Physical, Chemical, and Serological Properties of Cymbidium Mosaic Virus

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ABSTRACT

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Cymbidium mosaic virus (CyMV), which was isolated from *Cattleya* sp. in British Columbia, was multiplied in *Datura stramonium*. Purified virus was obtained using a citrate-chloroform extraction procedure and polyethylene glycol precipitation followed by sucrose density-gradient centrifugation. Virus yields averaged 66 mg/kg leaf tissue. Purified virus had a sedimentation coefficient of 121 S and an $A_{260/280}$ ratio of 1.11. Molecular weights of protein subunit and RNA determined by polyacrylamide gel electrophoresis, were 27,640 and 2.5×10^6 daltons, respectively. Amino acid composition data indicated that the protein subunit of CyMV had 257 amino acid residues, with a molecular weight of 27,610. The base percentage composition of viral RNA was G = 21.1, A = 28.9, C = 24.4, U = 25.6. Serological tests showed no relationships between CyMV and potato virus X or white clover mosaic virus.

Cymbidium mosaic virus (CyMV) has some properties similar to those of potexviruses; it has elongated particles approximately 490 nm in length (4) and no known vector (22). Occasional cross-reactions, suggesting a distant serological relationship between CyMV and potato virus X (PVX), have been reported (4). Francki (6) used CyMV purified from *Cattleya* orchids to determine the ultraviolet absorption spectrum and detailed particle structure by electron microscopy. Much of the available information on CyMV was summarized by Francki (7). Detailed determinations of the physical and chemical properties of CyMV have not been made. The object of the present investigation was to further characterize CyMV by a study of its physical and chemical properties, for a comparison with those of potexviruses, particularly PVX and white clover mosaic virus (WCMV), the two most-fully characterized members of the group.

MATERIALS AND METHODS

The viruses.—The CyMV used in this study was obtained from *Cattleya* sp. 'Alcimeda Alba' growing in Richmond, B. C. The plant was doubly infected with CyMV and the orchid strain of tobacco mosaic virus (O-TMV). CyMV was separated from O-TMV by repeated selection of local lesions in *Chenopodium amaranticolor* Coste & Reyn., and cultured in *Datura stramonium* L. An isolate of WCMV was obtained from red clover (*Trifolium pratense* L.) at Abbotsford, B. C., and cultured in *Phaseolus vulgaris* L. 'Top Crop'. A Vancouver isolate of PVX (27) was cultured in *Nicotiana tabacum* L. 'Haranova'.

Method of purification.—The highest yields of CyMV were obtained from *D. stramonium* leaves with local lesions, harvested 14 days after inoculation. Yields of virus from several purification methods were compared in the analytical ultracentrifuge. The following method gave the highest yield of purified virus. Infected *D. stramonium* leaves were homogenized in a mixture of 0.5 M sodium citrate buffer, pH 6.5, containing 0.002 M sodium ethylenediaminetetraacetate and 0.1% 2-mercaptoethanol, and chloroform, in the ratio 1:1:1 (w/v/v). After centrifugation at 1,800 rpm (540 *g*) for 15 minutes in an International SBV centrifuge, the aqueous phase was further clarified at 9,000 rpm (5,200 *g*) for 25 minutes in a Sorvall Superspeed RC2-B centrifuge (GSA rotor). Polyethylene glycol 6000 (4 g/100 ml) and NaCl (0.25 g/100 ml) were then added to the aqueous phase and the mixture stirred for 1 hour before centrifugation at 9,000 rpm for 25 minutes. Pellets of precipitated virus were resuspended in 1.0 ml of 0.1 M sodium borate buffer, pH 8.5, for each 10 ml of clarified extract. The resuspended pellets were pooled and allowed to stand overnight before centrifugation at 6,000 rpm (3,140 *g*) for 15 minutes. The supernatant fluid then was centrifuged at 28,000 rpm (68,000 *g*) for 1.5 hours in a Spinco No. 30 rotor, and pellets were resuspended in 2.0 ml of the borate buffer, and allowed to stand overnight. The suspension was clarified by centrifugation at 6,000 rpm (3,140 *g*) for 15 minutes and layered onto 10-40% sucrose density gradients in the borate buffer. The gradients were centrifuged at 23,000 rpm (46,000 *g*) for 1.5 hours in an SW 25 rotor, then the virus bands were removed with a hypodermic syringe and dialyzed overnight against 0.01

M Tris-HCl buffer, pH 7.4. The virus was pelleted by centrifugation at 28,000 rpm (68,000 *g*) for 1.5 hours in the Spinco No. 30 rotor and resuspended in the Tris buffer. All procedures were carried out at 3 C. Virus preparations were infective when assayed on *D. stramonium*. Average yields were 66 mg virus per kilogram of infected leaf tissue.

