Mapping of Epitopes for Citrus Tristeza Virus-Specific Monoclonal Antibodies Using Bacterially Expressed Coat Protein Fragments

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ABSTRACT

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Epitopes for a panel of 30 monoclonal antibodies (MAbs) specific for citrus tristeza virus (CTV) were mapped on the CTV coat protein (CP) expressed in bacterial cells. Expression constructs that generated different portions of the CTV CP were screened against MAbs by Western blotting and enzyme-linked immunosorbent assay. All MAbs analyzed could be placed into five groups (I to V), four of which have continuous sequential epitopes. Group I has an epitope within the nine C-terminal amino acids (aa), 215 to 223, that reacted only to MAb 4H6; the group II epitope was mapped between aa 173 and 215 and reacted to MCA-14; the group III epitope was mapped between aa 118 and 128 and reacted to five MAbs, including MCA-13; and the group IV epitope was mapped between aa 2 and 121 and reacted to four MAbs. Epitope(s) for a large group of MAbs (group V) either were conformational or included a conformational element, because they only reacted with the complete CP fusion protein and not with its fragments. Specific proteolytic cleavage of a CP fusion protein expressed in *Escherichia coli* as a peptide fused to a maltose-binding protein (MBP) released a full-size CP with essentially no reactivity with MAbs from group V. Additional studies will be needed to differentiate members of this group. The linear, continuous epitope for MCA-13 (aa 118 to 128), which distinguishes Florida quick decline-inducing (D-I) CTV isolates from mild isolates, was expressed in *E. coli* cells as a peptide fused to an MBP. This fusion protein was purified and used as antigen to generate a rabbit polyclonal antiserum. The aa 118 to 128-specific polyclonal antiserum had the same serological properties as MAb MCA-13 and reacted with Florida quick D-I CTV isolates but not with mild isolates.

Citrus tristeza virus (CTV), often referred to as citrus tristeza virus complex, is aphid-transmitted, with long, flexuous thread-like particles 2,000 nm in length and 11 nm in diameter (1,2,10). Isolates of CTV have been described that differ in serological properties (4); they can be broadly defined into four groups based on their pathology in different economically important citrus cultivars. The most important groups are those that cause incompatibility between sour orange rootstock and susceptible scion cultivars (decline-inducing isolates [D-I]) and those that cause stem pitting (SP isolates) of scion cultivars in decline-tolerant scion-rootstock combinations. These two symptoms are under independent genetic control. CTV isolates that do not produce decline or stem pitting are considered mild isolates. Some isolates cause severe seedling yellows (SY) on sour orange, lemon, and grapefruit. SY is economically important only when susceptible scions are topworked onto infected stocks, but the SY reaction is commonly used as a diagnostic symptom for severe CTV isolates.

Disease management differs significantly depending on which isolates are present. D-I isolates are important in areas where trees are on sour orange rootstock, whereas SP isolates are important in areas with trees on CTV-tolerant rootstocks. CTV isolates are now biologically characterized by indexing on several standard host indicator plants, hence a better, faster system is needed (5). A monoclonal antibody (MAb), MCA-13, has been successfully used to distinguish Florida D-I CTV isolates from mild isolates (15). However, MCA-13 does not differentiate D-I from SP isolates and may fail to detect some D-I isolates. Many MAbs have been produced to different CTV isolates in the search for isolate-specific and group-reactive antibodies (6,7,15,18,20,23,24), and evaluation of these continues (6–8,15,18,20,23,24; S. M. Garnsey, M. Cambra, D. J. Gumpf, C. A. Powell, M. C. Tsai, and M. Zebzami, *unpublished data*). However, the lack of information on the epitope specificity of the MAbs complicates this evaluation. A method to systematize CTV-specific MAbs based on mapping of their respective epitopes is needed.

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Epitopes may be classified in several ways, but there are two fundamentally different types of epitopes (22): linear or sequential (defined by a continuous stretch of amino acids [aa]) and conformational (requires that the native conformation of a protein be present). The coat protein (CP) of CTV has 223 aa and a molecular mass of about 25 kDa (17). Antibodies have been produced to native and denatured virus proteins (15), but the antigenic structure of the viral CP is generally unknown. Experiments utilizing site-directed mutagenesis of selected aa residues suggest that the epitopes for MAbs 3DF1 and MCA-13 are linear and close to aa 2 and 124, respectively (13,14). This approach has produced valuable information, but it is not convenient for rapid screening of the entire library of CTV-specific MAbs. Recently, the CTV CP fused to the bacterial maltose-binding protein (MBP) tag, MBP-CP, has been expressed in *Escherichia coli* cells (12), providing a convenient instrument for mapping the epitopes of CTV MAbs, because constructs can be made easily that represent different portions of the CTV CP gene.

