THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 264, No. 12, Issue of April 25, pp. 6655-6659, 1989
Printed in U.S.A.

Complete Amino Acid Sequence and Structure Characterization of the Taste-modifying Protein, Miraculin*

(Received for publication, July 21, 1988)

Sarroch Theerasilp‡, Hiromu Hitotsuya, Shigeo Nakajo§, Kazuyasu Nakaya§, Yasuharu Nakamura§, and Yoshie Kurihara¶

From the Department of Chemistry, Faculty of Education, Yokohama National University, Yokohama 240, Japan and the §Laboratory of Biological Chemistry, School of Pharmaceutical Sciences, Showa University, Tokyo 152 Japan

The taste-modifying protein, miraculin, has the unusual property of modifying sour taste into sweet taste. The complete amino acid sequence of miraculin purified from miracle fruits by a newly developed method (Theerasilp, S., and Kurihara, Y. (1988) *J. Biol. Chem.* 263, 11536–11539) was determined by an automatic Edman degradation method. Miraculin was a single polypeptide with 191 amino acid residues. The calculated molecular weight based on the amino acid sequence and the carbohydrate content (13.9%) was 24,600. Asn-42 and Asn-186 were linked *N*-glycosidically to carbohydrate chains. High homology was found between the amino acid sequences of miraculin and soybean trypsin inhibitor.

Richadella dulcifica is a native shrub of tropical West Africa. It yields red berries which have an unusual property in modifying sour taste into sweet taste. For example, lemons elicit sweet taste after chewing pulps of the berries. Because of this unusual property, the berry has been called "miracle fruit."

Kurihara and Beidler (1) first isolated the active principle of miracle fruit and showed that it is a basic glycoprotein. Brouwer *et al.* (2), Giroux and Henkin (3), and Kurihara and Terasaki (4) also isolated the active principle, and Brouwer *et al.* (2) named it "miraculin." The miraculin samples isolated in all these studies were not completely pure, and hence any attempt to determine miraculin's primary structure has not been made. Recently Theerasilp and Kurihara (5) established a new method to obtain miraculin in very pure form. They showed that it is a basic glycoprotein having a molecular weight of about 28,000 as estimated by SDS-PAGE,[1] while molecular weights reported in previous papers (1–4) ranged from 40,000 to 48,000. Miraculin contained as much as 13.9% of carbohydrate (5).

In the present study, we have determined the complete amino acid sequence of miraculin. It is a single polypeptide with 191 amino acid residues. The calculated molecular weight of miraculin based on the amino acid sequence and the carbohydrate content is 24,600.

EXPERIMENTAL PROCEDURES

Materials—Miracle fruits (*R. dulcifica*) were obtained from plants grown in the green house of Yokohama National University. Miraculin was purified from pulps of the fruits free from seeds and skins as described in the previous paper (5).

The sources of proteases are as follows. *Achromobacter lyticus* protease I (lysyl endopeptidase), Wako Pure Chemicals Industries, Ltd.; 1-chloro-3-tosylamido-7-amino-2-heptanone-chymotrypsin and carboxypeptidase A, Sigma; *Staphylococcus aureus* V8 protease, Miles Laboratories, Inc.; L-1-tosylamido-2-phenylethyl chloromethyl ketone-trypsin, Worthington.

All other chemicals used were of analytical grade.

Preparation of S-Carboxyamidomethylated Miraculin—Seven milligrams of the purified miraculin were dissolved in 5 ml of 0.4 M Tris buffer, pH 8.2, containing 6 M guanidine hydrochloride, 2 mM EDTA, and 60 mM dithiothreitol. The solution was incubated at 37 °C under atmosphere of nitrogen gas for 24 h. Iodoacetamide, 0.2 g, was added to the solution, mixed, let stand at room temperature for 10 min, and then placed in an ice bath for 60 min. The obtained *S*-carboxyamidomethylated miraculin was desalted by using a Sephadex G-25 column (1.6 × 5 cm) equilibrated with 50 mM EDTA.

Enzymatic Cleavage—Lysyl endopeptidase digestion of *S*-carboxyamidomethylated miraculin was performed in 50 mM ammonium bicarbonate buffer, pH 8.0, containing 2 M urea and 2 mM EDTA at 37 °C for 20 h. The protein concentration was 1 mg/ml, and the enzyme:substrate ratio was 1:100 (w/w). The reaction was terminated by addition of HCl to give a final pH 2.0. There was no insoluble material formed after digestion. The solution was injected into a HPLC to isolate the peptides.

