Nucleotide Sequences of the 3′ Regions of Two Major Viruses from Mosaic-Diseased Garlic: Molecular Evidence of Mixed Infection by a Potyvirus and a Carlavirus

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ABSTRACT

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Garlic viruses from 289 mosaic-diseased garlic plants, cultivar Howaito-roppen, were purified, and two different flexuous, rod-shaped viruses, one with a 33-kDa coat protein (CP) and the other with a 36-kDa CP, appeared to be the most common. We determined the partial amino acid sequences of these two CPs and obtained cDNA clones. The 1,413 nucleotides of the 3′ region of garlic virus 1 had a genome structure conserved in carlaviruses: a coding region for the 36-kDa CP and the 3′ open reading frame (ORF) for a 14.2-kDa putative protein followed by 51 nucleotides of the 3′ noncoding region. Phylogenetic comparison of the CP sequences between this virus and known carlaviruses and potexviruses suggested that the virus belongs to the carlavirus group. The 1,974 nucleotides of the 3′ terminal region of garlic virus 2 has one large ORF, 597 nucleotides of the 3′ noncoding region, and a genome structure characteristic of the Potyviridae. The ORF codes for the C terminal 1/3 region of nuclear inclusion protein b (NIb) and the full length of the 33-kDa CP, which have high homologies to NIb and CP of known potyviruses. Comparison of the amino acid sequence at the NIb-CP junction revealed phylogenetic conservation of S/A residues at the P′1 position as well as the F/L/H residues at the P2 position.

Additional keywords: garlic latent virus, garlic mosaic virus, NIa protease, 3′ palindrome.

Most garlic plants are virus infected because garlic is propagated vegetatively (51). Healthy plants thought to be free of known viruses have been produced through meristem tip culture. These plants produce larger bulbs, which are of greater commercial value (36,51,52). An efficient micropropagation method through in vitro bulblet formation was developed (34), but an effective method for virus indexing has not been available. Generally, virus-infected garlic plants display mosaic symptoms that seem to be caused by a single potyvirus, garlic mosaic virus. However, Walkey and coworkers showed that at least three potyviruses, onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), and unknown potyvirus(es) are widespread together with two or more carlaviruses, shallot latent virus (SLV) and unknown carlavirus(es) (51,53). It appears that mixed infection of garlic by two or more of these viruses is a common occurrence. Recently, mite-transmitted rymovirus(es) were detected in a number of garlic cultivars (50,56). However, identification of the viruses that cause garlic mosaic has yet to be made (5).

Carlaviruses have flexuous, filamentous virions that are 610–700 nm long. The viral genome is one molecule of single-stranded (+)RNA, which is encapsidated by a coat protein (CP) of 31–37 kDa (55). Recently, the complete genome sequence of potato virus M was published (57). Partial sequences of the 3′ region of other carlaviruses are also known (Fig. 1A). The carlaviruses sequenced so far have a CP gene and an open reading frame (ORF) for a protein of 10–14 kDa in their 3′ regions (33).

Potyviruses and rymoviruses are members of the family Potyviridae and have flexuous, rod-shaped particles of 680–900 nm (8,20). Characterization and classification of members of the family are incomplete because many of the viruses have a narrow host range and purification of some is difficult. However, data on the nucleotide sequences of the genomic RNA of potyviruses are increasing rapidly, especially for the 3′ region containing the CP gene, forming the largest database of plant virus groups (43). It is well established that amino acid sequences of CPs of potyviruses are highly conserved in the C terminal 2/3 region.

Data concerning the genome organization and the amino acid sequences provide useful information for virus classification (33,39,43). In the present work, we determined the partial amino acid sequences of two different CPs (36 and 33 kDa) of garlic viruses and isolated cDNA clones encompassing their respective CPs. The nucleotide sequences obtained indicate that garlic virus (GV) 1, with a CP of 36 kDa, is a member of the carlavirus group and that GV2, with a CP of 33 kDa, belongs to the potyvirus group. The genome organization and phylogenetic conservation of protein sequences of both viruses are discussed.

MATERIALS AND METHODS

Plant material. Two hundred eighty-nine garlic plants, cultivar Howaito-roppen, with mosaic symptoms were collected from a farmer's field in May 1990 and 1991 in Aomori Prefecture (northern Japan). The leaves of individual plants were stored separately at −80 C until used. Garlic bulbs, cultivar Isshu-wase, were purchased in Nagasaki Prefecture (southern Japan) and grown in a greenhouse in 1989 and 1990. Leaves with mosaic symptoms were collected and stored at −80 C until used.

