Methionine Content of the Polypeptides of Methionine-Rich Protein from Peanut

Rama Sri Sathanoori and Sheikh M. Basha*

Division of Agricultural Sciences, Florida A&M University, Tallahassee, Florida 32307

The six subunits of the methionine-rich protein (MRP) from peanut seed were separated using two-dimensional gel electrophoresis followed by western blotting of MRP. The PVDF membrane blots containing the MRP subunits were acid hydrolyzed and analyzed for amino acid composition. The results showed that of the six MRP subunits the low molecular weight (15 500) polypeptides, MRP-5 and MRP-6, contained the highest amounts (3.8% and 4.6%, respectively) of methionine, while the methionine content of the other four subunits was between 1.7% and 1.9%.

Keywords: *Methionine; peanut; protein; subunits*

Peanuts are deficient in sulfur-containing amino acids such as methionine and cystine. Studies on the identification of a peanut cultivar rich in sulfur-containing amino acids have failed to reveal a cultivar with desirable levels of these amino acids (Young, 1979; Pancholy et al., 1978; Young et al., 1973; Heinis, 1971). In this connection, Basha and Pancholy (1981) have isolated a methionine-rich protein (MRP) fraction from mature peanut. It was found (Basha, 1991) that maximum deposition of this protein occurred between the first (white) and third (orange) maturity stages, while it was minimal from the third (orange) to fifth (black) maturity stages. Recently, Bolques and Basha (1994) have purified the peanut MRP and found this to be an acidic protein with an apparent molecular weight of 118 000. The purified MRP was found to contain around 3.5% methionine and 3.1% cystine.

Previous studies using two-dimensional gel electrophoretic (Basha and Pancholy, 1981, 1984; Basha, 1991) analysis of MRP have revealed the presence of six subunits with molecular weights of 15 500, 18 000, and 20 000 and isoelectric points of 5.6 and 6.2. These subunits have been found to accumulate at varying rates during seed development (Basha, 1991). Since the MRP is a relatively large molecule and is composed of multiple subunits, it is necessary to identify the subunit containing the highest amount of methionine. This identification is critical for further work aimed at improving the methionine content of peanut by means of genetic engineering. The objective of this study was to isolate the MRP subunits and determine the amino acid composition of individual subunits for identifying the subunit(s) rich in methionine.

MATERIALS AND METHODS

Seed Material. Cotyledons from peanut (*Arachis hypogaea* L.) cv. Florunner were ground into a powder and defatted with hexane as described earlier (Basha et al., 1976). The defatted powder was stored at −20 °C until use.

Protein Extraction and Fractionation. Defatted peanut meal (3 g) was extracted with 10 mL of 0.01 M Tris-HCl/2 M NaCl, pH 8.2, using a Polytron homogenizer. The homogenate was centrifuged at 20000*g* for 20 min, and the supernatant was collected and used for protein fractionation. Protein fractionation and MRP purification were carried out according to the method of Bolques and Basha (1994) using a Sephacryl S-200 column (2.5 cm × 135 cm). Following the chromatography, the MRP fractions (55−66) were collected, dialyzed, and further purified by ion-exchange chromatography on DEAE (Bolques and Basha, 1994). The purified MRP was dialyzed and concentrated using a Speedvac dryer.

Two-Dimensional Polyacrylamide Gel Electrophoresis (2-D PAGE). The MRP was dissolved in a solution containing 9.3 M urea, 1.5% (w/v) dithiothreitol, 0.005 M K_2CO_3, and 2% Nonidet P-40 and then subjected to 2-D PAGE following the method described earlier (Basha, 1979). The first dimension was isoelectric focusing (IEF), and the second dimension was SDS−gel electrophoresis.

Western Blotting. Following 2-D PAGE, the gel was placed on a PVDF membrane soaked in 2-(4-morpholino)-ethanesulfonic acid (MES) buffer, pH 6.0. The protein was then electroblotted onto the membrane using MES as the transfer buffer at 60 V for 1.8 h. The protein was stained with 40% Coomassie Blue in methanol and destained with 50% methanol. The six polypeptide spots corresponding to the six MRP subunits were cut and used for amino acid analysis.

Amino Acid Analysis. The PVDF membrane containing the MRP polypeptides were cut into 1−2 mm strips, placed in 6 × 50 mm Pyrex test tubes, and hydrolyzed with 6 N HCl containing 1% phenol at 110 °C for 24 h in a Pico-Tag work station (Waters, Milford, MA). The hydrolysates were dried and derivatized with phenyl isothiocyanate (PITC). An aliquot of the derivatized sample was analyzed using an HPLC system equipped with a Pico-Tag stainless steel column, a UV−VIS detector, two Model 510 pumps, and an 820 data station (Basha, 1989). The amino acids were identified and quantified using an external amino acid standard and expressed as relative percentage of total amino acids.

RESULTS AND DISCUSSION

The MRP from peanut has been purified and found to contain relatively higher levels of methionine and cystine compared to other seed proteins (Bolques and Basha, 1994). The MRP was shown to contain six subunits that are accumulated in varying amounts during maturation (Basha, 1991). Our research is aimed at cloning the cDNA(s) corresponding to the MRP with an objective to increase the methionine content of peanut by genetic engineering. The large size (118 kDa) and multiple subunit nature of MRP made amino acid sequencing difficult. Hence, it was necessary to isolate the MRP subunit(s) and obtain their amino acid com-

* Author to whom correspondence should be addressed [telephone (904) 561-2218; fax (904) 561-2221].

