Deposition Pattern of Methionine-Rich Protein in Peanuts

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The methionine-rich protein (MRP) accumulation pattern in peanut (*Arachis hypogaea* L. cv. Florunner) seed was studied by using seeds of different maturities from plants harvested at weekly intervals for 9 weeks 95 days after of planting. Immature seed contained less MRP than the mature seed. Maximum MRP accumulation occurred between the first and third maturity stages, and accumulation from the third to sixth maturity stage was minimal. In general, seeds from later diggings contained higher amounts of MRP than the seeds from early diggings. Examination of MRP polypeptide maps from maturing seeds showed that these polypeptides accumulated in varying amounts during maturation.

INTRODUCTION

Like other legumes, peanuts are low in sulfur-containing amino acids such as methionine and cystine (Young et al., 1973; Young, 1979; Pancholy et al., 1978). Considerable variation in the levels of methionine, lysine, and cystine has been found among the various protein fractions of peanut seed (Basha and Cherry, 1976; Horn and Blum, 1956; Sauborlich et al., 1948; Woodham and Dawson, 1968). In their efforts to detect nutritionally rich proteins, Basha and Pancholy (1981a) identified and isolated a methionine-rich protein (MRP) from Florunner peanut seed having 2.9% methionine and 10.8% cystine. This protein was found to contain six polypeptides of molecular weights between 15 500 and 20 000 with isoelectric points between pH 5.6 and 6.2. Analysis of seeds of different species of the genus *Arachis* for methionine-rich proteins showed significant genetic variation in the methionine-rich protein composition (Basha and Pancholy, 1984). Studies on protein deposition pattern in maturing seeds have shown differential accumulation of arachin (the major storage globulin of peanut) and non-arachin proteins (Cherry, 1974; Basha et al., 1976; Basha and Pancholy, 1981b), indicating selective synthesis and modification of certain proteins during seed maturation.

Most of the previous studies were concerned with the major seed globulins of peanut seed. Although MRP from the mature peanut seed has been isolated and characterized, little is known about its synthesis and deposition patterns during seed maturation. Hence, this study was conducted to determine the accumulation pattern of MRP and its polypeptide components during seed maturation.

MATERIALS AND METHODS

Seed Material. Peanuts (*Arachis hypogaea* L. cv. Florunner) were grown during the 1988 season in experimental plots at Florida A&M University, Tallahassee, FL, according to the recommended cultural practices. Randomly selected plants were dug at weekly intervals for 9 weeks between 90 and 160 days after planting; pods were harvested and separated into six maturity categories on the basis of the hull–scrape method of Williams and Drexler (1981). Following the classification pods were shelled, seed coats removed, and the cotyledons freeze-dried. The samples were ground into a meal and defatted with hexane as described earlier (Basha et al., 1976). The defatted meals were stored at −20 °C until use.

Methionine-Rich Protein Quantitation. Defatted peanut meal (50 mg) was extracted with 2 mL of 0.01 M sodium phosphate buffer, pH 7, containing 0.5 M NaCl by using a Polytron homogenizer. The homogenate was centrifuged at 20000*g* for 20 min, and an aliquot (20 μL) of the supernatant was used for protein fractionation using HPLC. The HPLC system consisted of a Waters Model 510 pump, a UV/vis detector, a Model 820 data station, and a Protein-Pak SW 300 column. The run conditions were the same as described earlier (Basha, 1988). The position of the MRP in the column eluates was identified by injecting purified methionine-rich protein prepared from the peanut seed according to the method of Basha and Pancholy (1981a). Comparative analysis of the samples of various maturities for MRP was achieved by summing area counts for peak corresponding to MRP.

Isolation of Methionine-Rich Protein. Defatted peanut meal (2 g) was homogenized in 10 mL of 0.01 M Tris-HCl buffer (pH 8.2) containing 2 M NaCl and 0.002% (w/v) sodium azide by using a Polytron homogenizer. The homogenate was centrifuged at 20000*g* for 20 min, and the protein extract was fractionated on a Sephacryl S-300 column (2.5 × 135 cm), which was equilibrated with 0.5 M NaCl, 0.01 M Tris-HCl (pH 8.2), and 0.002% sodium azide, to obtain the methionine-rich protein (MRP) as described earlier by Basha and Pancholy (1981a). The fractions under the MRP peak were pooled, dialyzed, and used in further studies.