Purification of virus particles and genomic RNA. Viruses were purified by the method of Shirako and Ehara (45). Frozen leaves were homogenized at 4 C in 5 volumes of 0.1 M sodium borate buffer (pH 8.0) containing 0.1 mM EDTA, 0.1% (w/v) sodium diethyldithiocarbamic acid, and 0.25% 2-mercaptoethanol. The homogenate was passed through four layers of gauze and centri-

fuged at 12,000 *g* for 10 min at 4 C. The supernatant was clarified by addition of Triton X-100 to 2% (v/v) and centrifuged at 156,000 *g* for 60 min through a 20% sucrose cushion suspended in distilled water. The pellet was resuspended in distilled water, and the viruses were further purified by three differential centrifugations. Purified virus particles were used for analyses of CPs. Virus RNA was extracted from virus particles as described by Vance and Beachy (49), and poly(A)$^+$ RNA was purified by oligo(dT) cellulose chromatography (5 Prime →3 Prime Inc., West Chester, PA).

Preparation of antiserum. Purified viruses from cultivar Isshuwase were injected into rabbits (60 μg of protein per 1 ml of adjuvant [Ribi ImmunoChemical Research Inc., Hamilton, MT] and 60 μg of protein with adjuvant per rabbit per injection). The rabbits were injected again after a 2-wk interval. The antiserum was prepared 2 wk after the second injection.

SDS-PAGE and Western blotting. Purified viruses were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (25), and proteins were transferred onto nitrocellulose sheets (47). After electroblotting, proteins were visualized by enzyme-linked immunostaining as described by Shirako and Ehara (45). Virus proteins were detected with antiserum diluted at 1:2,000 followed by alkaline-phosphatase conjugated anti-rabbit goat immunoglobulin G diluted at 1:1,000. Usually, adequate color development occurred within 5 min.

Protein analysis. Virus particles were partially digested by trypsin to yield trypsin-core CPs, as described by Allison et al (3). A portion of the reaction mixture was subjected to SDS-PAGE and transferred to a polyvinylidene difluoride membrane, as described by Matsudaira (31). The desired protein band (100–300 pmole) was cut from the membrane and sequenced with a model 473A peptide sequencer (Applied Biosystems, Foster City, CA).

cDNA cloning and nucleotide sequencing. Oligo(dT)-primed cDNA synthesis was performed from poly(A)$^+$ viral RNA with a cDNA synthesis kit (Amersham, Arlington Heights, IL). Blunt-ended cDNA was ligated to an *Eco*RI-*Not*I-*Bam*HI adaptor (Takara Co., Kyoto, Japan) and cloned into *Eco*RI cleaved λ-ZAPII arms to produce a library of recombinants in *Escherichia coli* XL1 blue cells.

