Identification and Characterization of a Potexvirus from California Barrel Cactus

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ABSTRACT

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A rod-shaped virus, 520 nm long was isolated from California barrel cactus plants, *Ferrocactus acanthodes,* showing severe external symptoms. The virus was transmitted to and maintained in *Chenopodium quinoa* plants for purification and characterization. The purification procedure included clarification with butanol, precipitation with polyethylene glycol, and differential centrifugation. Purified virus had a maximum ultraviolet absorption at 260 nm, a minimum at 246 nm, and an $A_{260/280}$ ratio of 1.26. One infectious, single-stranded RNA species was isolated from purified virus preparations. The molar percentages of nucleotides in the viral nucleic acid were A = 26.4, C = 29.2, G = 20.8, and U = 23.6. The coat protein had 216 amino acid residues, electrophoresed as a single component in SDS-polyacrylamide gels, and its molecular weight was estimated at 22,000. The virus reacted with antisera against Chessin's isolate of cactus virus X and d-protein of potato virus X. Ultrastructure of infected *C. quinoa* and *Amaranthus caudatus* leaves revealed abundant large aggregates of flexuous virus particles. It is concluded that this virus is a strain of cactus virus X.

Surveys of natural distribution of cactus viruses in the USA have been made by Chessin and Lesemann (9), and Milbrath et al. (22). Several elongated viruses were found to infect cultivated and wild cactus plants but none was reported to naturally infect barrel cactus plants. In 1974, barrel cactus plants, *Ferrocactus acanthodes* (Lemaire) Britton & Rose showing distorted areoles, malformed spines, necrosis, and systemic mottle were observed in a cactus forest in San Bernardino County, California (Fig. 1). We isolated a virus from several of these plants and prospectively determined that the virus belonged to the potexvirus group (3, 5).

This paper characterizes the viral agent and defines its relation to other members of the potexvirus group. Preliminary reports have been published (3, 4, 5).

MATERIALS AND METHODS

Virus culture.—A virus isolate was obtained from a naturally infected barrel cactus plant in the Clark mountains. The virus, partially purified by a modification of the method of Makkouk and Gumpf (20), was used as inoculum. The virus was mechanically inoculated on leaves of *Chenopodium quinoa* Willd. seedlings. Chlorotic local lesions developed on primary inoculated leaves approximately 6 days after inoculation followed by systemic necrosis. The virus was maintained in *C. quinoa* plants for all experimental uses. Plants of *C. quinoa* also served as the biological assay host. Cactus plants were inoculated by injecting a small amount of virus suspension (~1 mg/ml) to the base of the plants with a hypodermic needle. After inoculation, new growth on inoculated cactus plants was observed for presence of virus particles by electron microscopy.

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Virus purification.—A modification of the method of Makkouk and Gumpf (20) was used. Frozen leaf tissue of systemically infected *C. quinoa* plants was homogenized in 0.5 M sodium citrate, pH 7.0, containing 0.2% ascorbic acid, 0.2% sodium sulfite, and 1% 2-mercaptoethanol (1 ml complete buffer per gram of tissue). The extract was expressed through four layers of cheesecloth, clarified by shaking with 6% *n*-butanol, and centrifuged at 12,000 *g* for 20 min. The supernatant fluid was stored overnight at 4 C and centrifuged again at 12,000 *g* for 20 min. Virus was precipitated from the resulting supernatant fluid in the presence of 4% polyethylene glycol (molecular weight 6000) and 3% sodium chloride, and collected by low-speed centrifugation. Virus-containing pellets were resuspended in 0.02 M phosphate buffer, pH 7.0, containing 0.5 M urea, and centrifuged at 10,000 *g* for 15 min. Partially purified preparations were incubated with DNAse (50 μg/ml) for 16 hr at room temperature to hydrolyze host DNA that becomes adsorbed to the virus particles during extraction before sucrose density gradient centrifugation. The density gradient columns (100-400 mg/ml sucrose in resuspension buffer) were centrifuged in a Beckman SW27 rotor at 25,000 rpm for 90 min. Virus zones were collected with an ISCO density gradient fractionator. Ultraviolet absorption of the purified virus preparations was measured in a Beckman ACTA CII spectrophotometer.