Here, we present data on the screening of 30 CTV-specific MAbs, using different bacterially expressed fragments of the CTV CP.

MATERIALS AND METHODS

Sources of CTV isolates, polyclonal antiserum, and MAbs. D-I isolate T36 and mild isolates T30 and T55 (16) were maintained in Mexican lime plants under greenhouse conditions at Lake Alfred, FL. Rabbit polyclonal antiserum (#1052) was produced to T36 purified by the method of Lee et al. (11). The MAbs used are described in Table 1.

Plasmid construction. All plasmid constructs were based on the initial MBP-CP plasmid described previously (12). They contained various fragments of the CTV CP gene cloned into the pMAL-c2 expression vector (New England Biolabs, Beverly, MA) between *Eco*RI and *Bam*HI sites. Plasmids 2-173 and 2-121 were generated after digestion of the MBP-CP plasmid with *Eag*I and *Bam*HI or *Aat*I and *Bam*HI, respectively, treatment with T4 DNA polymerase to create blunt ends, and self-ligation. The resulting con-

TABLE 1. Sources of citrus tristeza virus (CTV)-specific monoclonal antibodies (MAbs) used

MAb	Original immunogen[a]	Biological properties of immunogen[b]	MAb source	Reference
18H9	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	7
17H9	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	7
17A4	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	7
17G11	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	7
1D3	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	7
2F7	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	7
16G11	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	7
19C1	T36+B185	Dec+GSP+OSP+SY	Fort Pierce, FL	C. A. Powell, *unpublished data*
ECTV176	OSP	Dec+GSP+OSP+SY	Orlando, FL	S. M. Garnsey, *unpublished data*
ECTV175	OSP	Dec+GSP+OSP+SY	Orlando, FL	S. M. Garnsey, *unpublished data*
MCA-13	T36	Dec+SY	Orlando, FL	15
MCA-14	T36	Dec+SY	Orlando, FL	T. A. Permar et al.[c], *unpublished data*
IIA1	SY568	Dec+GSP+OSP+SY	Riverside, CA	D. J. Gumpf et al.[d], *unpublished data*
IIIAD5	T514	OSP	Riverside, CA	6
3DF1	T308	Dec	Spain	23
3CA5	T308	Dec	Spain	23
4H5	OSP	Dec+GSP+OSP+SY	Australia	P. Barkley, *unpublished data*
20E9	OSP	Dec+GSP+OSP+SY	Australia	P. Barkley, *unpublished data*
6B7	OSP	Dec+GSP+OSP+SY	Australia	P. Barkley, *unpublished data*
3E10	CTV-D	Dec+GSP	Taiwan	20, 21
5D8	CTV-D	Dec+GSP	Taiwan	20, 21
7F2	CTV-D	Dec+GSP	Taiwan	20, 21
11B1	CTV-D	Dec+GSP	Taiwan	20, 21
4G12	CTV-D	Dec+GSP	Taiwan	20, 21
4H6	Tm-1	Dec+SY	Taiwan	20, 21
10E3	Tm-1	Dec+SY	Taiwan	20, 21
4E5	CTV-SS	Dec+SY	Morocco	24
4C1	CTV-SS	Dec+SY	Morocco	24
1D12	CTV-SS	Dec+SY	Morocco	24
4D3	CTV-SS	Dec+SY	Morocco	24

[a] Parent isolates: B185 = Dec+GSP+OSP+SY; OSP (Australian orange stem pitting) = Dec+GSP+OSP+SY; B6 (SY568) = Dec+GSP+OSP+SY; T36 = Dec+SY; Tm-1 (B211) = Dec+SY; CTV-SS = Dec+SY; CTV-D = Dec+GSP; T514 = OSP; and T308 = Dec.
[b] Dec = decline; GSP = grapefruit stem pitting; OSP =orange stem pitting; and SY = seedling yellows.
[c] T. A. Permar, S. M. Garnsey, D. J. Gumpf, M. Cambra, and M. C. Tsai, *unpublished data.*
[d] D. J. Gumpf and J. Diaz, *unpublished data.*

