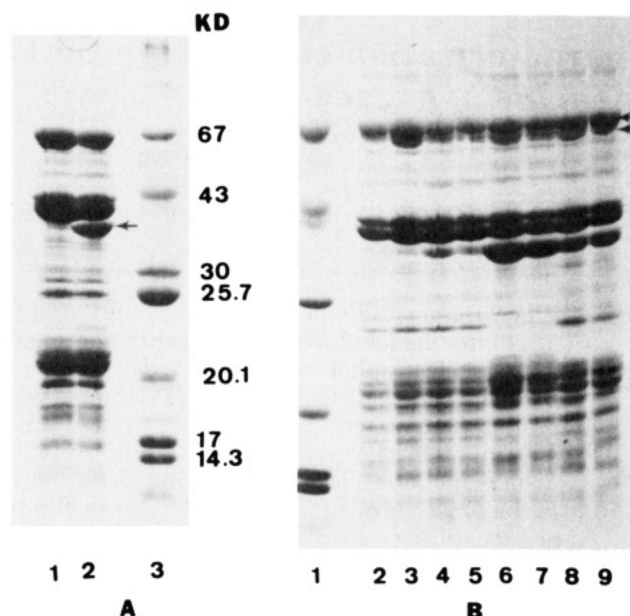

Figure 1. Comparison of the pattern of the peanut seed protein (reduced by 2-ME) from selected cultivars and breeding lines as revealed by SDS–PAGE in (A) homogeneous (12%) and (B) gradient gels (8–15%). (A) (Lane 1) Florigiant; (lane 2, Early Bunch; lane 3) molecular weight markers (bovine serum albumin, ovalbumin, glyceraldehyde-3-phosphate dehydrogenase, carbonic anhydrase, trypsinogen, soybean trypsin inhibitor, and lactalbumin). (B) (Lane 1) molecular weight markers; (lane 2) Florigiant; (lane 3) NC 2; (lane 4) NC9; (lane 5) VC 1; (lane 6) NC-Fla 14; (lane 7) NC 17; (lane 8) NC 7; (lane 9) Early Bunch. The protein profile is similar in all cultivars except for the presence or absence of a 36-kDa polypeptide (arrow) in some cultivars. Note the conarachin band (67 kDa) separates into two distinct bands in the gradient gel, and also the low molecular weight polypeptides (14–22 kDa) are resolved better in the gradient gel.

Earlier studies by Yamada et al. (1980) using sucrose density gradient centrifugation showed conarachin forms a monomer fraction which upon homogeneous SDS–PAGE and electrophoresis in the absence of denaturant always produces one band but which breaks down to its subunit only when subjected to IEF.

Polypeptide profiles after SDS–PAGE under nonreducing and reducing conditions were compared to identify all of the protein components whose subunits were held together by intermolecular disulfide bonds. Under nonreducing conditions the major storage proteins, arachin and conarachin, separated as two major components of 60 000–67 000, four smaller components with estimated molecular weights of 14 000, 17 000, 19 000, and 34 000 (Figure 2, lanes 5 and 6, arrows), and several minor bands. The major components all appeared to be composed of disulfide-linked subunits because, when the reducing agent was added to the sample, the protein profile changed dramatically with the disappearance of these major bands and the appearance of numerous new bands (Figure 2, lanes 2 and 3). The shift in the protein pattern after reduction was in fact readily evident in lane 4, which received unreduced sample but was run adjacent to a lane (lane 3) with reduced sample. In this case, the reducing agent diffused laterally from lane 3 to the adjacent lane 4 containing the unreduced sample and reduced the disulfide bonds on that half of the lane facing the 2-ME-treated sample. This resulted in the production of two different patterns in lane 4, the right side corresponding to that of the unreduced samples and the left to that of the fully reduced samples. The comparison of the protein profiles in the two adjacent lanes allowed the clear deduction of the extent of disulfide-linked oligomeric