Viral nucleic acid.—Nucleic acid was isolated from the purified virus preparation by the method of Brakke and Van Pelt (7). The isolated nucleic acid was resuspended in TKM buffer, pH 7.4, consisting of 0.01 M Tris, 0.01 M KCl, and 0.1 mM $MgCl_2$. Nucleic acid preparations were treated at room temperature with RNAse (3 μg/ml for 30 min), DNAse (50 μg/ml), or pronase (10 μg/ml for 30 min), and assayed on *C. quinoa* plants or electrophoresed in 2.2% polyacrylamide gel containing 0.5% agarose. Buffer used for electrophoresis was 0.36 M Tris,

0.18 M $NaC_2H_3O_2 \cdot 3H_2O$, 0.009 mM EDTA, and 1.8% SDS, pH 7.2 (adjusted with acetic acid). Electrophoresis was for 2 hr at 6 mA/gel, and the gels scanned at 260 nm.

Nucleotides from hydrolyzed viral nucleic acid were separated by paper chromatography (21) and molar ratios of eluted nucleotides determined using the extinction coefficients given by Sober (25).

Viral protein.—Viral coat protein was isolated from purified virus by the guanidine lithium chloride method of Damirdagh and Shepherd (12). The molecular weight was estimated by SDS-polyacrylamide gel (7.5%) electrophoresis (19), using the following markers (molecular weight in daltons in parentheses): cytochrome c (11,700); tobacco mosaic virus coat protein (17,500); cowpea chlorotic mottle virus coat protein (19,400); and ovalbumin (43,000). Gels were fixed and stained with Coomassie blue in trichloroacetic acid (10). Destained gels were scanned at 580 nm with a Beckman ACTA CII spectrophotometer equipped with a gel scanner.

Samples of viral protein for amino acid analysis were hydrolyzed with 6 N HCl for 12, 24, 36, and 72 hr, and the amino acid composition determined with the Beckman model 120 C amino acid analyzer. Cysteine was assayed by the method of Anderson and tryptophan by the method of Spies and Chamber, both as modified by Knight (16).

Serology.—Antiserum to the virus was prepared by first injecting rabbits three times, at 5-day intervals, intramuscularly with a mixture (1:1, v/v) of 1 mg/ml purified virus suspension and Freund's complete adjuvant. After 5 days, two intravenous injections were given 5 days apart with 0.5 ml of 1 mg/ml purified virus suspension. Collection of blood from immunized rabbits was started 1 wk after the last injection. Serological studies were done by mycroprecipitin and agar double diffusion tests.

Electron microscopy.—Negatively stained and shadowcast preparations of virus-infected cactus tissue were used for studying particle morphology. Freshly cut surface tissue was placed in contact with a drop of distilled water for 2-3 sec on formvar-coated grids. Grids were air dried and stained with a solution of 2.0% potassium phosphotungstate, pH 7.0, or shadowed with palladium.

Small portions of systemically infected leaves of *C. quinoa* and *A. caudatus* were fixed at 25 C for 2 hr in a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0. Tissues were washed in the same buffer, postfixed in a 1.0% solution of osmium tetroxide (w/v) at 25 C for 2 hr, dehydrated through graded acetone, and embedded in Spurr's medium (26). Pieces of healthy leaves were similarly prepared to serve as controls. Ultrathin sections were cut with a diamond knife on a Porter-Blum microtome and stained with 2.0% aqueous uranyl acetate for 15-20 min and 0.02% lead citrate (w/v) for 2 min. All observations were made with an Hitachi HU-12 electron microscope operating at 75 kV.

RESULTS

Host range and symptomatology.—Fifty-nine species of plants were mechanically inoculated with barrel cactus virus. Forty-six species of plants were not susceptible. Thirteen species of nine genera in four families were susceptible to this virus (Table 1). Reactions among susceptible species ranged from symptomless infection to chlorotic and necrotic localized and systemic infections.

