by Morrison (1980) and Theander and Aman (1980) support this hypothesis. It may be impossible to distinguish chemically between cellulose polymers and linear xylans that remain in the dilute acid insoluble "cellulose" residues. As a result, two-step hydrolysis commencing with 12 M sulfuric acid may be the only rapid means of estimating total hemicellulose in terms of total noncellulosic sugars. From a nutritional standpoint the "hemicellulosic" polymers contained in the insoluble residues may have characteristics similar to cellulose. The need for quantitating hemicellulose for nutritional purposes should proceed with this in mind.

Registry No. Xylan, 9014-63-5; cellulose, 9004-34-6; hemicellulose, 9034-32-6.

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Conformational Stability and Dissociation of a Peanut Storage Protein (Arachin) Exposed to Organic Solvents

Thomas J. Jacks,* Thomas P. Hensarling, Navin J. Neucere, and Elena E. Graves

Arachin (the major peanut storage protein) was exposed to hexane, acetone, hexane–acetone–water, and acidic hexane (mixtures of hexane and acetic acid), and its antigenicity, electrophoretic mobility, ultraviolet and infrared absorbances, and circular dichroism were examined. Samples exposed to hexane, acetone, and hexane–acetone–water were virtually identical with native arachin in these properties, indicating no effects of these solvents. Exposure to acidic hexane, however, resulted in loss of reactivity to antiarachin and increased electrophoretic mobility in nondenaturing gels but no corresponding change of migrational pattern in dissociating sodium dodecyl sulfate gels and no changes in infrared and circular dichroic spectra. Results were interpreted as irreversible dissociation of arachin by acidic hexane into subunits, each of which maintained the native secondary structure of the multimeric form. Antigenic determinants embraced separate subunits and required quaternary structure for subsequent antibody reactivity.

About one-fourth of the peanut crop produced in the United States and over three-fourths produced elsewhere is processed for oil. Mechanical pressing, solvent extraction, or a combination of the two is employed to obtain the oil. Hexane is generally used for commercial solvent extraction, but other solvents, such as hexane–acetone–water and acidic hexane, offer several advantages over hexane for thorough oil removal (Frampton and Pepperman, 1967; Hensarling et al., 1974; Jacks et al., 1970). After oil removal, the residual oil-free meal had previously received scant consideration as a source of either food-grade or feed-grade protein; however, in view of the current worldwide need for protein, attention has recently focused on peanut meal as a source of edible protein (Lusas, 1979). Consequently, research concerning the preparation and properties of peanut protein for edible usage has greatly increased in magnitude (Lusas, 1979; Martinez, 1979).

In this communication, we describe the response of the major peanut storage protein (arachin) to exposure to hexane, acetone, hexane–acetone–water, and acidic hexane at the temperatures used commercially for oil extraction (60 °C) and for removal of residual solvent from the oil-free meal (68.5 °C). Structural features of arachin were assessed from examination of antigenicity, electrophoretic mobility, ultraviolet and infrared absorbances, and circular dichroism.

MATERIAL AND METHODS

Arachin was isolated from quiescent peanut seeds (*Arachis hypogeae* L., Virginia 56-R variety) as described previously (Neucere, 1969, 1974). Fifty-milligram samples

Southern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 19687, New Orleans, Louisiana 70179.

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were finely dispersed in 4.5 mL of one of the following solvents: hexane (H), acetone (A), hexane–acetone–water (HAW), which is hexane–acetone (31:20 v/v) that contains 1.8% water by volume (King et al., 1961), and hexane–acetic acid (H–HOAc), which is hexane that contains either 5% or 20% glacial acetic acid by volume (Hensarling et al., 1974). Each suspension was heated at 60 °C for 2 h in a sealed tube and the solvent was evaporated at 68.5 °C overnight followed by 68 °C in vacuo for 1 h. The dried samples were stored at room temperature in a vacuum desiccator over paraffin shavings until used.

