The Effect of Limited Proteolysis on the Amino Acid Composition of Five Potyviruses and on the Serological Reaction and Peptide Map of the Tobacco Etch Virus Capsid Protein

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ABSTRACT

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The capsid protein subunits of the selected potyviruses (tobacco etch [TEV], pepper mottle [PeMV], soybean mosaic, and watermelon mosaic 1 and 2) were studied after limited proteolysis in situ. The molecular weights of the protein subunits of these five viruses, estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, were in a range from 32,000 daltons (32 kd) to 35 kd before proteolysis and from 26 to 29 kd after proteolysis. Comparisons of amino acid analyses of nonproteolyzed and proteolyzed viral capsid protein for each virus showed that the cleaved portion of the subunit (~6 kd) had a high content of lysine. Electrophoresis of the capsid proteins of TEV cleaved by cyanogen bromide revealed the loss of two peptides after the limited proteolysis. Reaction of TEV with a lysyl-specific reagent 2,4,5-trinitrobenzenesulfonate before and after limited proteolysis, indicated that at least six lysyl residues per protein subunit were lost during the limited proteolysis of TEV. Serological tests indicated that some serological determinants are lost after limited proteolysis of TEV and PeMV. The amino acid compositions of the five potyviruses are compared with each other as well as with 10 other potyviruses from the literature, using Pearson's correlation coefficients.

Additional key words: capsid protein heterogeneity.

Molecular weight, antigenic properties, and amino acid composition of the capsid protein are commonly used as criteria in classifying plant viruses. Analyses of the capsid protein of potyvirus group members by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) show protein heterogeneity (2,6–9,13,14,16). This capsid protein heterogeneity affects virion properties such as sedimentation coefficient, buoyant density, and serological reaction (1,7). However, many reports on the chemical properties of potyviruses (2,11,14,20) have not considered the possible effect of capsid protein heterogeneity on these properties.

In the study reported here, we examined the capsid protein heterogeneity in five different potyviruses and checked for evidence of the proteolytic nature of the capsid protein heterogeneity by comparing their amino acid compositions, antigenic properties, peptide maps, and reactions with a lysyl-specific reagent before and after limited proteolysis.

MATERIALS AND METHODS

Virus purification. Tobacco etch virus (TEV) (American Type Culture Collection No. 69) was cultured in *Nicotiana tabacum* L. 'Havana 425.' Tobacco leaf tissue (100 g) 4–6 wk after inoculation was homogenized for 2 min at 4 C in 200 ml of 0.5 M potassium phosphate buffer (PPB), pH 7.5, containing 0.5 g of sodium sulfite. The homogenate was expressed through cheesecloth, and the filtrate was centrifuged at 13,200 *g* (max) for 15 min. The supernatant was stirred for 15 hr after *n*-butanol was added to a final concentration of 8% (v/v). The mixture was centrifuged at 11,700 *g* (max) for 10 min. The supernatant solution was filtered through glass wool and Miracloth (Chicopee Mills, Inc., New York, NY 10018). Polyethylene glycol 6,000 (PEG) (final concentration 8% w/v) was added to the supernatant solution and the mixture was stirred for 1–2 hr. The mixture was centrifuged at 11,700 *g* for 10 min. The pellets were resuspended (with the aid of a tissue grinder) in 6 ml of 0.05 M PPB, pH 8.3, containing 0.1% mercaptoethanol. The resuspended material was layered onto 4.5 ml of 30% CsCl containing 0.05 M PPB, pH 8.3, in a Beckman SW 41 rotor tube and was centrifuged at 33,000 rpm for 16 hr. The virus band, ~17–22 mm (measured from the bottom of the tube), was collected by droplet fractionation, diluted with an equal volume of H_2O, and centrifuged at 12,100 *g* for 10 min. The virus was removed from the CsCl in the supernatant solution either by centrifugation at 80,000 *g* for 90 min or by PEG precipitation. The final virus pellets were resuspended in 1–2 ml 0.02 M tris-HCl, pH 8.3.

The pepper mottle virus (PeMV) isolate (17) was cultured in *N. tabacum* L. 'White Burley' and purified as described for TEV above. The isolates of watermelon mosaic virus 1 (WMV-1) and watermelon mosaic virus 2 (WMV-2) were cultured and purified as described by Purcifull and Hiebert (18). Soybean mosaic virus (SMV) (American Type Culture Collection PV 94) was cultured in *N. benthamiana* and purified by the procedure described for WMV-2 (18). The TEV and PeMV preparations were purified from cultures grown in Vancouver and in Florida. The WMV-1, WMV-2, and SMV were prepared in Florida and mailed to Vancouver for further study.