Virus purification.—High yields of partially purified virus (20-40 mg/100 g tissue) were obtained by resuspension of the polyethylene glycol precipitate collected by low speed centrifugation. When purified virus preparations were subjected to sucrose density gradient centrifugation, two light scattering bands in sucrose density gradients were formed; a sharp band approximately 4 cm below the meniscus contained particles 520 nm long and a broad band below the first containing aggregated particles primarily dimers, and aggregated fragments of various lengths. Virus collected from both bands was infectious. Purified virus preparations had ultraviolet absorption spectra typical of elongated nucleoproteins, with a maximum at 260 nm, a minimum at 246 nm, and a 260/280 ratio of 1.26. The extinction coefficient of this virus as calculated by correlating absorption at 260 nm with dry weight was $E = 2.57\ cm^{-1}\ (mg/ml)^{-1}$.

Viral nucleic acid.—Isolated nucleic acid migrated as a single component in polyacrylamide-agarose gel electrophoresis (Fig. 6). The RNAse-treated preparation lost its infectivity and showed the presence of faster migrating components suggesting the presence of hydrolyzed fragments of viral nucleic acid (Fig. 6). DNAse and pronase had no effect on infectivity and electrophoretic mobility indicating that the nucleic acid of barrel cactus virus is single stranded RNA. The average ratio of bases of isolated RNA was: A = 26.4, C = 29.2, G = 20.8, and U = 23.6.

TABLE 1. Reactions of plants susceptible to barrel cactus virus

Family, genus, species	Reaction[a]
Amaranthaceae:	
Amaranthus caudatus L.	SM, SN
Celosia cristata L.	SN
Celosia cristata f. *plumosa* (Voss.) Back	SM, SN
Gomphrena globosa L.	L
Cactaceae:	
Cereus peruvianus L.	SS
Coryphanta vivipara (Nutt.) Britton & Rose	SS
Ferrocactus acanthodes (Lemaire) Britton & Rose	SS, SM
Opuntia basilaris Engelm. & Bigelow	SS
O. erinacea Engelm. & Bigelow	SS
O. ramosissima Engelm.	SS
Caryophyllaceae:	
Saponaria vacaria L.	S, Lp
Chenopodiaceae:	
Chenopodium amaranticolor Coste & Reyn	L
C. quinoa Willd.	L, S

[a]Abbreviations: S = systemic infection, SS = systemic infection, symptomless, SM = systemic mosaic, SN = systemic necrosis, L = local infection, Lp = local infection of primary inoculated leaves.