Chymotrypsin digestion of the modified miraculin was performed under the same conditions as those for lysyl endopeptidase digestion except that the digestion time was 90 min. The reaction was also terminated in the same manner. There was no insoluble material formed, and the solution was injected into a HPLC to isolate the peptides.

S-Carboxyamidomethylated miraculin was digested by *S. aureus* V8 protease in 50 mM ammonium bicarbonate buffer, pH 7.8, containing 2 M urea, and 2 mM EDTA at 37 °C for 3 h. The protein concentration was 1 mg/ml, and the enzyme:substrate ratio was 1:30, w/w. Precipitates formed after digestion. Solid urea was added to the reaction mixture until the solution became clear. The solution was injected in a HPLC to isolate the peptides.

Peptide Isolation—The peptides in hydrolysates of the enzymatic digestion were separated by HPLC (Tosoh PC 8000) with a TSK-ODS-120T column (0.46 × 25 cm) (Tosoh). The peptides were eluted from the column by a linear gradient of acetonitrile containing 0.05% trifluoroacetic acid at a flow rate of 1 ml/min. The eluted peptides were monitored by measuring absorption at 210 nm, and each peak was collected manually.

Amino Acid Analysis—Amino acid compositions of the peptides were determined by a Waters Picotag system (6). The peptide was hydrolyzed by HCl vapor at 110 °C for 22 h. The obtained amino acids were converted to phenylthiocyanate derivatives, and their contents were analyzed by HPLC using a TSK-ODS-80TM column

* This work was supported by grants from the Ministry of Education of Japan, Mitajiri Chemical Industry Co., and Mishima Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Chemistry, Faculty of Science and Technology, Sri-Ayudhya United Colleges at Pranakornsri-Ayudhya, Ayudhya 13000, Thailand.

¶ To whom all correspondence should be addressed: Dept. of Chemistry, Faculty of Education, Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama, 240 Japan.

[1] The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; LEP, lysine endopeptidase; Ch, chymotrypsin.

(0.46 × 15 cm) (Tosoh). Phenylthiocyanate-amino acid derivatives were eluted from the column by a linear gradient from solution A (3% acetonitrile in 50 mM phosphate buffer of pH 7.0 containing 0.1 M sodium perchlorate) to a mixture of solution A and 40% acetonitrile (1:4, v/v) for 20 min. The elution was carried out at column temperature of 40 °C and at a flow rate of 1 ml/min. The eluted phenylthiocyanate-amino acid derivatives were monitored by measuring absorption at 254 nm.

Sequence Analysis of Protein and Peptides—Amino-terminal amino acid sequence analysis by automatic Edman degradation was performed with an Applied Biosystem Protein Sequencer (model 470A). Phenylthiohydantoin amino acid derivatives were analyzed by HPLC using a TSK-ODS-120T column, 0.46 × 25 cm, as described by Tsunasawa *et al.* (7) or by an Applied Biosystem on-line HPLC system.

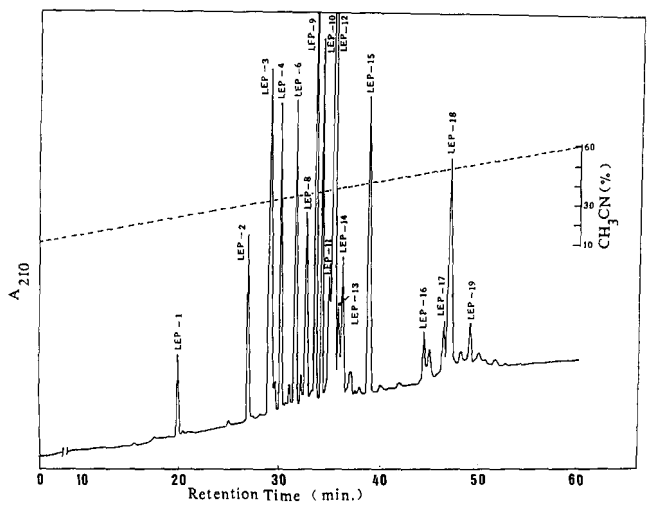

FIG. 1. **HPLC separation of peptides obtained by lysine endopeptidase digestion of *S*-carboxyamidomethylated miraculin.** Separation was carried on a reverse phase column, TSK-ODS-120T (0.46 × 25 cm). The peptides were eluted by a linear gradient of acetonitrile containing 0.05% trifluoroacetic acid from 10 to 60% at a flow rate of 1 ml/min for 1 h. The eluted peptides were monitored by absorption at 210 nm.