Limited proteolysis. Partially purified TEV (the resuspended material after initial PEG precipitation [see the Virus purification section above]) was stored at 4 C for 4–16 wk before further purification by equilibrium density-gradient centrifugation in CsCl. During this storage, the capsid protein changed in estimated molecular weight from 32,000 daltons (32 kd) to 26 kd. This preparation is referred to as storage-degraded TEV.

Purified virus preparations were also partially digested with trypsin (A-grade; Calbiochem, San Diego, CA 92112). Virions were incubated with trypsin (2 μg of enzyme per milligram of virus) for 0.5 hr at room temperature (10). The tryptic peptides and the virions with limited proteolyzed capsid protein were separated by equilibrium density gradient centrifugation in CsCl.

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Amino acid analyses. The amino acid composition of the viruses before and after limited proteolysis was determined as described by Stace-Smith and Tremaine (22).

Polyacrylamide gel electrophoresis. Electrophoresis of sodium dodecyl sulfate (SDS)-dissociated capsid proteins was performed as described previously (6). The cyanogen bromide (CNBr) peptides were analyzed by electrophoresis in a 12.5% polyacrylamide gel (PAGE) with a discontinuous buffer system (pH 3.8) composed of acetic acid and β-alanine (12, buffer system #7). Air-dried samples were dissolved in the electrode buffer containing 8 M urea and 20% sucrose, then 20–40 μl of sample, at a concentration of 1 mg/ml, was applied to the gels. The gels were stained with Coomassie brilliant blue R250 in 10% acetic acid and 50% methanol, and destained in 7% acetic acid containing 10% methanol. The gels were maintained in 5% acetic acid and scanned at 565 nm in a Beckman DU spectrophotometer with a Gilford model 252 update, model 2520 gel scanner, and model 6051 recorder.

Reaction with TNBS. TEV was reacted with TNBS at room temperature in a Beckman DU spectrophotometer with a Gilford model 252 update. Nondegraded and trypsin-degraded TEV at 300 μg/ml in 20 mM borate buffer, pH 8.2, were reacted with 0.05% TNBS (Pierce Chemical Co., Rockford, IL 61105). Absorbance measurements on the virus preparations were made at 345 nm with 0.05% TNBS in the same buffer as a blank. The degree of trinitrophenylation per protein subunit molecule was calculated according to the method of Goldfarb (4) and using the appropriate protein molecular weight determined in this study.

Cyanogen bromide cleavage. Formic acid (99%) was added to preparations of nondegraded TEV, storage-degraded TEV, and trypsin-degraded virions to obtain 66% formic acid, and the preparations were kept overnight at room temperature. A 30-fold molar excess of CNBr was added to each preparation, and the containers were capped and left at room temperature for 1–3 days (5). Four volumes of water were added and the mixture was lyophilized.

Serology. Serological tests were done by the agar double diffusion method in 10-cm-diameter plastic petri dishes. The medium contained 0.8% agar, 1% sodium azide, and 0.5% SDS. The antiserum to nondegraded TEV protein was prepared by Batchelor (1). The PeMV antiserum was prepared by D. E. Purcifull. Tissue extracts were heated at 100 C for 1 min in 0.05 M sodium phosphate buffer, pH 7.0, containing 1% SDS. The antiserum wells were filled with 50 μl of appropriate antiserum and the antigen wells with either purified virus at 1 mg/ml or dissociated tissue extracts.

TABLE 1. Estimated molecular weights of the potyviral capsid proteins before and after limited proteolysis

Virus	Molecular weight (kd)[a]		
	Freshly prepared	After trypsin	After storage
TEV[b]	32	26	26
PeMV	33	28	ND[c]
SMV	33	26	ND
WMV-2	34	26	ND
WMV-1	35	ND	29

[a] Molecular weights in kilodaltons (kd) were estimated by SDS-PAGE as described for Fig. 1.
[b] TEV = tobacco etch, PeMV = pepper mottle, SMV = soybean mosaic, WMV-2 = watermelon mosaic-2, and WMV-1 = watermelon mosaic-1.
[c] ND = not determined.