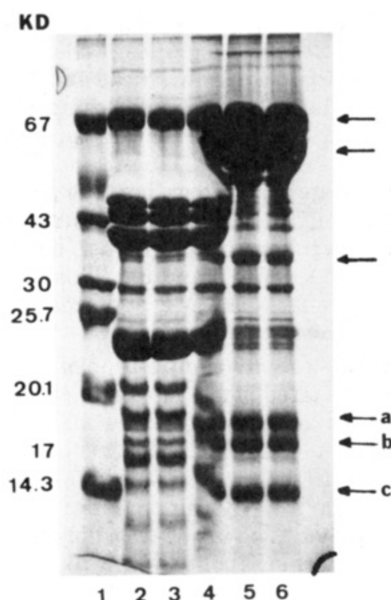

Figure 2. SDS-PAGE (12% homogeneous) profiles of the reduced and unreduced protein of Early Bunch. (Lane 1) Molecular weight markers; (lane 2) 2-ME treated; (lane 3) 2-ME and 6 M urea treated; (lane 4) unreduced sample which is partially reduced by the lateral diffusion of 2-ME from lane 3; (lanes 5 and 6) unreduced sample (in the absence and presence of 6 M urea, respectively). Note the electrophoretic mobility of bands a, b, and c, (19, 17, and 14 kDa, respectively) decreases upon reduction. In lane 4 the shift in electrophoretic mobility of these three bands is clearly evident on the two sides of the bands. The presence of 6 M urea does not affect the polypeptide profile in either the reduced (lanes 2 and 3) or unreduced states (lanes 5 and 6).

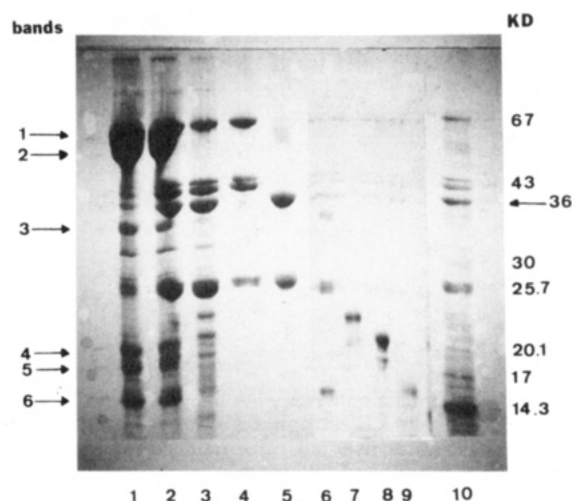

Figure 3. SDS-PAGE profile of peanut protein (Early Bunch) under reducing and nonreducing conditions showing the composition of each unreduced protein component upon reduction with 2-ME. (Lanes 1 and 2) Unreduced sample; (lane 3) reduced sample (the lateral diffusion of 2-ME from lane 3 to lane 2 is evident from the production of the 25-, 36-, and 43-kDa polypeptides); (lane 4) polypeptide composition of band 1 from the unreduced sample after electrophoresis in the presence of 2-ME; (lane 5) polypeptide composition of band 2 (unreduced) upon reduction with 2-ME; (lanes 6, 7, 8, and 9) polypeptide compositions of bands 3, 4, 5, and 6, respectively, upon reduction; (lane 10) molecular weight marker. Note there is a decrease in the electrophoretic mobility of the last three bands upon reduction when compared with their original unreduced state (lane 1, bands 4–6).

associations in these proteins. Addition of 6 M urea to the sample had no effect on the polypeptide profiles under reduced (Figure 2, lanes 2 and 3) or unreduced (Figure 2, lanes 5 and 6) conditions. This observation does not agree with those of Krishna and Mitra (1987), who found that the addition of urea to SDS-PAGE changed the polypeptide pattern in the region of 21–45 kDa. This difference must be due to differences in their experimental conditions including procedures followed for sample preparation.