TABLE 2. Primers used and the respective citrus tristeza virus (CTV)-specific plasmid constructs generated

Primer ID	Sequence[a]	Polarity	Construct (amino acids)
D1988	ccgaattcGACGACGAAACAAAGAAATTG	+	2–223, 2–128, 2–121, 2–173
C117	ccgaattcATGGACGACGAAACAAAGAAATTG	+	1–223
#524	ggtgaattcCTTTGGACTGACGT	+	118–223, 118–128
M104	ggtgaattcGTGTTTAACTCTAAGGGT	+	123–223
C116	ggtgaattcCTTGGGAAATTCAACACA	+	215–223
D2085	gttggatCCGGGAATCGGAACGCAACAGATCAA	–	1–223, 2–223, 118–223, 123–223
#556	gctggatccACCCTTAGAGTTAAACA	–	2–128, 118–128
C115	ccaggatcCTTCATTAGGAACGTGT	–	215–223

[a] Virus-specific sequences are presented in uppercase letters; restriction sites introduced to facilitate cloning are underlined.

structs lacked 50 and 102 codons at the 3′ terminus of the gene, respectively. Other constructs were generated by polymerase chain reaction (PCR). The primers used and the fragments of the CP gene cloned are described in Table 2. The CTV CP-specific clones generated previously (12) were used as templates for PCR. Although the original construct was generated on the template from the plant infected with the SY568 isolate, the CP sequence was identical to the prototype isolate T36 (12), and thus, we refer to all our derivative constructs as T36 specific.

Expression of fusion proteins, purification, and generation of polyclonal antisera. The plasmids generated expressed fusion proteins in bacterial cells after induction by isopropyl-β-D-thiogalactopyranoside. The N-terminal portion of every fusion protein was the MBP of *E. coli* with a molecular mass of 42 kDa, and the C-terminal portion represented a CTV-specific fragment. In some experiments, fusion proteins were treated with factor Xa protease (New England Biolabs) according to the manufacturer's instructions. Growth of the transfected cells, induction, and affinity column chromatography purification of the fusion proteins were performed as described previously (12). Polyclonal antisera against the proteins obtained were produced by Cocalico Biological (Reamstown, PA) in rabbits after a series of subcutaneous and intramuscular injections of the expressed protein (12).

Immunoblotting and enzyme-linked immunosorbent assay (ELISA). Sweet orange bark tissue or bacterial cells containing induced fusion proteins were heated in Laemmli's sample buffer, and proteins were subjected to electrophoretic separation in 8 to 20% gradient polyacrylamide gels in a discontinuous Tris-glycine-sodium dodecyl sulfate (SDS) system (9). After separated proteins were transferred to nitrocellulose membranes (19), they were probed with different MAbs or polyclonal antisera as previously described (12).

For the ELISA experiments, immunoglobulin G (IgG) fractions from rabbit polyclonal antiserum #1052 were isolated by protein A-Sepharose column chromatography. These IgGs were used for coating at 1 μg/ml. Different CTV-CP fusion proteins were affinity-purified by amylose resin (New England Biolabs) column chromatography and used as antigens at a concentration of 1 μg/ml. MAbs were tested unlabeled as intermediate antibodies. Antigen-bound MAbs were detected with rabbit anti-mouse antibodies conjugated to alkaline phosphatase (Sigma Chemical Company, St. Louis). All ELISA procedures followed the general protocol of Clark and Adams (3).

RESULTS

MAbs specific to linear or sequential epitopes (groups I to IV). Nine overlapping CP gene fragments were expressed in bacterial cells (Table 2). The resulting fusion proteins were tested by immunoblotting and ELISA with 30 MAbs generated against different CTV isolates (Table 1). The results of these tests are summarized in Figure 1. Eleven MAbs reacted to linear epitopes and were divided into four groups—hereafter referred to as I to IV. MAbs 4H6, MCA-14, MCA-13, and 18H9 were designated as type MAbs for the respective groups. A single MAb (4H6) representing group I reacted with all CP fragments retaining the exact C-terminal region, including the short 9-aa C-terminal peptide (Fig. 2A). The corresponding 4H6 MAb epitope is apparently located within the last 215 to 223 aa of the CP. A single MAb (MCA-14) representing group II reacted with CP fusion proteins that retained 42 residues between aa 173 and 215 (Fig. 2B), implying that at least a portion of the respective epitope is located within this 42-aa stretch. Five MAbs in group III, with MCA-13 as the type MAb, reacted with CP fusion proteins that retained an internal 11-residue fragment of CP between aa 118 and 128 (Fig. 2C). Four MAbs comprised group IV, with 18H9 as the type MAb. These MAbs reacted with the N-terminal half of the CP between aa 2 and 118 (data not shown). All 11 MAbs demonstrated strong reactions in immunoblotting against every CP fusion protein that had a respective epitope, indicating their linear or sequential specificity (Fig. 2).