Purification of WCMV was by the method of Miki and Knight (21); PVX was purified by method IV of Tremaine and Agrawal (27).

Sedimentation coefficients.—The sedimentation properties of purified preparations of CyMV in Tris buffer were determined in the Spinco Model E analytical ultracentrifuge at 35,600 rpm and 20 C. Sedimentation coefficients were estimated by the graphical method of Markham (20). Virus concentrations were estimated using an assumed extinction coefficient of 3.0.

Serological tests.—Virus antisera were prepared by injecting rabbits intramuscularly with 1 mg of purified virus in 1 ml of buffer emulsified with 1 ml of Freund's incomplete adjuvant. These injections were repeated 1 week later. Rabbits were bled at weekly intervals starting 7 days after the final injection.

Serological relationships were determined in tube precipitin tests with purified virus at concentrations of 0.33 and 0.033 mg/ml. In these tests, 1 ml of virus antigen was mixed with an equal volume of twofold serial dilutions of antiserum in 0.85% NaCl. Tubes were partly immersed in a water bath at 37 C for 4 hours to obtain convective mixing of the contents. The tubes were assessed for precipitation after overnight storage at room temperature.

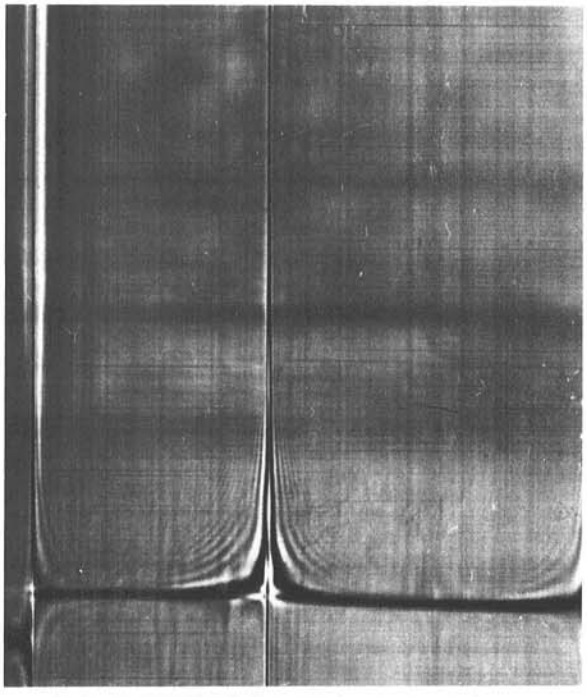

Fig. 1. Analytical ultracentrifugation photography of a purified cymbidium mosaic virus containing approximately 1.5 mg/ml 8 minutes after reaching 35,600 rpm.

Nucleotide and amino acid composition.—Preparations of CyMV at 2 mg/ml were mixed with an equal volume of 2 N HCl. This mixture was allowed to stand at room temperature for 16 hours, then centrifuged at 15,000 rpm (15,000 *g*) for 15 minutes. The supernatant liquid, containing the nucleic acid, then was sealed in a test tube, heated in boiling water for 1 hour, evaporated to dryness and dissolved in a few drops of 0.1 N HCl. Aliquots were transferred quantitatively to a Whatman No. 1 filter paper and nucleotides were separated by descending chromatography using a solvent system composed of isopropanol-HCl and water. The molar ratios of nucleotides were determined by the method of Markham (19).

For amino acid analysis, the protein pellet was dissolved in 12 N HCl and aliquots were pipetted into two hydrolysis tubes. An equal volume of distilled water was added to each tube. The tubes were evacuated, sealed, and placed in an oven at 107 C for 24 and 72 hours. After hydrolysis, the excess HCl was removed in a flash evaporator and the samples were dissolved in 5.0 ml citrate buffer, pH 2.2. Aliquots of 1.0 ml were analyzed in a Spinco amino acid analyzer. For cysteine and

