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Isolation and Characterization of Two Cryoproteins from Florunner Peanut (*Arachis hypogaea* L.) Seed

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Two cryoproteins (CP I and CP II) from the peanut (*Arachis hypogaea* L.) seed were isolated following the fractionation of a 2 M NaCl, 0.01 M Tris-HCl (pH 7.5), and 0.002% sodium azide protein extract on a Sephacryl S-300 column. The cryoproteins I and II had molecular weights of 500 000 and 380 000 ± 20 000, respectively. The protein composition of the cryoproteins was found to be different after ion-exchange chromatography and gel electrophoresis. Addition of 2-mercaptoethanol did not alter the molecular weights and electrophoretic patterns of the cryoproteins. Amino acid compositions of the cryoproteins I and II were similar. The critical temperature for cryoprecipitation was between 2 and 15 °C. Cryoprecipitation increased with increasing protein concentration and reached a maximum at 40 mg/mL. Cryoprotein I showed higher cryoprecipitation with lower protein concentration compared to the cryoprotein II. Maxium cryoprecipitation was obtained around pH 7.5 and at an ionic concentration of 0.4 M (CP I) and 0.2 M (CP II). Limited protease treatment, although it destroyed the cryoprecipitation property, did not cause a major change in the molecular weights of the cryoproteins.

Cooling of certain protein solutions brings about the precipitation of specific proteins. Such precipitation phenomenon is known as "cryoprecipitation" and is thermally reversible. The occurrence of such phenomenon in seed extracts of certain species is known (Ghetie and Buzila, 1962). Daussant et al. (1969) have suggested that in peanut seed the cryoprecipitation property occurs almost exclusively for α-arachin. Neucere (1969) has employed this property for purification of α-arachin. Although peanut proteins are known to possess the cryoprecipitation property, very little information is available on the process of cryoprecipitation, the number of proteins involved, and the factors affecting the cryoprecipitation of peanut proteins. Hence, a study was initiated to isolate the peanut cryoproteins and to determine their characteristics and the factors influencing the cryoprecipitation property. This paper describes isolation of two cryoproteins from peanut seed and examines various factors affecting the cryoprecipitation property.

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MATERIALS AND METHODS

Seed Material. Seeds of cv. Florunner are a gift from Dr. A. J. Norden of the University of Florida. After removal of the seed coats and embryonic axes, the cotyledons were ground into a meal and defatted with diethyl ether. Defatted meal was stored at −20 °C.

Protein Extraction. Protein from defatted peanut meal (3 g) was extracted with 10 mL of 2 M NaCl, 10 mM Tris-HCl buffer (7.5), 0.002% (w/v) sodium azide (NaN_3), and 2 mM phenylmethanesulfonyl fluoride (PMSF). The homogenate was centrifuged at 20000*g* for 20 min at 20 °C. The clear supernatant was used for further analysis.

Separation of Proteins. An aliquot (8 mL) of the seed protein extract was placed on a Sephacryl S-300 column (125 × 2.5 cm) equilibrated at room temperature with 0.5

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M NaCl, 0.01 M Tris-HCl, pH 7.5, and 0.002% (w/v) NaN_3. The column was eluted in 5-mL fractions by using a peristaltic pump.

Isolation of Cryoproteins. The column fractions were allowed to stand at 2 °C for 24 h, and the cryoprecipitates were collected by centrifugation at 20000*g* for 20 min at 0 °C. The supernatant was discarded, and pellets were dissolved in 5 mL of 0.5 M NaCl and 10 mM Tris-HCl buffer, pH 7.5, containing 0.002% NaN_3 and analyzed for protein content. On the basis of the protein data, fractions 20–30 and 31–50 were pooled and designated as cryoprotein I (CP I) and cryoprotein II (CP II), respectively. The pooled materials were allowed to recryoprecipitate, and the resulting precipitates of CP I and CP II were rechromatographed on a Sephacryl S-300 column. The major protein fractions were pooled and used for further characterization of CP I and CP II proteins. Total cryoprotein was obtained following refrigeration of a 0.5 M NaCl, 10 mM Tris-HCl (pH 7.5), 0.002% NaN_3, and 2 mM PMSF extract of the defatted peanut meal.

