in the presence of chelators (EDTA and/or phytic acid) accelerate the formation of DMA. These observations are consistent with those of Craig et al. (1961), who discussed tertiary amine oxide rearrangement in the presence of Fe^{2+}, Fe^{3+}, and organic constituents, and Ferris et al. (1967), who found that Fe^{2+} catalyzed the degradation of TMAO but that Fe^{3+} did not. In this respect, no means (except severe heating) has been found to inhibit the formation of DMA in frozen gadoid muscle. On the contrary, several accepted food additives such as EDTA and SO_2 accelerated its formation—the same may be true for some antioxidants. Because the presence of DMA has been related to fish quality problems in frozen fish (Babbitt et al., 1972; Castell et al., 1973a) and potential problems of public health significance (Singer and Lijinsky, 1976; Ember, 1980), more work is required to define its formation and, in particular, its inhibition in processed fishery products.

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Identification of Methionine-Rich Polypeptides in Peanut (*Arachis hypogaea* L.) Seed

Shaik-M. M. Basha* and Sunil K. Pancholy

Peanut seed protein was extracted from the defatted meal with a buffer containing 2 M NaCl, 0.01 M Tris-HCl (pH 8.2), and 0.002% (w/v) NaN_3. The protein extract was then separated into 10 fractions by gel filtration on a Sephacryl S-300 column. Amino acid analysis of the protein eluants from the column showed 1.4%, 2.9%, and 1.2% methionine in fractions V, VI, and VII, respectively. In addition to methionine, fractions VI and VII were found to be rich in cystine (10.75% and 6.52%, respectively). Methionine-containing proteins were also identified by labeling the seed proteins with [^{35}S]methionine, 18 weeks after planting. The [^{35}S]methionine incorporation data was found to be consistent with the methionine content of the fractions which also showed the highest amount of radioactivity in fraction VI. Gel electrophoresis revealed that all the proteins of fraction VI contained [^{35}S]methionine radioactivity. However, the amount of radioactivity varied among the polypeptides of fraction VI. Autoradiographs of two-dimensional polypeptide maps from fraction VI indicated the presence of four major and two minor radioactive polypeptides with different isoelectric points and molecular weights.

Peanut seeds have an excellent potential as a source of plant protein for incorporation into a variety of foods (Dechary and Altschul, 1966; Johnson and Lay, 1974). However, like other legumes, peanuts are also low in sulfur-containing amino acids like methionine and cystine. Many studies have shown that the nutritional value of peanut proteins (arachin and conarachin) differs considerably because of higher amounts of methionine, lysine, and cystine in the conarachin fraction than in the arachin fraction (Sauberlich et al., 1948; Horn and Blum, 1956; Woodham and Dawson, 1968; Basha and Cherry, 1976). These observations suggest that identification of cultivars with higher amounts of conarachin would lead to an improvement in the nutritional level of peanut proteins. Limited exploratory research has shown that there are genotypes within the species *hypogaea* with a better balance of nutritionally important amino acids than that

Peanut Protein Laboratory, Division of Agricultural Sciences, Florida Agricultural & Mechanical University, Tallahassee, Florida 32307.

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found in the commercial cultivars (Heinis, 1972; Young et al., 1973; Pancholy et al., 1978). Although the arachin and conarachin fractions have been subjected to numerous studies (Jones and Horn, 1930; Johnson and Shooter, 1950; Dechary et al., 1961; Evans et al., 1962; Dausant et al., 1969; Dawson, 1968; Shetty and Rao, 1974; Basha and Cherry, 1976; Neucere and Conkerton, 1978), very little information is available on their polypeptide composition and relative nutritional importance of individual polypeptides.

Since, methionine is an essential amino acid and is deficient in peanuts, a study was initiated to identify and isolate the peanut polypeptides that are rich in methionine. For this purpose, peanut proteins were fractionated by gel filtration and the individual protein fractions were tested for methionine content. Methionine distribution in seed proteins were also determined by labeling the seed proteins with [^{35}S]methionine.