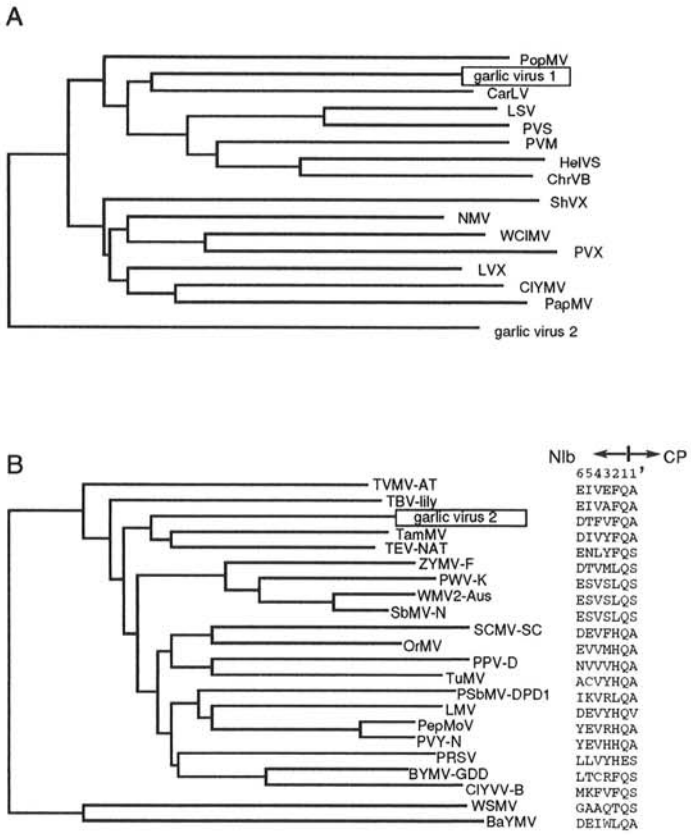

Fig. 1. Phylogenetic analyses of coat proteins (CPs) of garlic virus (GV) 1 and GV2. **A,** A phylogenetic tree of carlaviruses and potexviruses. Amino acid sequences of CPs of seven carlaviruses, seven potexviruses, and GV1 were aligned by using the CLUSTAL V program, and the tree was calculated by the neighbor-joining method. GV2 was included as an outgroup. PopMV = poplar mosaic carlavirus; CarLV = carnation latent carlavirus; LSV = lily symptomless carlavirus; PVS = potato S carlavirus; PVM = potato M carlavirus; HelVS = Helenium S carlavirus; ChrVB = chrysanthemum B carlavirus; ShVX = shallot virus X; NMV = narcissus mosaic potexvirus; WClMV = white clover mosaic potexvirus; PVX = potato X potexvirus; LVX = lily virus X; ClYMV = clover yellow mosaic potexvirus; and PapMV = papaya mosaic potexvirus. **B,** A phylogenetic tree and septipeptide around the nuclear inclusion protein b (NIb)-CP junction of Potyviridae. A tree of 19 potyviruses and GV2 was calculated with one rymovirus, wheat streak mosaic (WSMV), and one bymovirus, barley yellow mosaic (BaYMV), as outgroups. Seven amino acid residues around the NIb-CP cleavage site are shown. TVMV-AT = tobacco vein mottling potyvirus AT; TBV-lily = tulip breaking potyvirus; TamMV = tamarillo mosaic potyvirus; TEV-NAT = tobacco etch potyvirus NAT; ZYMV-F = zucchini yellow mosaic potyvirus F; PWV-K = passionfruit woodiness potyvirus K; WMV2-Aus = watermelon mosaic potyvirus 2; SbMV-N = soybean mosaic potyvirus N; SCMV-SC = sugarcane mosaic potyvirus SC; OrMV = Ornithogalum mosaic potyvirus; PPV-D = plum pox potyvirus D; TuMV = turnip mosaic potyvirus; PSbMV-DPD1 = pea seedborne mosaic potyvirus DPD1; LMV = lettuce mosaic potyvirus; PepMoV = pepper mottle potyvirus; PVY-N = potato V potyvirus, strain N; PRSV = papaya ringspot potyvirus P; BYMV-GDD = bean yellow mosaic potyvirus GDD; and ClYVV-B = clover yellow vein potyvirus B.

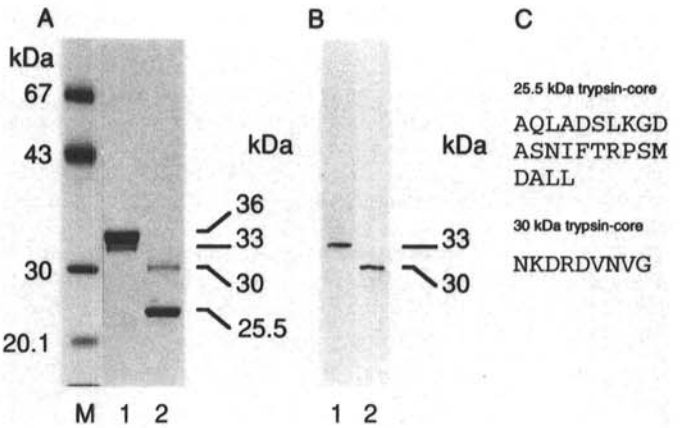

Fig. 2. A, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, **B,** Western blotting of the coat proteins (CPs) of viruses of the garlic cultivar Howaito-roppen, and **C,** partial amino acid sequences of these CPs. Proteins were visualized by Coomassie brilliant blue staining (**A**) or by enzyme-linked immunostaining with an antiserum to garlic viruses of cultivar Isshu-wase (**B**). Lane 1: virus fraction purified from a garlic plant, cultivar Howaito-roppen, infected by a mixture of viruses but mainly by the 36-kDa CP virus; lane 2: the virus fraction of lane 1 digested by trypsin.