Carboxyl-terminal amino acid sequence was determined by using carboxypeptidase A as described by Ambler (8). Miraculin, 200 μg, was dissolved in 0.9 ml of 0.1 M *N*-ethylmorpholine acetate buffer, pH 8.0. Carboxypeptidase A, 1 μg, was added, and the reaction mixture was incubated at room temperature. Aliquots were taken at 15, 30, 60, and 120 min, and the proteins were precipitated by addition of trichloroacetic acid to give a final concentration of 10%. The precipitates were removed by centrifugation, and the supernatant was subjected to analysis of the released amino acids by a Waters Picotag system as described above.

Detection of Carbohydrates—Carbohydrates in peptides were detected by orcinol-sulfuric acid reaction (9). In addition, the carbohydrate-containing peptides (LEP-6 and LEP-12) were subjected to acid hydrolysis and the presence of carbohydrates in the hydrolysate was confirmed by HPLC on an ISA-07/S2504 column (5).

RESULTS

Amino acid sequence from the amino terminus of miraculin to the 46th amino acid residue has been determined with the whole molecule of *S*-carboxyamidomethylated miraculin (Fig. 3). Aspartic acid is the amino-terminal amino acid.

Several peptides were obtained by digestion of the modified miraculin with lysyl endopeptidase (Fig. 1). The peptides were well separated on HPLC except LEP-3 which contained two peptides, LEP-3A and LEP-3B. These peptides were separated from each other upon rechromatography on the same column but using a narrow gradient of acetonitrile and a longer running time.

The sequences of minor peaks (LEP-11, LEP-13, and LEP-14) from the amino-terminal to at least 10 residues toward their carboxyl terminus were identical with that of LEP-12. Therefore these minor peaks seem to be either a part of LEP-12 peptide or LEP-12 peptide with some extending amino acid sequences from its carboxyl terminus. Similarly, peptides from the minor peaks, LEP-16, LEP-17, and LEP-19, had the same sequence as LEP-18 at least 10 residues from their amino terminus toward the carboxyl terminus. Amino acid sequences and amino acid compositions of all major peaks were determined as shown in Fig. 3 and Table I, respectively.

The modified miraculin was cleaved by lysyl endopeptidase

TABLE I

Amino acid compositions of miraculin and peptides obtained from digestion of S-carboxyamidomethylated miraculin with lysyl endopeptidase

Amino acids[a]	LEP-1[b] (170–179)	LEP-2 (159–169)	LEP-3A (1–14)	LEP-3B (145–156)	LEP-4 (134–144)	LEP-6 (180–191)	LEP-8 (170–187)	LEP-9 (57–72)	LEP-10 (105–120)	LEP-12 (15–56)	LEP-15 (121–133)	LEP-18 (73–104)	Miraculin
							residues/molecule[c]						
Asx(D/N)	0.9(1)	1.9(2)	3.4(4)			0.6(1)	1.7(2)	2.7(3)	0.9(1)	2.6(3)	0.9(1)	4.7(5)	20.1(21)
Glx(E/Q)		1.0(1)	1.2(1)		1.8(2)	0.7(1)	0.8(1)	2.1(2)	2.1(2)	1.0(1)	1.2(1)	1.4(1)	11.8(12)
Cys(C)		0.8(1)		2.6(3)	0.8(1)					0.9(1)		0.9(1)	6.5(7)
Ser(S)	0.8(1)		0.9(1)	0.9(1)	0.9(1)		0.9(1)		0.8(1)	0.9(1)	1.8(2)	4.5(5)	12.8(13)
Gly(G)	0.9(1)	1.8(2)	1.1(1)	1.1(1)	1.9(2)		1.4(1)		3.0(3)	4.3(5)	2.1(2)		15.6(17)
His(H)								0.9(1)		1.2(1)			1.8(2)
Thr(T)				0.8(1)		1.4(1)			1.6(2)	6.4(7)	0.9(1)	2.8(3)	14.6(15)
Ala(A)	0.9(1)		1.3(1)			0.6(1)	1.9(2)	1.1(1)		1.3(1)		1.3(1)	6.2(6)
Pro(P)			2.1(2)	1.0(1)		0.5(1)	0.9(1)	2.9(3)		4.4(4)	2.2(2)	1.2(1)	13.9(14)
Arg(R)	2.7(3)						2.8(3)	0.9(1)		4.2(4)		2.7(3)	10.8(11)
Tyr(Y)		0.8(1)			0.7(1)	1.2(1)			1.5(2)	1.7(2)			7.3(7)
Val(V)		1.0(1)	0.9(1)	2.1(2)		1.1(1)		1.2(1)	2.2(2)	7.4(6)		3.7(4)	21.3(19)
Met(M)												1.2(1)	0.9(1)
Ile(I)		2.3(2)	1.0(1)		1.5(1)				1.4(1)	0.8(1)	1.1(1)	1.3(1)	8.4(8)
Leu(L)	2.2(2)		0.9(1)	0.9(1)			2.4(2)	0.9(1)		2.6(3)		2.0(2)	9.5(10)
Phe(F)				0.9(1)	2.1(2)	3.6(4)	2.7(3)	1.9(2)	1.2(1)	1.7(1)	1.0(1)	2.1(2)	14.5(14)
Lys(K)	1.3(1)	1.4(1)	0.9(1)	1.0(1)	1.2(1)	1.1(1)	2.1(2)	1.4(1)	1.1(1)	1.1(1)	0.9(1)	1.2(1)	12.4(12)
Trp(W)											ND(1)[d]	ND(1)	ND(2)
Total	(10)	(11)	(14)	(12)	(11)	(12)	(18)	(16)	(16)	(42)	(13)	(32)	(191)