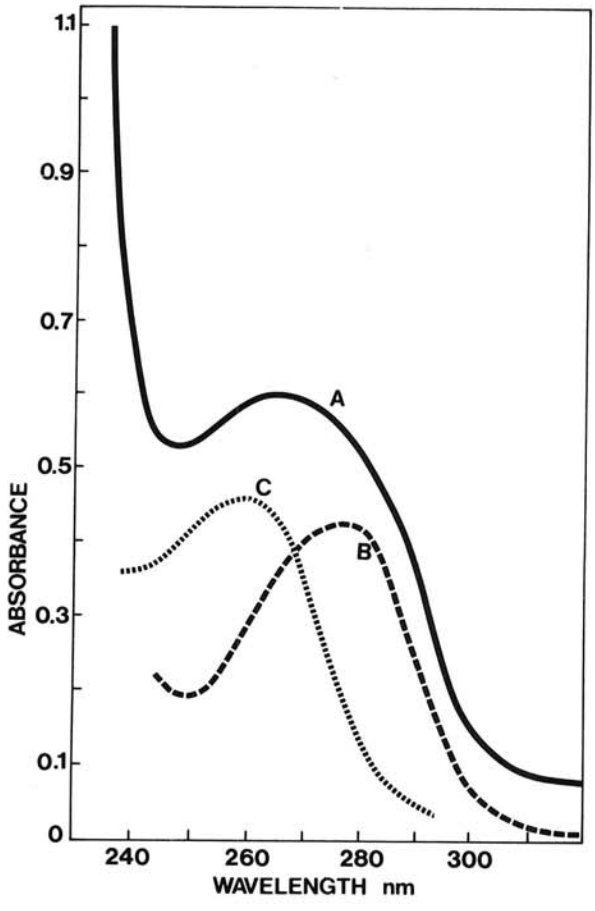

Fig. 2. Ultraviolet absorption curves for (curve A) purified cymbidium mosaic virus at 0.2 mg/ml in 0.01 M Tris-HCl buffer, pH 7.4; (curve B) cymbidium mosaic virus protein in 0.1 N HCl; and (curve C) cymbidium mosaic virus RNA in 0.1 N HCl.

methionine determinations, separate virus protein preparations were oxidized with performic acid before hydrolysis for 24 hours in 6 N HCl. Cysteic acid and methionine sulfone were estimated by reference to aspartic acid, which was determined in the same amino acid analysis.

Tryptophan content was determined by the ultraviolet absorption method of Bencze and Schmid (2) using the protein dissolved in water.

Polyacrylamide gel electrophoresis of protein and nucleic acid.—Molecular weight of CyMV protein was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Virus preparations and protein standards were boiled for 1 minute in 0.1 M sodium phosphate buffer, pH 7.2, containing 4 M urea, 1% SDS, and 1% 2-mercaptoethanol. The proteins used as markers were bovine serum albumin, ovalbumin, carbonic anhydrase, alcohol dehydrogenase, and myoglobin. The markers and dissociated virus were applied separately to 2.5%, 3.5%, and 5.0% gels. After electrophoresis at 6 mA per gel for 3 hours, the gels were fixed in 15% trichloroacetic acid, stained overnight in 0.25% Coomassie blue in 10% trichloroacetic acid, and destained in 10% trichloroacetic acid. The CyMV protein molecular weight was determined from plots of logarithms of molecular weights of marker proteins and their migration distances.

The molecular weight of the nucleic acid was estimated by electrophoretic movement relative to standards in 2.4% polyacrylamide gels (18). The nucleic acids were prepared for the gels by dissociation of the viruses in a buffer (3) modified by Pring (23). Gels were stained in 0.02% toluidine blue O in 40% ethylene glycol monomethyl ether in the dark for 12 hours and destained in distilled water (1). The standards were RNA from brome mosaic virus (16), tobacco mosaic virus (18), and echtes Ackerböhnemosaik virus (Blevings and Stace-Smith, *unpublished*).

RESULTS

Sedimentation rate.—The CyMV sedimented as a single peak (Fig. 1) at 115 S and 118 S at concentrations of 1.5 mg/ml and 0.75 mg/ml, respectively. The sedimentation coefficient at infinite dilution was calculated to be 121 S.

Ultraviolet absorption.—A typical absorption spectrum of CyMV preparations is shown in Fig. 2. Maximum absorption was at 265 nm and minimum absorption was at 248 nm. The average ratio of absorbances at 280/260 for nine preparations was 0.91 ± 0.02, which, after correction for light scattering, indicated an RNA content of 5.5%. The $A_{260/245}$ was 1.14 ± 0.04.