Molecular Weight Estimation. The Sephacryl S-300 column was calibrated by using the following molecular weight markers: immunoglobulin M (970 000), thyroglobulin (669 000), ferritin (500 000), catalase (232 000), alkaline phosphatase (140 000), and transferrin (76 000). The void volume and salt volume of the column were determined by using Blue Dextran 2000 and sucrose, respectively. The molecular weight of the unknown protein was estimated according to the method of Andrews (1964).

Assay of Cryoprecipitation. Stock solutions of cryoproteins I and II were pipetted into centrifuge tubes followed by appropriate addition of buffers to give a final concentration of 10 mg/mL. The tubes were incubated at 23 °C for 5 min and then refrigerated for 24 h. Following incubation, the tubes were transferred to a centrifuge rotor prechilled at 0 °C and centrifuged at 20000*g* for 20 min at 0 °C. The supernatant was removed and its protein content was measured. Cryoprecipitate is defined as percent cryoprecipitate = A_{650}(total) − A_{650}(sup)]/A_{650}(total) × 100 where A_{650}(total) is the total protein in assay mixture at 23 °C and A_{650}(sup) is the protein in the supernatant following centrifugation. All the assay mixtures contained 0.002% NaN_3 and 2 mM PMSF. Thermal reversibility of cryoprecipitates was checked by resuspension of individual solutions at 23 °C and subsequent determination of its protein content after centrifugation at 20000*g* for 20 min at 23 °C. Unless stated otherwise, cryoprecipitation was routinely carried out in a buffer containing 0.5 M NaCl, 10 mM Tris-HCl, pH 7.5, 0.002% NaN_3, and 2 mM PMSF.

Temperature Dependence. The dependence of cryoprecipitation on temperature was tested by incubating cryoprecipitates for 24 h at temperatures between 2 and 40 °C. Following the incubation, the assay tubes were spun at the appropriate temperatures (between 2 and 40 °C) for 20 min at 20000*g*. An aliquot of the supernatant was used for the determination of nonprecipitated protein.

Effect of pH on Cryoprecipitation. Aliquots from the stock solutions of cryoproteins were transferred into two sets of centrifuge tubes, followed by the addition of appropriate buffers of pH 2–11. The pH of the assay mixture was measured by using a microelectrode, and if necessary, the pH was adjusted. One set of assay tubes was incubated at room temperature (23 °C) while the other at 2 °C. After 24 h, the tubes were centrifuged and the protein content in the supernatant was determined.

Effect of Ionic Strength. Aliquots from the stock solutions of cryoproteins (150 mg/mL) were pipetted into centrifuge tubes. Prior to making up to the volume (1 mL), solid NaCl was added to each assay tube to obtain desired ionic strength (0.01–3 M). The contents of the tubes were thoroughly mixed and incubated at 2 °C for 24 h. Following the incubation, the tubes were centrifuged at 20000*g* for 20 min at 0 °C. The supernatant was assayed for soluble protein.

Effect of Protein Concentration. The stock solutions of cryoprecipitates were diluted with a buffer containing 0.5 M NaCl, 10 mM Tris-HCl, pH 7.5, 0.002% NaN_3, and 2 mM PMSF to obtain a final protein concentration of 0.02–40 mg/mL. The tubes were cooled (2 °C) for 24 h and centrifuged, and the protein content of the supernatant was measured.

Protein Determination. Protein content was determined by following the method of Lowry et al. (1951), using bovine serum albumin as the standard.

DEAE-cellulose Chromatography. The cryoproteins were dissolved in 10 mM Tris-HCl buffer, pH 7.5, and loaded on a DEAE-cellulose column (1 × 30 cm) which was equilibrated with 10 mM Tris-HCl, pH 7.5, containing 0.002% NaN_3. The column was washed with the column equilibration buffer and then eluted with a linear gradient of 0–0.4 M NaCl in 10 mM Tris-HCl, pH 7.5, and 0.002% NaN_3.

Amino Acid Composition. Fifty milligrams of lyophilized cryoproteins was hydrolyzed with 6 N HCl for 18 h at 110 °C, neutralized, and then analyzed in a JEOL amino acid analyzer (Pancholy et al., 1978).

Purification of Arachin. Defatted peanut meal was extracted with 2 M NaCl, 10 mM Tris-HCl, pH 7.5, 0.002% NaN_3, and 2 mM PMSF. After centrifugation, the supernatant was saturated to 30% with solid ammonium sulfate, and the resulting crude arachin was recovered by centrifugation. The crude arachin was dialyzed against 10 mM Tris-HCl, pH 7.5, and fractionated on a DEAE-cellulose column (1 × 30 cm) by using a NaCl gradient of 0–0.4 M (Dechary et al., 1961). The major protein fractions were pooled and used for analysis.