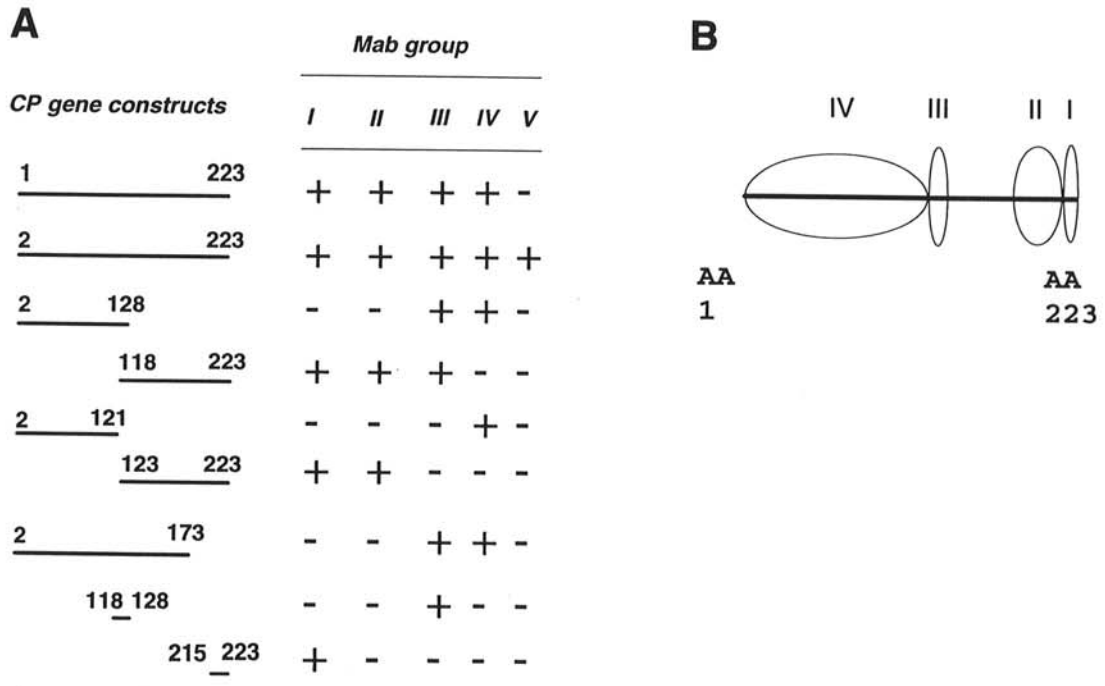

Fig. 1. A, Schematic representation of the citrus tristeza virus (CTV) coat protein (CP) gene constructs expressed in *Escherichia coli* and their reactions with monoclonal antibodies (MAbs) from five groups. Plus and minus signs mark positive and negative reactions, respectively, in Western blot and enzyme-linked immunosorbent assays. MAbs were grouped as follows: group I (4H6, a single representative); group II (MCA-14, a single representative); group III (MCA-13, 1D3, 2F7, 16G11, and 10E3); group IV (18H9, 17A4, 17H9, and 19C1); and group V (3E10, 3DF1, 17G11, 6B7, 5D8, 7F2-4, 11B1, 4G12, 3CA5, IIA1, IIIAD5, 4H5, 20E9, ECTV 175, ECTV 176, 4E5, 4C1, 1D12, and 4D3). **B,** A diagram of the CTV CP molecule showing the positions of the continuous epitopes mapped in this work. Ellipses designate the positions of the continuous epitopes: group I, amino acids (aa) 215 to 223; group II, aa 173 to 215; group III, aa 118 to 128; and group IV, aa 2 to 121.

