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Characterization of a Chimeric Cauliflower Mosaic Virus Isolate That Is More Severe and Accumulates to Higher Concentrations than Either of the Strains From Which It Was Derived

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We have previously shown that cauliflower mosaic virus (CaMV) isolate H30, a chimeric virus in which most of gene VI and the large intergenic region are derived from the mild CaMV strain CM1841 and genes I–V are derived from the severe strain W260, was significantly more severe and attained higher virion antigen concentrations than either W260 or CM1841 (E. J. Anderson, S. G. Qiu, and J. E. Schoelz, Virology 181:647-655, 1991). We have now determined that sequences within the 5′ half of CM1841 gene VI, rather than the 35S promoter or translational regulatory sequences present in the large intergenic region, were responsible for the increases in virion antigen concentration and symptom severity. The levels of H30 DNA and RNA in infected turnip leaves were also higher than either CM1841 or W260. Although CM1841 inclusions were less stable than W260 inclusions, gene VI did not determine the difference in inclusion stability, demonstrating that the enhanced severity of H30 was not attributed to the role of gene VI in inclusion body structure. Instead, the increased concentration and severity of H30 may be related to the function of the gene VI product in post-transcriptional transactivation. Additionally, the purification and stability characteristics of wild type and chimeric viruses indicated that purified CM1841 virions were less stable than purified W260 virions. CM1841 virions were recovered from infected tissue at a much lower level than W260 virions, were less infectious than W260 virions in a local lesion assay, and were more heterogenous in CsCl gradients.

Additional keywords: hyperseverity, inclusion body stability, virion stability.

Cauliflower mosaic virus (CaMV) is a DNA plant virus that has been used extensively in recombinant DNA studies to identify viral genes that influence symptomatology and host specificity. Several research groups have attempted to identify regions of CaMV that influence stunting severity in turnips (cv. Just Right). The results of these studies are not in agreement, probably because different virus strains have been used by each group and different chimeric viruses have been tested. Results with chimeric CaMV isolates constructed between the mild strain CM4-184 and the severe strain Cabb S have suggested that the 5′ portion of gene VI of Cabb S was responsible for the characteristic degree of stunting (Vaden and Melcher 1990). The use of chimeric viruses consisting of portions of the severe strain Cabb B-JI and the mild strain Bari I have indicated that two separate loci, one that contained portions of genes I and II and a second that consisted of the 5′ half of gene V, influenced stunting severity (Stratford and Covey 1989). Chimeric viruses composed of the mild strain CM1841 and the severe strain W260 demonstrated that at least a portion of every CaMV gene influenced stunting severity (Anderson *et al.* 1991). In one test that involved 10 chimeric viruses derived from CM1841 and W260, the infected turnips could be divided into nine distinct size classes. An enzyme-linked immunosorbant assay (ELISA) of turnips infected with CM1841, W260, and the chimeric viruses revealed that the concentration of CM1841 in infected plants was higher than that of W260, and that several regions of CaMV influenced virus concentration in infected turnips.

It is clear from these studies that the stunting phenotype of CaMV is very complex. This complexity may be resolved only by a greater understanding of the CaMV strains that have been used in mapping stunting determinants. At least two factors may govern the severity of plant virus infections. First, the actions of individual viral genes may disrupt host metabolism, resulting in a characteristic degree of stunting. Second, any viral sequences that influence replication, transcription, or translation should also influence the degree of stunting. For example, an increase in the levels of viral gene products might be expected to lead to a greater degree of stunting. To identify specific CaMV genes that influence stunting severity, primarily by disrupting host metabolism, it is first necessary to determine whether a difference in severity of a recombinant virus is merely a reflection of a difference in viral gene expression.

In the present study, we have further characterized a chimeric virus, H30 constructed from CM1841 and W260, that induces a significantly greater degree of stunting than either of the wild type viruses. The increased severity of H30 is correlated with high levels of virus antigen in infected plants, indicating that the increase in severity of H30 relative to W260 may be a result of increases in viral gene expres-

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sion. We have mapped the region of CaMV responsible for the hypersevere symptoms of H30 more precisely and have also measured concentrations of viral DNA, RNA, and virus antigen to further characterize the H30-induced phenotype.

During the course of this work, we also discovered that we could consistently purify W260 virions from infected turnips in greater amounts than CM1841 virions, contradicting the ELISA and dot blot tests that indicated that CM1841 viral antigen and DNA were present at higher concentrations. We present several lines of evidence to show that purified W260 virions are more stable than purified CM1841 virions, suggesting that virion stability may explain the difference between the ELISA tests and virus purification patterns.