[a] One-letter symbols are given in parentheses.
[b] Name of peptides and the sequence position (in parentheses).
[c] Determined by amino acid analysis or from the sequence (in parentheses).
[d] ND, not determined.

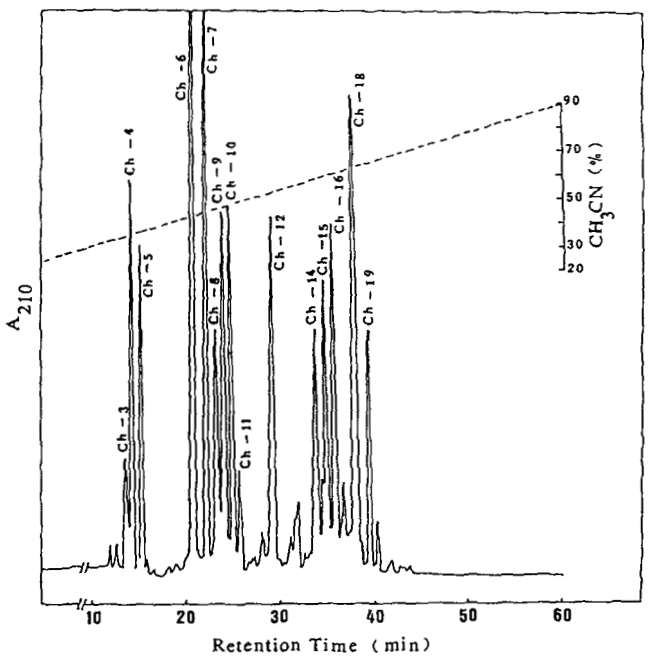

FIG. 2. **HPLC separation of peptides obtained from digestion of *S*-carboxyamidomethylated miraculin by chymotrypsin.** Separation was carried on a reverse phase column, TSK-ODS-120T (0.46 × 25 cm). The peptides were eluted by a linear gradient of acetonitrile containing 0.05% trifluoroacetic acid from 20 to 90% at a flow rate of 1 ml/min for 1 h. The eluted peptides were monitored by absorption at 210 nm.