MAbs with conformational epitopes (group V). The remaining 19 MAbs comprised group V, reacting only with the CP fusion protein that contained the complete CP sequence, except for the first methionine residue (Fig. 1). Surprisingly, any change in the CP gene construct, i.e., removal of the N- or C-terminal halves, "trimming" from the C terminus, or even an addition of the first Met codon, abolished the reaction (Figs. 1 and 3A). MAbs in this group reacted in immunoblots only with the presumably intact 25-kDa CTV CP from the infected plant tissue and did not react with products of the CP proteolysis (13; data not shown). To check whether the N-terminal tag of the *E. coli* MBP contributed to the reactivity of our CP fusion proteins with the 3E10-type MAbs, we utilized the factor Xa protease, which specifically cleaved the MBP-CP fusion protein and released the complete CP with four additional N-terminal aa residues derived from the vector. After releasing the CP from the MBP-CP construct, its reactivity to the group V MAbs was almost completely lost, whereas reactivities to the MAbs with linear or continuous epitopes from groups I to IV and polyclonal CTV antisera remained unchanged (Fig. 3; Table 3). The immunoblot (Fig. 3) and ELISA (Table 3) data suggest that the group V MAbs recognize a conformational epitope or epitopes.

Production of polyclonal antiserum to the 118–128 peptide. Based on the data obtained (Figs. 1 and 2C), we concluded that the linear epitope for the MCA-13–type MAbs is located between aa 118 and 128 (Fig. 1B), residues 121 and 123 are within the epitope, the N-terminal (left) border of the epitope lies between residues 118 and 121, and the C-terminal (right) border lies between residues 123 and 128. The 118–128 construct containing the epitope for the MCA-13–type MAbs was expressed in *E. coli* cells, purified to homogeneity by affinity column chromatography, and used as an antigen for immunizations. The resulting rabbit polyclonal antiserum was tested in immunoblots for Florida CTV isolates differing in symptomatology. This polyclonal antiserum demonstrated the same isolate specificity as the MCA-13 MAb and readily distinguished D-I isolate T36 from mild isolates T30 and T55 (Fig. 4).

DISCUSSION

We demonstrated that the expression constructs encoding different fragments of the CP represent a convenient tool for mapping large numbers of MAbs with linear or sequential epitopes and, consequently, for systematization of available MAb libraries. The 30 MAbs tested were placed in five groups, with one group (group V) apparently comprising MAbs with conformational epitope(s). Diversity among the MAbs with linear epitopes tested was surprisingly limited, with two groups (I and II) having only one representative apiece, 4H6 and MCA-14, respectively. In this work we mapped two of four groups of linear epitopes (groups I and III) to fairly tight regions of 9 and 11 aa, respectively. Using the same approach, positions of the epitopes for groups II and IV also may be narrowed. Unfortunately, the CP fusion proteins described cannot be used for analysis of the conformational MAbs (group V), for which an alternative method needs to be found. These CP fusion proteins provide a source of uniform antigen for repeated production of polyclonal antisera with essentially indistinguishable specificities or for production of polyclonal antibodies with select antigenic specificities (Fig. 4).

Two initial studies addressed the mapping of two CTV-specific MAbs by site-directed mutagenesis of codons of selected aa residues (13,14). The linear epitope for MCA-13 was suggested to be located close to and probably include aa 124 of the CP (14). Our data also delineate the epitope to the region between aa 118 and 128. Nevertheless, more accurate analysis of the region is needed to confirm that residue 124 is, in fact, in the epitope, because construct 123–223 did not react with MCA-13 (Fig. 2C). The epitope for another MAb, 3DF1, was reported to be linear and located close to and probably including aa 2 of the CP (13). This suggestion seemed to corroborate initial evidence that MAb 3DF1 reacted only with the intact, full-length CP but not with proteolytic fragments lacking the N terminus (17). However, in contrast to the previous report (13), our data show that 3DF1, like 18 other MAbs, has a conformational epitope (Figs. 1 to 3; Table 3). We suggest that after electroblotting onto nitrocellulose membrane the 2–223 fusion protein can be (at least partially) refolded into a conformation resembling the native CTV CP. Partial renaturation of the electroblotted proteins was observed after electrophoretic removal of SDS (19). We also might suggest that in the case of eight other fusion proteins (Fig. 1) such refolding did not produce a native conformation.