MATERIALS AND METHODS

Preparation of wild type and chimeric viruses. Wild type CaMV strains CM1841 (Howarth *et al.* 1981) and W260 (Gracia and Shepherd 1985) have been cloned in infectious form (Howarth *et al.* 1981; Schoelz and Shepherd 1988). The chimeric viruses H30, H31, H33, H28, and H29 were constructed previously (Schoelz and Shepherd 1988; Anderson *et al.* 1991). Hybrid viruses H82 and H72 were constructed by first subcloning the 2,335 base pair (bp) *Sac*I (nucleotide 5822)–*Bst*EII (nucleotide 126) DNA segments of CM1841 and W260 into the plasmid vector pUCD9X (Close *et al.* 1984). The appropriate *Pvu*II (nucleotide 6318)–*Bst*EII exchanges were made and the recombinant *Sac*I–*Bst*EII fragments were then exchanged back into the cloned viruses W260 or H30. Viruses were propagated in turnips and stored in freeze-dried tissue at 4° C. Growth, inoculation, and maintenance of turnip plants with wild type and chimeric viruses has been described (Daubert *et al.* 1984; Anderson *et al.* 1991).

Detection of viral DNA. The amount of viral DNA in infected turnip leaf tissue was quantitated by a modification of the method of Maule *et al.* (1983). Tissue samples prepared for ELISA were mixed (10 μl) with 90 μl of solution 1 (1× PBS, 0.02 M EDTA, pH 8.0) and 400 μl of solution 2 (12.5% sodium dodecyl sulfate (SDS), 6.25 mg/ml of proteinase K, 12.5 mM EDTA, pH 8.0). The samples were held at 37° C for 30 min to release encapsidated DNA, extracted once with 500 μl of phenol/chloroform/isoamyl alcohol (1:1:0.02), and the aqueous phase was collected. An aliquot of this DNA preparation (20 μl) was mixed with 380 μl of solution 3 (0.1 M Tris-HCl, pH 7.4, 6 μg/ml of herring sperm DNA). To this was added 71 μl of 2 M NaOH and 235 μl of 20× SSC (1× SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0). Samples were incubated at 80° C for 10 min to denature DNA and degrade any RNA, and then placed immediately on ice. The samples were neutralized with 96 μl of 2 M Tris-HCl, pH 7.4, and applied in 340-μl vol to two wells each of a Minifold dot blot apparatus (Schleicher and Schuell, Keene, NH) and vacuum-dotted onto Nytran membrane (Schleicher and Schuell). The membrane was air-dried and baked at 80° C under vacuum for 1–2 hr as described by Sambrook *et al.* (1989). CaMV DNA standard curves were generated by digesting known amounts of purified virions as described above. Dot blot membranes were blocked, washed, and probed according to Sambrook *et al.* (1989). The probe was prepared by mixing equal amounts of full-length CM1841 and W260 viral DNAs excised from pBR322 vectors and labeled using a random primed DNA labeling kit (United States Biochemicals Corp., Cleveland, OH).

RNA analysis. Total leaf RNA was isolated by the method of Chomczynski and Sacchi (1987) and quantitated by spotting onto nitrocellulose (Sambrook *et al.* 1989). For the dot blot analysis, 20 μg of RNA was diluted in 100 μl of sterile, diethylpyrocarbonate-treated glass-distilled water. To this was added 300 μl of 6.15 M formaldehyde and 10× SSC. The samples were heated at 65° C for 15 min and then chilled on ice. These were applied in two 200-μl aliquots (10 μg each) to a Minifold dot blot apparatus and bound to nitrocellulose membrane (Schleicher and Schuell). The membrane was air-dried and baked at 80° C for 1–2 hr. Because it was impractical to generate CaMV RNA standards, all samples to be compared were prepared at the same time and blotted to the same membrane. A 1,203-bp *Sac*I (nucleotide 5822)–*Hgi*AI (nucleotide 7025) CaMV DNA fragment containing sequences common to both the 19S and 35S CaMV RNA transcripts was excised from the plasmid vector pUC13 and used as a probe by labeling with a random primed DNA labeling kit (United States Biochemicals Corp.). Relative viral RNA concentrations were then determined by scanning the autoradiogram with a densitometer (Hoefer model GS300, Hoefer Scientific Instruments, San Francisco, CA).

Viral coat protein quantitation. One-gram samples were taken from inoculated and various systemically infected leaves at given times after inoculation, and ELISA was used to determine relative concentrations of virus (Anderson *et al.* 1991). To make direct comparisions of protein concentrations between groups of plant samples taken and analyzed at different times, it was necessary to develop a standard curve for each ELISA using a dilution series of purified CaMV virions. From these curves, the approximate virus concentrations in nanograms per milliliter of sample and micrograms per gram fresh weight of starting tissue were calculated.