MATERIALS AND METHODS

Seed Material. Cotyledons from peanut (*Arachis hypogaea* L.) cultivar Early Bunch were ground in a mortar with a pestle. The ground meal was defatted with cold diethyl ether and the resulting meal was stored at −20 °C until used.

Protein Extraction. Two grams of defatted peanut meal was homogenized in 10 mL of 2 M NaCl, 0.01 M Tris-HCl (pH 8.2), and 0.002% (w/v) NaN_3 by using a mortar and pestle. The homogenate was stirred for 15 min, squeezed through a layer of miracloth (Calbiochem, CA) and cheesecloth, and centrifuged at 20000*g* for 20 min at 10 °C. The resulting clear supernatant was decanted and used for further analysis.

Protein Separation. Protein was separated by gel filtration on a Sephacryl S-300 (Pharmacia, Uppsala, Sweden) column (2.5 × 135 cm) which was equilibrated at room temperature with 0.5 M NaCl, 0.01 M Tris-HCl, pH 8.2, and 0.002% NaN_3. The flow rate (10 mL/h) of the column was regulated by using a peristaltic pump. About 8 mL of the protein extract was applied on the column and eluted in 5-mL fractions. Protein content of the fractions was monitered by measuring their absorption at 280 nm. The fractions under each protein peak were pooled and designated as fractions I–X. The 10 pooled fractions were then dialyzed extensively against distilled deionized water, lyophilized, and stored at −20 °C.

Amino Acid Analysis. Protein (5 mg) from the above 10 fractions was hydrolyzed by using 6 N HCl at 110 °C for 18 h. The hydrolysates were neutralized, diluted (500 μg/mL), and analyzed for amino acid composition on a dual-column JEOL automated amino acid analyzer (Pancholy et al., 1978).

[^{35}S]Methionine Incorporation. [^{35}S]Methionine (1046 Ci/mmol; 5 μCi/cotyledon) was injected into the peanut cotyledons (18 weeks after planting) while the pods were still attached to the plant. After injection, the pods were bagged and shaded with the branches. After a 4-h incubation, the plants were harvested, and the pods collected and transported to the lab on ice. The pods were shelled, and after removing the testae and embryonic axes, the cotyledons were lyophilized. The lyophilized cotyledons were ground into a meal and defatted with cold diethyl ether. The defatted meal was stored at −20 °C.

One-Dimensional Polyacrylamide Gel Electrophoresis. Protein from the defatted peanut meal was extracted with 0.01 M Tris-HCl buffer (pH 8.2) containing 2 M NaCl. After centrifugation, the supernatant was diluted and electrophoresed in 7.5% (w/v) acrylamide gels under nondenaturing conditions (Davis, 1964). For sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis, the protein extracts were made to 2% (w/v) $NaDodSO_4$, 1.5% (w/v) dithiothreitol, and 1.2% (w/v) Tris, and the proteins were dissociated by boiling for 3 min. The dissociated samples were dialyzed against 0.01 M Tris-HCl (pH 6.9) and 0.1% (w/v) $NaDodSO_4$ for 16 h. The dialyzed material was electrophoresed in 10% (w/v) acrylamide gels containing 0.1% $NaDodSO_4$ according to the method of Laemmli (1970). After electrophoresis the proteins were stained with Coomassie blue R-250 and destained with 7% acetic acid and 10% methanol. The gels were scanned at 600 nm in a Beckman Model 25 spectrophotometer equipped with a gel scanner using a 0.05-mm slit.

Radioactivity Determination. The amount of radioactivity in the samples was measured in a Packard scintillation counter using a scintillation fluid containing 0.33% (w/v) 2,5-diphenyloxazole (PPO) and 0.008% (w/v) 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl-POPOP) in toluene and Triton X-100 (2:1 v/v). Radioactivity in the polyacrylamide gels was determined by sectioning them into 1 mm thick slices using a Bio-Rad Model 190 gel slicer. Each slice was transferred to a scintillation vial and digested for 24 h with 0.1 mL of H_2O_2 (30%) at 70 °C. The gel digests were taken up in 0.5 mL of water and the radioactivity was measured after the addition of 5 mL of scintillation fluid (Basha et al., 1979).