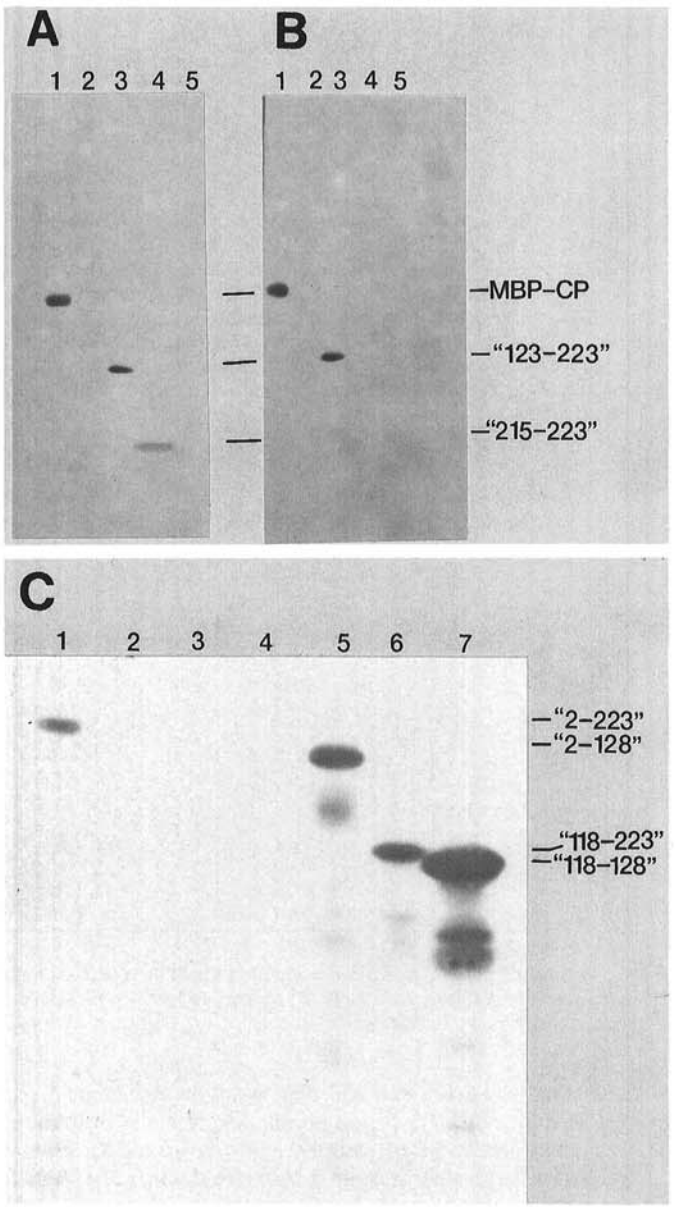

Fig. 2. Immunoblotting of total proteins from *Escherichia coli* cells expressing different citrus tristeza virus coat protein-specific fusion proteins after electrophoretic analysis in 8 to 20% polyacrylamide gels. The immobilized proteins were treated with ascitic fluids containing monoclonal antibody **A,** 4H6 (dilution 1:10,000), **B,** MCA-14 (dilution 1:10,000), or **C,** MCA-13 (dilution 1:20,000). Constructs in **A and B**: lane 1, amino acids (aa) 2 to 223; lane 2, aa 2 to 173; lane 3, aa 123 to 223; lane 4, aa 215 to 223; and lane 5, maltose-binding protein (MBP) alone (negative control). Constructs in **C**: lane 1, aa 2 to 223; lane 2, MBP alone (negative control); lane 3, aa 2 to 121; lane 4, aa 123 to 223; lane 5, aa 2 to 128; lane 6, aa 118 to 223; and lane 7, aa 118 to 128.