Polyacrylamide Gel Electrophoresis. About 100 μg of the cryoproteins and cryosupernatants was subjected to nondenaturing gel electrophoresis in 7.5% acrylamide gels (Davis, 1964). For sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis, the samples were boiled for 3 min in a buffer containing 2% (w/v) $NaDodSO_4$, 1.5% (w/v) dithiothreitol, and 1.2% (w/v) Tris. The dissociated proteins (100 μg) were electrophoresed in 10% (w/v) acrylamide gels containing 0.1% $NaDodSO_4$ (Laemmli, 1970). Following electrophoresis, the proteins were stained with Coomassie Blue R-250 and destained with 7% acetic acid and 10% ethanol. The gels were scanned in a Beckman Model 25 spectrophotometer equipped with a gel scanner at 600 nm by using a 0.05-mm slit.

RESULTS AND DISCUSSION

Isolation of Cryoproteins. Total peanut seed proteins were first separated into 10 peaks on a Sephacryl S-300 column (Figure 1). For comparison, proteins from cultivars Florunner (Figure 1a) and Early Bunch (Figure 1b) were examined for cryoprecipitability. Some differences were present in the protein elution profiles of the two cultivars between fractions 20 and 60. Since, exposure of peanut protein extracts to low temperatures causes cryoprecipitation, protein extraction, centrifugation, and fractionation were carried at room temperature. So that microbial and protease activity could be prevented during the protein fractionation, 0.002% sodium azide and 2 mM PMSF were included in all the buffers. Upon incubation of the column fractions at 2 °C, fractions between 20 and

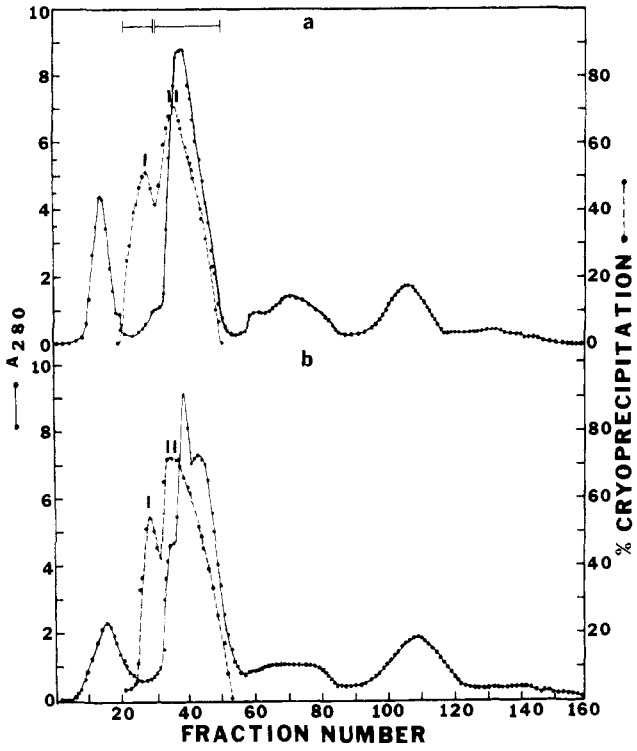

Figure 1. Protein (—) and cryoprecipitation (---) patterns of the salt-soluble extracts from cultivars Florunner (a) and Early Bunch (b), following gel filtration on a Sephacryl S-300 column (2.5 × 125 cm). After gel filtration, the fractions were cooled (2 °C for 24 h) and centrifuged, and the protein content of the pellets was determined. The precipitates in the column fractions between 20 and 30 and 31 and 50 were pooled and designated as cryoprotein I and cryoprotein II, respectively.