Qualitative analysis by double diffusion of antiarachin and arachin was performed according to Ouchterlony (1949) with either 20 or 30 μg of protein from each sample in phosphate buffer, pH 7.9, μ = 0.2. Semiquantitative analysis of arachin was peformed in 1.5% agar in 0.025 M veronal buffer, pH 8.2, according to Laurell (1966). All samples contained 10 μg of protein. The agar contained 2% antiserum against arachin. Electrophoresis proceeded for 16 h at 150 V and 10 mA. The dried plate was stained with 0.1% amido black and destained with 4% acetic acid. Antiserum against native arachin (antiarachin) was prepared by Antibodies, Inc., Davis, CA, according to their protocol.

Gel electrophoresis was performed as described earlier (St. Angelo et al., 1977). In brief, the gels were 10% polyacrylamide with 0.27% cross-linking; the buffer was 0.1 M Tris–glycine, pH 8.3; electrophoresis proceeded at 4 °C with 3 mA/tube until the bromophenol blue tracking dye migrated to the end of each tube; gels were stained with 0.5% amido black and destained with 7% acetic acid.

Sodium dodecyl sulfate (NaDodSO$_4$) gel electrophoresis was performed with NaDodSO$_4$–polyacrylamide gels in 0.1 M phosphate buffer, pH 7.2, according to Weber and Osborn (1969) except that 5 mA was applied per tube until the tracking dye was about 1 cm from the bottom of the tube. The gels were stained with Coomassie Blue and destained with an aqueous mixture of 7.5% acetic acid–5% methanol.

Ultraviolet spectra of arachin samples dissolved in 0.5 M NaF were obtained with a Beckman Model DK-2A ratio-recording spectrophotometer.

Infrared spectra of arachin samples in KBr pellets were obtained with a Perkin-Elmer Model 621 spectrophotometer.

Circular dichroic spectra of protein samples dissolved in 0.3 M NaF were obtained with a Cary Model 60 spectropolarimeter equipped with a 6001 CD attachment. Amounts of α-helical, pleated sheet, and unordered conformational modes in each protein sample were calculated by comparing ellipticity values for the protein with values for conformationally known polypeptides at several wavelengths as determined and described by Greenfield and Fasman (1969). The mean residue weight of arachin was calculated as 132 g/mol from previous data (Neucere, 1969).

Protein concentrations were determined by the method of Lowry et al. (1951).

RESULTS

The effects of exposure of arachin to five organic solvents on the antigenic reactions of treated arachin with antiarachin are shown in Figure 1. Qualitative analysis by double diffusion in part A shows that the precipitin reaction vanished after exposure of the antigen to acidic hexane (wells 5 and 6). Exposures to the other solvents had no effect (wells 2–4).

Semiquantitative analysis in part B (Figure 1) is based on the electrophoretic migration of protein in agar that
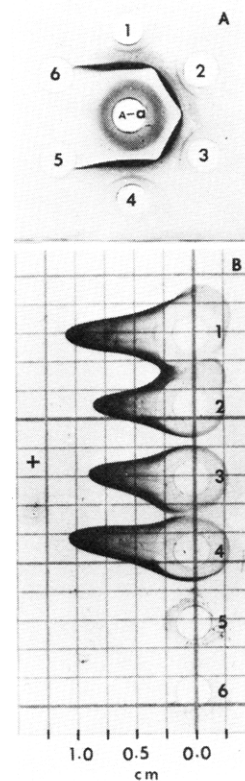

Figure 1. Double diffusion in agar (A) and antibody in gel electrophoresis (B) of arachin exposed to organic solvents. Well 1 contained unexposed, native arachin. Wells 2, 3, 4, 5, and 6 contained arachin exposed to hexane (H), acetone (A), hexane–acetone–water (HAW), hexane–acetic acid (H–HOAc, 5%), and H–HOAc (20%), respectively. A-a refers to antiarachin. Antigen wells of (A) and (B) each contained 30 and 10 μg of protein, respectively.

contains its antibody. The results show virtually no effect after exposure to H, A, or HAW (wells 2–4), but loss of migration occurred after exposure to acidic hexane (wells 5 and 6). Since similar protein concentrations were applied to the gels, these observations are indicative of a loss of determinant groups on arachin available to the antibody.