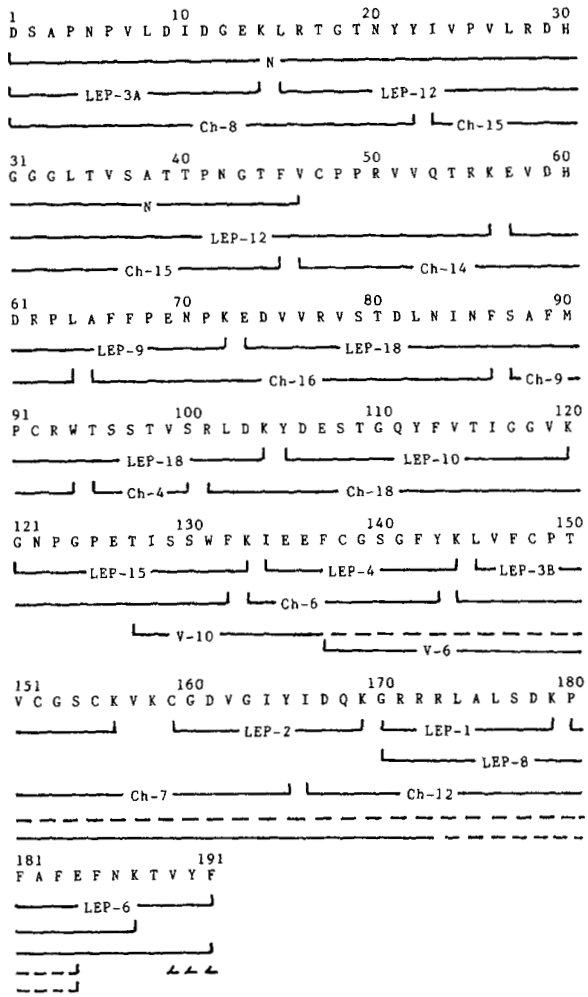

FIG. 3. **The proof amino acid sequence of miraculin.** The sequence of the peptides is represented by a *one-letter code*. *LEP* and *Ch* denote peptides derived from lysyl endopeptidase and chymotrypsin digestions of *S*-carboxyamidomethylated miraculin, respectively. Their numbers also correspond to those in Figs. 1 and 2. *V* denotes peptides derived from V8-protease digestion, and their numbers correspond to those in Fig. 4. *Solid lines* indicate the amino acid sequence obtained from the automatic sequencing, and *dashed lines* indicate the sequence which was not determined by the sequencing. The *arrow* (←) indicates the sequence from the carboxyl terminus determined by carboxypeptidase A digestion.

at the carboxyl-terminal site of all lysine residues. Only peptide bonds at two positions (Lys-Pro at position 179–180 and Lys-Thr at position 187–188) showed partial resistance to the digestion as indicated by amino acid sequences of LEP-1, LEP-6, and LEP-8 (Fig. 3 and Table I). Amino acid compositions of all LEP-peptides were in agreement with their compositions as determined from the sequence. Miraculin contained 2 tryptophan residues, one in LEP-18 and the other in LEP-15. Only LEP-6 had no lysine as the carboxyl-terminal amino acid. There was no phenylthiohydantoin amino acid observed after phenylalanine. This result suggests that phenylalanine is the carboxyl-terminal amino acid of miraculin.

LEP-6 and LEP-12 showed orcinol-sulfuric acid reaction, suggesting that these peptides contain carbohydrates. In addition, the peptides were subjected to acid hydrolysis, and the presence of carbohydrates in the hydrolysates was confirmed by HPLC. Amino acid residue at position 186 in LEP-6 could not be identified by the automatic sequencing. It was, however, identified as aspartic acid by determination of amino acid composition of LEP-6. Thus the amino acid at position 186 is asparagine to which a carbohydrate chain is linked. LEP-12 was a long peptide composed of 42 amino acid residues; and hence, to identify the amino acid residue to which a carbohydrate chain is linked, LEP-12 isolated was digested with chymotrypsin, and a carbohydrate-containing peptide was collected. The automatic sequencing of this peptide indicated that its sequence is Thr-Val-Ser-Ala-Thr-Pro-*X*-Gly-Thr-Phe. The results of determination of the amino acid composition was consistent with this sequence if *X* (position 42) is aspartic acid. Therefore it was concluded that the amino acid at position 42 is asparagine to which a carbohydrate chain is linked.

Attempt to overlap LEP-peptides was performed by digestion of the modified miraculin with chymotrypsin. The results obtained are shown in Fig. 2. The peptides, Ch-4 to Ch-9, Ch-12, Ch-14, Ch-16, and Ch-18 were subjected to determination of amino acid sequences and amino acid compositions (Fig. 3 and Table II). Amino acid compositions of the Ch-peptides were in agreement with their compositions as determined from the sequences. Ch-14 linked LEP-12 to LEP-9, which extended the sequence from 1st to 72nd positions. Ch-16 overlapped LEP-9 and LEP-18. Ch-18 overlapped three peptides, *i.e.* LEP-18, LEP-10, and LEP-15. Ch-7 clearly linked LEP-3B to LEP-2, indicating that there is a Val-Lys peptide (position 157–158) between the two peptides. This linkage and amino acid sequences of Ch-6 and Ch-7 suggested that LEP-15 links to LEP-4 which further links to LEP-3B. Ch-12 overlapped LEP-2, LEP-1, and LEP-6. Thus the Ch-peptides overlapped LEP-peptides to give the complete amino acid sequence from the amino-terminal amino acid (Asp-1) to the carboxyl-terminal amino acid (Phe-191).