Analysis of inclusion body stability. To analyze inclusion body stability, 1.0 g of infected turnip leaves was homogenized in 10 ml of grinding buffer (phosphate-buffered saline, pH 7.4, 1.0% polyvinyl pyrrolidone [mol. wt. 40,000], 0.1% bovine serum albumin, 0.05% Tween-20). One milliliter of the homogenized sample was then separated into supernatant and pellet fractions by low-speed centrifugation in Eppendorf tubes (5 min at 15,000 × *g*). The pellet fraction was subsequently resuspended in 1.0 ml of grinding buffer. To disrupt inclusions, 1.0 ml of the homogenized sample, supernatant fraction, and pellet fraction was treated with 1.0 M urea and 2.5% Triton X-100 (final concentrations) overnight at 4° C. For direct ELISA, samples were diluted in grinding buffer to a final dilution of 1:800 (w/v).

Purification of virus. Virions were isolated as described by Hull *et al.* (1976), and the concentration of purified virus was determined employing an extinction coefficient of $E^{01\%}_{260nm} = 7.1$ (Hull *et al.* 1976). Purified virions were stored in 50% sterile glycerol at 4° C or −20° C.

For isopycnic CsCl centrifugations, two-step CsCl gradients, $\rho = 1.50$ g/ml and $\rho = 1.25$ g/ml, were prepared according to Sehgal *et al.* (1970). Aliquots of partially purified virions (30 μl containing 40–50 μg), were either left untreated or fixed with 1% (v/v) formaldehyde for 2 hr at room temperature. The samples were mixed with the lighter density CsCl solution, which was then layered onto the heavier density CsCl solution. The gradients were centrifuged at 140,000 × *g* for 24 hr at 15° C, after which they were fractionated into 100-μl amounts by a density gradient fractionator (ISCO Model 640, Isco, Lincoln, NE). The refractive indices of the various fractions were determined by a Bausch and Lomb Abbe 3L refractometer at 25° C and converted to density values.

RESULTS

Gene VI influences disease severity and the concentration of viral structural antigen in infected turnip leaves. We have previously shown through the use of chimeric viruses H30 and H31 that a 2,335-bp *Sac*I (nucleotide 5822)–*Bst*EII (nucleotide 126) DNA segment that contains most of gene VI and the large intergenic region influences virus accumulation and disease severity (Anderson *et al.* 1991). The large intergenic region of CaMV contains the 35S promoter and upstream transcriptional enhancer sequences, as well as translational regulator sequences in the 600 nucleotide leader of genes VII and I (Odell *et al.* 1985, 1988; Baughman and Howell 1988). Results with chimeric viruses H30 and H31 might reflect 35S promoter or leader sequence effects rather than a gene VI influence. By constructing and testing hybrid viruses H33, H72, and H82 (Fig. 1), we divided the gene VI coding sequence roughly in half, allowing us to identify the region within the *Sac*I–*Bst*EII DNA segment that influences stunting severity and virus concentration.

The disease severity and concentration of H72 was indistinguishable from H30 (Table 1, lines 1 and 2), demonstrating that sequences within the 5′ half of gene VI have a significant role in influencing both stunting severity and virus concentration. Furthermore, the reciprocal virus H33 was consistently as mild as H31 and also accumulated to levels that were intermediate between CM1841 and W260 (Table 1, lines 3, 5–7). In contrast, chimeric virus H82 was indistinguishable from W260 in stunting severity (Table 1, lines 3 and 4), demonstrating that the 3′ half of gene VI and large intergenic region were not responsible for the hyperseverity of H30. The level of H82 virion antigen in infected turnip leaves was intermediate between the parentals. Although the concentration of H82 could not be statistically distinguished from either W260 or CM1841 (Table 1, lines 3–5), it was significantly lower than H30, indicating that the 3′ half of gene VI and large intergenic region did not play as important a role in influencing virus concentration as the 5′ half of gene VI. The chimeric viruses H72, H82, and H33 demonstrated that differences between CM1841 and W260 in the 5′ half of gene VI most strongly influence disease severity and virus accumulation.