Absorption spectra for CyMV protein and RNA (Fig. 2) have absorbance maxima at 278 and 260 nm, respectively.

Polyacrylamide gel electrophoresis.—The molecular weight of CyMV protein (Fig. 3-A) was determined by the Shapiro et al. plot (25) of SDS-PAGE results. An average value of 27,640 was obtained in 14 plots of this type on 5% gels; separate determinations ranged from 26,600 to 30,400.

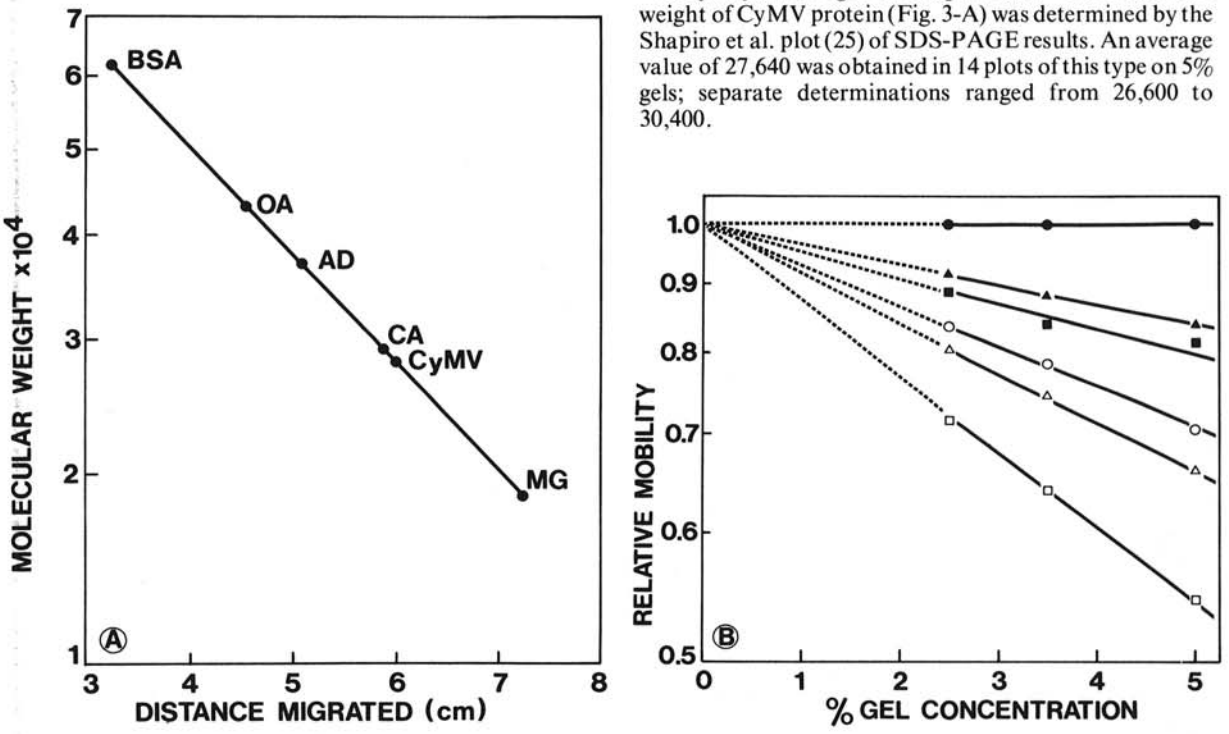

Fig. 3-(A, B). **A)** Determination of molecular weight of the protein subunit of cymbidium mosaic virus by electrophoresis on 5.0% polyacrylamide gel. Abbreviations are: BSA, bovine serum albumin; OA, ovalbumin; AD, alcohol dehydrogenase; CA. carbonic anhydrase; CyMV, cymbidium mosaic virus protein; MG, myoglobin. **B)** Effect of different gel concentrations on the relative mobilities of the protein standards and cymbidium mosaic virus in SDS-polyacrylamide gel electrophoresis. Legend: ● myoglobin; ▲, cymbidium mosaic virus protein; ■, carbonic anhydrase; ○, alcohol dehydrogenase; △, ovalbumin; □, bovine serum albumin.