Precise characterization of the individual components with respect to their specific subunits held together by disulfide bonds was accomplished by excising each band from nonreducing preparative gels and subjecting it to electrophoresis under reducing conditions. For example, when the upper half of the major protein component (67 000) shown in Figure 3 (band 1) was excised from the nonreducing (preparative) gel and then electrophoresed under reducing conditions, it yielded four different polypeptides with molecular weights of 67 000, 45 000, 43 000, and 25 000 (Figure 3, lane 4). The lower half of the same component (band 2, 60 000) yielded two distinct bands, with molecular weights of 36 000 and 25 000 (Figure 3, lane 5). Similarly, the 34 000 component (band 3) from the nonreducing gel separated as two subunits, 25 000 and 15 000 (Figure 3, lane 6). However, the three low molecular weight components (19 000, 17 000, and 14 000 or bands 4, 5, and 6, respectively) each produced only a single polypeptide band after reduction (Figure 3, lanes 7–9), and the resulting polypeptides exhibited slower electrophoretic mobility than the parental unreduced proteins. This was in contrast to the behavior of the other components in which the addition of the reducing agent invariably yielded polypeptides with smaller size than that of the parental unreduced protein. In this case there was an apparent decrease of mobility from 19, 17, and 14 kDa to 22, 20, and 15 kDa, respectively. Apparently these three subunits are monomeric polypeptides which in unreduced forms occurred as compact structures held together through intramolecular disulfide bonds. Once these bonds were reduced, the structure would unfold to linear chain, hence an increase in its hydrodynamic volume and slower mobility in the electric field.

The above data clearly suggested that three of the storage protein components (67, 60, and 34 kDa) consisted of heterodimers of various sizes held together by intermolecular disulfide bonds and three low molecular weight components (19 000, 17 000, and 14 000) which are not dimeric and have greater mobility in nonreduced form. These results, when compared with those previously reported (Tombs, 1965; Singh and Dickert, 1973; Cherry et al., 1973), show the applicability of our technique of excising protein components from nonreducing gels and using them as sample sources for electrophoresis under reducing conditions. These data suggest an improved method for identification of the number and nature of disulfide-containing components because we were able to identify in a clear way the number of subunits which are held together by inter- or intramolecular disulfide linkages. In addition, the transition of each polypeptide from nonreduced to reduced state, the identity of the number of subunits in each band, and the existence of the monomeric low molecular weight subunits which are linked together by intermolecular disulfide linkages were also monitored.

Results from the special two-dimensional studies (Figure 4) supported the observations made in one-dimensional gels. These results also showed the usefulness of this special 2-D separation by SDS-PAGE in which a 0.5 cm wide strip from a nonreducing preparative gel was excised and used as the sample for the second-dimension gel in the presence of a reducing agent. This method of 2-D, in contrast to the conventional IEF-SDS-PAGE technique

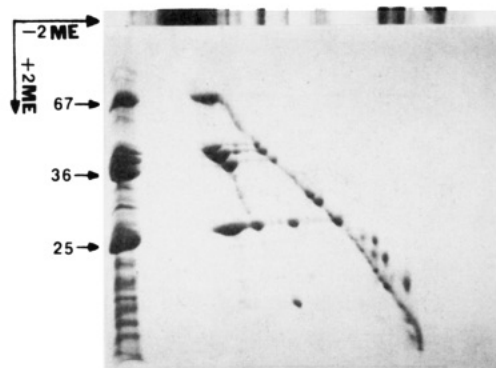

Figure 4. Two-dimensional SDS–PAGE profile of the peanut protein (Early Bunch). Electrophoresis was performed preparatively in the first dimension using unreduced sample. A strip (0.5 cm) of the first dimensional gel was treated with 2-ME and applied as sample for the second dimensional electrophoresis. Note the protein bands from the first dimension produce additional bands upon reduction by 2-ME prior to electrophoresis in the second dimension (for details refer to the text).