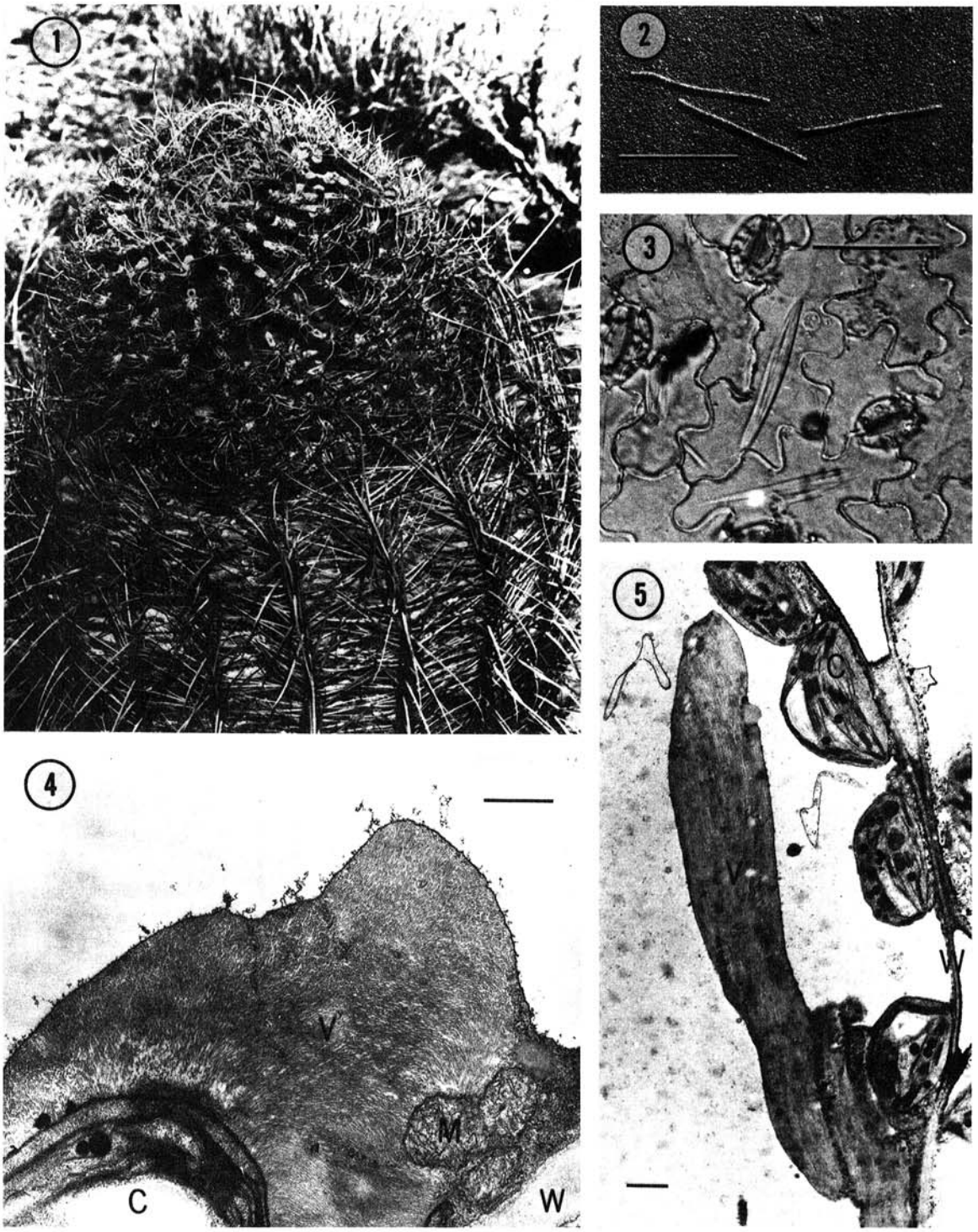

Fig. 1-5. 1) Symptoms of a virus-infected California barrel cactus, *Ferrocactus acanthodes* (Lemaire) Britton & Rose, showing the disorganization of areoles and malformed spines. **2)** Electron micrograph of a shadowcast preparation of the virus from diseased California barrel cactus. Bar = 500 nm. **3)** Spindle-shaped inclusions in virus-infected epidermal cells of *Amaranthus caudatus*. Bar = 50 μm. **4)** Portion of virus aggregate in infected leaf cell of *Chenopodium quinoa*. Bar = 0.5 μm. **5)** A longitudinal section through a spindle-shaped inclusion from *A. caudatus*. Bar = 1 μm. Legend: C = chloroplast, M = mitochondria, V = virus, and W = cell wall.

Viral protein.—Purified viral protein migrated as a single band in SDS-polyacrylamide gel electrophoresis with no indication of heterogeneity (Fig. 7), and its molecular weight was estimated at 22,000. Analyses of amino acids indicated that the viral protein contained 216 amino acid residues, corresponding to a molecular weight of 23,536 (Table 2).

Serology.—The virus was strongly immunogenic. The titer of antiserum in the homologous reaction was 1:4,096 as determined by the microprecipitin tests. Homologous reactions were readily observed in microprecipitin tests. Sharp precipitation zones were observed in agar double diffusion tests only when SDS-treated particles were used. The virus reacted with antisera against Chessin's isolate of cactus virus X and d-protein of potato virus X. No reaction was observed when the virus was reacted with potato virus Y and tobacco etch virus antisera.

Electron microscopy.—Negatively stained and shadowed preparations revealed rod-shaped, 520 nm long particles (Fig. 2). The most striking feature of infected cells of *C. quinoa* and *A. caudatus* plants is the presence of aggregated virus particles (Fig. 4, 5). Virus aggregates sometimes formed crystalline inclusions containing mainly virus particles. These inclusions corresponded to spindle-shaped inclusions observed in the light microscope (Fig. 3). No other anomalous structures were detected within infected tissue and healthy tissues contained none of these virus particles and aggregates.