Connection between LEP-15 with LEP-4 with LEP-3B was established by amino acid sequences of V-6 and V-10 peptides which were obtained from digestion of the modified miraculin

TABLE II

Amino acids compositions of peptides obtained from digestion of S-carboxyamidomethylated miraculin with chymotrypsin

Amino acids[a]	Ch-4[b] (95–100)	Ch-5 (166–181)	Ch-6 (133–143)	Ch-7 (144–165)	Ch-8 (1–22)	Ch-9 (87–94)	Ch-12 (166–191)	Ch-14 (46–64)	Ch-15 (23–45)	Ch-16 (65–86)	Ch-18 (101–132)
						residues/molecule[c]					
Asx(D/N)		1.9(2)		0.7(1)	3.8(5)		2.2(3)	2.3(3)	1.7(2)	4.8(5)	3.5(3)
Glx(E/Q)		1.3(1)	1.7(2)		1.0(1)		2.1(2)	2.2(2)		1.6(2)	2.9(3)
Cys(C)			0.9(1)	3.5(4)		0.8(1)		0.8(1)			
Ser(S)	2.7(3)	1.1(1)	1.1(1)	0.7(1)	1.2(1)	0.9(1)	1.2(1)		1.2(1)	1.3(1)	2.2(2)
Gly(G)		1.2(1)	2.4(2)	3.6(3)	2.4(2)		1.1(1)		3.3(4)		3.8(5)
His(H)								0.8(1)	0.8(1)		
Thr(T)	2.1(2)			1.2(1)	2.2(2)		1.1(1)	0.9(1)	3.2(4)	1.2(1)	2.5(3)
Ala(A)		0.9(1)			1.3(1)	1.2(1)	1.8(2)		1.1(1)	1.1(1)	
Pro(P)		0.9(1)		1.3(1)	2.4(2)	1.4(1)	1.1(1)	2.5(3)	2.1(2)	2.2(2)	2.4(2)
Arg(R)		2.9(3)			1.2(1)	1.3(1)	2.6(3)	2.8(3)	1.2(1)	1.1(1)	1.3(1)
Tyr(Y)			1.0(1)	1.0(1)	2.0(2)		1.0(1)				1.9(2)
Val(V)	1.2(1)			4.4(4)	1.4(1)		1.4(1)	4.3(3)	3.5(3)	2.8(3)	2.1(2)
Met(M)						0.9(1)					
Ile(I)		0.8(1)	1.3(1)	0.7(1)	0.5(1)		0.8(1)		0.9(1)	0.8(1)	1.9(2)
Leu(L)		1.7(2)		0.7(1)	1.6(2)		1.6(2)	0.9(1)	2.1(2)	1.3(1)	1.5(1)
Phe(F)		1.2(1)	1.5(2)	1.4(1)		1.5(1)	3.6(4)		1.5(1)	2.8(3)	2.9(2)
Lys(K)		1.9(2)	1.0(1)	2.5(3)	0.6(1)		3.3(3)	1.2(1)		1.4(1)	2.2(2)
Trp(W)						ND(1)[d]					ND(1)
Total	(6)	(16)	(11)	(22)	(22)	(8)	(26)	(19)	(23)	(22)	(32)

[a] One-letter symbols are given in parentheses.
[b] Name of peptides and the sequence position (in parentheses).
[c] Determined by amino acid analysis or from the sequence (in parentheses).
[d] ND, not determined.