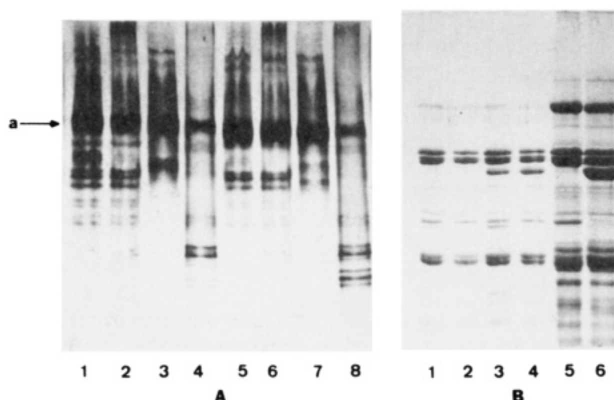

Figure 5. (A) Native gel profiles of peanut protein after electrophoresis in a 7% polyacrylamide gel. (Lanes 1–4) Profiles of the extracts from Florigiant; (lanes 5–8) profiles of the extracts from Early Bunch. Lane 1 and 5 extracts were prepared with no additive; lanes 2 and 6 are extract plus 6 M urea; lanes 3 and 7 are extract plus 5% 2-ME; lanes 4 and 8 are extract plus 6M urea plus 5% 2-ME. Note that addition of 6 M urea (lanes 2 and 6) does not change the protein profiles, but 2-ME (lanes 3 and 7) does have some effect. Addition of urea and 2-ME together causes substantial change in the profile (lanes 4 and 8). (B) SDS–PAGE profiles of the protein components isolated from a slice of band a (arrow) from the native gel. (Lanes 1 and 2) Band a isolated from Florigiant sample which was extracted in the absence and presence of 2-ME, respectively; (lanes 3 and 4) band a isolated from Early Bunch sample also extracted in the absence and presence of 2-ME; (lanes 5 and 6) whole protein profile of Florigiant and Early Bunch, respectively. Comparison of lanes 1–4 with 5 and 6 shows the polypeptides which are not subunits of arachin and belong to conarachin and nonstorage (basic) protein fractions.

(O'Farrel, 1975), allowed an easier and probably more accurate study of the effect of an additive (e.g., 2-ME) in situ.

Nondenaturing Gel Analysis. The results of electrophoretic studies with nondenaturing (native) gels under different conditions are shown in Figure 5A. For electrophoresis under nondenaturing conditions, the acrylamide concentration in the gel had to be reduced to 7% to allow the native proteins to pass through the bis(acrylamide) matrix. In the 7% native gel the major portion of the protein occurred in one low-mobility band (band a), and the rest was divided into several minor bands with higher electrophoretic mobility (Figure 5A). When a 5% gel was used, resolution of these minor bands was poor, and most of the protein moved as a single, broad band (not shown). Addition of 6 M urea to the extraction medium did not result in a significant change in protein profiles in native gels (Figure 5A, lanes 2 and 6). Similarly, addition of 2-ME to the extraction medium did not make a major change and only caused the disappearance of the minor components (Figure 5A, lanes 3 and 7). But when urea and 2-ME were added together, the general profile showed drastic changes (including band a); all major bands broke into smaller bands with higher electrophoretic mobility (Figure 5A, lanes 4 and 8). This would be due to the synergistic effects of 2-ME and urea in disrupting the structure of the native protein.

The polypeptide subunit composition of band a (native arachin) was elucidated when this band was used as the sample source for analysis by SDS–PAGE. The result (Figure 5B, lanes 1–4) showed that this band was made up of at least six different polypeptides in Florigiant (lanes 1 and 2) and seven in Early Bunch (lanes 3 and 4). The extra band in Early Bunch is a 36-kDa polypeptide which was previously reported as contributing to peanut blanchability (Shokraii et al., 1985). All of these bands were apparently held together in the native state by noncovalent polypeptide interactions in addition to interchain disulfide bonds which were dissociated in the presence of SDS and 2-ME into six or seven polypeptide bands. This observation also suggested that the polypeptides forming the native arachin (band a) were not exclusively held together by disulfide bonds alone, because 2-ME was not able to disrupt significantly the structure of native arachin in the absence of urea. It appeared that the intermolecular disulfide bonds in band a (native arachin) were not accessible to reduction by 2-ME until after exposure to urea. This explains the earlier conclusion made by Yamada (1979), who claimed "no intersubunit disulfide bond probably takes part in the subunit association of intact arachin" as the effect of the reducing agent was not clearly detected. When the arachin fraction was purified by precipitation in 45% $(NH_4)_2SO_4$ and recovered for use in SDS–PAGE studies, the results showed (not shown) that this purified arachin had a polypeptide profile similar to that obtained from band a. Comparisons of the profile of the arachin fractions in two samples with that of the whole peanut protein of the same samples (Figure 5B, lanes 1–4 vs 5 and 6) readily identify the non-arachin subunits (conarachin and nonstorage proteins and enzymes).