Two-Dimensional Polyacrylamide Gel Electrophoresis. The protein fractions obtained after Sephacryl S-300 column chromatography were dissolved (10 mg/mL) in an alkaline–urea solution containing 9.3 M urea, 0.005 M K_2CO_3, 0.5% (w/v) dithiothreitol, and 2% (v/v) Nonidet P-40. The proteins (200-300 μg) were then subjected to two-dimensional gel electrophoresis according to the method of Basha (1979) and Horst and Roberts (1979). The acrylamide concentration of IEF gels was 4% (w/v) and of $NaDodSO_4$ gels was 10% (w/v). After electrophoresis, the proteins were stained with Commassie blue R-250 and destained with 7% acetic acid containing 10% methanol.

Autoradiography. The destained two-dimensional gels were dried by using a Bio-Rad slab gel dryer, and autoradiographs of gels were prepared by exposing them to Kodak XRP-1 X-ray film. After a 1–2-week exposure at −20 °C, the film was processed and autoradiographs were obtained.

RESULTS AND DISCUSSION

Gel Filtration. Fractionation of salt-soluble proteins on Sephacryl S-300 column yielded five major and five minor protein peaks (Figure 1). Protein fractions under each peak were pooled, dialyzed, and analyzed. Our preliminary studies based on the electrophoretic analysis of the protein fractions, obtained after gel filtration of purified arachin (Johns and Jones, 1916), showed that arachin eluted mainly in fractions III and IV. Small amount of high molecular weight (~800 000) arachin was also found in the region of fraction II and may represent the aggregated form of arachin. These results also indicate that the proteins eluted in fractions I and V–X contained mainly the nonarachin proteins. A detailed study on the characterization of these fractions will be presented elsewhere and does not constitute part of this study.

Amino Acid Composition. As seen in Table I, the amino acid composition of fractions II–IV were similar and resembled that of total peanut seed protein having high amounts of aspartic acid (12.4%), glutamic acid (18.8%), and arginine (13.8%). Amino acid composition of these fractions is similar to the one reported for arachin by Dawson (1968). Only fractions V–VII contained any significant amount of methionine. For example, fraction VI

Table I. Amino Acid Composition of the Ten Peanut Protein Fractions

amino acid	composition, g/100 g of protein										
	I	II	III	IV	V	VI	VII	VIII	IX	X	total
lysine	5.74	3.46	2.82	4.19	6.94	2.38	5.33	7.00	7.15	6.48	3.49
histidine	3.56	3.08	2.31	2.81	2.81	1.56	2.22	2.34	1.67	2.38	2.46
NH_3	3.27	3.36	2.15	2.05	1.46	2.84	1.54	3.90	13.26	5.26	2.37
arginine	8.27	13.64	13.87	12.89	6.81	15.61	8.85	10.26	8.58	8.64	12.39
aspartic acid	8.11	11.90	12.42	12.48	9.86	10.57	10.11	11.50	11.55	11.80	11.98
threonine	8.26	3.97	3.81	4.19	7.37	1.72	5.31	4.83	3.08	4.68	4.33
serine	4.70	4.07	3.84	4.21	5.22	3.87	3.84	6.21	2.81	4.03	4.81
glutamic acid	11.23	18.36	18.83	19.28	12.29	22.52	14.72	17.16	12.43	16.34	18.79
proline	4.35	4.91	6.11	6.26	6.31	4.30	5.94	1.52	0.88	0.94	5.16
glycine	7.47	4.21	4.09	4.48	13.06	3.28	7.46	5.80	4.73	6.05	6.00
alanine	7.11	4.03	4.26	4.16	4.33	2.08	4.08	4.37	7.70	7.27	4.62
cystine	T[a]	0.10	T	T	1.12	10.75	6.52	0.74	T	T	T
valine	6.24	4.43	3.56	5.12	5.18	1.66	4.00	4.37	3.96	4.03	4.21
methionine	0.17	0.06	0.08	0.54	1.40	2.93	1.29	0.51	T	T	0.74
isoleucine	4.22	3.60	3.78	4.23	3.30	1.82	3.10	3.31	1.71	3.31	3.18
leucine	8.40	6.67	7.32	7.35	5.44	6.18	7.62	8.01	7.92	8.21	6.38
tyrosine	3.99	4.10	4.51	3.58	3.77	3.12	3.98	4.55	3.96	3.17	3.90
phenylalanine	5.18	5.53	6.31	6.01	3.75	1.89	4.22	3.45	4.73	5.76	5.19

[a] T = trace.