Two-Dimensional Polyacrylamide Gel Electrophoresis (2-D PAGE). Defatted peanut meals from seeds of various maturities were extracted with a solution containing 9.3 M urea, 1.5% (w/v) dithiothreitol, 0.005 M K_2CO_3, and 2% Nonidet P-40. The homogenate was centrifuged at 20000*g*, and the clear supernatant was subjected to 2-D PAGE by the method of Basha (1979). The first dimension consisted of isoelectric focusing, while the second dimension was 10% SDS slab gel. After electrophoresis, the gels were stained with Coomassie Blue R-250 and destained with 7% acetic acid and 10% ethanol.

RESULTS AND DISCUSSION

Peanuts are a good source of plant protein. However, their nutritional value is poor because of the deficiency of the essential amino acid methionine. Although total peanut protein is low in sulfur amino acids, Basha and Pancholy (1981a) identified a protein in peanuts that contains as much as 3% methionine and 10% cystine. Therefore, a potential exists for improving the nutritional value of peanuts by either conventional plant breeding methods or genetic engineering. Since the seed proteins have been found to accumulate at varying rates during seed development (Cherry, 1974; Basha et al., 1976; Basha and Pancholy, 1981b), it was of interest to determine the capacity of the seeds of different maturities to accumulate MRP. For this purpose, peanut pods at harvest are divided into six categories of increasing maturity on the basis of the inner pod colors of white, yellow 1, yellow 2, orange, brown, and black, according to the hull–scrape method of Williams and Drexler (1981). The white category contains the most immature seeds, while the black category contains

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Table I. Changes in the Methionine-Rich Protein (MRP) Content during Peanut Seed Maturation

maturity	peak area, μV s	area %[a]
	Digging 1	
white	34 821	0.54
yellow 1	272 429	3.28
yellow 2	282 122	4.97
orange	293 861	5.12
	Digging 2	
white	60 570	1.05
yellow 1	410 501	4.35
yellow 2	456 757	5.44
orange	755 673	6.85
brown	709 020	5.98
	Digging 3	
white	118 165	2.09
yellow 1	572 837	4.15
yeloow 2	666 792	5.33
orange	650 983	6.61
brown	657 108	6.41
black	672 931	6.07
	Digging 4	
white	127 209	1.56
yellow 1	464 021	5.07
yellow 2	658 686	6.30
orange	697 946	6.27
brown	725 111	6.63
black	773 243	6.28
	Digging 5	
white	234 956	1.09
yellow 1	452 143	4.92
yellow 2	425 325	5.04
orange	720 146	6.48
brown	740 447	6.67
black	786 802	6.99
	Digging 6	
white	198 040	2.69
yellow 1	673 177	5.85
yellow 2	732 619	6.81
orange	752 633	6.99
brown	732 184	6.84
black	754 401	6.61
	Digging 7	
yellow 2	747 203	6.79
orange	767 178	6.46
brown	752 632	6.65
black	758 755	6.39
	Digging 8	
yellow 2	672 994	6.97
orange	739 091	6.38
brown	775 257	6.82
black	690 147	6.51
	Digging 9	
yellow 1	618 093	7.51
yellow 2	603 494	7.93
orange	638 324	7.64
brown	636 494	7.24
black	689 120	6.82

[a] Percent of total peak area.

the most mature seeds. This system of maturity classification is necessary because of the indeterminate growth characteristics of the peanut plant, which produces pods continuously and has pods of varying maturities at a given time. Plants from diggings 1–9 contained, respectively, most immature to most mature pods. It should be noted that the plants from early (1 and 2) and late (7–9) diggings normally do not contain pods of all the maturity categories.