TABLE 1. The virus population of mosaic-diseased Howaito-roppen garlic in a field determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using purified virus fractions from mosaic-diseased garlic plants[a]

Molecular weight of viral coat protein (kDa)	No. of plants	Frequency (%)
36 and 33	207	71.6
36 alone	30	10.4
33 alone	49	17.0
None	3	1.0
Total	289	100

[a] Mosaic-diseased garlic plants (289 individual plants) were collected from a farmer's field in May 1991 and stored at −80 C until use. Virus fractions were prepared by three differential centrifugations, loaded on SDS-PAGE, and analyzed by Coomassie brilliant blue staining or electroblot immunoassay.

CP sequence analysis. For sequence comparisons of the garlic viruses and other viruses, the CLUSTAL V program (19) was used for multiple alignment of sequences, and phylogenetic trees were calculated by the neighbor joining method (44). Amino acid sequences of CPs of the following viruses were used; carlaviruses: poplar mosaic (PopMV [18]), carnation latent (CarLV [32]), lily symptomless (LSV [33]), potato S (PVS [29]), potato M (PVM [42]), Helenium S (HelVS [14]), and chrysanthemum B (ChrVB [28]); potexviruses: shallot X (ShVX [22]), Narcissus mosaic (NMV [58]), white clover mosaic (WClMV [15]), potato X (PVX [30]), lily X (LVX [33]), clover yellow mosaic (ClYMV [2]), and papaya mosaic (PapMV [46]); potyviruses: tobacco vein mottling AT (TVMV-AT [4]), tulip breaking virus (TBV-lily [26]), tamarillo mosaic (TamMV [12]), tobacco etch NAT (TEV-NAT [43]), zucchini yellow mosaic F (ZYMV-F [38]), passionfruit woodiness K (PWV-K [43]), watermelon mosaic 2 (WMV-2 [43]), soybean mosaic N (SbMV-N [13]), sugarcane mosaic SC (SCMV-SC [43]), Ornithogalum mosaic (OrMV [9]), plum pox D (PPV-D [43]), turnip mosaic (TuMV [24]), pea seedborne mosaic DPD1 (PSbMV-DPD1 [21]), lettuce mosaic (LMV [10]), pepper mottle (PepMoV [43]), potato Y, N strain (PVY-N [40]), papaya ringspot P (PRSV-P [37]), bean yellow mosaic GDD (BYMV-GDD [17]), and clover yellow vein B (ClYVV-B [48]); rymovirus: wheat streak mosaic (WSMV [35]); and bymovirus: barley yellow mosaic (BaYMV [23]).

RESULTS

Virus purification from mosaic-diseased garlic. Pooled frozen leaves of mosaic-diseased garlic were used to optimize conditions for virus purification. Usually 40–80 μg of virus proteins was obtained from 100 g (fresh weight) of leaves. The purified virus fraction contained flexuous, rod-shaped particles 600–750 nm long (data not shown). The purified virus fraction of the cultivar Howaito-roppen contained 36- and 33-kDa CPs (Fig. 2A). We next analyzed independent virus purifications from 289 mosaic-diseased plants of the cultivar Howaito-roppen obtained by two to three cycles of differential centrifugations. The 36- and 33-kDa CP viruses were found in 82.0–88.4% of the total diseased plants collected, indicating that most of these plants were infected by a mixture of viruses with CPs of 36 and 33 kDa (Table 1). Other viruses with CPs of different molecular weights were not found. These data suggest that viruses with the 36- and 33-kDa CPs are the most common ones found in the mosaic-diseased garlic cultivar Howaito-roppen, although reintroduction of these viruses into garlic was not done.