To investigate the possibility that protein dissociation or protein scission (breakage of primary valence bonds) occurred upon exposure of arachin to organic solvents, we subjected control and exposed samples to gel electrophoresis in nondenaturing (Figure 2, top) and dissociating (NaDodSO$_4$; Figure 2, bottom) solvents. Figure 2 shows that electrophoretic migration in either system was not affected by exposure to H, A, or HAW. Exposure of arachin to acidic hexane, however, had a pronounced effect on migration in the nondenaturing system (Figure 2, top), but the migrational pattern was similar to that of native arachin in the NaDodSO$_4$ gel (Figure 2, bottom). The increased migration in nondenaturing gels results from an increased ratio of charge to hydrodynamic volume. Such a change in ratio can result from increased net negative charge, a change in size or shape, or a combination of these. The somewhat similar pattern of migration of acidic hexane-treated arachin in NaDodSO$_4$ gels (Figure 2, bottom, gels 5 and 6) compared to that of the other samples indicates that dissociation of arachin occurred on exposure to acidic hexane.

Ultraviolet spectra of untreated and solvent-treated samples are shown in Figure 3. Native arachin and arachin exposed to H, A, and HAW yielded typical protein spectra. However, an absorbance maximum at 310 nm appeared with samples exposed to acidic hexane. The

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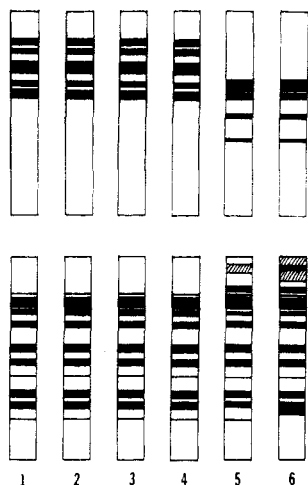
Figure 2. Nondenaturing polyacrylamide gel electrophoretic patterns (top) and dissociating NaDodSO₄ gel electrophoretic patterns (bottom) of arachin exposed to organic solvents. The origin is at the top of each pattern; the anode is at the bottom of each. 1, unexposed, native arachin; 2, 3, 4, 5, and 6, arachin exposed to H, A, HAW, H–HOAc (5%), and H–HOAc (20%), respectively. Diagonally striped areas represent smearing.
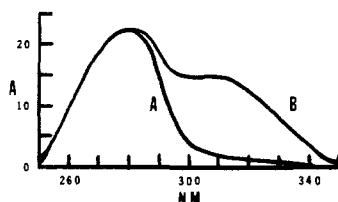
Figure 3. Ultraviolet spectra of arachin. (A) Unexposed, native arachin; (B) arachin exposed to H–HOAc (5%). Cuvettes contained 0.28 mg of protein/mL. Spectra of other solvent-treated samples of arachin were similar to (A) except for H–HOAc (20%), which was similar to (B).

amplitude of the peak suggests that the unidentified chromophore, which is not accounted for by aromatic amino acid residues, is produced rather than unveiled by acidic hexane. The spectra of the acidic hexane treated samples also reflected the physical appearance of the dried powders: they were tan whereas the others were white.

Infrared spectra were obtained for the unexposed control and the solvent-treated samples. Absorbancies assigned to amide I, II, and V bands occurred at 1645–1655, 1510–1525, and about 710 cm⁻¹, respectively. Bands in these spectral regions indicate the conformation of each sample is principally from pleated sheet and unordered structures (Miyazawa and Blout, 1961; Miyazawa et al., 1962). Conformational changes sufficient to affect the spectral locations of these amide bands were not induced by exposure to the solvents. Furthermore, the relative intensities of amide IV and VI bands were stable, contrary to the thermally induced change in the bands accompanying the conformational transition in heated arachin (Jacks et al., 1975).

Figure 4 shows the circular dichroic spectra of samples of native arachin and arachin exposed to the organic solvents. Minimization of differences between each experimental spectrum and spectra of model polypeptides yielded the conformational modes of each sample as shown in Table I. The data indicate that essentially no changes of conformational modes were induced by exposure to any of the solvents.