Immunological Studies. The results of the ELISA studies revealed the production of high-titer antisera against the polypeptide antigens present in the injected total storage protein. Dilutions of up to 1/3600 of these antisera were capable of yielding positive reactions when tested in a checkerboard scheme against decreasing amounts of antigen (Shokraii et al., 1991). Antigenicities of the proteins in unreduced vs reduced states appeared to be similar because almost every component in immunoblots after SDS–PAGE performed under reducing and nonreducing conditions was immunoreactive. It was also observed that the antiserum against the reduced total protein recognized the proteins of 40 to 67 kDa resolved under nonreducing conditions (Figure 6, lane 1). The antiserum naturally reacted with all of the 14–20-kDa polypeptides resolved by SDS–PAGE under reducing conditions (Figure 6, lane 2). These low molecular weight polypeptides were all derived from homodimer and heterodimers linked by disulfide bonds. The identical antigenicity of all of the reduced and unreduced proteins on the immunoblots suggested that in these proteins the antigenic epitopes were mostly continuous type rather than discontinuous (Atassi, 1984) which were recognizable both

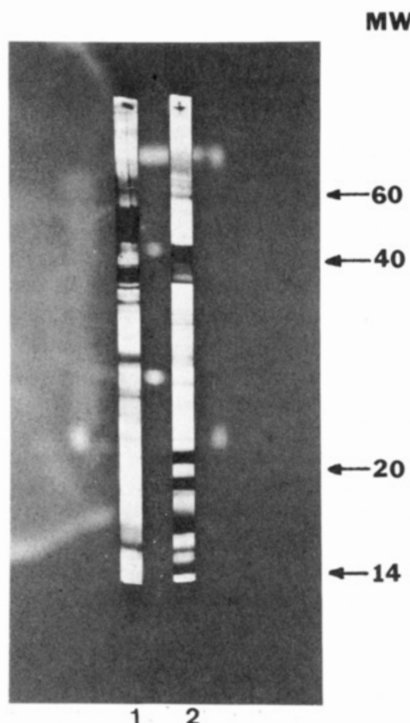

Figure 6. Immunoblots of peanut protein (Early Bunch) after electrophoresis (SDS-PAGE) under nonreducing (lane 1) and reducing (lane 2) conditions. Note that the antibodies recognize and react similarly with both unreduced and reduced components.

in the native and in the reduced protein. This was not surprising in view of the fact that reduced and denatured protein was used for the immunization.

In conclusion, the data obtained show with clarity the extent of involvement of disulfide bonds in different polypeptide subunits of arachin which were not clearly reported before. The more novel aspect of this study is the properties and characteristics of the lower molecular weight species (14 000–19 000) which contain intramolecular disulfide bonds and have greater mobility under nonreduced state. The effect of various additives on the storage proteins under native conditions, the separation of conarachin into two components on nonhomogeneous SDS gel, and the similar immunological reactivity of the storage proteins under reduced and unreduced states add to our understanding of the physicochemical properties of peanut storage proteins.

LITERATURE CITED

Atassi, M. Z. Antigenic Structures of Proteins. Their Determination Has Revealed Important Aspects of Immune Recognition and Generated Strategies for Synthetic Mimicking of Protein Binding Sites. *Eur. J. Biochem.* **1984**, *145*, 1–20.

Basha, S. 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.; Pancholy, S. K. Polypeptide Composition of Arachin and Non-arachin Proteins from Early Bunch Peanut (*Arachis hypogaea* L.) Seed. *Peanut Sci.* **1981**, *8*, 82–88.

Basha, S. M.; Pancholy, S. K. Isolation and Characterization of Two Cryoproteins from Florunner Peanut (*Arachis hypogaea* L.) Seed. *J. Agric. Food Chem.* **1982a**, *30*, 36–41.

Basha, S. M.; Pancholy, S. K. Composition and Characteristics of Basic Protein from Peanut (*Arachis hypogaea* L.) Seed. *J. Agric. Food Chem.* **1982b**, *30*, 1176–1179.