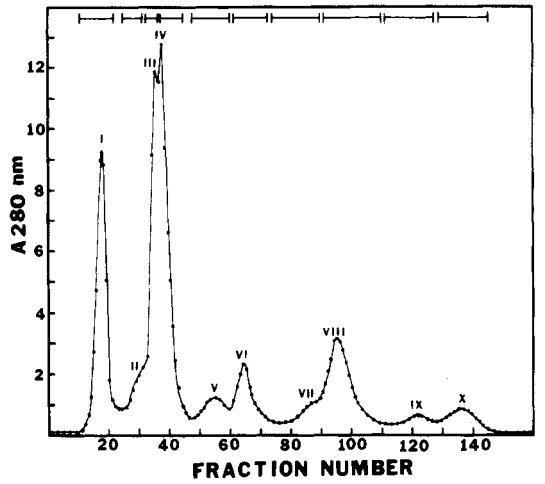

Figure 1. Elution profile of peanut salt-extractable proteins on a Sephacryl S-300 column (2.5 × 135 cm). The column was equilibrated with 0.5 M NaCl and 0.01 M Tris-HCl (pH 8.2) buffer at room temperature. Eight milliliters of the protein extract was loaded on the column and eluted in 5-mL fractions. Protein under each peak was pooled and assigned the following fraction numbers: 11–22 (I), 25–31 (II), 32–35 (III), 36–45 (IV), 48–60 (V), 61–73 (VI), 74–90 (VII), 91–110 (VIII), 111–128 (IX), and 129–146 (X).

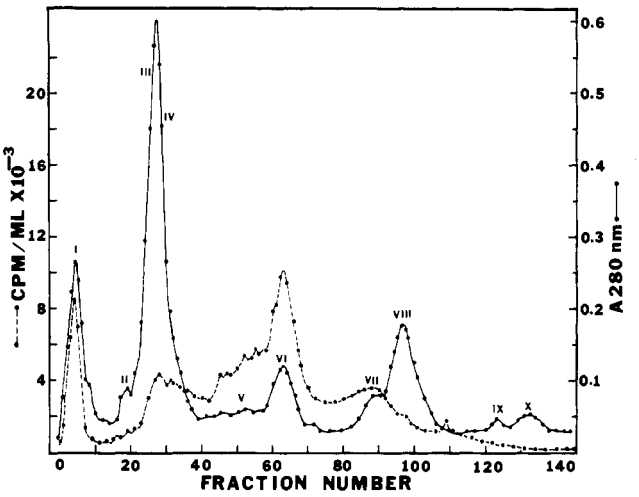

Figure 2. Gel filtration of [^{35}S]methionine-labeled peanut proteins on a Sephacryl S-300 column (2.5 × 135 cm). Seed proteins were extracted with 2 M NaCl and 0.01 M Tris-HCl (pH 8.2) buffer and fractionated on the column. An aliquot (0.1 mL) of the sample was taken from each fraction and counted for its radioactivity content.