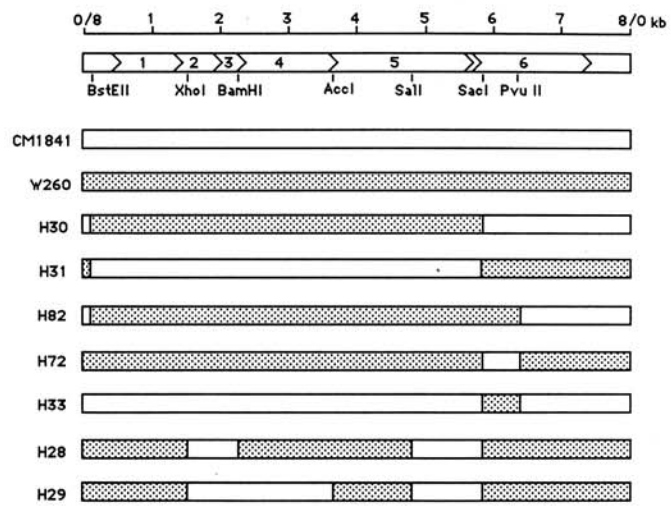

Fig. 1. The genetic composition of chimeric viruses constructed between wild type viruses CM1841 and W260. The genomes are presented linearly with the 0/8,031-bp position of the sequences at each end of the diagram. The genetic map at the top shows the position of the six major cauliflower mosaic virus open reading frames and important restriction enzyme sites.

Inclusion body stability is not correlated with gene VI. The CaMV gene VI product may have at least two functions in infections of crucifers. It transactivates the expression of genes on the 35S RNA (Bonneville *et al.* 1989; Gowda *et al.* 1989) and it also has a structural role in the formation of the amorphous inclusions characteristic of CaMV infections (Covey and Hull 1981; Xiong *et al.* 1982). Because inclusion body stability may differ between CaMV isolates (Givord *et al.* 1984), it is possible that gene VI of CM1841 might be responsible for the hyperseverity of H30 by altering the stability of its inclusions in plant cells. To determine whether inclusion body stability was correlated with gene VI of CM1841, we homogenized infected turnip leaves and compared by ELISA the amount of viral antigen present in the supernatant and pellet fractions after a low-speed centrifugation. If inclusions remain intact, all of the virus should be found in the pellet fraction. However, if inclusions fall apart upon homogenization, then significant amounts of virus may be found in the supernatant. A similar test was developed by Givord and co-workers (1984) to show that gene II influences inclusion body stability.

Table 1. Fresh weight of infected turnip plants and endogenous concentrations of cauliflower mosaic virus antigen for parental strains and chimeric viruses

Virus	Mean fresh weight (g)	ELISA results (A_{450} absorbance)
H30 (10)[y]	43.4 a[z]	0.786 a
H72 (9)	52.8 a	0.706 ab
W260 (10)	112.8 b	0.331 c
H82 (8)	114.9 b	0.504 cde
CM1841 (10)	165.9 c	0.600 bd
H33 (3)	194.6 cd	0.482 de
H31 (10)	194.3 d	0.404 ce
Healthy (10)	243.9 e	0

[y] Ten plants were inoculated with each virus. In three cases it was necessary to discard some plants that had become infected with a root rotting organism during the course of the study. The number of plants analyzed in the test are indicated within parentheses.

[z] Values are the means for plants tested in this representative experiment. ANOVA and LSD mean separation procedure was performed using raw data. Means followed by the same letter are not statistically different at $P \leq 0.05$.

In a representative experiment presented in Table 2, virtually no W260 virus was detected in the supernatant after low-speed centrifugation, indicating that W260 inclusions remained intact. In contrast, CM1841 virions were divided equally between the supernatant and pellet fractions, suggesting that CM1841 inclusions were not as stable as W260 inclusions. Results obtained with H30 and H31 demonstrated that gene VI was not responsible for differences in inclusion body stability. The inclusions of H30 were as stable as W260 inclusions, whereas H31 inclusions had the characteristic stability of CM1841 inclusions (Table 2).

Accumulation of viral DNA, RNA, and structural antigen for wild type and chimeric viruses. We have demonstrated that CM1841 and H30 viral antigens can accumulate to higher levels in systemically infected turnip leaves than W260 and H31. To characterize the molecular basis for differences in the virus antigen concentration *in planta*, we determined the amounts of viral DNA and RNA, as well as virus concentration in the 10th systemically infected leaf above the inoculated leaf of turnips infected with CM1841, W260, and the reciprocal chimeric viruses H30 and H31 (Fig. 1) at 4 and 6 wk after inoculation.