50 turned turbid due to protein precipitation. So that complete cryoprecipitation was attained, the fractions were refrigerated for 24 h. Following the 24-h incubation, cryoprecipitate buttons appeared at the bottom of the tubes. Analyses of the cryoprecipitates showed (Figure 1) the presence of two protein peaks (I and II) between the fractions 20 and 50 (shown with dashed lines). Hereafter, these two peaks will be referred as cryoprotein I (CP I) and cryoprotein II (CP II). Only about 50 and 70% of the protein were precipitated from the CP I and CP II regions of the Sephacryl column fractions, respectively. Recooling of the supernatants (resulted after removing the cryoprecipitates) did not give any more precipitation. This would indicate that all the proteins present in the Sephacryl column fractions between 20 and 50 are not capable of cryoprecipitation but only certain proteins of that region exhibit cryoprecipitation. CP I was amorphous and white, while CP II was gelatinous and yellow. The cryoproteins retained thermal reversibility even after repeated cooling and warming and also freezing. The cryoproteins were highly soluble and yielded a protein concentration of at least 300 mg/mL. Rechromatography of cryoproteins I and II on the Sephacryl S-300 did not change their elution patterns (Figure 2). Similarly, addition of 2-mercaptoethanol (10 mM) to the samples and chromatography in presence of 2-mercaptoethanol had no effect on protein elution patterns. Thus, the observed differences in the elution volumes of the cryoproteins were real and not an artifact due to protein aggregation involving disulfide bonding.

For comparison, a 30% ammonium sulfate precipitate (arachin-rich fraction; Jones and Horn, 1930; Dawson, 1971) was obtained, and the precipitate (in 0.5 M NaCl, 10 mM Tris-HCl, pH 7.5, and 0.002% NaN_3) was incubated at 2 °C for 24 h. The resultant cryoprecipitate was then fractionated on the Sephacryl S-300 column, and the protein distribution was examined. The protein composition of this cryoprecipitate was found (data not shown) to be similar to that of total seed protein (Figure 1) containing 10 protein peaks. In addition, cooling of the above protein fractions failed to show any cryoprecipitation even at a protein concentration of 25 mg/mL. This suggest that unlike the CP I and CP II, the cryoprecipitates obtained from the ammonium sulfate precipitate are nonspecific and unstable.

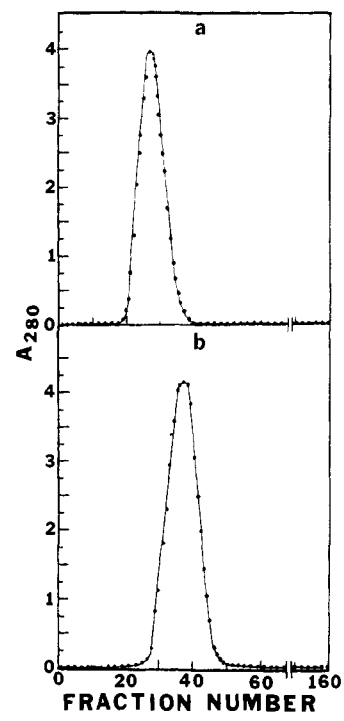

Figure 2. Rechromatography of the cryoproteins I (a) and II (b) on the Sephacryl S-300 column (2.5 × 125 cm). The chromatographic conditions were the same as described in Figure 1.

Molecular Weight Estimation. The molecular weights of the cryoproteins I and II were found to be 380 000 and 500 000 (±20 000), respectively. Although, CP II appears to have a similar molecular weight as the α-arachin, the electrophoretic patterns of these two proteins were quite different. For example, CP II contained at least three major proteins, while α-arachin had a single protein band (Neucere, 1969), indicating that it is not similar to α-arachin in its protein composition.

DEAE-cellulose Chromatography. The DEAE-cellulose chromatography patterns of cryoproteins I and II are shown in Figure 3. In the case of cryoprotein I, the majority of the protein eluted in two peaks at ionic strengths of 0.23 M (B) and 0.27 M (C), respectively. CP II showed (Figure 3b) two major and three minor protein peaks. The major protein peaks of CP II eluted at ionic strengths of 0.20 M (A) and 0.23 M (B). Similarly, the total cryoprotein showed (Figure 3c) one major peak (at an ionic strength of 0.23 M) with a trailing edge (ionic strengths between 0.24 and 0.3 M). Thus the ion-exchange chromatography data showed that except for protein "B", CP I and CP II have a slightly different protein composition.