The results showed (Table I) that the MRP content was higher in more mature seed than in the less mature seed. Thus, the white (immature) seed contained a relatively smaller amount (0.5–2.7%) of MRP than the more mature brown and black categories (5.7–7.7%). The MRP content of the seed increased 2-fold (digging 6) to 9-fold (digging 1) between the white and yellow 2 maturity stages, while it increased less than 1.25-fold between the yellow 2 and black stages. This would indicate that maximum accumulation of MRP occurred between the white and yellow 2 stages, and afterward the MRP accumulation was minimal. Comparison of MRP content of seeds of the same maturity category obtained from plants dug at different intervals showed (Table I) that the seeds of the plants obtained from later diggings contained higher amounts of MRP than the seed from earlier diggings. For example, the yellow 2 and orange categories from digging 1 contained 4.97% and 5.12% MRP, respectively, while the seeds of these maturity categories obtained from digging 7 contained 6.79% and 6.46%, respectively. Similar results were observed for the seeds of other maturities. However, these differences in the seeds of similar maturities from different diggings became smaller after digging 2. For example, the MRP content of the orange category from diggings 3–9 was 6.61%, 6.27%, 6.48%, 6.99%, 6.46%, 6.38%, and 7.64%, respectively. These variations, although small, might be due to the differential developmental pattern among the pods because of the changing plant age and indeterminate growth habit of the peanut plant. Moreover, environmental and agronomical factors may also have some impact on MRP accumulation. The data indicated that, in general, seeds from older plants contain higher amounts of MRP than seeds from younger plants. These differences may be due to the more efficient protein sythetic capacity of older plants than younger plants, since the seeds of younger plants contained higher amounts of free amino acids than the seeds from older plants (data not shown). Previous work (Basha et al., 1976; Basha and Pancholy, 1981b) has also shown the presence of a larger free amino acid pool in immature seed than in mature seed.

The accumulation pattern of MRP in relation to other seed proteins is shown in Figures 1 and 2. As seen in Figure 1 and MRP (peak V) peak of white (immature) seed is relatively smaller than the other peaks. However, the MRP peak increased with increasing maturity, with rapid increase occurring between the white and yellow 2 categories. Comparison of protein profiles of seeds of the same maturities obtained from plants of different diggings showed that the MRP peak was larger in seeds from older plants than from younger plants. Thus, the MRP content of the yellow 1 and yellow 2 categories from diggings 2 and 4 (Figure 1) was relatively lower than from diggings 6 and 9 (Figure 2). Examination of developmental changes in other seed proteins showed that the arachin (peak II, the major storage globulin of peanut seed) content of the seed also increased rapidly during seed maturation. This is consistent with our previous findings (Basha et al., 1976; Basha and Pancholy, 1981b). Like the MRP, arachin (peak II) content of the seeds from older plants was relatively higher (Figure 1) than from younger plants (Figure 2), suggesting that seeds from older plants have a higher protein accumulating capacity than seeds from young plants. Seeds from other diggings also showed similar developmental changes in the protein (not shown). Since the MRP content of the seed increased with seed maturation, and also differed among the plants of different ages, it was of interest to determine the compositional differences, if any, in the MRP from seeds of different maturities and from plants of different ages. Our previous studies have shown that the MRP is composed of six poly-

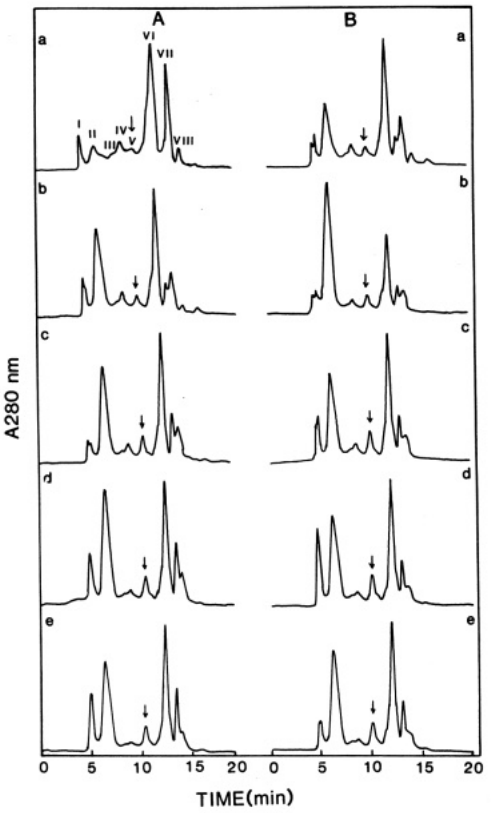

Figure 1. HPLC protein profile of seeds of different maturities from diggings 2 (A) and 4 (B). Peak V represents the methionine-rich protein. a, White; b, yellow 1; c, yellow 2; d, orange; e, brown.