The DNA concentrations determined by dot blot analysis mirrored the virion antigen concentrations determined by ELISA (Fig. 2A,B). At 4 wk after inoculation, CM1841 virus antigen and DNA were detected at higher concentrations than W260, whereas the concentrations of H31 DNA and virus antigen were intermediate between the two wild type viruses. By the sixth week, the DNA and virus antigen concentrations of CM1841 and H31 had decreased, whereas W260 DNA and viral antigen concentrations had increased slightly. At 6 wk, the DNA and viral antigen concentrations of CM1841, W260, and H31 were essentially indistinguishable from each other. In contrast, H30 DNA and virus antigen concentrations were significantly higher than the other viruses at both 4 and 6 wk. The DNA dot blot analysis confirmed the differences in virus concentration that had been detected by ELISA and also revealed differences in the rates of replication between the four viruses.

The viral RNA concentrations of the four viruses in infected tissue generally agreed with DNA and virion antigen concentrations (Fig. 2C). Most importantly, H30 viral RNA was detected at higher concentrations than the other viruses at both 4 and 6 wk after inoculation. One notable exception was that RNA concentrations for all four viruses increased from 4 to 6 wk, in contrast to DNA and viral antigen concentrations, which generally peaked and dropped as the leaf aged. A similar increase in RNA levels over 2–6 wk was observed in a time course study of virus infections in the fifth systemically infected leaf above the inoculated leaf (data not shown).

Purified CM1841 virions are not as stable as W260 virions. Although W260 structural antigen and DNA were detected at concentrations lower than CM1841 in systemically infected turnip leaves, the yield of W260 virions from equivalent amounts of tissue was always four to five times higher than that of CM1841 virions. In three representative experiments, the average yield of W260 virions

Table 2. Inclusion body stability of CM1841, W260, H30, and H31 viruses

Treatment	Virus			
	CM1841	W260	H30	H31
Crude extract	0.629[z]	0.256	0.665	0.380
Low-speed centrifugation supernatant	0.328	0.013	0.033	0.190
Low-speed centrifugation pellet	0.339	0.214	0.467	0.213

[z] Absorbance at 450 nm used to represent relative virus concentrations in various fractions of homogenized leaf samples for inclusion body stability evaluation. Each value presented is the average of three absorbance readings.

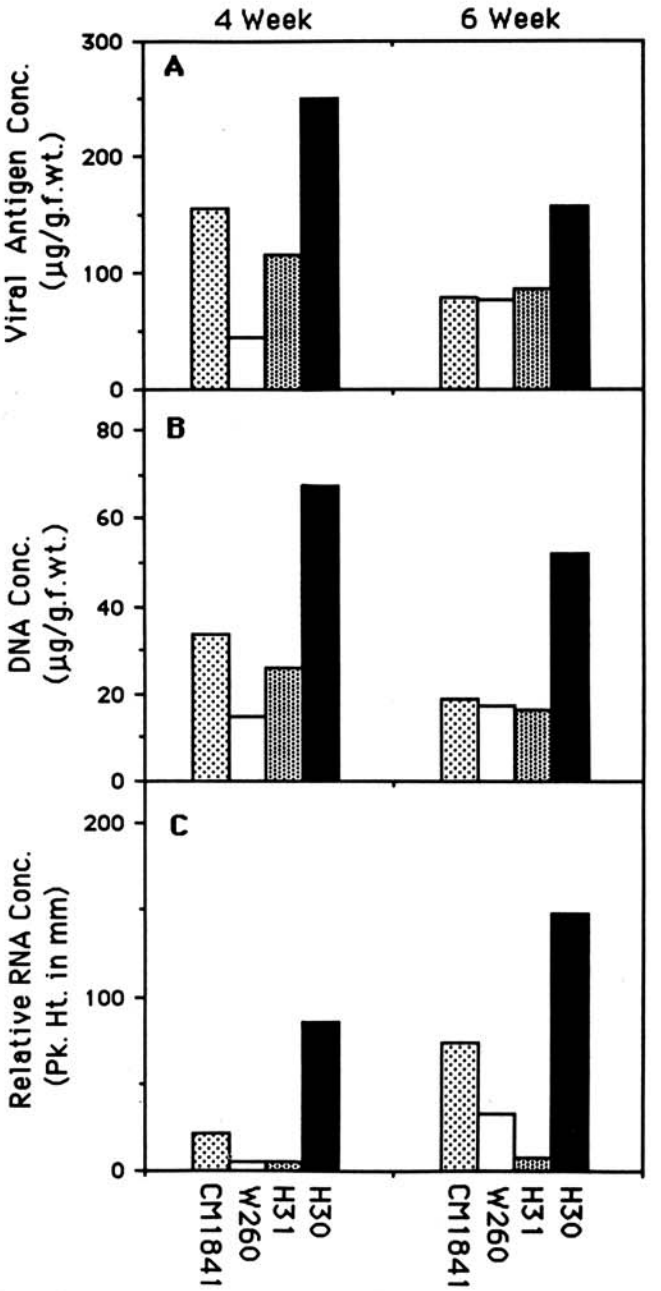

Fig. 2. The accumulation patterns of **A,** viral antigen; **B,** viral DNA; and **C,** viral RNA in the 10th leaf above the inoculated leaves of turnips infected with strains CM1841, W260, H31, and H30 sampled from five plants for each virus at 4 and 6 wk after inoculation.