DISCUSSION

Evidence presented herein indicates that barrel cactus virus is a member of the potexvirus group and closely related to cactus virus X (6), differing only in its host range. Cactus virus X infects *Beta vulgaris* L. (Chenopodiaceae) and *Ocimum basilicum* (Labiatae) (24) whereas barrel cactus virus does not. It also differs from Zygocactus virus (8) and Zygocactus virus X (15) in both host range and physical properties.

Cactus virus X has strong tendencies to aggregate (18) as does barrel cactus virus. Purified nonaggregated virus

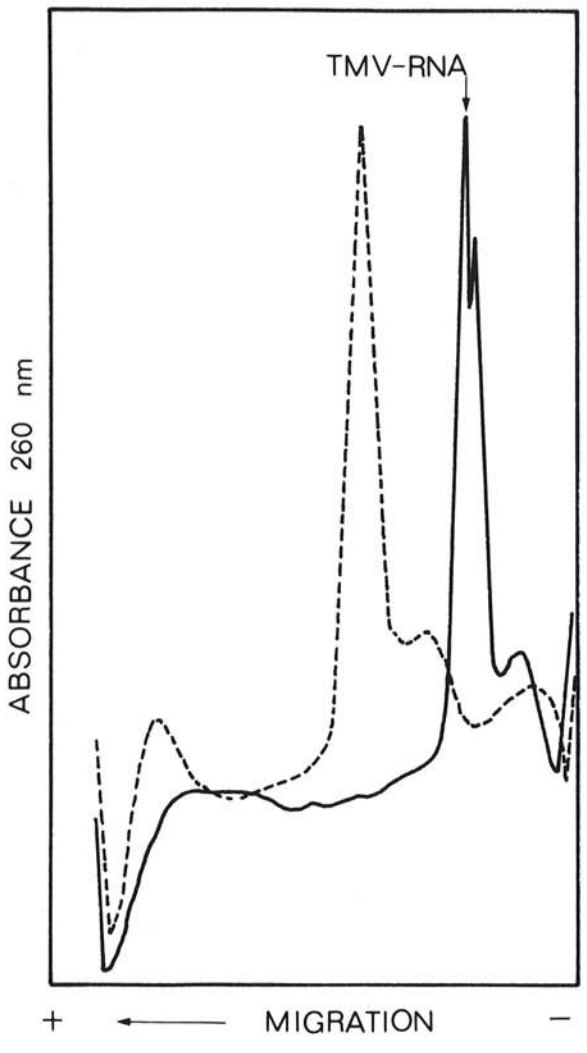

Fig. 6. Scanning profiles of nonstained RNA isolated from barrel cactus virus in 2.2% polyacrylamide gel containing 0.5% agarose. Electrophoresis was for 4 hr at 6 mA/gel, and the gels were scanned at 260 nm. Arrow indicates position of tobacco mosaic virus - RNA. Legend: (- - - - - -) = RNA preparation treated with RNAse, 3 μg/ml for 30 min. (————) = non-treated control.

