Purification and characterization of seed storage proteins from Momordica charantia

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Summary. 2 storage proteins have been isolated and purified from seeds of Momordica charantia. These proteins appear to consist of 4 subunits of 5500 daltons and they contain a very high concentration of glutamic acid plus glutamine and arginine.

Plant seed storage proteins usually contain a large amount of glutamine residues to provide a source of nitrogen for the developing seedling[2,3]. 2 low-molecular-weight storage proteins have been isolated and purified from the seeds of Momordica charantia, and shown to conatin a very high concentration of glutamic acid, glutamine and arginine. This report describes the isolation and some characterization of these proteins.

Material and methods. The seeds of Momordica charantia were obtained from Chan Man Hop Seeds Co., Hong Kong. The proteins were extracted from the decorticated, defatted meal with 0.9% NaCl, and precipitated by 95% ammonium sulfate. After centrifugation at 25,500 × g for 1 h the precipitate was dissolved and dialyzed in water. The crude protein extract was further purified by ion-exchange chromatography and gel filtration as described in the results section.

Samples of isolated storage proteins were run on SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoresis in Trisglycine buffer, pH 8.3[4]. The gels were stained with Coomassie brilliant blue. The purified proteins were hydrolyzed in 6 N HCl at 110 °C for 24, 48 and 72 h, and the hydrolysates analyzed with an automatic Beckman amino acid analyzer[5]. The total number of half-cystine residues was determined as cysteic acid after performic acid oxidation[6].

Results. The crude protein extract was passed over a column of DEAE-Sephadex (figure 1a) followed by chromatography of a Sephadex G-150 column. The proteins under peak G2 (figure 1b) were further separated into fractions I and II on a CM-cellulose column (figure 1c). The proteins under peak G1 in figure 1b were shown to be galactose-binding lectins, and will be described elsewhere.

Samples of protein fractions I and II were reduced with β-mercaptoethanol and run on 12.5% and 20% polyacrylamide gel electrophoresis in SDS. Both preparations showed single bands corresponding to polypeptides of an apparent mol. wt of 5500 daltons. As the proteins under peak G2 (figure 1b) were estimated to have a mol. wt of approximately 19,000 daltons on a calibrated Sephadex G-150 column, they appear to consist of four subunits of 5500 daltons.

The amino acid composition of the 2 proteins was calcu-

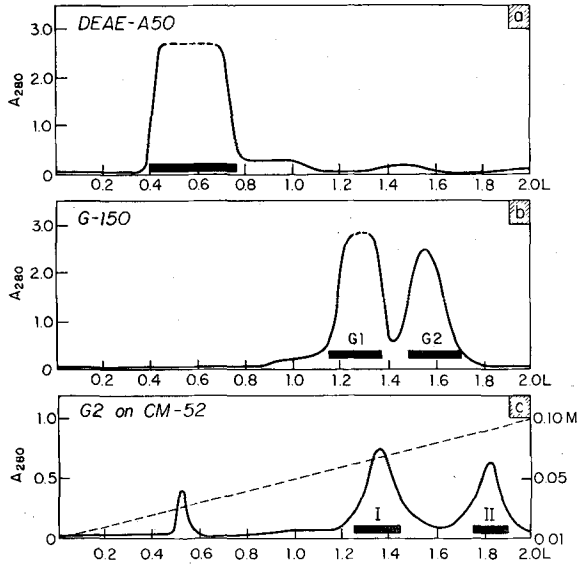

Fig. 1. Purification of storage proteins. a) DEAE-Sephadex chromatography: The column (5 × 70 cm) of DEAE-Sephadex A-50 was pre-equilibrated and eluted with a buffer containing 5 mM sodium acetate, pH 5.8. b) Sephadex G-150 column: The pooled fractions from Figure 1a were chromatographed on a column (5 × 90 cm) of Sephadex G-150 in 0.05 M Tris-HCl/0.1 M NaCl, pH 7.6 buffer. c) CM-cellulose chromatography: The column (5 × 50 cm) was pre-equilibrated and first eluted with 100 ml of the initial gradient. The column was then developed with a linear gradient of sodium posphate buffer (pH 6.5, 0.01 M – 0.10 M, 950 ml each).

Fig. 2. SDS-polyacrylamide gel (12.5%) electrophoresis of protein fractions I (left) and II (right).

1 Acknowledgment. The author wishes to thank Ms M. Diane Forde for skillful assistance, Drs J. Michael Kehoe and Thomas T.-S. Lin for discussion and reading the manuscript. This investigation was supported by NIH grants CA18621 and AI09810.