Amino Acid Composition. The amino acid compositions of the cryoproteins and total protein are shown in Table I. The amino acid compositions of the CP I and CP II were very similar and contained relatively large amounts of arginine, glutamate, and aspartate and trace amounts of cystine and methionine. Compared to the total protein, cryoproteins contained lower amounts of lysine

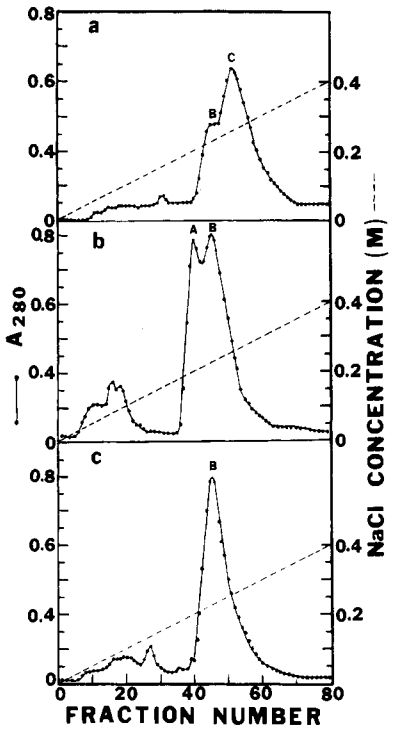

Figure 3. DEAE-cellulose chromatography patterns of the cryoprotein I (a), cryoprotein II (b), and total cryoprecipitate (c). The protein isolates (50 mg) were loaded on the column (1 × 30 cm), washed with 0.01 M Tris-HCl buffer (pH 7.5), and then eluted with 500 mL of a linear NaCl gradient (---) of 0–0.4 M, in 6-mL fractions.

Table I. Amino Acid Composition[a] of Peanut Cryoproteins I and II

amino acid	amino acid composition, g/100 g of protein			
	total cryoprotein	cryoprotein I	cryoprotein II	Florunner
lysine	2.26	2.85	2.23	5.34
histidine	2.13	2.16	2.34	2.89
NH_3	2.38	2.50	2.71	3.18
arginine	13.40	13.38	13.35	15.26
aspartic acid	13.38	12.59	13.04	12.29
threonine	3.95	4.28	3.81	4.28
serine	4.08	4.13	4.09	3.97
glutamic acid	20.16	19.63	20.22	18.64
proline	4.44	4.67	4.61	4.38
glycine	3.81	4.13	4.00	5.10
alanine	3.37	3.28	4.49	3.49
cystine	T	T	T	T
valine	5.21	4.65	5.17	3.95
methionine	0.53	0.11	T	T
isoleucine	3.81	4.61	4.05	2.62
leucine	7.17	7.45	7.39	6.00
tyrosine	3.73	3.15	3.41	3.14
phenylalanine	5.76	5.68	5.42	5.21

[a] Average of three replications.

(2.2–2.8 vs. 5.3%) and glycine (3.8–4.1 vs. 5.1%) but were relatively high in valine, methionine, isoleucine, and leucine. A possible correlation between the solubility of the proteins and their glutamate content has been suggested (Neucere, 1969).

Gel Electrophoresis. Protein composition of cryoprecipitates and cryosupernatants was examined by polyacrylamide gel electrophoresis under nondenaturing conditions. The results are shown in Figure 4. As seen, the protein composition of CP I is distinct from that of CP II and contained six major and four minor components