DISCUSSION

This study addresses the question of what happens to oilseed protein when the oilseeds have been subjected to
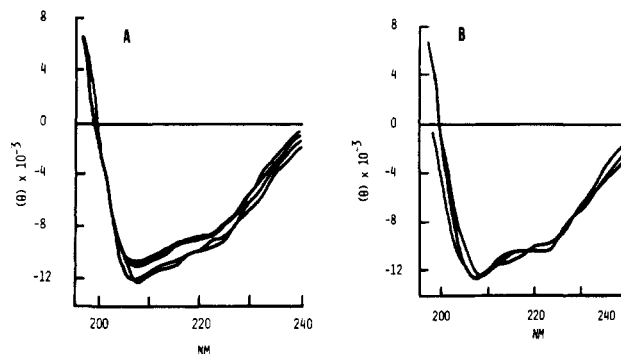
Figure 4. Far-ultraviolet circular dichroic spectra of native and solvent-treated arachin. Values on the abscissa refer to deg cm²/dmol. (A) Native arachin and arachin exposed to H, A, and HAW; (B) native arachin and arachin exposed to H–HOAc (5%) and H–HOAc (20%).

Table I. Contents of α-Helical, Pleated Sheet, and Unordered Structures in Conformations of Treated and Untreated Arachin

| | conformational modes[a] | | |
treatment	α helical	pleated sheet	un-ordered
control	27.1	21.6	51.3
hexane	24.9	23.5	51.6
acetone	20.4	25.4	54.2
HAW	20.5	26.7	52.8
hexane-HOAc (5%)	25.5	22.5	52.0
hexane-HOAc (20%)	28.0	18.6	53.4

[a] Values were computed from Figure 4 as described in the text and are given as percentages of the total conformation. The closeness of fit of each spectrum shown in Figure 4 to the theoretical spectrum for each sample of the conformational modes given above is estimated by the following standard deviations (θ × 10⁻³ deg cm²/dmol): control, ±0.82; hexane, ±0.65; acetone, ±0.50; HAW, ±0.45; hexane-HOAc (5%), ±0.45; hexane-HOAc (20%), ±0.75.

solvents used to extract their oils commercially. Isolated peanut storage protein (arachin) was used as the model because it was easily obtainable, its physicochemical properties have been thoroughly investigated, and protein-rich peanut meal is prepared with solvents commercially.

Any effect observed in this study was not due to temperature per se. The temperatures (60 and 68.5 °C) used in industry and in this study were much lower than those (above 140 °C) needed to modify the solubility, electrophoretic mobility, antigenicity, and conformation of arachin (Jacks et al., 1975; Neucere, 1972, 1974; Thomas and Neucere, 1973). Also, acetic acid did not become concentrated during evaporation of acidic hexane; the mixtures of hexane with acetic acid used in this study are azeotropic (Horsley, 1952; Horsley and Tamplin, 1962).

Three of the five solvents used in this study had virtually no effect on the conformational properties of arachin. After exposure of arachin to H, A, or HAW, its antigenicity (Figure 1), electrophoretic migration (Figure 2), and ultraviolet, infrared, and circular dichroic spectra (Figures 3–4) were identical with those of native arachin. Similarly, contact of arachin with acetone–hexane (Thomas and Neucere, 1973), acetone, carbon tetrachloride, and ether (Yamada et al., 1979), hexane and chloroform–methanol (St. Angelo and Ory, 1975), or chloroform–methanol followed by methanol (Jacks et al., 1982) produced no effect. However, the elution pattern of arachin from DEAE-cellulose changed slightly when protein was prepared from

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peanuts extracted at room temperature with carbon tetrachloride or heptane and significantly with acetone (Neucere and Ory, 1968). Apparently, exposure of arachin to certain polar and nonpolar organic solvents at mild temperatures can slightly affect its anionic character without a corresponding dissociative or conformational change.

Exposure of arachin to acidic hexane, however, produced entirely different results. The antigenicity of arachin was completely abolished (Figure 1) and its patterns of electrophoretic migration were indicative of a decrease in molecular weight caused by protein dissociation (Figure 2). However, corresponding shifts in infrared and circular dichroic spectra (Figure 4), concomitant with transitions of conformational modes in arachin, did not occur, implying solvent effects independent of conformational considerations. The significance of the ultraviolet peak at 310 nm, unrelated to conformational transitions, that developed when arachin was treated with acidic hexane (Figure 3) remains for future investigation.