contained as much as 2.93% methionine, while fractions V and VII had 1.4% and 1.29% methionine, respectively. Further, fraction VI had 4-fold more methionine than the total peanut seed protein (0.74% methionine). In addition to methionine, fraction VI also contained a large amount of cystine (10.75%), followed by fraction VII (6.5%). As seen in Table I, fractions I, III, IV, IX, and X contained only trace levels of cystine. These data thus suggest that in peanut seed, mainly fraction VI proteins are enriched with sulfur amino acids such as methionine and cystine. Although the fraction VI proteins contained higher amounts of sulfur amino acids, the levels of other amino acids such as histidine, threonine, glycine, alanine, valine, isoleucine, and phenylalanine were lower compared to those of the total peanut seed protein. On the other hand, fractions V and VII appeared to contain a favorable amino acid composition, with relatively high amounts of the methionine, cystine, and other essential amino acids. However, higher amounts of cystine and methionine found in fractions V and VII could be due to their contamination with fraction VI proteins. Thus, two-dimensional electrophoretic examination revealed (data not shown) the presence of a small amount of fraction VI proteins in fractions V and VII. Because of the desirable amino acid composition of fractions V–VII, it is possible to obtain a large quanity of protein (19% of total protein) having balanced amino acid composition by mixing the three fractions together. It is interesting to note that fractions V–VII constituted the nonarachin proteins of peanut seed. These findings are in agreement with the previous studies (Sauberlich et al., 1948; Horn and Blum, 1956; Woodham and Dawson, 1968; Basha and Cherry, 1976) which reported that nonarachin proteins are nutritionally more valuable than the arachin.

[^{35}S]Methionine Incorporation. The methionine distribution in peanut poteins was also examined by labeling the seed proteins with [^{35}S]methionine. The [^{35}S]methionine-labeled peanut seed proteins were separated by gel filtration on a Sephacryl S-300 column, and the individual fractions were counted for their radioactivity content. The radioactivity data showed (Figure 2) the presence of large amounts of [^{35}S]methionine mainly in the regions of fractions I and VI. The high level of [^{35}S]-methionine in fraction VI is consistent with the amino acid composition data (Table I) which showed high methionine

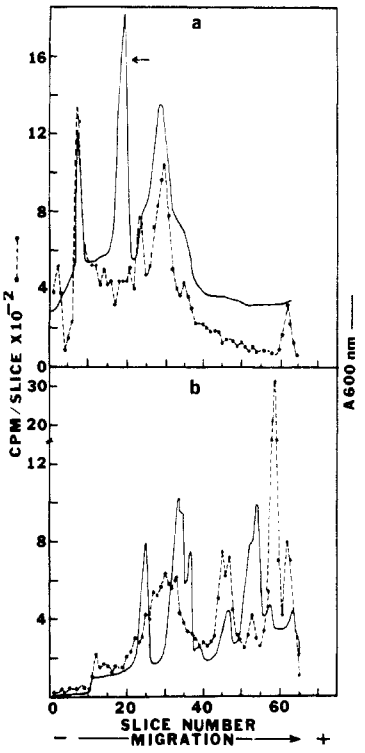

Figure 3. Radioactivity pattern along polyacrylamide gels after electrophoretic analysis of total peanut proteins labeled with [^{35}S]methionine. Part a is a nondenaturing gel of the total seed protein. Part b is a $NaDodSO_4$–polyacrylamide gel of total seed protein. After electrophoresis, the gels were sliced and digested with H_2O_2, and their radioactivity content was determined. Note the lack of radioactivity in the arachin band (arrowed) in part a.

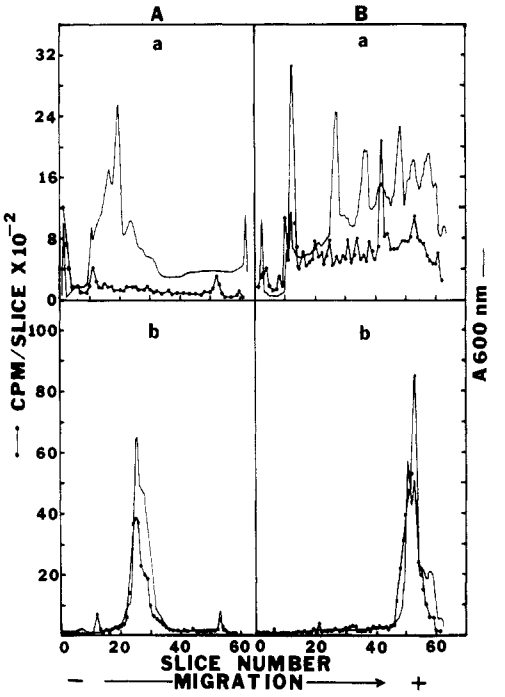

Figure 4. Protein and radioactivity profiles of fractions I and VI after polyacrylamide gel electrophoresis under nondenaturing (A) and denaturing (B) conditions. Part A represent the nondenaturing gels of fraction I (a) and fraction VI (b). Part B represents the $NaDodSO_4$ gels of fraction I (a) and fraction VI (b). Note that in fraction VI [A(b) and B(b)] radioactivity coincided with the stained protein bands. All the gels were sliced and digested with H_2O_2, and their radioactivity content was estimated.