from 100 g of tissue was 0.733 mg, whereas CM1841 virions yielded an average of 0.158 mg from the same amount of tissue, a ratio of 4.6:1. When bulked leaf tissue was sampled just before purification, ELISA results showed that the concentration of CM1841 viral antigen was higher than, or at least equal to W260 (data not shown). ELISA failed to reveal more significant loss of CM1841 than W260 at any step during the purification procedure (data not shown).

The representative experiment presented in Table 3 shows that the yields of the viruses encapsidated by W260 coat protein (W260, H30, H28) were greater than those encapsidated with CM1841 coat protein (CM1841, H31, H29). The difference in the yields of chimeric viruses H28 and H29 is important, because these viruses differ from each other only in the origin of their coat proteins (gene IV) (Fig. 1). A previous ELISA study has shown that the concentrations of H28 and H29 in infected turnip leaves are equivalent (Anderson *et al.* 1991), suggesting that the difference in yield resulted from a difference in virion stability. These results indicated that the coat protein of CM1841 was responsible for the decrease in yield of CM1841 virions.

To resolve the discrepancy between the ELISA studies and the comparative yields of CM1841 and W260 virions, we examined viral stability by infectivity assays and sedimentation properties in CsCl gradients. Purified W260 virions were at least 10 times more infectious than CM1841 when assayed on the local lesion host, *Datura stramonium* L. (Table 4). Indeed, if these viruses were held for more than 3 mo at 4° C or −20° C in sterile glass-distilled water or TE buffer, the infectivity of CM1841 generally dropped to zero (data not shown). The difference in the infectivity was significantly less if virions were maintained in 50% glycerol after purification (Table 4), indicating that CM1841 virions were less stable *in vitro* than W260 virions.

In isopycnic CsCl gradients, W260 virions that had been fixed with formaldehyde or left untreated banded at a density of 1.350 g/ml, approximately 1.6 cm from the meniscus (Fig. 3C,D). The density of W260 virions fell within the values of 1.35–1.37 g/ml reported for CaMV (Shepherd *et al.* 1970; Hull 1984). Untreated CM1841 virions banded

Table 3. Yields of parental and chimeric cauliflower mosaic virus virions from infected turnip tissue 5 wk after inoculation

Virus isolate	Virion yield (mg)
W260	0.9[z]
CM1841	0.3
H30	3.3
H31	0.7
H28	1.1
H29	0.6

[z] Virions were purified from 100 g of tissue by banding in sucrose gradients as described in Materials and Methods.

Table 4. Infectivity of purified CM1841 and W260 virions stored in Tris-EDTA, pH 8.0, or 50% glycerol as determined by the number of lesions formed on *Datura stramonium* half leaves

Inoculum (μg/ml)	Necrotic lesions per half leaf			
	Tris-EDTA[x]		50% glycerol[y]	
	W260	CM1841	W260	CM1841
0.1	1.0 ± 0.8[z]	0	6.8 ± 2.6	2.3 ± 1.0
1.0	93.5 ± 30.1	0.5 ± 0.5	18.0 ± 5.7	12.5 ± 6.1
10.0	359.8 ± 182.8	31.0 ± 40.7	95.0 ± 33.2	65.0 ± 37.0

[x] Inocula were prepared by diluting virions stored for 4 wk at −80°C in TE (following the spectrophotometric determination of concentrations immediately after purification).

[y] Inocula were prepared by diluting virions in TE that were stored for 2 wk at 4° C in 50% sterile glycerol (after the concentrations had been determined following purification).

[z] Values are the mean number of lesions formed on four half leaves inoculated with each inoculum concentration ± S. E.