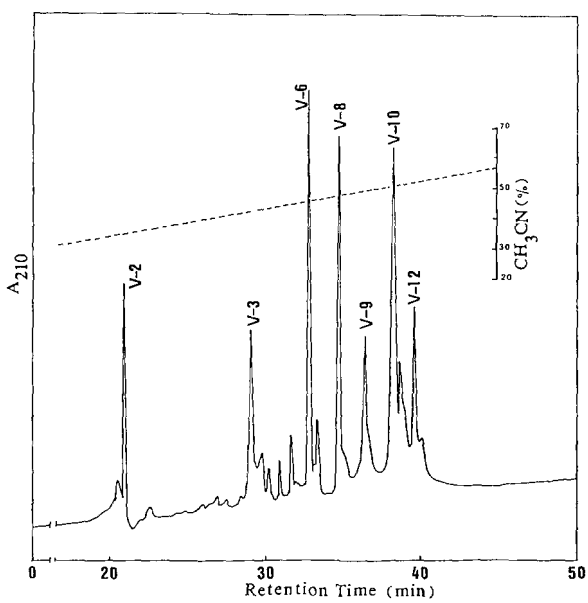

FIG. 4. **HPLC separation of peptides obtained from *S. aureus* V8 protease digestion of *S*-carboxyamidomethylated miraculin.** Separation was carried on a TSK-ODS-120T column. The peptides were eluted by a linear gradient of acetonitrile containing 0.05% trifluoroacetic acid from 20 to 70% at a flow rate of 1 ml/min for 1 h. The eluted peptides were monitored by absorption at 210 nm.

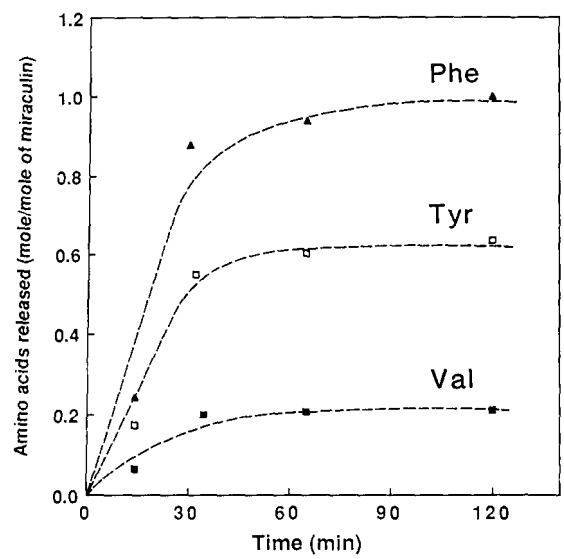

FIG. 5. **Amino acids released from the carboxyl terminus of miraculin after digestion with carboxypeptidase A.** Native miraculin was digested by carboxypeptidase A in 0.1 M *N*-ethylmorpholine acetate buffer of pH 8.0 at room temperature with an enzyme:substrate ratio of 1:200 (w/w). 8 nmol of miraculin was used for the digestion. The *ordinate* represents moles of amino acids released/mol of miraculin.

with *S. aureus* V8 protease (Figs. 3 and 4).

Determination of the amino acid sequence from the carboxyl-terminal was performed by digestion of native miraculin with carboxypeptidase A. The results are shown in Fig. 5. The rate of release of amino acids is decreased in the order: phenylalanine > tyrosine > valine. There was no other amino acid released during 2 h digestion. These results indicate that the amino acid sequence from the carboxyl terminus of miraculin is Phe-Tyr-Val which is in accordance with the sequence determined by automatic sequencing of Ch-12 and LEP-6 peptides.

The complete sequence of 191 amino acid residues (Fig. 3) is approximately in accordance with the amino acid composition of the whole protein (Table I). The sum of the molecular weights of amino acid residues is 21,257. In Fig. 6, the hydropathic profile (10) is plotted, which suggests that there are a number of hydrophobic domains in the miraculin molecule.

DISCUSSION

In the present study, we have determined the complete sequence of 191 amino acid residues of miraculin. It is a single

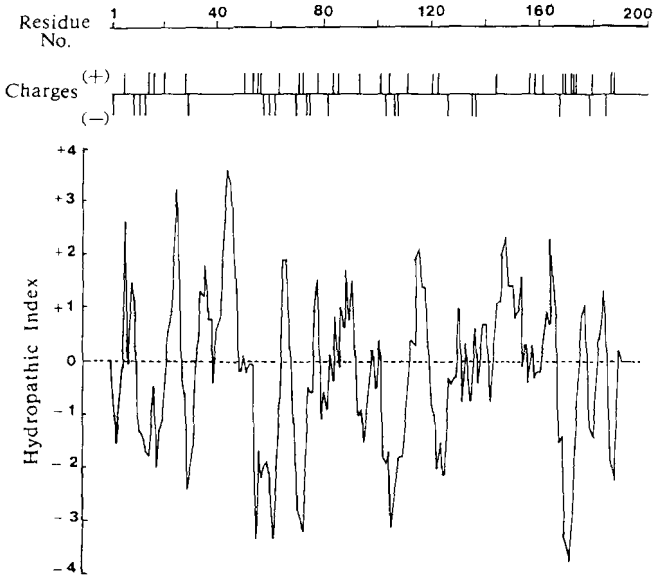

FIG. 6. **Hydropathic profile calculated by the method of Kyte and Doolittle (10).** The global average hydropathic index is indicated by the *horizontal line.* In the *upper part,* the location of positive and negative charges along the polypeptide chain of miraculin is represented.