Cherry, J. P. Electrophoretic Observations on Protein Changes and Variability during Development of the Peanut. *J. Agric. Food Chem.* **1974**, *22*, 723–725.

Cherry, J. P. Potential Sources of Peanut Seed Proteins and Oil in the Genus *Arachis*. *J. Agric. Food Chem.* **1977**, *25*, 188–193.

Cherry, J. P.; Ory, R. L. Gel Electrophoretic Analysis of Peanut Proteins and Enzymes. II. Effects of Thiol Reagents and Frozen Storage. *J. Agric. Food Chem.* **1973**, *21*, 656–660.

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.

Conroy, J. M.; Esen, A. An ELISA Assay for Zein and Other Proteins Using Unconventional Solvents for Antigen Absorption. *Anal. Biochem.* **1984**, *137*, 182–187.

Daussant, J.; Neucere, N. J.; Yatsu, L. Y. Immunochemical Studies of *Arachis hypogaea* Proteins with Particular Reference to the Reserve Proteins. I, Characterization, Distribution and Properties of a-arachin and a-conarachin. *Plant Physiol.* **1969**, *44*, 471–479.

Davis, B. J. Disc Electrophoresis II. Methods and Application to Human Serum Proteins. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 404–427.

Jones, D. B.; Horn, M. J. The Properties of Arachin and Conarachin and Their Proportionate Occurrence in the Peanut. *J. Agric. Res.* **1930**, *40*, 672–682.

Krishna, T. G.; Mitra, R. Arachin Polymorphism in Groundnut (*Arachis hypogaea* L.). *Phytochemistry* **1987**, *26*, 897–902.

Krishna, T. G.; Pawar, S. E.; Mitra, R. Variation and Inheritance of the Arachin Polypeptides of Groundnut (*Arachis hypogaea* L.) *Theor. Appl. Genet.* **1986**, *73*, 82–87.

Laemmli, U. K. Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Neucere, N. J. Effect of Heat on Peanut Proteins. I. Solubility Properties and Immunochemical-Electrophoretic Modifications. *J. Agric. Food Chem.* **1972**, *20*, 252–255.

O'Farrel, P. H. High Resolution Two Dimensional Electrophoresis of Proteins. *J. Biol. Chem.* **1975**, *250*, 4007–4021.

Ornstein, L. Disc electrophoresis—I. Background and theory. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 321–349.

Shokraii, E. H.; Esen, E.; Mozingo, R. W. Relation of a 36000-Dalton Arachin Subunit to Blanchability in Peanuts (*Arachis hypogaea* L.). *J. Agric. Food Chem.* **1985**, *33*, 1114–1116.

Shokraii, E. H.; Esen, E.; Mozingo, R. W. Immunological Characterization of a 36KD Polypeptide in Peanut (*Arachis hypogaea* L.). *Peanut Sci.* **1991**, *18*, 11–15.

Singh, J.; Dieckert, J. W. Types and Molecular Weights of Subunits of Arachin-P6. *Prep. Biochem.* **1973**, *3*, 73–82.

Tombs, M. P. An Electrophoretic Investigation of Groundnut Proteins: The Structure of Arachin A and B. *Biochem. J.* **1965**, *96*, 119–133.

Towbin, H.; Staehelin, T.; Gordon, J. Electrophoretic Transfer of Proteins from Polyacrylamide Gels to Nitrocellulose Sheets. Procedure and Some Applications. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4350–4354.

Webber, K.; Osborn, M. The reliability of molecular weight determinations by dodecyl-sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **1969**, *244*, 4406–4412.

Yamada, T.; Aibara, S.; Morita, Y. Isolation and Some Properties of Arachin Subunits. *Agric. Biol. Chem.* **1979**, *43*, 2563–2568.

Yamada, T.; Aibara, S.; Morita, Y. Accumulation Pattern of Arachin and its Subunits in Maturation of Groundnut Seeds. *Plant Cell Physiol.* **1980**, *21*, 1217–1226.

Received for review September 29, 1991. Revised manuscript received February 3, 1992. Accepted May 27, 1992.