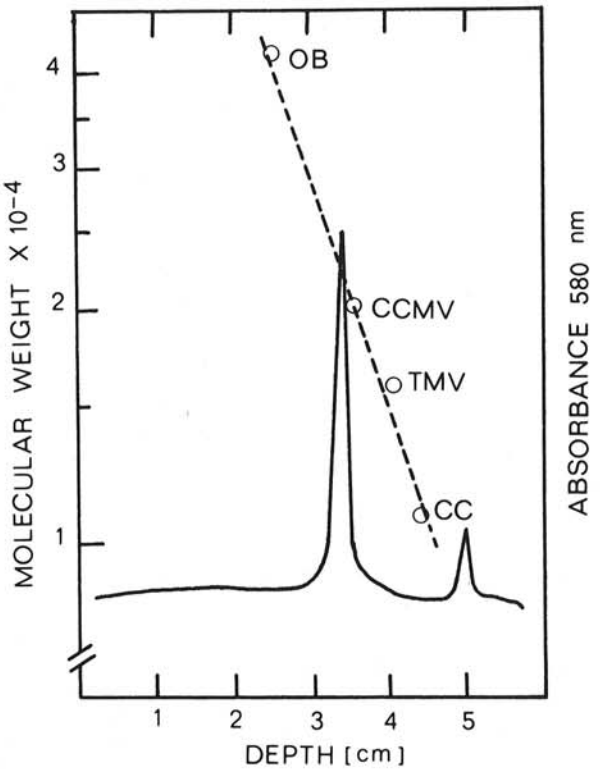

Fig. 7. Scanning pattern at 580 nm of protein isolated from barrel cactus virus electrophoresed on 7.5% SDS-polyacrylamide gel. The front component is a dye marker. Legend: OB = ovalbumin, CCMV = cowpea chlorotic mottle virus coat protein, TMV = tobacco mosaic virus coat protein, CC = cytochrome c.

preparations invariably developed aggregates in vitro similar to those observed in ultrathin sections of virus infected cells. Aggregated virus preparations can, however, be used in some characterization studies. For instance, nucleic acid extracted from aggregated virus obtained from the lower band in sucrose density gradient showed only a single RNA species as did that extracted from virus monomers.

The molar percentage of cytidylic acid in barrel cactus virus exceeds that of adenylic acid, whereas with other potexviruses, the reverse is true (14). The significance of this difference in nucleotide composition is difficult to appraise.

The molecular weight of 22,000 for subunit protein estimated from SDS-polyacrylamide gel electrophoresis was in good agreement with the 23,536 calculated from amino acid composition, and is within the 18,000 to 27,000 range determined for other potexviruses (14). Our values, however, were higher than the 17,500 reported by Koenig (17) and Frowd and Tremaine (14).

Barrel cactus virus produces spindle-shaped inclusions in infected plant cells similar to those produced by some strains of cactus virus X (1, 23). These inclusions were helpful for positive diagnosis of infected plants where external symptoms were not pronounced. Correlation of the morphology of virus particles in infectious sap and purified virus preparations with those seen in the ultrathin sections of virus infected cells, and the absence of the aggregates in healthy cells leads to the conclusion that spindle-shaped inclusions are aggregates of barrel cactus virus. Although we agree with Amelunxen and Thaler (2) that cellular components such as small vacuoles and ribosomes can be trapped during inclusion formation, we believe the crystalline inclusions are aggregated virus particles, which are typical of potexvirus (11, 13).

TABLE 2. Amino acid residues per subunit of barrel cactus virus protein

Amino acid	Residues per subunit recovered after hydrolysis for:[a] 12 hr	24 hr	36 hr	72 hr	Average	Integer value
Lys	7.52	7.80	8.23	7.97	7.88	8
His	2.14	2.00	2.06	2.13	2.08	2
Arg	8.19	8.22	8.69	8.31	8.35	8
Asp	17.35	17.45	17.16	17.40	17.34	17
Thr	16.38	15.92	14.87	13.47	(17)[b]	17
Ser	22.87	21.25	19.90	13.53	(25)[b]	25
Glu	23.81	24.09	23.68	23.80	23.85	24
Pro	15.19	15.34	14.41	14.87	14.95	15
Gly	11.97	11.91	12.24	12.29	12.10	12
Ala	21.68	21.72	21.85	21.78	21.76	22
Val	11.85	12.23	12.24	12.46	(12)[c]	12
Met	0.85	0.84	0.80	0.79	0.82	1
Ile	4.86	5.38	5.72	5.78	(6)[c]	6
Leu	23.96	23.56	23.79	23.52	23.71	24
Tyr	4.25	4.01	4.00	3.93	4.05	4
Phe	11.09	11.07	11.09	11.22	11.12	11
Cys					(2)[d]	2
Trp					(6)[d]	6
				Total		216
				Molecular weight		23,536

[a]Values listed are the average of three separate determinations.
[b]Calculated by extrapolation to zero hydrolysis time.
[c]Calculated from 72-hr hydrolysis time.
[d]Determined by the methods described in Materials and Methods.

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