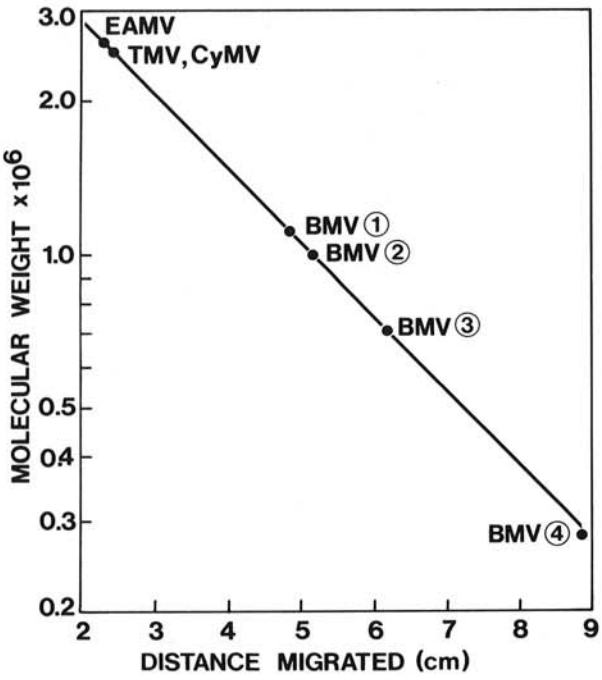

Fig. 4. Relationship between molecular weight and electrophoretic migration of cymbidium mosaic virus RNA and marker RNA's. Abbreviations are: EAMV, echtes Ackerböhnemosaikvirus; TMV, tobacco mosaic virus; CyMV, cymbidium mosaic virus; BMV-1, -2, -3, -4, brome mosaic virus, components 1, 2, 3, and 4, respectively.

The mobilities of CyMV protein and protein standards relative to the mobility of myoglobin in 2.5%, 3.5%, and 5.0% gels were determined. Hedrick and Smith (8) plots of the logarithm of their relative mobilities (Rm) against gel concentration (Fig. 3-B) gave straight lines which extrapolated to a common intercept, Rm = 1.0, at zero gel concentration. This result demonstrated that CyMV protein behaved normally in gel electrophoresis, and that molecular weights determined from Shapiro plots are independent of gel concentration. Results with PVX protein from preparations purified by method IV (27) yielded a linear plot which intercepted at an Rm value of 0.9. This result agrees with Koenig's result (12) for the "S" component of PVX and demonstrated an anomolous behavior attributed to a "charge isomer" (9).

A single component was observed when RNA from dissociated CyMV was electrophoresed in polyacrylamide gels. The molecular weight of CyMV-RNA was estimated to be 2.5×10^6 (Fig. 4) by comparing its mobility with those of RNA from tobacco mosaic, echtes Ackerböhnemosaik, and brome mosaic viruses. The molecular weight of PVX-RNA was 2.2×10^6 in similar tests.

Amino acid and nucleotide composition.—Analyses of amino acids in 24- and 72-hour hydrolyzates of protein and 24-hour hydrolyzates of protein oxidized with performic acid are shown in Table 1. The amounts of threonine and serine decreased as hydrolysis time increased, and their values were calculated by extrapolation of 24- and 72-hour hydrolyzate values to zero hydrolysis time. The amounts obtained in the 24-hour hydrolyzates were 98.7% and 96.2% of the extrapolated values for threonine and serine, respectively.

TABLE 1. Amino acid composition of cymbidium mosaic virus protein

Amino acid	Amino acid recovery after hydrolysis for: 24 hours[a] (nmoles)	72 hours[a] (nmoles)	Average (nmoles)	Relative molar ratio	Integer value
Lys	64	69	67	10.1	10
His	14	15	15	2.3	2
Arg	80	85	83	12.5	13
Asp	167	166	167	25.1	25
Thr[b]	151	148	153	23.0	23
Ser[b]	75	70	78	11.7	12
Glu	128	127	128	19.3	19
Pro	152	146	149	22.4	22
Gly	82	83	83	12.5	13
Ala	322	312	317	47.7	48
Cys[c]	...	...	16	2.4	2
Val[d]	70	81	81	12.2	12
Met[c]	...	...	7	1.1	1
Ile[d]	63	73	73	11.0	11
Leu[d]	130	129	129	19.4	19
Tyr	52	52	52	7.8	8
Phe	58	59	59	8.9	9
Trp[e]	...	...	52	7.8	8
					257
				Molecular weight	27,614

[a]Average values of two analyses on each of two virus preparations.
[b]Extrapolated to zero hydrolysis time.
[c]Determined from performic acid-oxidized protein.
[d]Only 72-hour hydrolysis analyses included in average.
[e]Determined by the method of Bencze and Schmid (2).