RESULTS

Limited proteolysis of the potyviruses. Aliquots of purified preparations of TEV, PeMV, SMV, WMV-1, and WMV-2 were treated with trypsin for limited proteolysis. Insufficient amounts of WMV-1 were recovered from the CsCl gradients for further study after the trypsin treatment. Therefore the results for WMV-1 were limited to proteolysis which occurred during storage of partially purified preparations at 4 C. The molecular weights of the capsid protein subunits from the nonproteolyzed viruses ranged from 32 to 35 kd, depending upon the virus, and from 26 to 29 kd for the proteolyzed viruses (Table 1). Between 15 and 20% (~6 kd) of the capsid protein subunit of each potyvirus was readily susceptible to proteolysis.

The amino acid analyses of the proteolyzed and nonproteolyzed virus proteins are summarized in Table 2. The number of amino

TABLE 2. Amino acid composition of the capsid proteins of potyviruses before and after limited proteolysis

Amino acid	Residues per subunit[a]										
	TEV			PeMV		SMV		WMV-1		WMV-2	
	N[b]	S[b]	T[b]	N	T	N	T	N	S	N	T
Lys	15	8	9	23	14	22	14	26	18	29	17
His	9	9	9	6	5	5	5	6	6	6	5
Arg	21	20	20	20	16	17	15	21	19	16	15
Asp	37	29	29	32	27	44	37	44	39	42	34
Thr[c]	19	18	16	25	20	15	13	20	16	19	15
Ser[c]	13	11	11	20	13	18	10	20	17	19	13
Glu	37	29	32	36	30	36	30	37	27	36	29
Pro	14	12	13	13	12	15	12	13	13	14	12
Gly	20	17	15	19	17	21	16	16	15	23	17
Ala	26	22	20	19	17	21	19	17	15	18	17
Cys[d]	4	ND	3	4	3	1	1	3	2	4	4
Val	21	17	16	20	16	19	15	17	13	18	15
Met[d]	16	15	15	16	14	16	14	13	11	10	10
Ile	10	8	8	11	11	11	11	14	12	11	10
Leu	22	19	20	18	17	22	21	16	13	20	19
Tyr	10	9	10	9	8	10	9	9	8	9	9
Phe	8	8	8	9	8	8	8	8	7	7	7
Total[e]	302	251	254	300	248	301	250	300	251	301	248
MW[f]	34.1	28.4	29.1	33.9	28.0	33.9	28.4	34.3	28.6	33.7	28.0

[a] Integer values derived from the means of at least two analyses of 24- and 72-hr hydrolysates of one preparation or at least one analysis of 24- and 72-hr hydrolysates from two preparations.
[b] N = nondegraded protein, S = storage-degraded protein, and T = trypsin-degraded protein.
[c] Extrapolated to zero hydrolysis time.
[d] Determined from performic acid oxidized protein. ND = not determined.
[e] Total amino acid residues were adjusted to about 300 for the nondegraded protein and to about 250 for degraded protein.
[f] Molecular weight × 10^{-3} of protein subunits calculated from analyses.

acid residues was adjusted to ~300 for the nonproteolyzed viral protein subunits and ~250 for the proteolyzed viral protein subunits. The amino acid compositions of the proteolyzed virus preparations indicated a disproportionate loss in certain amino acid residues in the cleaved, ~6-kd polypeptide. For example, the 26-kd protein subunit of TEV contained only 60% of the lysyl residues found in the 32-kd protein subunit of TEV. The other potyviruses also showed a disproportionate loss of lysyl residues. The most hydrophobic of the amino acids (methionine, isoleucine, leucine, tyrosine, and phenylalanine) appeared to be affected the least during the limited proteolysis.

Pearson's correlation coefficients (21) of the amino acid compositions and those reported for other potyviruses by other authors (14) are presented in Table 3. The correlations between the amino acid compositions (molar percentages) of all viruses in Table 3 average 0.87 ± 0.22, but the correlations between the compositions of the proteolyzed and nonproteolyzed protein of each virus average 0.96 ± 0.04.