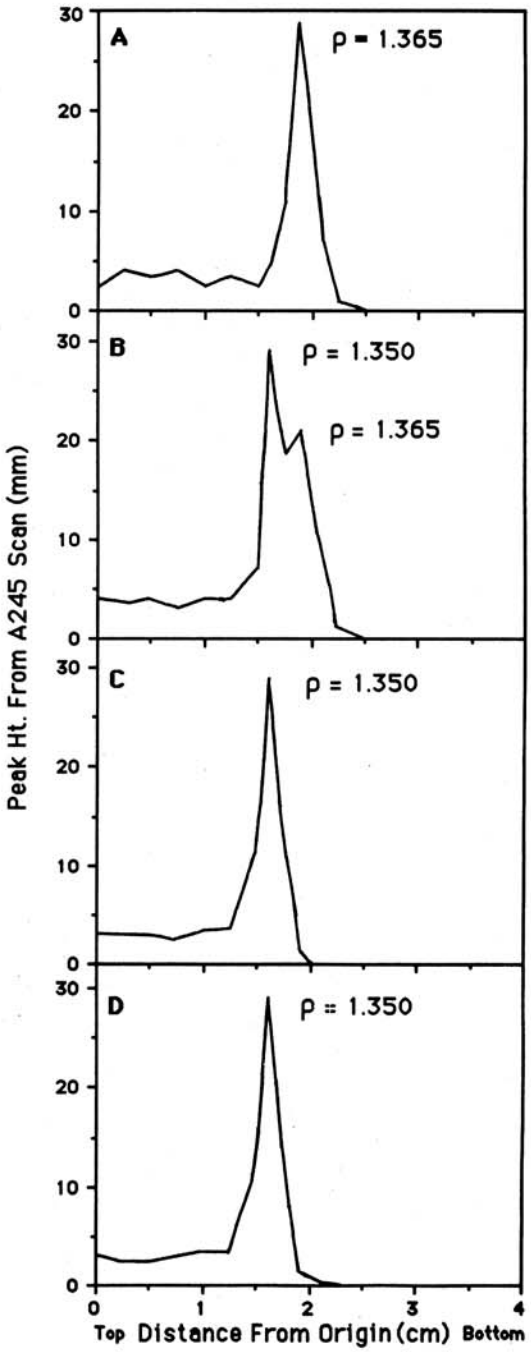

Fig. 3. Banding patterns in CsCl of unfixed and formaldehyde-fixed virions of cauliflower mosaic virus strains CM1841 and W260. **A,** CM1841 virions untreated. **B,** CM1841 virions fixed in 1% (v/v) formaldehyde. **C,** W260 virions untreated. **D,** W260 virions fixed in 1% (v/v) formaldehyde.

at $\rho = 1.365$ g/ml, approximately 1.9 cm from the meniscus (Fig. 3A). In contrast to W260, formaldehyde-fixed CM1841 yielded two virion populations; a major peak at $\rho = 1.350$ g/ml and a minor peak at $\rho = 1.365$ g/ml (Fig. 3B). These results further implied that there were structural and/or stability differences between CM1841 and W260 virions.

DISCUSSION

We previously reported that H30, a chimeric CaMV isolate constructed from W260 and CM1841, was significantly more severe than either of the parental viruses, and that H30 attained concentrations in turnip leaves greater than CM1841 (Anderson *et al.* 1991). In the present study, we have demonstrated that CaMV sequences responsible for the hyperseverity and increased concentration of H30 mapped within a *Sac*I (nucleotide 5822)–*Pvu*II (nucleotide 6318) DNA segment that makes up the 5′ half of gene VI. The role for this region is actually the opposite of that described by Vaden and Melcher (1990). These authors found that the 5′ region of gene VI of the severe strain Cabb S influenced disease severity in chimeric viruses between Cabb S and the mild strain CM4-184. In contrast, the increased severity of H30 relative to W260 was due to the presence of sequences from the mild strain CM1841.

The localization of the hypersevere phenotype of H30 to the 5′ half of gene VI strongly suggests that the gene VI coding region, rather than the 35S promoter, has been altered. Previous studies that have mapped the upstream limits of the 35S promoter indicate that it extends from the transcription start site at nucleotide 7435 to nucleotide 7092, a distance of 343 bp (Odell *et al.* 1985, 1988; Ow *et al.* 1987; Benfey *et al.* 1989). Because the region that regulates disease severity and virus concentration is at least 750 bp upstream from the limit of the 35S promoter, it is unlikely that changes in the 35S promoter could be responsible for those phenotypes. The gene VI coding region could influence disease severity through the interaction of its protein product with host components. However, at this point we cannot eliminate the possibility that gene VI DNA or RNA sequences are responsible for the hyperseverity of H30 and H72.