Partial amino acid sequences of 36- and 33-kDa CPs. We were unable to determine the amino acid sequences of the 36- and 33-kDa CPs when untreated protein samples were used. The amino terminal ends of proteins may be blocked, as is the case with CPs of other viruses. Therefore, trypsin-digested samples of the purified virus fractions were used to determine the amino acid sequences. Some of the virus fractions contained a large amount of 36-kDa CP (more than 90% of the total) and a much lesser amount of 33-kDa CP (Fig. 2A, lane 1). Trypsin digestion of the fraction generated a distinct 25.5-kDa trypsin core band and a faint 30-kDa band, suggesting that the 25.5-kDa peptide should be derived from 36-kDa CP (Fig. 2A, lane 2). Western blotting of these proteins and trypsin cores was done to confirm the relationship of these CPs and trypsin cores. We used an antiserum raised against garlic viruses purified from Isshu-wase garlic. Consequently, the antiserum specifically detected the 33-kDa CP but did not react with the 36-kDa CP of viruses extracted from cultivar Howaito-roppen (Fig. 2B, lane 1). Furthermore, the antiserum detected only the 30-kDa trypsin core (Fig. 2B, lane 2). These data indicate that the 30-kDa peptide should be derived from the 33-kDa CP. Amino acid sequences of trypsin-digested peptides are shown in Figure 2C.

Nucleotide sequences of the 3′ region. The nucleotide sequence of the 3′ region of GV1 is a carlavirus coding the 36-kDa CP. The cDNA library was screened with 5′ radiolabeled mixed oligonucleotides (32 mer). After the second screening, eight clones containing a common insert of the *Eco*RI fragment (930 bp) hybridizing with the synthetic oligonucleotide were obtained. The largest insert (4.3 kb) was subcloned into pBluescript SK(−) and sequenced by the primer-extension method. We determined the sequence of the 1,413 nucleotides of the 3′ terminal region, excluding the poly(A)$^+$ tail, which contained at least two ORFs (Fig. 3A). The first ORF coded a protein with a calculated molecular weight of 33,084 Da. This protein had an amino acid sequence identical to that determined from Edman degradation of the 25.5-kDa trypsin core or the 36-kDa CP. A homology search with the EMBL database showed that the predicted amino acid sequence of the 36-kDa CP has the highest homology to that of carlaviruses, a 34.6% homology to chrysanthemum virus B, 43.5% to carnation latent virus, and similar levels of homologies with those of other known carlaviruses. The 36-kDa CP also has homology (to a lesser extent) to potexviruses (20.8% to narcissus mosaic virus and 28.3% to potato virus X). The second ORF started within the 36-kDa CP gene and coded a protein with a calculated molecular weight of 14,212 Da and a high homology to the putative nucleotide binding protein coded by the 3′ ORF of the known carlaviruses (33). The 14-kDa protein had an arginine-rich region and a zinc finger motif, which are conserved in nucleotide binding proteins. The 14-kDa ORF was followed by 51 nucleotides of noncoding region. On the basis of the many similarities in the CP and the genome structure, the garlic virus with the 36-kDa CP, tentatively named garlic virus 1 (GV1), should be classified in the carlavirus group. Recently, nucleotide sequences of several "garlic viruses" have been entered into the EMBL database. However, these sequence data are based on unpublished results, and only sequence data are available. One sequence (AC D11161) contains a sequence closely related to GV1. Although a high homology was observed in both sequences, three and 14 amino acid deletions as well as several amino acid substitutions were found in the CP and 14-kDa ORF of the unpublished data (Fig. 3A).

The nucleotide sequence of the 3′ region of GV 2 is a potyvirus coding the 33-kDa CP. Analyses of the remainder of the cDNA library revealed that several clones have the same 320-bp *Eco*RI fragment. The nucleotide sequence of the 3′ region, except for the poly(A)$^+$ tail (1,974 bases), was determined. It had one large ORF coding a protein of 458 amino acids followed by a long 3′ noncoding region of 597 nucleotides (Fig. 3B). A portion of the predicted amino acid sequence is identical to the sequence of the 30-kDa trypsin-core peptide derived from the 33-kDa CP. Alignment of the amino acid sequence of the putative protein with polyproteins of known potyviruses revealed that the predicted sequence has high homology with corresponding portions of the polyprotein of other potyviruses, starting at the C terminal 1/3 of the nuclear inclusion protein b (NIb), beginning 5′ to the GDD motif, and containing full-length CP. The NIb-CP junction of the predicted polyprotein was estimated to be Q168/A170 on the basis of the alignment. The prediction was supported by the presence of the DAG motif at six residues downstream from the putative cleavage position, a motif commonly found near the N terminus of potyviral CPs (4). The predicted CP is 289 amino acids with a calculated molecular weight of 32,351 Da. The extent of the CP homology was 49.5% with plum pox potyvirus D and 59.2% with potato virus Y (N strain). The homology was low in the N terminal 1/3 region but high in the C terminal 2/3 region. These results strongly suggest that the garlic virus with the 33-kDa CP is a member of the potyvirus group and is tentatively named garlic virus 2 (GV2).