The capsid protein of TEV in a partially purified preparation from infected tissue grown in Florida was almost completely converted from 32 to 26 kd during storage at 4 C for 4 wk (Fig. 1). However, this conversion of TEV protein was not detected during storage at 4 C for 3 wk or at room temperature for 3 days in partially purified preparations from infected tissue grown in Vancouver. The protein of TEV purified from tissue grown in Vancouver was converted to the 26-kd form by incubation with trypsin. The apparent subunit molecular weight of trypsin-proteolyzed TEV was similar to that of storage-proteolyzed TEV (Fig. 1). The amino acid composition of storage-degraded TEV protein (Table 1) was similar to that of trypsin-treated virus. The correlation coefficient between the storage-degraded and trypsin-degraded TEV protein was 0.995.

Preparations of trypsin-degraded TEV (three samples), PeMV (one), SMV (one), and storage-degraded WMV-1 (one) were placed on CsCl gradients and centrifuged overnight at 33,000 rpm in a SW41 rotor. The top 1-ml of each tube was withdrawn and the amino acid composition was determined (Table 4). The amino acid compositions of the nonsedimented material from each of the virus preparations resembled, but were not identical to, the difference between the amino acid compositions of the nondegraded and degraded virus.

Chemical properties of nondegraded and degraded TEV. To confirm the proteolytic nature of the molecular weight change in the virus capsid protein subunits we chemically cleaved preparations of storage-degraded, trypsin-degraded, and

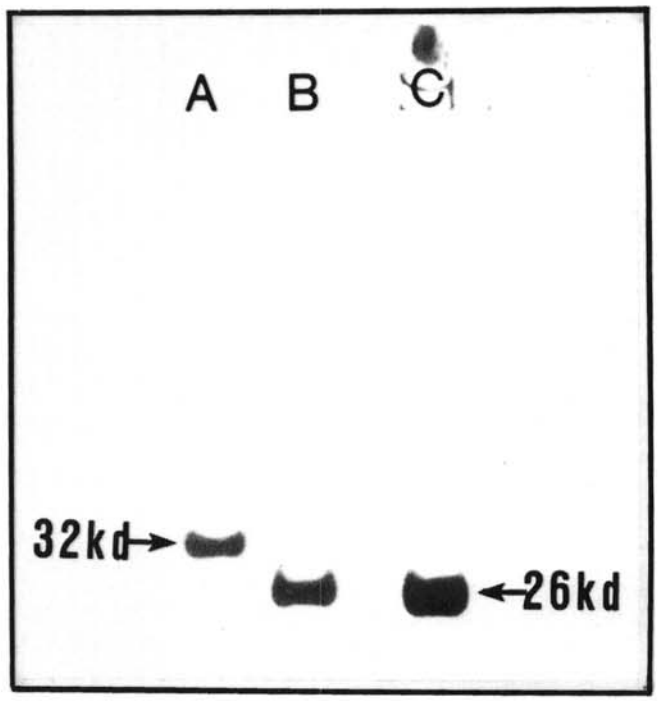

Fig. 1. Polyacrylamide gel electrophoresis of **A,** sodium dodecyl sulfate-dissociated capsid protein of nondegraded TEV; **B,** trypsin-degraded TEV; and **C,** storage-degraded TEV. The numbers are in daltons (d). Electrophoresis was for 4 hr at 160 V and 90 mA in 10% gel. The molecular weight of the capsid protein was estimated by comparison with the electrophoretic mobility of bovine serum albumin (67 kd), glutamate dehydrogenase (53 kd), carbonic anhydrase (29 kd), and tobacco mosaic virus capsid protein (17.5 kd).

TABLE 3. Calculated Pearson's correlation coefficients of potyvirus[a] amino acid composition