The gene VI product might modulate disease severity and virus concentration in one of three ways. First, the gene VI product might influence disease severity and virus concentration through its effect on inclusion body structure. Nearly all CaMV virions are localized within amorphous inclusions in the cytoplasm (Shalla *et al.* 1980), and the gene VI product is a major component of inclusions (Al Ani *et al.* 1980; Odell and Howell 1980; Covey and Hull 1981; Xiong *et al.* 1982). If one consequence of CaMV inclusion body formation is that virus remains sequestered within the cell, then a decrease in stability of inclusions might result in a release of virus or viral DNA into the cytoplasm. Viral DNA might be cycled back to the nucleus, leading to a greater accumulation of viral DNA, RNA, and proteins and an increase in disease severity. Inclusions of the mild isolate CM1841 were in fact less stable than W260 inclusions. However, the chimeric viruses H30 and H31 demonstrated that the gene VI product was not responsible for the differences in inclusion body stability. Therefore, the increased severity of H30, a trait that had been mapped to gene VI, was not correlated with inclusion stability.

Secondly, the gene VI product might be directly responsible for the degree of stunting in turnips by disrupting host metabolism in some manner. The evidence to support this hypothesis is inconclusive. Transgenic tobacco that express either the CaMV or figwort mosaic virus (FMV) gene VI products are chlorotic and stunted (Baughman *et al.* 1988; Takahashi *et al.* 1989; Balazs 1990; Goldberg *et al.* 1991), demonstrating that the gene VI product by itself is capable of stunting plants. However, Goldberg and co-workers (1991) have found that not all transgenic solanaceous species that express gene VI have viruslike symptoms. Transgenic *Datura innoxia* Mill. that expressed the FMV gene VI product did not develop any symptoms, whereas *D. innoxia* transformed with gene VI of either CaMV strains D4 or CM1841 were stunted and chlorotic. The observation that some solanaceous plants may remain symptomless upon transformation and expression of gene VI suggests that development of symptoms in transgenic plants might be unrelated to the severity of systemic symptoms in turnips.

Lastly, the gene VI product could influence disease severity in turnips by regulating the expression of genes on the 35S RNA. Recent studies have shown that the gene VI product transactivates the expression of genes VII and I (Bonneville *et al.* 1989; Gowda *et al.* 1989), whereas further research has shown that gene VI also transactivates the expression of genes II–V (Scholzhof *et al.* 1990). We previously demonstrated that W260 genes on the 35S RNA, in particular gene IV, are responsible for the increased severity of W260 relative to CM1841 (Anderson *et al.* 1991). In the present study, we have shown that sequences within the 5′ half of gene VI influence virus concentration and disease severity. Therefore, a possible explanation for the hyperseverity of H30 is that the gene VI product of CM1841 is more efficient at transactivation than the corresponding W260 gene VI product, leading to a greater accumulation of the W260 gene products responsible for the stunting phenotype. Further research with H30-infected turnips will be necessary to determine whether other W260 gene products on the 35S RNA have increased in concentration in addition to gene IV.

An increase in translation of viral gene products may explain the concomitant increases in viral DNA and RNA detected in H30-infected leaves. Higher levels of reverse transcriptase could lead to greater amounts of viral DNA. Increases in coat protein might result in a greater number of particles in which reverse transcription could occur (Marsh and Guilfoyle 1987) and/or less degradation of viral DNA. Saunders *et al.* (1990) have suggested that a portion of the viral DNA synthesized in the cytoplasm might be cycled back into the nucleus. Therefore an increase in viral DNA in the cytoplasm could result in a greater level of DNA in the nucleus and an increase in transcription. An alternative mechanism may be that the gene VI product directly regulates transcription, although the research of Bonneville *et al.* (1989) indicates that the gene VI product only regulates translation.

During the course of this study we discovered that

purified CM1841 virions are less stable than W260 virions. Native CM1841 virions possessed a somewhat higher buoyant density ($\rho = 1.365$ g/ml) in CsCl relative to W260 virions ($\rho = 1.350$ g/ml), suggesting that CM1841 virions are structurally more relaxed (or swollen) than W260 virions. Due to the relaxed conformation, Cs ions may interact with the coat protein or diffuse through the coat subunits and bind with the virion DNA and/or accumulate at the core, resulting in a net increase in the CM1841 buoyant density. In an earlier study, Al Ani *et al.* (1979) demonstrated that alkali-induced swelling of the Cabb S strain of CaMV was accompanied by an increase in virion buoyant density in CsCl gradients; this change was reversed fully upon capsid recompaction. Whereas treatment with formaldehyde transformed a proportion of CM1841 virions to the lower density form ($\rho = 1.350$ g/ml), no such change was observed for W260 virions. The basis for this formaldehyde-induced change in CM1841 virions is unclear. However, it indicates that formaldehyde treatment somehow alters the Cs+-binding characteristics of CM1841 virions. Whether the possible structural differences between W260 and CM1841 are manifested in the plant or only during purification and storage is yet to be determined. However, it is tempting to speculate that the increased level of expression of CM1841 may compensate for the decrease in stability of CM1841 virions.

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