Increases of 15% for valine and isoleucine with extended hydrolysis time are due to the greater stability of peptide bonds involving those amino acids. The molecular weight of CyMV protein was determined to be 27,640 in SDS-PAGE runs. Multiplication of the yield in nanomoles in Table 1 by 0.1507 gave relative molar ratios with integer values totaling 257 residues, corresponding to a molecular weight of 27,614.

The molar percentages of recovered nucleotides based on 18 analyses of four CyMV-RNA preparations are shown in Table 2.

Serology.—Serological relationships of CyMV were determined in tube precipitin tests, by titration with homologous and heterologous antisera. The optimum concentration of CyMV for reaction with its homologous antiserum was 0.33 mg/ml. These preparations also reacted with CyMV antisera obtained from R. Francki, Waite Agricultural Research Institute, Adelaide, Australia, and from R. Lawson, U. S. Department of Agriculture, Beltsville, Maryland. No cross-reactions were observed between CyMV and antisera to WCMV or PVX, nor between WCMV or PVX and antiserum to CyMV at any dilution from 1/4 to 1/8,192. Dilution end point titers for homologous reactions were CyMV, 1/4,096; PVX, 1/4,096; WCMV, over 1/8,192.

DISCUSSION

A comparison of the chemical and physical properties of CyMV with those of potexviruses provides evidence for its inclusion in the potexvirus group. The sedimentation coefficient that we obtained, after correction to infinite dilution, is slightly higher than our uncorrected values and previously reported values (5, 17). Varma et al. (29) stated that the sedimentation coefficients of potexviruses can be readily distinguished from those of carlaviruses and potyviruses. The sedimentation coefficient of CyMV is within the range of values reported for potexviruses (29). The RNA content of CyMV is similar to that of potexviruses (29).

TABLE 2. Base composition of nucleic acids of cymbidium mosaic virus (CyMV), potato virus X (PVX), white clover mosaic virus (WCMV), and papaya mosaic virus (PMV)

Base	CyMV (%)	PVX[a] (%)	WCMV[b] (%)	PMV[c] (%)
Guanine	21.1	22	16.1	20.7
Adenine	28.9	32	33.1	33.8
Cytosine	24.4	24	28.4	23.4
Uracil	25.6	22	22.4	22.1

[a]By the method of Knight (10).
[b]By the method of Miki and Knight (21).
[c]By the method of Purcifull and Hiebert (24).

TABLE 3. Molecular weight values reported for the RNA's of potexviruses

Potexvirus	Molecular weight (daltons × 10^6)	Reference
Cymbidium mosaic virus	2.5	(this study)
Potato virus X	2.2	(this study)
Potato virus X	2.1	(11)[a]
Cactus virus X	2.1	(11)
Papaya mosaic virus	2.2	(11)
Clover yellow mosaic virus	2.4	(11)
White clover mosaic virus	2.4	(11)
White clover mosaic virus		
Pea latent strain	2.8	(9)[b]
Pratt 'C' strain	3.1	(9)

[a]Koenig. 1971. J. Gen. Virol. 10:111-114.
[b]Hill and Shepherd. 1972. Virology 47:807-816.

The molecular weight values of CyMV-RNA and PVX-RNA we obtained in gel electrophoresis (Table 3) are similar to values obtained for five potexviruses by Koenig (11). The values reported by Hill and Shepherd (9) for the RNA's of WCMV strains were significantly greater than Koenig's value (Table 3). The extrapolation of a plot of molecular weight versus migration distance, the lack of well-defined standards, and the short migration distance obtained with large RNA molecules, make accurate determination difficult and make comparisons with values obtained in other laboratories subject to conjecture.

The base compositions of nucleic acids of potexviruses differ markedly (Table 2). The guanine content of WCMV is lower and the cytosine content higher than those of CyMV, PVX, and papaya mosaic virus (PMV). The adenine content of CyMV is lower, and the uracil content higher, than those of PVX, WCMV, and PMV. The significance of these differences in virus classification is difficult to assess because even though PVX and WCMV are serologically related (4), their base compositions differ.