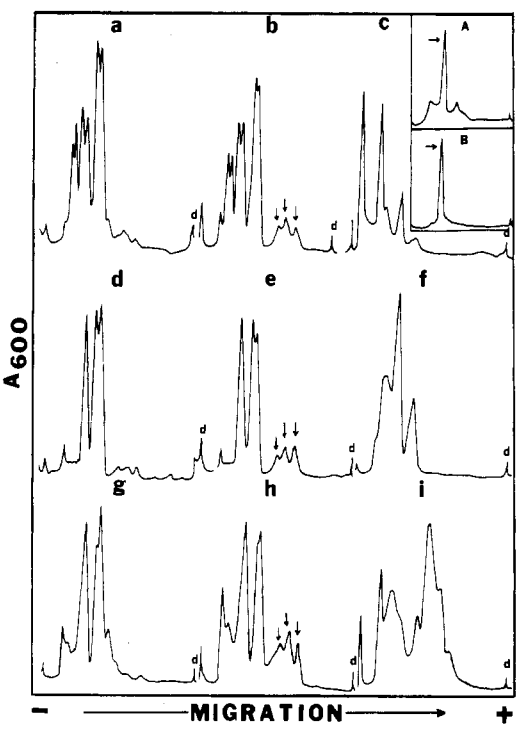

Figure 4. Gel electrophoretic profiles of the cryoprecipitates and cryosupernatants with and without the addition of 2-mercaptoethanol (20 mM): (a) cryoprotein I (−mercaptoethanol); (b) cryoprotein I (+mercaptoethanol); (c) cryosupernatant I; (d) cryoprotein II (−mercaptoethanol); (e) cryoprotein II (+mercaptoethanol); (f) cryosupernatant II; (g) total cryoprecipitate (−mercaptoethanol); (h) total cryoprecipitate (+mercaptoethanol); (i) total cryosupernatant. The proteins that increased following the 2-mercaptoethanol addition are indicated with arrows. The insert in the figure shows the electrophoretic patterns of the total peanut seed proteins (A) and purified arachin (B). The arrows in the insert show a protein with an R_m value of 0.34 that is found in all the cryoproteins. About 100 μg of protein was loaded on each gel. d = tracking dye.

(Figure 4a). CP II showed (Figure 4d) three major and six minor proteins. Addition, of 2-mercaptoethanol (20 mM to the samples prior to electrophoresis did not cause a significant change in the electrophoretic patterns of the cryoproteins (Figure 4b,e). However, a slight increase in the amounts of three faster migrating proteins (shown with arrows) was noticed as a result of 2-mercaptoethanol addition. The protein composition of the cryosupernatants was different between the two cryosupernatants (Figure 4c,f). The electrophoretic profiles of the total cryoprecipitate (Figure 4g–i) were similar to that of the CP II. For comparison, electrophoretic patterns of total seed and arachin are also included (insert A and B, respectively). It is evident that the total protein and arachin patterns differ from those of cryoproteins. However, the cryoproteins did contain a component with an R_m value of 0.34, found in the total protein and arachin (arrow in the insert). Examination of the polypeptide composition of cryoprecipitates I and II by sodium dodecyl sulfate gel electrophoresis also showed variation in their polypeptide composition (Figure 5). The polypeptide compositions of cryoproteins I and II were found to be much alike except for the quantitative differences in certain polypeptides. For example, CP I contained relatively small amounts of component A compared to CP II. In addition, the ratio of components B to C in CP I was 1.1 while it was 0.8 in CP II. This suggests that CP I and CP II, although qualitatively similar, differ in their polypeptide quantities.

Effect of Temperature on Cryoprecipitation. The influence of temperature on cryoprecipitation is shown in

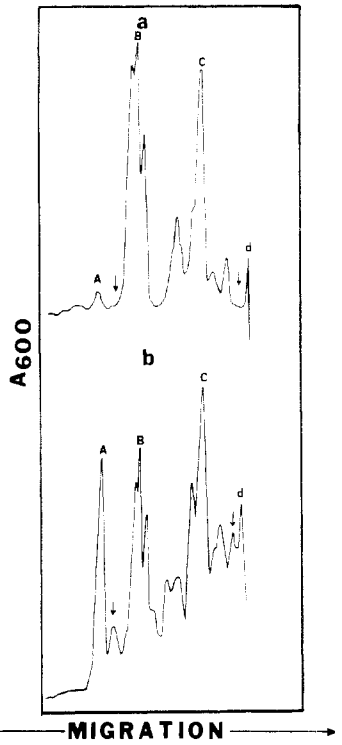

Figure 5. Sodium dodecyl sulfate gel electrophoretic patterns of cryoprotein I (a) and cryoprotein II (b). Each gel was loaded with 100 μg of protein. The arrows show other differences in the polypeptide composition between two cryoproteins.

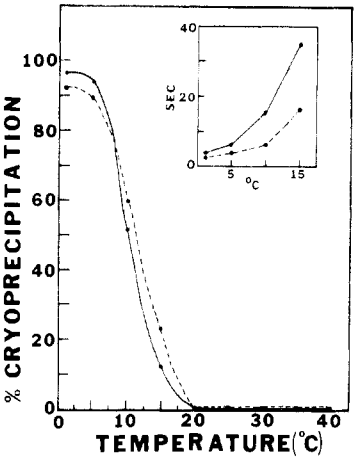

Figure 6. Effect of temperature of percent protein precipitation of cryoprotein I (—) and cryoprotein II (---). The insert in the figure shows the relationship between the temperature and time requirement for cryoprecipitation, as measured by the turbidity development in the cryoproteins I (—) and II (---).