polypeptide chain with molecular weight of 21,257. Carbohydrate content was as much as 13.9% (5), and hence the total calculated molecular weight is 24,600 which is 88% of the molecular weight (28,000) estimated by SDS-PAGE (5). Apparent molecular weight of a glycoprotein on SDS-PAGE is often larger than the actual one (11), and hence SDS-PAGE of miraculin seems to have given a larger molecular weight than the actual one. Resistance of Lys-Thr at position 187–188 to lysyl endopeptidase may be due to the presence of the carbohydrate chain at Asn-186. Similar effect of the carbohydrate chain on the proteolytic action was observed in carboxypeptidase A digestion.

One characteristic of the miraculin structure is that the amino-terminal half of the molecule is enriched in proline residues; it contains 10 of 14 proline residues. Another characteristic of miraculin structure is that 5 half-cystines of a total 7 half-cystines are located between positions 138 and 159. That is, 2 half-cystine residues are located at positions 47 and 92, and 5 residues are at positions 138, 148, 152, 155, and 159. The fact that the region containing 5 half-cystine residues is highly resistant to the protease digestion unless dithiothreitol is applied to miraculin suggests that these half-cystine residues form disulfide bonds.

The amino acid sequences of thaumatin and monellin, which are sweet-tasting proteins, were already determined (12, 13). We looked for homology of amino acid sequence between miraculin and these sweet-tasting proteins using homology search program (IDEAS) developed by Kanehisa (14), but there was no particular homology between miraculin and the sweet proteins. This is consistent with the fact that the antibody to miraculin did not exhibit cross-reactivity with thaumatin (data not shown). However, high homology was found between the sequences of miraculin and soybean trypsin inhibitors A and C (Kunitz) was 36.3% (the sequence of miraculin from the amino terminus to 60th residue) and 51.1% (the sequence of miraculin from 143rd to the carboxyl terminus). It is interesting to note that both proteins are produced in plants and have similar molecular weights of about 20,000.

Acknowledgments—We are indebted to Professor Lloyd M. Beidler of The Florida State University for supply of seeds of miracle fruit and for constant encouragement. We also thank Professor Takeo Aso of Yokohama National University who cultured miracle fruit. We thank Professor Noriko Takahashi of Nagoya City University for valuable suggestions on carbohydrate chains of miraculin.

REFERENCES

1. Kurihara, K., and Beidler, L. M. (1968) *Science* **161,** 1241–1242
2. Brouwer, J. N., Van der Wel, H., Francke, A., and Henning, G. J. (1968) *Nature* **220,** 373–374
3. Giroux, E. L., and Henkin, R. I. (1974) *J. Agric. Food Chem.* **22,** 594–601
4. Kurihara, Y., and Terasaki, S. (1982) *Biochim. Biophys. Acta* **719,** 444–449
5. Theerasilp, S., and Kurihara, Y. (1988) *J. Biol. Chem.* **263,** 11536–11539
6. Bidlingmeyer, B. A., Cohen, S. A., and Tarvin, T. L. (1984) *J. Chromatogr.* **336,** 93–104
7. Tsunasawa, S., Kondo, J., and Sakiyama, F. (1985) *J. Biochem. (Tokyo)* **97,** 701–704
8. Ambler, R. P. (1967) *Methods Enzymol.* **11,** 155–166
9. Francois, C., Marshall, R. D., and Neuberger, A. (1962) *Biochem. J.* **83,** 335–341
10. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132
11. Segrest, J. P., and Jackson, R. L. (1972) *Methods Enzymol.* **28,** 54–63
12. Frank, G., and Zuber, H. (1976) *Hoppe-Seyler's Z. Physiol. Chem.* **357,** 585–592
13. Iyengar, R. B., Smits, P., Van der Ouderaa, F., Van der Wel, H., van Brouwershaven, J., Ravestein, P., Richters, G., and Van Wassenaar, P. D. (1976) *Eur. J. Biochem.* **96,** 193–204
14. Kanehisa, M. (1982) *Nucleic Acids Res.* **10,** 183–196