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Amino acid composition

Protein fractions Amino acids	I Calculated values[a]	integer	moles (%)[f]	II Calculated values	integer	moles (%)
Lysine	–	–	–	0.78	1	1.4
Histidine	1.53	2	3.0	1.64	2	3.0
Ammonia	6.67	7	12.7	5.82	6	10.7
Arginine	9.95	10	19.5	10.62	11	19.5
Aspartic Acid	2.13	2	4.2	1.96	2	3.6
Threonine	–	–	–	–	–	–
Serine	1.95[b]	2	3.8	1.80	2	3.3
Glutamic Acid	17.48	17	34.2	17.49	17	32.1
Proline	1.13	1	2.2	1.98	2	3.6
Glycine	4.47	4	8.8	3.96	4	7.3
Alanine	1.94	2	3.8	2.12	2	3.9
$^1/_2$ Cystine	4.16[c]	4	8.1	4.38	4	8.0
Valine	1.56[d]	2	3.1	1.66	2	3.0
Methionine	0.98	1	1.9	0.96	1	1.8
Isoleucine	0.87[d]	1	1.7	1.10	1	2.0
Leucine	1.97	2	3.9	2.36	2	4.3
Tyrosine	–	–	–	0.61	1	1.1
Phenylalanine	0.93	1	1.8	1.10	1	2.0
Tryptophane[e]						
Total	51.05	51	100.0	54.52	55	99.9

a) Average of values for 24-, 48- and 72-h hydrolysis except where indicated. b) Extrapolated to zero time. c) Determined as cysteic acid. d) From 72-h hydrolysis. e) Not determined. f) Based on calculated values.

lated from the molar ratio and the assumed mol. wt of 5500 daltons (table). They have an almost identical composition except that protein fraction II possesses 4 additional residues of lysine, arginine, proline and tyrosine.

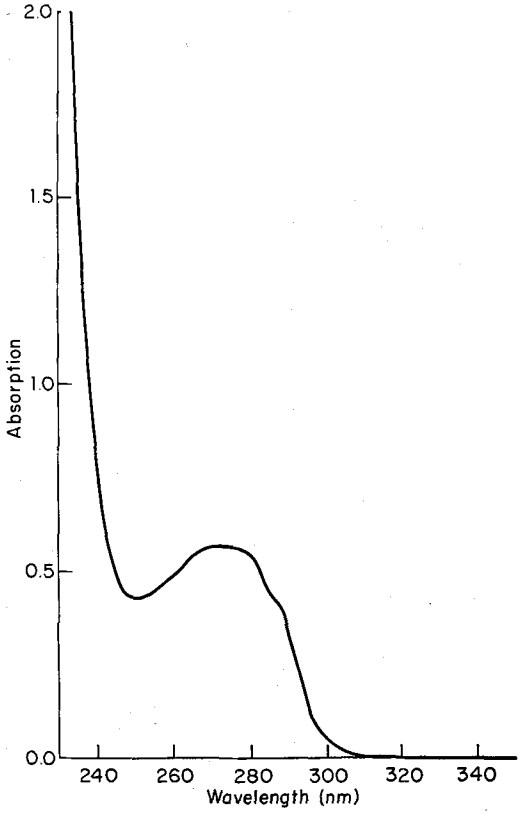

Fig. 3. Near-UV absorption spectrum of protein fraction I.

Both proteins contain 32–34% glutamic acid and 20% arginine. On the basis of ammonia content one third of glutamic acid residues are present as glutamine. The half-cystine residues are also present in a fairly high amount (8%). No threonine residues were detected at all, and no amino-terminal residues were identifiable by automatic Edman degradations[7] on either protein, presumably because of cyclization of N-terminal glutamine to pyrrolidone glutamic acid.

The near-UV absorption spectra of both proteins are very similar, although protein fraction II contains 1 more residue of tyrosine. The absorption spectrum of protein fraction I is shown in figure 3. Its A280 to A260 absorbance ratio is 1.10.

Discussion. 2 storage proteins purified from the seeds of Momordica charantia appear to exist as tetramers composed of subunits being approximately 5500 daltons. Protein fraction I could conceivably be a derivative of protein fraction II, because the 2 proteins showed the same amino acid composition except for the presence of 4 additional residues in fraction II. Furthermore, both proteins showed very similar UV absorption spectra.

Glutamine-rich storage proteins have been isolated from the seeds of many plant species such as wheat[8], rice[9] and rapeseed[10]. Although the seeds are not eaten, the fruit of Momordica charantia is widely used in the Orient.

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