Figure 6. The cryoproteins I and II were exposed to temperatures between 2 and 40 °C for 4 h, and the amount of precipitated protein was estimated. As seen in the figure, cryoprecipitation was highest between 0 and 5 °C and dropped rapidly at 15 °C. No cryoprecipitation was obtained at and above 20 °C. The insert in the figure shows the relationship between the temperature and time required for cryoprecipitation. This was determined based on the time required for turbidity development in the samples when exposed to temperatures between 2 and 15 °C. Cryoprecipitation was rapid (3–6 s) at temperatures between 2 and 10 °C. However, at 15 °C, 35 and 13 s were required to precipitate CP I and CP II, respectively. The cryoproteins retained the cryoprecipitation property even after exposure to temperatures of up to 80 °C, indicating their heat stability.

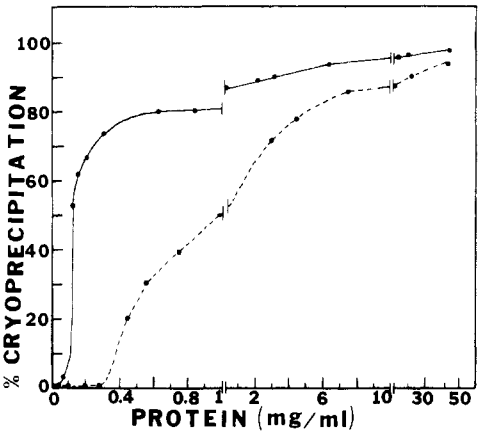

Figure 7. Effect of protein concentration on the cryoprecipitation amounts of cryoprotein I (—) and cryoprotein II (---) in 0.5 M NaCl, 0.01 M Tris-HCl, pH 7.5, 0.002% NaN_3, and 0.002% phenylmethanesulfonyl fluoride.

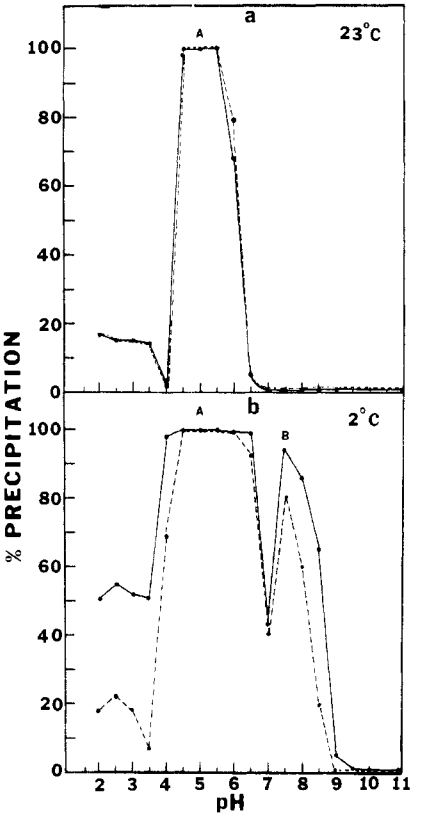

Figure 8. Effect of pH on the precipitation patterns of cryoprotein I (—) and cryoprotein II (---) at 23 °C (a) and 2 °C (b). The samples were adjusted to pH between 2 and 11 and then incubated at 23 and 2 °C for 24 h. The samples were then centrifuged, and the supernatants were analyzed for their protein content.

Concentration Dependence of Cryoprecipitation. Figure 7 illustrates the dependence of cryoprecipitation on the protein concentration and the differences in the cryoprecipitation amounts between CP I and CP II. As seen in the figure, a 50% cryoprecipitation was obtained at 0.1 mg/mL for CP I, while 1 mg/mL was needed for CP II. Similarly, so that 80% cryoprecipitation was obtained, a protein concentration of 0.5 mg/mL was required of CP I and 5 mg/mL of CP II, indicating that 10 times more protein is required in the case of CP II to obtain similar cryoprecipitation values given by CP I. The increase in the cryoprecipitation amounts beyond 80% was slow and found to be only 92–98%, even at a protein concentration of 40 mg/mL.

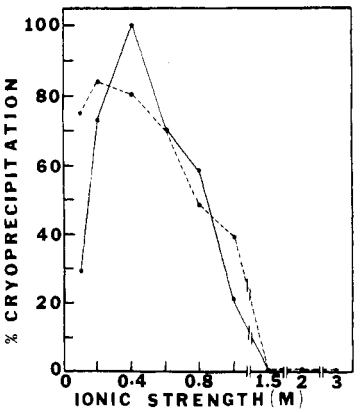

Figure 9. Effect of ionic strength on the cryoprecipitation amounts of cryoprotein I (—) and cryoprotein II (---). The samples were made to different ionic strengths by using solid NaCl and incubated at 2 °C for 24 h. The resulting precipitates were separated by centrifugation, and the supernatants were analyzed for their protein content.