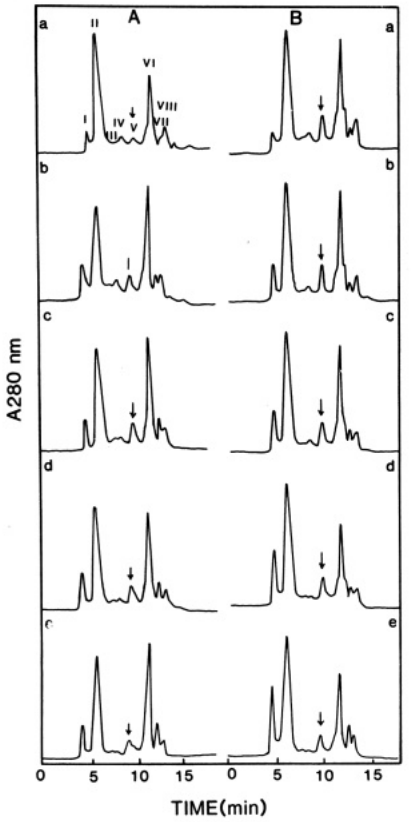

Figure 2. HPLC protein profile of seeds of different maturities from diggings 6 (A) and 9 (B). Peak V contains the methionine-rich protein. a, Yellow 1; b, yellow 2; c, orange; d, brown; e, brown.

peptides with p*I* values between 5.6 and 6.2 and molecular weights between 15 000 and 20 000 (Basha and Pancholy, 1981a, 1984). Figure 3 shows changes in the polypeptide profiles of MRP (boxed) as well as the other seed proteins during seed maturation in seeds obtained from digging 5. The data showed that the immature (white) seed contained four major (a, b, c, f) and two minor (d, e) polypeptides, while in the mature (black) seed all six components (a–f) were present in relatively major amounts. Increase in the intensity of the two minor components (d and e) appeared to be rapid between the white and yellow 2 stages, and afterward the change was minimal. This observation is consistent with the data in the Table I and Figures 1 and 2, which showed rapid MRP accumulation between the white and yellow 2 maturity stages. Comparison of polypeptide profiles of seeds of the same maturity obtained from plants dug at different intervals showed (data now shown) that the seeds from older plants contained more d and e polypeptides than the seeds from younger plants. These data indicate that in the immature seed all the MRP polypeptides do not accumulate simultaneously but are deposited in varying amounts. However, as the seed matures and the plant gets older, all six MRP components are accumulated simultaneously and reach major proportions by the time seed reaches full maturity. Additional studies using [^{35}S]methionine labeling of seeds of different maturities are in progress to determine changes in the synthetic pattern of MRP in maturing peanut seed.

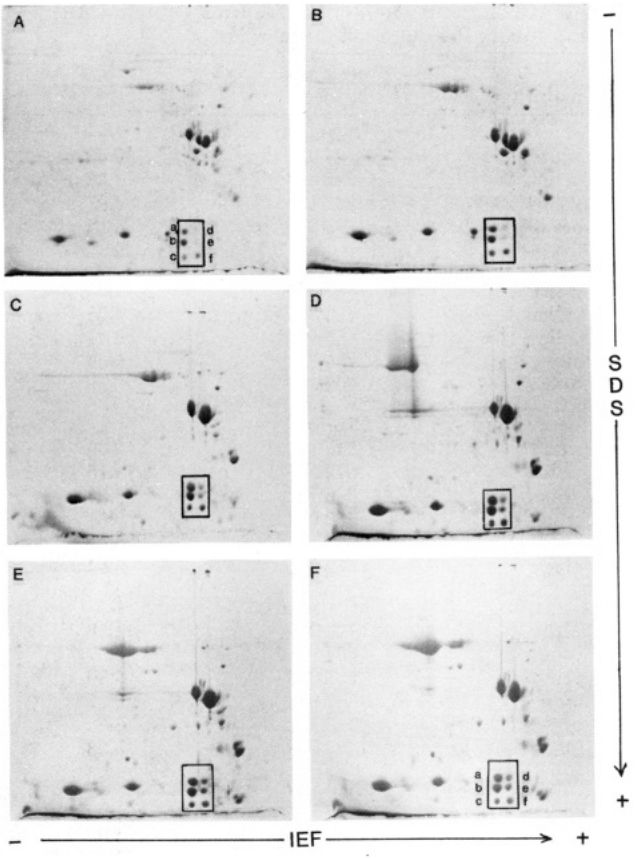

Figure 3. Two-dimensional gel electrophoretic profiles of protein from seeds of various maturities obtained from digging 5. About 300 μg of protein was loaded on each gel. The boxed area in each gel contains the six polypeptides (a–f) of methionine-rich protein. A, White; B, yellow 1; C, yellow 2; D, orange; E, brown; F, black.

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