(2.9%) in fraction VI. Interestingly, fraction I, which had only 0.17% methionine, contained a second peak of [^{35}S]methionine radioactivity. Since fraction I contained mainly the nucleic acids and ribosomes (data not shown), it is possible that the radioactivity found in this fraction may be due to the nascent polypeptides of fraction VI still associated with the protein synthesizing system. The radioactivity levels found in fractions V and VII are in agreement with their amino acid composition (1.4% and 1.29%, respectively). Similarly, trace levels of methionine found (Table I) in fractions II, III, IX, and X are consistent with the observed low levels of radioactivity in these fractions.

Gel Electrophoresis of Total Seed Proteins. Distribution of [^{35}S]methionine radioactivity into various protein components was also determined by electrophoresis of [^{35}S]methionine-labeled proteins. After electrophoresis, the gels were sliced and counted for their radioactivity content. Thus, electrophoresis of the seed protein extracts under nondenaturing conditions showed (Figure 3a) three radioactivity bands with R_m values of 0.1, 0.38, and 0.48. Most of the radioactivity found in the gels was associated with the nonarachin proteins while none was found with arachin (arrowed). The radioactivity found on top of the nondenaturing gels may be associated with the proteins that failed to enter the gel or with the high molecular weight components (fraction I). However, upon dissociation of the seed proteins with $NaDodSO_4$ and dithiothreitol and electrophoresis in $NaDodSO_4$ gels, no radioactivity or protein was found on top of the gel (Figure 3b), suggesting that $NaDodSO_4$ and dithiothreitol effectively dissociated the proteins and protein complexes and enabled their entry into the gel. The $NaDodSO_4$ gels showed (Figure 3b) several protein bands with varying amounts of radioactivity. Further, the polypeptides that migrated (arrowed) slightly behind the tracking dye contained 6-fold more radioactivity than the other polypeptides.

Gel Electrophoresis of Protein Fractions. Protein composition and [^{35}S]methionine distribution in different proteins/or polypeptides of the major radioactive fractions (I and VI) was determined by polyacrylamide gel electrophoresis under nondenaturing and denaturing conditions. As seen in Figure 4A(a), after electrophoresis under nondenaturing conditions, most of the radioactivity from fraction I (nucleic acid fraction) remained on top of the gel. Coomassie blue stained gels of fraction I, however, indicated that fraction I did contain some proteins that were able to enter the gel. Lack of radioactivity in these protein bands suggests that the radioactivity in fraction I is mainly associated with the proteins that failed to enter the gel. Failure of fraction I proteins to enter the 7.5% acrylamide gels is apparent since fraction I which eluted in the void volume of the Sephacryl S-300 column (exclusion limit greater than 1.5×10^6) mainly contains high molecular weight nucleic acids and protein associated with the nucleic acids. Thus, under the $NaDodSO_4$ gel electrophoresis system, most of the radioactivity from fraction I entered the gel and showed one major and several minor radioactivity peaks [Figure 4B(a)]. The $NaDodSO_4$ and dithiothreitol used in this sytem effectively dissociated the proteins and any nucleic acid–polypeptide associations and facilitated the polypeptide entry into the $NaDodSO_4$ gels. Fraction VI, on the other hand, showed two major radioactivity peaks and two protein bands with identical mobilities [Figure 4A(b)], indicating that all of the proteins of fraction VI were radioactive. Similarly, $NaDodSO_4$ gel electrophoresis of fraction VI showed four radioactive peaks (two major and two minor) and four protein bands with identical mobilities [Figure 4B(b)]. The observed