Studies on the comparison and size of potexvirus proteins have yielded complex results and comparison with data on CyMV protein is difficult. Koenig (12) reported that certain potexvirus proteins behave anomalously in SDS-PAGE; values of molecular weights estimated for these proteins by the method of Shapiro et al. (25) increased with decreasing gel concentration and therefore were unreliable. Nevertheless, Koenig (12) stated that reliable molecular weights for these proteins could be determined from plots of the logarithm of relative mobility against gel concentration as used by Hedrick and Smith (8) (e.g., Fig. 3-B). The SDS-PAGE results for CyMV and seven other potexviruses, some of which have more than one protein component are given in Table 4. Five of these proteins, including CyMV, moved normally; the molecular weights of four of the proteins that moved anomalously were determined from Hedrick and Smith (8) plots. The molecular weights of the three remaining proteins were studied at only one gel concentration and therefore the values listed for their molecular weight may not be reliable. Table 4 also lists molecular weights obtained by gel chromatography; this method is not affected by anomalous electrophoretic behavior.

The multiple protein components of PVX have been studied by at least four laboratories (15, 26, 27, 28). Limited proteolysis of PVX protein occurs in plant extracts (15, 26), with trypsin (15, 26, 27, 28), and on incubation of preparations at 37 C (28). Table 4 lists some of the range in the molecular weights of the multiple protein components of PVX as well as some other potexviruses. Both CyMV and undegraded PVX appear

to have a protein subunit molecular weight of about 27,000 daltons (28). It is possible that the protein subunit molecular weights of other potexviruses which range from 25,000 down to 17,000 daltons (Table 4) may be the result of limited proteolysis. If this is correct, CyMV and PVX may be the only potexviruses that have been isolated and characterized with their protein in the native condition. Therefore, amino acid composition comparisons between proteins of potexviruses will not be meaningful until this problem is resolved.

In our experiments, CyMV did not show any serological relation to either PVX or WCMV. However, the use of many high-titered antisera from different animals sometimes is required to establish distant relations (4). Some of the physical and chemical data presented here indicate that CyMV is a potexvirus. Other parameters vary greatly among known potexviruses and are not useful in classification at present.

LITERATURE CITED

1. BANCROFT, J. B. 1971. The significance of the multicomponent nature of cowpea chlorotic mottle virus RNA. Virology 45:830-834.
2. BENCZE, W. L., and K. SCHMID. 1957. Determination of tyrosine and tryptophan in proteins. Anal. Chem. 29:1193-1196.
3. BRAKKE, M. K., and N. VAN PELT. 1970. Properties of infectious ribonucleic acid from wheat streak mosaic virus. Virology 42:699-706.
4. BRANDES, J., and R. BERCKS. 1965. Gross morphology and serology as a basis for classification of elongated plant viruses. Adv. Virus Res. 11:1-24.
5. CORBETT, M. K. 1967. Some distinguishing characteristics of the orchid strain of tobacco mosaic virus. Phytopathology 57:164-172.
6. FRANCKI, R. I. B. 1966. Isolation, purification and some properties of two viruses from cultivated Cymbidium orchids. Aust. J. Biol. Sci. 19:555-564.
7. FRANCKI, R. I. B. 1970. Cymbidium mosaic virus. No. 27 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Applied Biol., Kew, Surrey, England. 3 p.
8. HEDRICK, J. L., and A. J. SMITH. 1968. Size of charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. Arch. Biochem. Biophys. 126:155-164.
9. HILL, J. H., and R. J. SHEPHERD. 1972. Biochemical properties of turnip mosaic virus. Virology 47:807-816.
10. KNIGHT, C. A. 1963. Chemistry of viruses. Protoplasmatologia IV 2:1-177.

TABLE 4. Electrophoretic behavior in, and molecular weights calculated from, SDS-polyacrylamide gel electrophoresis and gel chromatography of potexvirus proteins

	Polyacrylamide gel electrophoresis				
Virus	Number and name of components	Electrophoretic behavior[a]	Molecular weight[b]	Gel chromatography molecular weight	Reference
Cymbidium mosaic	1	Normal	27,640		
Cactus X	2 Slow	Anomalous	17,500		(12)[c]
	Fast	Normal	17,500		(12)
Clover yellow mosaic	1	Normal	18,800		(12)
Hydrangea ringspot	2	?	22,000	24,400	(13)[d]
		?	19,000	24,400	
Narcissus mosaic	2	Anomalous	20,800		(14)[e]
		Anomalous	18,300		
Papaya mosaic	1	Normal	20,300		(24)[f]
White clover mosaic	1	?	23,500	23,500	(28)[g]
Potato X	2 Slow	Anomalous	22,900	23,600	(12)
	Fast	Normal	22,900	23,600	
Potato X	3 PVX	?	27,600	27,000	(28)
	TD	?	23,900	23,500	
	PPD	?	24,500	25,000	

[a]Normal means the molecular weight value obtained is independent of gel concentration; anomalous means these values are dependent on gel concentration; ? means behavior in different gel concentrations is not known.