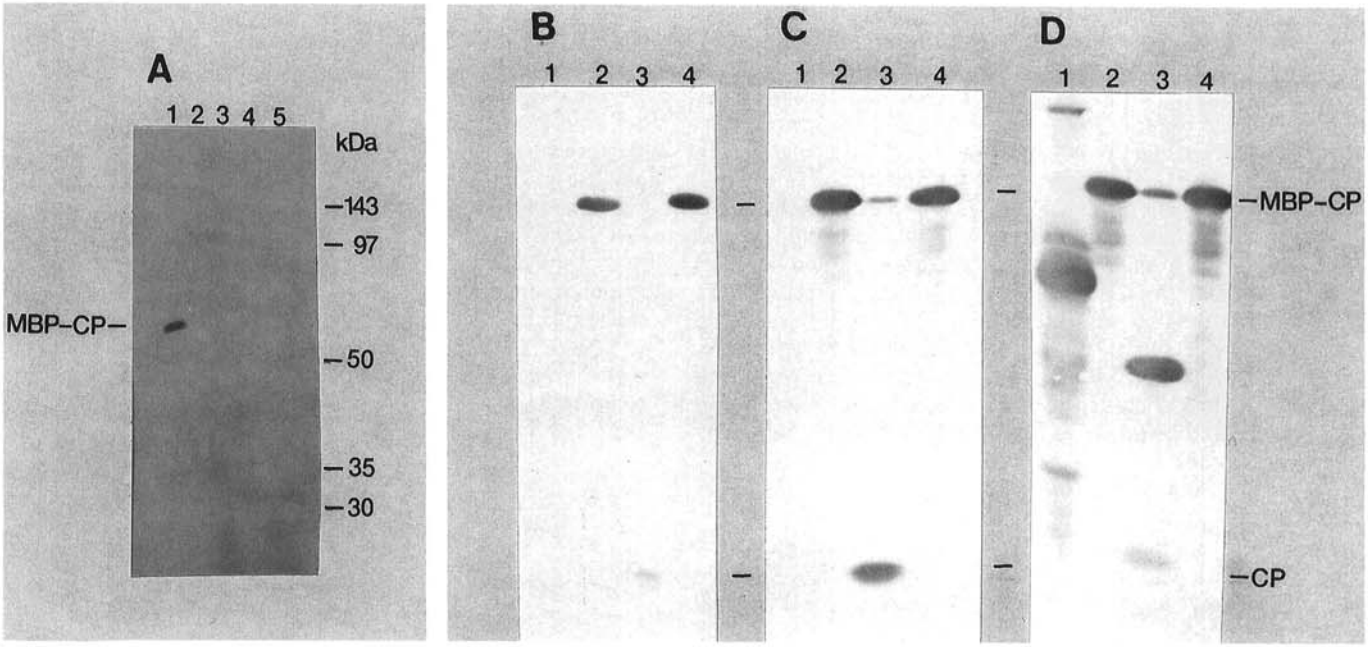

Fig. 3. A, Immunoblotting of total proteins from the *Escherichia coli* cells expressing different citrus tristeza virus coat protein (CP)-specific constructs after treatment with ascitic fluid containing monoclonal antibody (MAb) 3E10 (dilution 1:10,000). CP fragments: lane 1, amino acids (aa) 2 to 223; lane 2, aa 1 to 223; lane 3, aa 2 to 128; lane 4, aa 118 to 223; and lane 5, maltose-binding protein (MBP) alone (negative control). Positions of marker proteins are indicated. **B through D,** Immunoblotting of the affinity-purified fusion protein containing CP fragment aa 2 to 223 after electrophoretic separation in 8 to 20% polyacrylamide gels before and after treatment with factor Xa protease. The immobilized proteins were treated with ascitic fluid containing **B,** MAb 3E10 (dilution 1:10,000); **C,** polyclonal antiserum #1052 raised against purified virions (dilution 1:2,000); or **D,** polyclonal antiserum against a MBP-CP fusion protein ([12]; dilution 1:80,000). Fusion protein/CP fragments: lane 1, MBP alone; lane 2, fusion protein/aa 2 to 223; lane 3, fusion protein/aa 2 to 223 after treatment with factor Xa for 4 h at room temperature; lane 4, fusion protein/aa 2 to 223 after incubation for 4 h at room temperature without factor Xa. When expressed in the original pMAL-c2 construct, MBP is fused to a lacZ protein and, thus, has a larger molecular mass (lane 1) than when it is proteolytically released by factor Xa protease (lane 3).