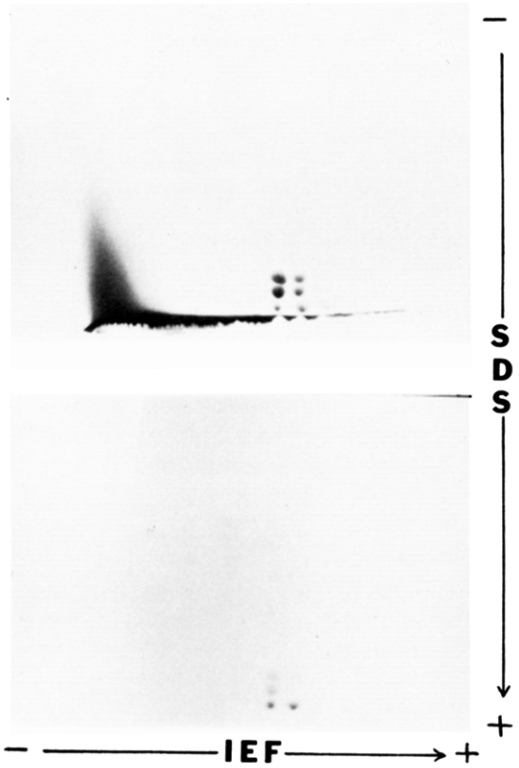

Figure 5. Two-dimensional gel electrophoretic separation of fraction VI proteins labeled with [^{35}S]methionine. Part a (top) shows the polypeptide composition of fraction VI as detected by Coomasie blue staining of the slab gel. Part b (bottom) is an autoradiograph of the two-dimensional gel of fraction VI proteins. Note that all polypeptides of fraction VI contained radioactivity to various degrees.

variations in the radioactivity amounts among the protein bands suggest that all the polypeptides of fraction VI may not contain similar amounts of methionine. However, since the labeling was done at one stage of seed maturation, the possibility of different synthetic rates for various polypeptides of fraction VI during seed development cannot be precluded.

Autoradiography. Although one-dimensional gel electrophoresis showed quantitative differences in the radioactivity content of fraction VI proteins, it failed to completely resolve the proteins of fraction VI due to the similarities in their electrophoretic mobilities and molecular weights. Hence, polypeptides of fraction VI were resolved by two-dimensional gel electrophoresis, and then the presence of [^{35}S]methionine in the individual polypeptides was determined by autoradiography of the gel. Unlike the one-dimensional gel electrophoresis, the two-dimensional gels clearly resolved the polypeptides of fraction VI and showed four major and two minor radioactive spots with different isoelectric points (pI's) and molecular weights (M_r's) (Figure 5). The autoradiographs and Coomasie blue stained protein patterns of fraction VI were very similar, but the amount of radioactivity in the polypeptides varied. The radioactivity distribution in the total peanut protein was also tested by autoradiography of their two-dimensional polypeptide gels. The autoradiographs of total protein showed (data not shown) that except for trace levels of radioactivity in other polypeptides, most of the radioactivity was present in fraction VI polypeptides. This would again suggest that the second peak of radioactivity found in fraction I may be due to the nascent polypeptides of fraction VI still associated with the nucleic acids. The results of this study indicate that in peanut seed a large amount of methionine is concentrated in fraction VI proteins. Further, the fractionation method employed in obtaining the methionine-rich proteins (fraction VI) is simple and will enable isolation of large amounts of this (these) protein(s) for possible food application. In view of their amino acid composition, the fraction VI proteins should improve the nutritional properties of food systems in which they can be incorporated. However, information on the physicochemical and functional properties of these proteins is necessary for their successful utilization in food systems. Numerous studies have been conducted on the utilization of plant proteins in the development of food products (Wolf, 1970; Circle and Smith, 1972; Glicksman, 1976; Hermansson, 1978; Kinsella, 1979; Lucas, 1979; Bookwalter et al., 1979; Hellendoorn, 1979). Further, identifiction of methionine-rich proteins would be also of great value for screening the peanut germ plasm to identify lines with high amounts of nutritionally desirable proteins.

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