	TEV[b]	PeMV[b]	SMV[b]	WMV-2[b]	WMV-1[b]	BYMV	PMV	SPMV	LMV	BCMV	PWV	PVY	SCMV	TuMV	MDMV	TEV[c]	PeMV[c]	SMV[c]	WMV-2[c]	WMV-1[d]
TEV[b]	1	0.89	0.93	0.84	0.86	0.91	0.90	0.90	0.90	0.90	0.93	0.88	0.81	0.93	0.75	0.97	0.95	0.95	0.96	0.86
PeMV[b]		1	0.90	0.90	0.93	0.89	0.87	0.87	0.81	0.84	0.86	0.88	0.80	0.84	0.77	0.82	0.96	0.84	0.87	0.88
SMV[b]			1	0.95	0.94	0.96	0.94	0.96	0.92	0.97	0.97	0.87	0.90	0.91	0.70	0.85	0.91	0.97	0.98	0.94
WMV-2[b]				1	0.94	0.92	0.91	0.93	0.86	0.90	0.91	0.87	0.87	0.83	0.69	0.72	0.85	0.87	0.92	0.92
WMV-1[b]					1	0.94	0.90	0.93	0.87	0.91	0.90	0.86	0.89	0.87	0.65	0.76	0.89	0.89	0.92	0.98
BYMV						1	0.99	0.99	0.96	0.92	0.96	0.91	0.92	0.95	0.75	0.83	0.91	0.94	0.98	0.93
PMV							1	0.98	0.95	0.85	0.94	0.92	0.90	0.94	0.76	0.82	0.91	0.92	0.96	0.90
SPMV								1	0.95	0.91	0.95	0.89	0.91	0.94	0.71	0.80	0.89	0.94	0.96	0.92
LMV									1	0.91	0.95	0.87	0.91	0.94	0.76	0.82	0.86	0.93	0.93	0.88
BCMV										1	0.97	0.80	0.93	0.88	0.65	0.82	0.85	0.95	0.94	0.93
PWV											1	0.86	0.94	0.91	0.72	0.82	0.87	0.95	0.96	0.90
PVY												1	0.84	0.85	0.77	0.81	0.91	0.84	0.90	0.84
SCMV													1	0.84	0.69	0.69	0.77	0.85	0.89	0.92
TuMV														1	0.69	0.89	0.91	0.94	0.95	0.88
MDMV															1	0.69	0.78	0.65	0.71	0.65
TEV[c]																1	0.92	0.91	0.90	0.77
PeMV[c]																	1	0.91	0.83	0.86
SMV[c]																		1	0.98	0.90
WMV-2[c]																			1	0.92
WMV-1[d]																				1

[a] Potyviruses: TEV = tobacco etch, PeMV = pepper mottle, SMV = soybean mosaic, WMV-2 = watermelon mosaic virus 2, WMV-1 = watermelon mosaic virus 1, this report. From Moghal and Francki (14), BYMV = bean yellow, PMV = pea mosaic, SPMV = sweet pea mosaic, LMV = lettuce mosaic, BCMV = bean common mosaic, PWV = passionfruit woodiness virus, PVY = potato virus Y, TuMV = turnip mosaic, MDMV = maize dwarf mosaic, and SCMV = sugarcane mosaic.

[b] Nondegraded viral protein.

[c] Trypsin-degraded viral protein.

[d] Storage-degraded viral protein.

nondegraded TEV with CNBr and compared their CNBr peptides on PAGE at pH 3.8 (Fig. 2). Seven peaks (four major and three minor) were resolved with the CNBr peptides from nondegraded TEV (Fig. 2a). Only five peaks (three major and two minor) were resolved with the CNBr peptides from storage-degraded TEV (Fig. 2b) or from trypsin-degraded TEV (*unpublished*). Assuming 100% CNBr reaction of methionine residues, one major and one minor CNBr peptide were lost by proteolysis either during storage or trypsin treatment. Based on the amino acid analysis data in Table 2, the CNBr digests of the nondegraded, storage-degraded, and trypsin-degraded protein should contain 17, 16, and 16 CNBr peptides, respectively. However, small peptides are not detected by staining with coomassie blue (24). The CNBr peptides of the preparations of WMV-2 and PeMV revealed a similar loss in CMBr peptides upon the limited proteolysis of these two viruses (*unpublished*). The SMV and WMV-1 preparations were not tested.

The availability of lysyl residues in the capsid protein of the nondegraded and trypsin-degraded TEV were compared by reaction with TNBS. During the course of the reaction the number of reacted lysyl residues in the nondegraded TEV was always greater than that in the trypsin-treated TEV (Fig. 3). The nondegraded TEV capsid subunit contained 11 reactive lysyl residues while the trypsin-degraded TEV capsid subunit had five. The amino acid analysis data in Table 1 indicated 15 lysyl residues in the nondegraded TEV capsid subunit and eight lysyl residues in the trypsin-degraded TEV capsid subunit. The difference between the two virus forms is seven lysyl residues in amino acid analysis and six lysyl residues in the TNBS reaction. It was possible that the loss of 50 amino acid residues upon trypsin treatment may have exposed an additional lysyl residue that was not available for TNBS reaction on the viral surface in the nondegraded virus protein.