These results can be satisfactorily explained on the hypothesis that arachin underwent irreversible dissociation without disorganization upon exposure to acidic hexane. The dissociated subunits maintained the parental conformational modes of the associated complex but antigenicity was lost. Furthermore, electrophoretic mobilities increased in nondenaturing gels but not in NaDodSO$_4$ (dissociating) gels. In H–HOAc, a strongly protic solvent of low dielectric constant, the ionic charges on arachin were extensively paired to the solvent counterions, and electrostatic repulsive interactions were of negligible importance in destabilizing the native conformation. Thus, in the acidic medium arachin was highly protonated for dissociation into subunits through loss of ionic bonding. Conformationally, the electrostatic tendency to disorder was absent in acidic hexane, and the hydrophobic interactions, which play the major role in stabilizing the native conformation of protein, re-formed upon removal of the organic solvent. Arachin remained protonated (and remained dissociated into subunits), but the possibility should not be overlooked that irreversibility was due to some scission or rearrangement of disulfide or other primary valence bonds within arachin. In arachin, however, disulfide bonds do not participate in association of subunits (Tombs, 1965; Yamada et al., 1979). The dissociation observed here might be comparable to the irreversible dissociation of arachin at strongly acidic pH values in aqueous systems (Yamada et al., 1979).

Arachin is a multimeric subunit protein of M_r 330 000–350 000 comprised of several subunits, the dissociated formation of which is dependent upon the pH value, ionic strength, and type of salt present in aqueous solutions (Daussant et al., 1969; Johnson and Shooter, 1950; Tombs, 1965; Yamada et al., 1979). Until now, dissociation of arachin or any plant protein by exposure to organic solvents has not been reported; rather, absence of dissociation has been noted (Jacks et al., 1982; Yamada et al., 1979). This study shows that dissociation of arachin occurred after exposure to acidic hexane, without protein scission (compare top and bottom of Figure 2). Also, arachin must have a fairly stable secondary structure without much conformational freedom because even its dissociated subunits retained the conformational modes in the parent multimer.

In earlier studies we found that whenever a given treatment affected the reactivity of arachin with antiarachin, a concomitant transition in the conformational modes in arachin occurred. For instance, both the antigenicity

and secondary structure of arachin were modified after exposure of arachin to high temperatures (Jacks et al., 1975), simple polyphenols (Neucere et al., 1978), or hydrogen peroxide (Jacks et al., 1982), and it was assumed that antibodies to native arachin were directed against conformational determinants. In this study, however, transitions of the conformational modes did not accompany the loss of antigenicity (Figure 1); the dissociated subunits were antigenically unreactive yet maintained the conformational modes of the undissociated multimer (Table I). Accordingly, more than secondary structure must be involved in arachin–antiarachin complexation. Since the subunits were unreactive, the recognition sites apparently embrace more than one subunit chain so that determinants reflect quarternary structure. The loss of antigenicity of arachin from exposure to hydrogen peroxide, on the other hand, was due to protein scission (Jacks et al., 1982).

Whether exposure of arachin to organic solvents affected its nutritive value was not assessed. Organic solvents at mild temperatures generally produce no deleterious effects on nutritive values of oilseed proteins. Extraction of cottonseed with acidic hexane, shown in this study to dissociate peanut protein, did not modify the protein efficiency ratio and digestibility of cottonseed protein (Hensarling and Jacks, 1982). Nor does exposure to mild temperatures; nutritional values were impaired only in meals prepared from peanuts subjected to temperatures above 110 °C (Neucere et al., 1972).