The 3′ noncoding region of GV2 is adenine-thymine rich (58.0%) and 597 nucleotides long. This 3′ region is presumably the longest reported so far (54). Although no typical secondary structure was found, this region may form extensive foldings with free energies of −11 to −15 kJ on the basis of an analysis that utilized the program Secst 1.1.1 of GENETYX MAC 6.0.1 (Software Development Inc., Tokyo, Japan). In addition, the 3′ noncoding region and the C terminal parts of the CP gene contained seven perfect palindromes (longer than eight bases with no gap or one gap). Interestingly, they were associated with a number of direct

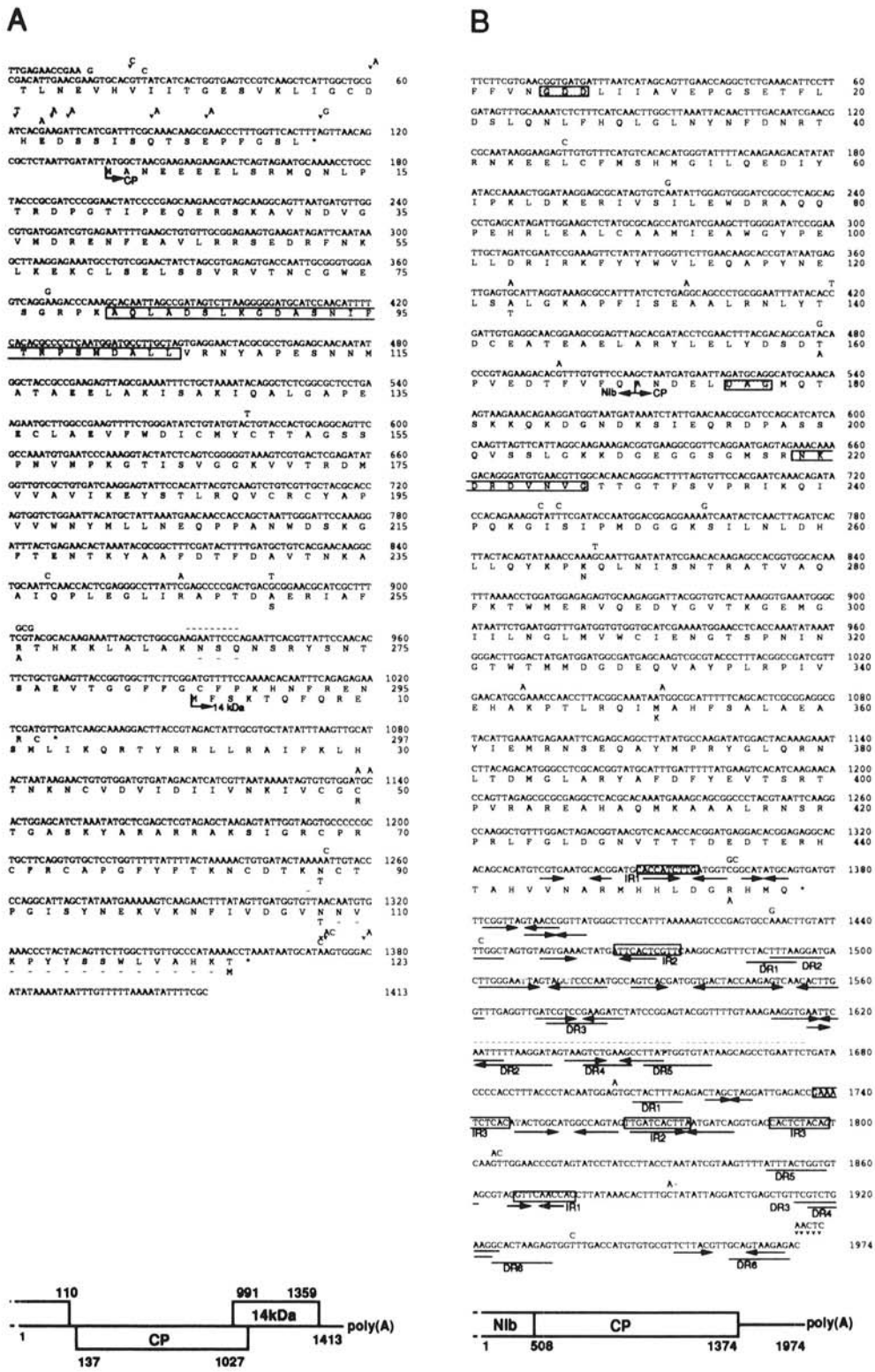

Fig. 3. The nucleotide and deduced amino acid sequences of garlic virus (GV) 1 and GV2, D28591 and D28590, respectively, in GSDB, DDBJ, EMBL, and NCBI DNA databases. **A,** Predicted amino acid sequences of the coat protein (CP) and the 3′ open reading frame for the 14-kDa protein of GV1 are shown. The amino acid sequence determined by Edman degradation of the trypsin-treated, 36-kDa CP is boxed. Nucleotide and amino acid sequences of the related garlic virus (*unpublished data,* EMBL AC D11161) that differed from GV1 are shown above and below the GV1 sequences. A dotted line represents a deletion, and small v indicates an insertion. Diagrammatic presentation of the genome structure of GV1 is also shown. **B,** The nucleotide and deduced amino acid sequences of GV2. Predicted junction for the nuclear inclusion protein b (NIb) and CP is also shown. The amino acid sequence determined by Edman degradation is boxed. The GDD motif of the NIb protein and the DAG motif of the CP are shown in shaded boxes. Palindromes, direct repeats (DR), and inverted repeats (IR) in the 3′ noncoding region are shown by a pair of arrows, underlines, or boxes, respectively. Nucleotide and amino acid sequences of the related garlic virus (*unpublished data,* EMBL AC D11118) that differed from GV2 are shown. A dotted line represents deletions of nucleotide sequences. Diagrammatic presentation of the genome structure of GV2 is also shown.

or inverted repeat sequences (Fig. 3B). In contrast, only three palindromes were found in the remainder of the CP coding region. No typical polyadenylation signal (AATAAA or TATGT) was found, but a possible signal (TATAAA) was observed 94 nucleotides upstream from the poly(A)$^+$ tail. A nucleotide sequence of the EMBL database (AC D11118, *unpublished results*) has high homology to GV2 but contains a large deletion in the 3′ noncoding region (nucleotides 1621–1675) as well as several substitutions and small deletions of nucleotide and amino acid sequences (Fig. 3B).