Serological reactions of nondegraded and degraded capsid protein of TEV. The virions in nondegraded, trypsin-degraded, and storage-degraded preparations of TEV were dissociated in SDS and compared in SDS-immunodiffusion tests with antiserum prepared against the nondegraded capsid protein. The nondegraded immunoprecipitation band of TEV spurred over the band of storage-degraded TEV, while the storage-degraded band fused with the band of trypsin-treated TEV (Fig. 4). In another test (not shown), the nondegraded TEV formed an immunoprecipitation band, which spurred over the band of trypsin-treated TEV. The protein from the nondegraded virus apparently had antigenic sites not present on the proteins from the trypsin- or the storage-degraded virions. A similar loss in serological determinants was detected with trypsin-treated PeMV (*unpublished*).

DISCUSSION

The amino acid compositions listed in Table 2 show a disproportionate loss in lysyl residues when the five potyviruses were subjected to limited proteolysis of the capsid protein. The loss of other hydrophilic amino acid residues was not as obvious. On the other hand, the most hydrophobic amino acid residues showed only limited losses during proteolysis. One would expect these residues to be more prevalent in the interior of the virion and less susceptible to proteolytic enzymes.

The proteolytic nature of the change in the molecular weight of the potyvirus capsid protein subunit (Table 1) and amino acid composition (Table 2) was supported by the peptide map analysis of the chemically cleaved TEV (Fig. 2) and by the TNBS reaction with the available lysyl residues in TEV (Fig. 3). The loss of CNBr fragments and the loss of reactive lysyl residues after storage-degradation or limited trypsin treatment of TEV also indicated that a portion of the TEV capsid protein subunit was cleaved. The similarity of the TEV properties after degradation either by storage or by trypsin imply that partially purified virus preparations may contain enzymes with specificities similar to those of trypsin. Tremaine and Agrawal (23) reported a similar limited proteolysis of potato virus X by either trypsin or plant protease in sap. The results presented here for four other potyviruses also indicated that capsid protein subunit heterogeneity in SDS-PAGE was due to limited proteolysis. The failure to induce storage degradation in preparations of TEV isolated from plants cultured in Vancouver indicated that the proteolytic activity in partially purified TEV preparations from Florida may be of microbial origin. Limited proteolysis of capsid proteins has been shown for other viruses such as cauliflower mosaic (3), cowpea mosaic virus (15), and potato virus X (10).

TABLE 4. Amino acid composition of nonsedimented material obtained by density gradient centrifugation of virions after limited proteolysis

Amino Acid	Relative molar ratio[a]				Amino acid composition of nondegraded minus degraded viruses[b]					
					TEV					
	TEV	PeMV	WMV-1	WMV-2	S[c]	T[d]	PeMV	SMV	WMV-1	WMV-2
Lys	9.3	9.0	8.0	8.0	7	6	9	8	8	12
His	0.4	0.2	1.0	0.3	0	0	1	0	0	1
Arg	1.1	1.5	0.6	0.4	1	1	4	2	2	1
Asp	8.0	7.1	7.0	8.3	8	8	5	7	5	8
Thr	2.6	4.1	3.2	5.7	1	3	5	2	4	4
Ser	3.4	8.1	3.3	5.7	2	2	7	8	3	6
Glu	5.4	7.8	7.3	8.1	8	5	6	6	10	7
Pro	2.4	0.5	0	2.3	2	1	1	3	0	2
Gly	5.3	3.5	1.9	6.9	3	5	2	5	1	6
Ala	6.3	3.4	2.5	1.6	4	6	2	2	2	1
Cys	ND[e]	ND	ND	ND	ND	1	1	0	1	0
Val	5.1	4.3	1.7	3.7	4	5	4	4	4	3
Met	0.4	1.0	1.0	0.4	1	1	2	2	2	0
Ile	0.8	0.3	1.3	0.3	2	2	0	0	2	1
Leu	1.6	2.1	2.2	1.6	3	2	1	1	3	1
Tyr	0.2	0.1	0.1	0.1	1	0	1	1	1	0
Phe	0.2	0.2	0.9	0.1	0	0	1	0	1	0
Totals					47	48	52	51	49	53

[a] Derived from one analysis of 24-hr hydrolysates, of three preparations of TEV, two preparations of PeMV, and one preparation each of WMV-1 and WMV-2.