[b]Molecular weights given in this column for proteins with anomalous electrophoretic behavior are from Hedrick and Smith plots (8) and hence are corrected for the anomaly.

[c]Koenig. 1972. Virology 50:263-266.

[d]Koenig. 1973. No. 114 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 3 p.

[e]Koenig et al. 1973. Intervirology 1:348-353.

[f]Purcifull and Hiebert. 1971. No. 56 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 p.

[g]Tung and Knight. 1972. Virology 49:214-223.

11. KOENIG, R. 1971. Nucleic acids in the potato virus X group and in some other plant viruses: comparison of the molecular weights by electrophoresis in acrylamide-agarose composite gels. J. Gen. Virol. 10:111-114.
12. KOENIG, R. 1972. Anomalous behavior of the coat proteins of potato virus X and cactus virus X during electrophoresis in dodecyl sulfate-containing polyacrylamide gels. Virology 50:263-266.
13. KOENIG, R. 1973. Hydrangea ringspot virus. No. 114 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc. Applied Biol., Kew, Surrey, England. 3 p.
14. KOENIG, R., D. LESEMANN, A. A. BRUNT, and H. KUHNE. 1973. Narcissus mosaic virus found in Nerine bowdenii. Identification aided by anomalies in SDS-PAGE. Intervirology 1:348-353.
15. KOENIG, R., H. STEGEMANN, H. FRANCKSEN, and H. L. PAUL. 1970. Protein subunits in the potato virus X group. Determination of the molecular weights by polyacrylamide electrophoresis. Biochim. Biophys. Acta 207:184-189.
16. LANE, L. C., and P. KAESBERG. 1971. Multiple genetic components of bromegrass mosaic virus. Nat. New Biol. 232:40-43.
17. LAWSON, R. H. 1970. Etiology of flower necrosis in Cattleya orchids. Phytopathology 60:36-40.
18. LOENING, U. E. 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. 113:131-138.
19. MARKHAM, R. 1955. Nucleic acids, their components and related compounds. Pages 265-266 *in* K. Peach and M. V. Tracey, eds. Modern methods of plant analysis, Vol. IV, Part 2. Springer-Verlag, Berlin. 776 p.
20. MARKHAM, R. 1960. A graphical method for the rapid determination of sedimentation coefficients. Biochem. J. 77:516-519.
21. MIKI, T., and C. A. KNIGHT. 1967. Some chemical studies on a strain of white clover mosaic virus. Virology 31:55-63.
22. NAMBA, R., and M. ISHII. 1971. Failure of aphids to transmit the Odontoglossum ringspot and Cymbidium mosaic viruses to orchid plantlets derived from meristem cultures. Phytopathology 61:582-583.
23. PRING, D. R. 1971. Viral and host RNA synthesis in BSMV-infected barley. Virology 44:54-66.
24. PURCIFULL, D. E., and E. HIEBERT. 1971. Papaya mosaic virus. No. 56 *in* Descriptions of plant viruses. Commonw. Mycol. Inst., Assoc.Applied Biol., Kew, Surrey, England. 4 p.
25. SHAPIRO, A. L., E. VIÑUELA, and J. V. MAISEL, JR. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28:815-820.
26. SHEPARD, J. F., and G. A. SECOR. 1972. The effects of enzymatic digestion on the molecular weight and antigenic specificity of potato virus X protein. Phytopathology 62:1154-1160.
27. TREMAINE, J. H., and H. O. AGRAWAL. 1972. Limited proteolysis of potato virus X by trypsin and plant proteases. Virology 49:735-744.
28. TUNG, J. S., and C. A. KNIGHT. 1972. The coat protein subunits of potato virus X and white clover mosaic virus, a comparison of methods for determining their molecular weights and some in situ degradation products of potato virus X protein. Virology 49:214-223.
29. VARMA, A., A. J. GIBBS, and R. D. WOODS. 1970. A comparative study of red clover vein mosaic virus and some other plant viruses. J. Gen. Virol. 8:21-32.