Phylogeny of the garlic viruses. CPs of carlaviruses are related to those of potexviruses. The phylogenetic status of the CP of GV1 was analyzed with several carlaviruses and potexviruses. CLUSTAL V (19) was used to align the amino acid sequence of the CP of GV1 with those of seven carlaviruses and seven potexviruses, and the phylogenetic tree was calculated by the neighbor-joining method. GV2 was used as an outgroup. Although these viruses had relatively limited areas of group-specific conserved regions, the predicted tree showed clear branching between carlaviruses and potexviruses while showing relatedness of the two virus groups (Fig. 1A). Thus, the phylogenetic analyses of the amino acid sequence of CPs is useful for classification of the carlaviruses and potexviruses. On the basis of this analysis, GV1 is most closely related to the carlavirus group.

Amino acid sequence of the CP of GV2 was aligned with those of 19 aphid-transmitted potyviruses; one bymovirus and one rymovirus were used as outgroups. Apparent groupings were observed for several viruses that are considered strains or closely related viruses, such as potato virus Y and pepper mottle potyvirus, watermelon mosaic virus 2 and soybean mosaic potyvirus, and bean yellow mosaic potyvirus and clover yellow vein potyvirus (Fig. 1B). The tree showed a good correlation with those of Rybicki and Shukla (43), grouping soybean mosaic potyvirus, watermelon mosaic virus 2, passionfruit woodiness potyvirus and zucchini yellow mosaic potyvirus and grouping tobacco etch potyvirus, tamarillo mosaic virus and tobacco vein mottling potyvirus as a possible ancestral potyvirus. According to this tree, GV2 appears to be a "typical" member of the potyvirus group (Fig. 1B). The cleavage site of the NIb-CP junction is associated by valine at the P4 (−4 from the cleavage site) position in most potyviruses (16,39). However, the predicted NIb-CP junction of GV2 did not have valine at the P4 site, as in the case of tobacco etch potyvirus. Because GV2 and tobacco etch potyvirus were grouped in the same branch in the phylogenetic tree, we examined the phylogenetic relation of amino acid sequences around the NIb-CP junction. Interestingly, a strong phylogenetic conservation at the P2 residue (F, L, or H) was observed (Fig. 1B). A specific residue F, which was found at the P2 position of GV2, was conserved in the viruses in earlier branches such as tobacco vein mottling potyvirus, tulip breaking virus, GV2, tamarillo mosaic virus, and tobacco etch potyvirus. The P2 residue was changed into L in the next branch containing zucchini yellow mosaic virus and then into H in most of the remaining branches. The substitution of F by L or of L by H can be generated by a single base change, whereas the substitution of F into H requires a simultaneous two-base change. The residue at P′1 was also conserved in each branch; it was A in the earlier branches containing GV2 and S in the next branch. These results support our estimate of the NIb-CP cleavage site of GV2.