[b] Calculated from Table 2 by subtraction of trypsin-degraded or storage-degraded protein amino acid composition from nondegraded protein amino acid composition.

[c] S = storage-degraded protein.

[d] T = trypsin-degraded protein.

[e] ND = not determined.

The nondegraded capsid protein of TEV and other potyviruses (6), SMV (8), turnip mosaic virus (13), and potato virus X (10) behave anomalously in SDS-PAGE; their migration rates relative to the migration rates of marker proteins decrease with decreasing polyacrylamide gel concentrations. However, the trypsin-degraded proteins of TEV and potato virus X react normally in SDS-PAGE. If anomalous behavior in SDS-PAGE is caused by a failure to bind SDS in proportion to the molecular weight, the portion of the proteins of TEV and potato virus X cleaved from the whole protein must be responsible for this failure. However, the 19 amino acids cleaved from the protein of potato virus X (10) differ greatly in composition from those cleaved from TEV (Table 2).

Hill and Benner (8) found two bands on SDS-PAGE with SMV protein, but only one band on discontinuous-PAGE with SMV protein after reduction and carboxymethylation. On the basis of these results and other electrophoresis experiments they proposed that the capsid protein heterogeneity may be due to either disulphide bridge variations, proteolysis, or a combination of the two. The protein of the SMV strain used in our study contains only one cysteine residue and thus the formation of different conformations by disulphide bridging seems improbable.

Moghal and Francki (14) reported Pearson's correlation coefficients between amino acid compositions of 10 potyviruses and discussed these in terms of the relationship between the viruses. Table 3 reports correlation coefficients between these amino acid compositions and those of five nondegraded and degraded viruses from our study. The correlations between the composition of the proteolyzed and the nonproteolyzed protein of each of the five potyviruses are greater than with any other potyvirus. This indicates that the loss of ~50 amino acids did not greatly affect the correlation coefficient. However, the compositions of the degraded and the nondegraded viral proteins (Table 2) can be readily distinguished because of the loss of a large proportion of some amino acids (eg, lysine). Maize dwarf mosaic virus has the most unusual composition of all the potyviruses (lowest correlation coefficients in Table 4). It seems improbable that this unusual composition was caused by a proteolysis of this virus, because of the results with the five potyviruses in our study. The correlation coefficients for SMV and WMV-2 amino acid compositions correspond with the close serological relationship of their capsid

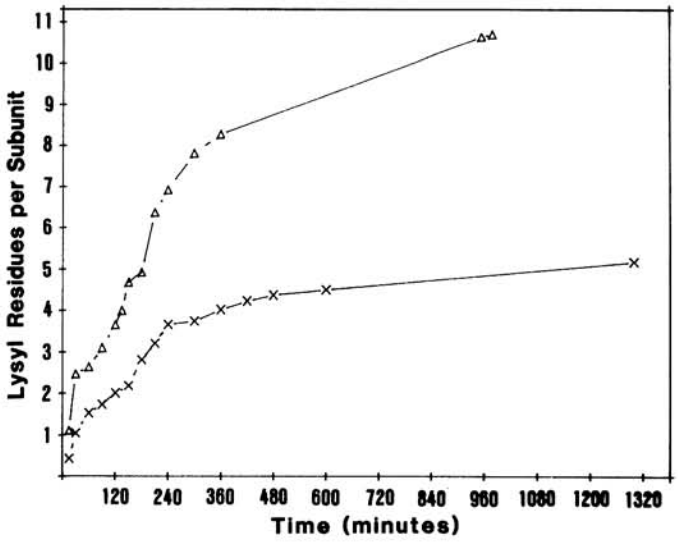

Fig. 3. Time-course of the reaction of TNBS with lysyl residues of nondegraded TEV (Δ——Δ) compared with trypsin-degraded TEV (×——×).