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Fatty Acids and Sensory Acceptance of a Dietary Sodium–Potassium Fish Sauce

Suphsorn Chayovan, Ramu M. Rao,* Joseph A. Liuzzo, and Mahmood A. Khan[1]

Two varieties of fish, (1) flounder, a lean fish with low fat (1.6%), and (2) trout, a fatty fish with 9.2% fat, were used in the preparation of fish sauce. The preparation of sauce consisted of fermenting a mixture of uneviscerated ground fish and sodium and potassium salts in different fish-to-salt ratios. Desired ratios of these salts in the sauce were obtained by mixing appropriate volumes of individual salt-based sauces. Identification of fatty acids was accomplished by gas chromatography. Results showed that all samples contained volatile fatty acids from C-2 to C-6 and nonvolatile fatty acids from C-8 to C-18 in various proportions. In all the samples, isovaleric acid was in the highest concentration followed by acetic and isobutyric acids. Sensory analyses indicated that a sodium–potassium dietary fish sauce could prove to be a possible replacement for an all sodium chloride sauce provided the salt ratios do not exceed 40:60.

Fermented fish products are manufactured in vast quantities for human consumption in Southeast Asia. These foods provide an excellent means of fish preservation in areas where few canning or freezing facilities are available. The protein diet of the people in these areas is closely linked with cured and fermented fish products. Fermented fish products are commonly used to brighten monotonous rice dishes, and somewhat more than 50 different kinds of fermented fish products are utilized in Southeast Asia (Amano, 1962).

Various sauces or pastes are prepared from a wide variety of both fresh and saltwater fishes, mainly clupeids, carangids, and cyprinids. Each country has its own national fermented fish products. For example, the Japanese have "Katsuo-Shiokara" made from the visceral organs of tuna; the Cambodians have "Nuoc-mam Gauca" made from fish liver; the Indonesians have "Trassi" made from planktonic shrimp which is similar to the Filipino "Bagoong" and the "Kapi" of Thailand. However, one of the most popular fermented products which is famous among the people of the Far East and distributed throughout the entire area is Nuoc-mam or Nam-pla, a fish sauce made in Vietnam, Cambodia, the Philippines, and Thailand.

Sodium chloride is a major ingredient in making fish sauce, its concentration ranging between 25 and 32% in the finished product. In fact, NaCl is essential for human and animal growth, but the level needed by human subjects has not been established with any degree of certainty. There is adequate evidence that the daily ingestion of 250–375 mg of NaCl (100–150 mg of Na) by adults can be maintained without any apparent signs of abnormalities (Dahl, 1972; Institute of Food Technologists, 1980). However, from this low requirement of the human body for NaCl, the average daily intake has been estimated as ranging from 10 g for Americans to 27 g for inhabitants of certain areas of Japan and most of Southeast Asia. Beneficial effects have been observed following the limitation of sodium salt intake by patients with a variety of diseases. Some investigators concluded that there exists a causative relationship between a high salt intake and hypertension (Dahl, 1960; Meneely et al., 1957; Meneely and Ball, 1958).

Therapeutical salt restriction was employed in 1901 in patients with edematous heart disease (Achard and Loeper, 1901; Bennett, 1979). The treatment was subsequently extended to congestive heart failure, hypertension, renal diseases, cirrhosis of the liver, toxemia of pregnancy, and Meniere's disease (Kempner, 1948; Jones, 1979). It has been reported that one-fourth of hypertensive patients respond by a decrease in blood pressure after salt restriction.

Man's requirement for salt is so low that most patients find it a hardship to be subjected to a salt-restricted diet. For this reason, efforts have been made to develop a seasoning agent to replace sodium salt. It has been suggested that high naturally occurring dietary KCl protects against human addition of excessive NaCl (Meneely et al., 1957; Michelsen et al., 1977). The use of NaCl and KCl mixtures in diets of populations susceptible to hypertension would be the most practical way to decrease the incidence of this disease.

Essentially no research has been reported on a dietary sodium–potassium fish sauce which can be used by people who are on a sodium-restricted diet. Therefore, the present investigation was undertaken with the following objectives: (1) to develop a dietary sodium–potassium fish sauce; (2) to make a sensory evaluation of the sauce in terms of color flavor, and overall quality; (3) to isolate and identify certain key flavor components such as volatile and nonvolatile

Department of Food Science, Louisiana Agricultural Experiment Station, Center for Agricultural Sciences and Rural Development, Louisiana State University, Baton Rouge, Louisiana 70803.
[1]Present address: Department of Food and Nutrition, University of Illinois, Urbana, IL 61801.