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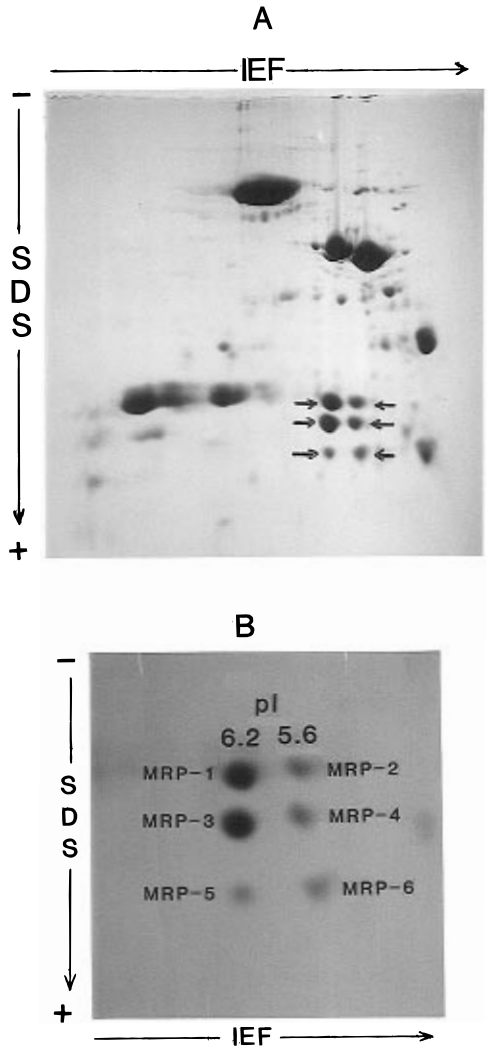

Figure 1. Two-dimensional polyacrylamide gel electrophoretic profile of total peanut seed protein (A) and methionine-rich protein (B). About 150 μg of protein was subjected to isoelectric focusing in the first dimension and SDS−gel electrophoresis in the second dimension. pI, isoelectric point.

position for identifying the subunit/s containing the highest amount of methionine. Identification of the subunit rich in methionine facilitates further characterization of the protein such as amino acid sequencing, and the data obtained can be used in designing the oligonucleotide probes/primers to screen the cDNA library.

2-D PAGE Polypeptide Profile. The polypeptide pattern of the total seed protein and the position of MRP in the seed protein profile are shown in Figure 1. As seen in Figure 1A, the six polypeptides of MRP (shown with arrows) migrated toward the acidic end of the IEF gel and are composed of three pairs of polypeptides. Figure 1B shows the polypeptide profile of the purified MRP following 2-D PAGE and western blotting. For identification purposes these components are labeled MRP-1, MRP-2, MRP-3, MRP-4, MRP-5, and MRP-6. The isoelectric points of these components are 5.6 (MRP-1, -3, -5) and 6.2 (MRP-2, -4, -6), and the molecular weights are 15 500 (MRP-5 and 6), 18 000 (MRP-3 and 4), and 20 500 ± 1000 (MRP-1 and 2).

Amino Acid Composition. The amino acid compositions of the six polypeptides obtained following acid hydrolysis of the western blots are shown in Table 1. In general, the amino acid compositions of the MRP polypeptides were very similar except for a few differences in the amounts of certain amino acids. The quantitative differences in the amino acids are reflective of the variations observed in the molecular weights and isoelectric points of the polypeptides. Differences in the isoelectric point of the MRP polypeptides can be attributed to the varying proportions of acidic and basic amino acids. Comparison of amino acid compositions of the six polypeptides showed that the low molecular weight (15 500) polypeptides, MRP-5 and MRP-6, contained higher amounts (3.8% and 4.6%, respectively) of methionine, compared to the polypeptides MRP-1 through MRP-4 (1.7% and 1.9%). In addition to the methionine, all six MRP polypeptides appear to be rich in glutamic acid, arginine, aspartic acid, and leucine. The histidine and threonine contents of the six MRP polypeptides were relatively lower compared to the other amino acids. The amount of cystine in all of the MRP polypeptides was considerably less than that of the total MRP (3.4%), indicating that the cystine is destroyed during the acid hydrolysis.

Additional studies are in progress involving determination of amino acid sequence and differential expression of these polypeptides during seed development.

Table 1. Amino Acid Composition[a] of MRP Polypeptides from Florunner Peanut

		polypeptides of MRP					
amino acid	total MRP	MRP-1	MRP-2	MRP-3	MRP-4	MRP-5	MRP-6
ASX[b]	12.1	10.5	9.6	10.7	9.8	8.5	9.0
Glx[c]	23.0	19.5	21.0	22.2	22.7	20.3	23.7
serine	7.1	7.6	8.0	7.2	7.9	6.4	7.4
glycine	6.7	7.3	9.0	6.6	9.9	8.3	8.2
histidine	1.2	1.0	1.1	1.1	0.8	T	T
arginine	12.7	13.7	12.0	15.2	12.2	11.8	13.0
theronine	0.9	T[d]	T	T	T	T	T
alanine	3.4	4.8	4.5	4.2	4.2	3.9	2.1
proline	4.8	6.3	6.8	5.8	6.5	4.3	4.4
tyrosine	3.5	5.1	5.1	5.3	5.0	3.9	3.3
valine	2.2	2.3	2.9	2.4	2.7	4.0	5.1
methionine	4.0	1.9	1.7	1.9	1.8	3.8	4.6
cystine	3.4	0.2	T	0.7	T	0.6	T
isoleucine	2.1	2.8	3.0	2.1	3.0	4.5	4.1
leucine	8.4	9.8	9.3	10.3	10.9	8.7	8.2
phenylalanine	1.9	2.5	2.7	1.8	2.7	3.1	2.8
lysine	2.2	2.9	2.9	2.6	3.2	2.1	2.7

[a] Relative percent to total. [b] Asx, asparagine plus aspartic acid. [c] Glx, glutamine plus glutamic acid. [d] T, trace.

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