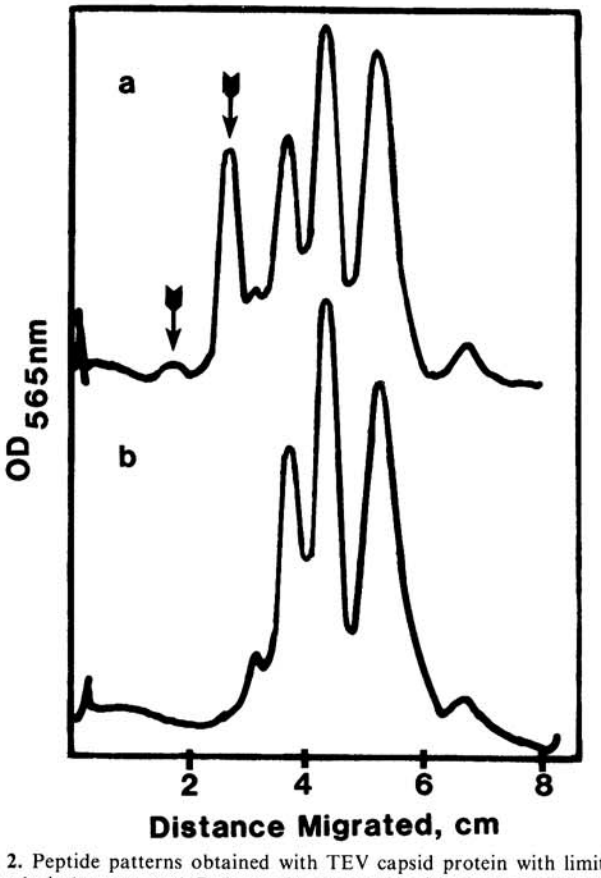

Fig. 2. Peptide patterns obtained with TEV capsid protein with limited proteolysis (storage at 4 C) (curve b) compared with nondegraded TEV (curve a), after cleavage with cyanogen bromide. The peptides were electrophoresed on 12.5% polyacrylamide gel at pH 3.8, buffer consisting of β-alanine and acetic acid. The two peptides missing in curve b are marked with arrows in curve a.

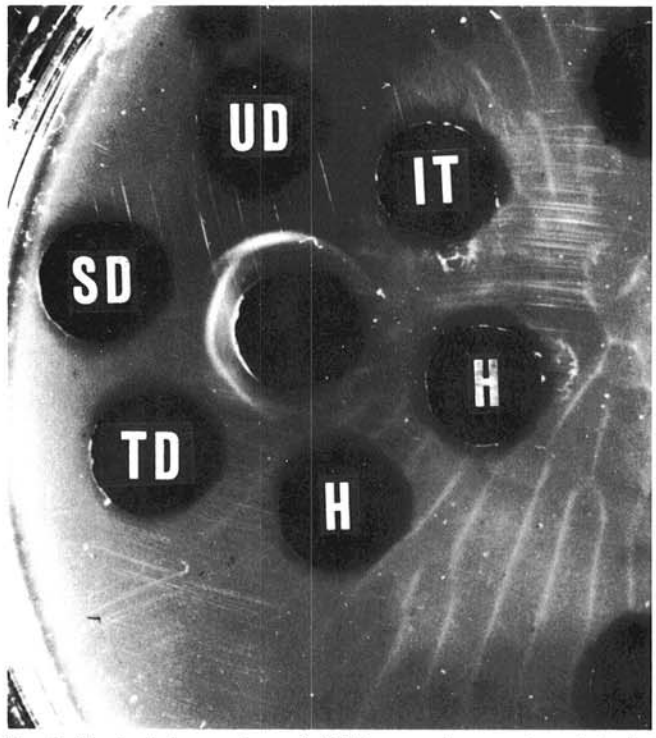

Fig. 4. Serological reactions of TEV comparing nondegraded viral preparations with viral preparations showing limited proteolysis. Antiserum prepared to nondegraded TEV is in the center well. Antigens in the peripheral wells are as follows: UD-nondegraded TEV; IT-infected tissue extract; H = healthy tissue extract; TD = trypsin-degraded TEV; and SD = storage-degraded TEV. The fused immunoprecipitation bands between storage-degraded and trypsin-degraded TEV indicated serological identity while the spur of the nondegraded TEV immunoprecipitation band over the storage-degraded TEV band indicated that the nondegraded TEV has serological determinants that are lacking in the degraded TEV.

proteins (Hiebert and Purcifull, *unpublished*). However, the similar correlation coefficients for WMV-1 and WMV-2 amino acid compositions are contrasted with a lack of a serological relationship between these two viruses (19). The resolution of more CNBr peptides for the nonproteolyzed TEV compared with proteolyzed TEV indicates that peptide mapping may be a sensitive probe for comparisons of potyviral capsid protein.

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