Effect of pH on Cryoprecipitation. pH effects on protein precipitation at 23 and 2 °C are shown in Figure 8. Most of the peanut seed proteins precipitate when exposed to pH values below 6.5 (Basha and Cherry, 1976). Hence, in order to distinguish the pH effects on protein solubility and cryoprecipitation, we carried out incubations at both 23 and 2 °C. At 23 °C almost all the protein precipitated between pH 4 and pH 5.5. Very little precipitation was found at other pH ranges. However, incubation at 2 °C showed two peaks of protein precipitation between pH 4 and pH 6.5 (A) and 7.5 and 9.0 (B), indicating that the precipitation between pH 7.5 and pH 9.0 is due to cryoprecipitation, while between pH 4 and pH 6.5 it is due to acidity. Further, it should be noted that the area under peak A increased at 2 °C compared to that at 23 °C. Except for the higher precipitation of CP I between pH 2 and pH 3.5 at 2 °C, no major differences were found between the CP I and CP II precipitation patterns.

Effect of Ionic Strength. Figure 9 illustrates the effects of ionic strength on cryoprecipitation of CP I and CP II. Maximum cryoprecipitation was obtained at an ionic strength of 0.4 M for CP I and 0.2 M for CP II. No cryoprecipitation was observed at and above a 1.5 M NaCl concentration. CP I showed a sharp decrease in the cryoprecipitation below and above 0.4 M, while CP II showed a gradual decrease below and above 0.2 M. Loss of cryoprecipitation at ionic strengths above 1.5 M and below 50 mM indicates the involvement of ionic interaction in the cryoprecipitation phenomenon of the proteins.

Action of Proteases. When the cryoproteins were incubated with trypsin (1:500 protein–trypsin), the cryoprecipitation property was lost within 20 s. Similarly, treatment of the cryoproteins with papain (1:10 protein–papain) also caused a loss in their cryoprecipitation property. Additional studies have indicated (data not shown) that limited proteolysis, although it destroyed the cryoprecipitation property, did not cause a significant change in the molecular weight of the cryoproteins. This suggest that limited proteolysis might have slighly altered the conformation of the protein molecules, preventing polypeptide interaction and leading to the loss of cryoprecipitation property. This would also indicate that the integrity of the native molecule is essential to maintain the cryoprecipitation property and requires polypeptide cleavage at specific points to destroy this property, as evidenced by the differential susceptibility to the protease treatment.

CONCLUSIONS

The results obtained in this study show that peanut seed contains two types of cryoproteins. These cryoproteins differ in their molecular weights, protein composition, appearance, and certain cryoprecipitation properties. However, except for slight quantitative differences, their polypeptide composition and amino acid content were similar. Daussant et al. (1969) and Neucere (1969) have suggested that the cryoprecipitation property occurs exclusively for α-arachin. Comparison of cryoproteins I and II with the arachin and conarachin enabled us to make the following obervations: (a) not all the proteins of arachin exhibit cryoprecipitation; (b) the protein composition of the cryosupernatant was different from that of the cryoprecipitate; (c) the protein and polypeptide compositions of the cryoprecipitate were different from those reported (Neucere, 1969; Dawson, 1971; Basha and Cherry, 1976) for α-arachin and conarachin; (d) unlike the cryoproteins I and II, the cryoproteins obtained following cooling of the 30% ammonium sulfate precipitate (arachin-rich fraction) were nonspecific and unstable; (e) further, the cryoproteins I and II remained highly soluble, even after repeated freeze–thawings and exposure to low ionic strength buffers (10 mM). We believe that extraction and fractionation of peanut proteins in high ionic strength buffer at 23 °C prevented any protein aggregation and nonspecific precipitation and facilitated separation of the two cryoprotein species.

Although the function of the cryoprecipitation property of the proteins in the peanut seed is not known, it is possible that this property might have a role in protecting the seed reserves under adverse environmental conditions and maintaining the seed viability and availability of seed reserves for the growing embryo. For example, in the animal system only certain classes of human immunoglobulins exhibit cryoprecipitation, and the amount of the cryoglobulin increases in response to certain diseases (Middaugh et al., 1978). Likewise, in peanut seed also only certain classes of arachin may possess the cryoprecipitation property.

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