TABLE 3. Immunoreactivity of maltose-binding protein-coat protein (MBP-CP) (amino acid 2–223 fusion protein) in enzyme-linked immunosorbent assay (ELISA) before and after treatment with the factor Xa protease

	Bacterially expressed protein[b] (OD_{405})			Sweet orange bark extract[c] (OD_{405})	
MAb[a]	MBP	MBP-CP	MBP-CP/Xa	T36-infected	Healthy
MAbs with conformational epitopes (group V)					
3E10	0.28[d]	1.26	0.30	2.97	0.23
5D8	0.42	2.20	0.47	1.85	0.41
11B1	0.15	1.27	0.21	2.70	0.18
17G11	0.11	0.45	0.10	0.85	0.12
MAbs with continuous epitopes (groups I to IV)					
4H6	0.12	0.89	1.00	0.76	0.13
MCA-14	0.05	0.45	0.43	0.42	0.07
MCA-13	0.20	1.95	2.10	2.15	0.15
18H9	0.14	1.56	1.61	0.95	0.13

[a] Monoclonal antibody.
[b] Affinity-purified proteins at 1 µg/ml were used as antigens before or after the Xa treatment.
[c] Sweet orange bark tissue extracts were prepared and used in ELISA as described previously (12).
[d] Values are an average for two wells.

Interestingly, the full-size CP that was synthesized in *E. coli* cells within the MBP-CP fusion protein and released from the reactive fusion protein after treatment with the factor Xa protease also seems to be unable to adopt a native fold either after transfer to nitrocellulose membrane (Fig. 3) or even in solution (Table 3). If our suggestion about partial refolding of the CP fusion proteins (or lack of it) on nitrocellulose membrane is correct, it may explain the loss of reactivity of conformational MAbs in immunoblotting after the truncation of CP (17) or even after its site-directed mutagenesis (13). An unexpected difference in reactivity with conformational (group V) MAbs was observed for the full-size CP proteolytically released with factor Xa protease from the MBP-CP fusion protein expressed in *E. coli* and the CP within the fusion protein containing a 42-kDa tag of the MBP (Fig. 3; Table 3). We propose that the presence of a 'carrier' may facilitate the spatial folding of the CP on the membrane, resulting in a conformation resembling native CTV CP.

Less than one-half of the MAbs studied had linear or sequential epitopes (Fig. 1). There may be two main reasons for this underrepresentation of MAbs with sequential epitopes: the CP may have only a few regions that are highly immunogenic, or the methods used to screen the hybridoma lines may have produced a bias toward conformational MAbs. We believe further work on expression of different fragments of the CTV CP and use of these fragments as immunogens for production of polyclonal antisera would probably help distinguish between these two possibilities.

Despite great biological diversity among CTV isolates, there are currently few means to distinguish them other than biological in-

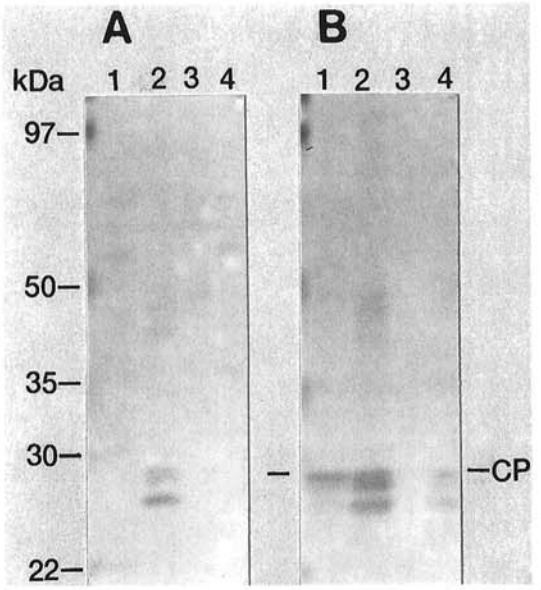

Fig. 4. Immunoblotting of total proteins from citrus tristeza virus-infected sweet orange bark tissue after electrophoretic analysis in 8 to 20% polyacrylamide gels. The immobilized proteins were treated with **A,** a polyclonal antiserum against the coat protein (CP) fusion protein containing amino acids (aa) 118 to 228 or **B,** a polyclonal antiserum against the maltose-binding protein-CP fusion protein ([12]; dilution 1:80,000). Lane 1, mild isolate T55; lane 2, decline-inducing isolate T36; lane 3, healthy plant tissue; and lane 4, mild isolate T30. Positions of marker proteins are indicated.

dexing. The MCA-13 MAb (15) has been widely used in serological assays to distinguish between D-I and mild isolates in Florida, demonstrating general applicability of MAbs to CTV isolate differentiation. A collection of MAbs that was generated over the past several years (6,7,18,20,21,23,24) created an opportunity to utilize MAb specificity for CTV isolate differentiation. We believe that mapping epitopes for all available CTV-specific MAbs will further expand the panel of isolate-specific MAbs and improve our ability to differentiate CTV isolates.

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