DISCUSSION

We collected 289 mosaic-diseased garlic plants, cultivar Howaito-roppen, from a farmer's field and purified filamentous viruses. Most of the virus fractions contained CPs of apparent molecular weights of 33 and 36 kDa. Thus, viruses with 33- and 36-kDa CPs were the most common in mosaic-diseased garlic plants. Nucleotide sequences of the 3′ regions of these viral RNAs were determined. The 1,413 nucleotides of GV1 coded for the 36-kDa CP and the 14,212-Da putative protein, both of which had high homology with corresponding proteins of known carlaviruses. The amino acid sequence of the CP of GV1 showed significant homology with those of potexviruses, as observed with known carlaviruses (29,33). However, phylogenetic analysis of the CP strongly suggested that GV1 should be classified as a carlavirus. On the other hand, 1,974 nucleotides of GV2 codes the C terminal region of the potyviral polyprotein, including the NIb and 33-kDa CP. The ORF is followed by 597 nucleotides of a noncoding sequence ending in a poly(A) tail. Phylogenetic analysis of the amino acid sequence of the CP indicated that GV2 is a member of the potyvirus group.

The CP of potyviruses is cleaved from the polyprotein by viral NIa protease. Septipeptide sequences around the cleavage sites have been analyzed by comparing sequences of known potyviruses and site-directed mutagenesis, and the general motif recognized by the NIa protease was considered to be V-X-X-Q|(S/G/A) (16,39). The predicted site of the NIb-CP junction of GV2 was not associated with valine at the P4 site. However, the prediction was supported by phylogenetic conservation of P2 and P′1 residues. Phylogenetic conservation of specific residues around the NIb-CP junction means that there is another way to predict the cleavage site between NIb and CP. It should also be noted that glycine is rare at the P′1 site of the NIb-CP junction, although it is common in the sites of other junctions recognized by the NIa protease (11,16,39).

Isolation and characterization of garlic viruses by traditional methods is time consuming because garlic is the only host to propagate garlic potyviruses. Cloning of garlic viruses directly from diseased plants is an alternative approach to characterize them. Nucleotide and amino acid sequences of GV1 and GV2 will be useful for the classification of garlic and *Allium* viruses. In addition, the cDNA from the 3′ noncoding region of potyviruses is useful in the development of probes to distinguish closely related viruses because this region has divergent sequences (48). One garlic latent carlavirus (GLV) (27) and two garlic mosaic potyviruses (GMV) (1,27) have been isolated from Japanese garlic. Host ranges of the Japanese GLV and shallot latent virus (7) are similar. GV1 may be either a strain of GLV (27) and shallot latent virus (7) or a previously unknown carlavirus. On the other hand, one of the Japanese GMV (1) and leek yellow stripe potyvirus (6) have similar host ranges. The other GMV (27) appears to be a rymovirus (50). Infection of an *Allium* potyvirus, onion yellow dwarf potyvirus, has not been reported for Japanese garlic, although this infection was found to be widespread in garlic in Europe and the Philippines. It is possible that the leek yellow stripe potyvirus, one of the Japanese GMV (1), and GV2 are strains of the same virus.

Recently, insertion of a direct repeat in the 3′ noncoding region was shown to alter pathogenicity of the tobacco vein mottling potyvirus (41). Interestingly, the 3′ noncoding region of GV2 has a number of palindromic sequences, direct and inverted repeats. It is likely that the 3′ noncoding region has a regulatory function for virus genome transcription and translation.

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