Identification of Citrus Tristeza Virus Strains by Peptide Maps of Virion Coat Protein

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ABSTRACT

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Virion coat proteins (CP) from six strains of citrus tristeza virus (CTV) were purified by gel electrophoresis (SDS-PAGE) of semipurified extracts and electroelution of the specific protein band from the gels. Peptide maps obtained by digestion of CPs with five endopeptidases (papain, trypsin, V8 protease, chymotrypsin, and thermolysin) and separation by SDS-PAGE distinguished the six CTV strains. Fewer differences among strains were observed in Western blots when the same digests were reacted with monoclonal antibodies, but Western blots provided additional information about the antigenic properties of peptides. Peptide maps and Western blot analysis enabled differentiation of CTV strains having similar biological properties and the same double-stranded RNA pattern.

Additional keywords: electrophoresis, serology.

Citrus tristeza virus (CTV), a closterovirus about 2,000 nm long and 12 nm wide, causes one of the most important diseases of citrus in the world (4). In Spain, CTV has caused the death of about 15 million sweet orange, mandarin, and grapefruit trees grafted on sour orange (*Citrus aurantium* L.) rootstock and the progressive decline of uncounted additional trees of the same combinations. There are numerous strains of CTV that differ in host range, symptom intensity on susceptible host species, and aphid transmissibility. Virus strains currently present in Spain only cause decline and death of citrus trees grafted on sour orange, the predominant rootstock (1). Stunting or stem pitting of grapefruit (*C. paradisi* Macf.) or sweet orange (*C. sinensis* (L.) Osb.), caused by severe strains of CTV (11,16,18,24), has not been observed in Spain. Tristeza damage can be prevented by propagating commercial varieties on tolerant rootstocks like citranges (*C. sinensis* × *Poncirus trifoliata* (L.) Raf.) or Cleopatra mandarin (*C. reshni* Hort. ex Tan.). Recently, a severe CTV strain was discovered in an illegally introduced satsuma (*C. unshiu* (Mak.) Mark.) cultivar (2). This strain, named as T-388, is a great threat to the Spanish citrus industry, and methods for quick identification of this strain for eradication are needed.

Over the last years, progress has been made in serological detection of CTV (5,6,22) and monoclonal antibodies specific to CTV have been obtained (23). Nevertheless, these antibodies have not unequivocally distinguished virus strains.

Traditionally, CTV strains have been characterized by symptoms induced on selected indicator citrus species, but this is a time-consuming method, and separation of strains is not always clear-cut (1). Some CTV isolates can be distinguished in hybridization procedures by using cloned cDNA (20). Analysis of double-stranded RNA (dsRNA) components in extracts of infected plants has been proposed as a reliable method to identify CTV strains (8,13). We have found seven dsRNA patterns among 24 CTV isolates collected from most citrus areas in Spain (17). However, differences in the biological characteristics of CTV isolates did not always correlate with the differences in dsRNA patterns.

Recently, a comparison of peptide maps obtained from virion coat protein has been used to distinguish several strains of red clover necrotic mosaic virus (19). Lee and Calvert (14) and Lee et al (15) used a similar procedure to compare several Florida CTV strains. Even though some differences were observed, these authors concluded that differences in peptide maps were not sufficient to distinguish the isolates they tested. In this paper we report the discrimination of several CTV isolates (differing in biological characteristics or dsRNA pattern) by peptide mapping of virion coat proteins and by Western blot analysis using a monoclonal antibody.

MATERIALS AND METHODS

Virus strains. CTV strains T-300, T-308, T-344, T-362, T-385, and T-388 from the Instituto Valenciano de Investigaciones Agrarias (IVIA) collection (1,2) and strains T-3, T-4, and T-36 from Florida (20) were used. T-300, T-344, and T-362 are moderate strains representative of the most common type of tristeza present in Spain (1) and they induce medium intensity veinclearing and stem pitting on Mexican lime (*C. aurantifolia* (Christm.) Swing). T-385 is a very mild strain inducing very light or inconspicuous veinclearing and stem pitting on Mexican lime. T-308 is a severe strain inducing strong veinclearing and stem pitting on Mexican lime, but does not cause stunting, seedling yellows or stem pitting on grapefruit (1). T-388 is a severe strain, inducing very intense symptoms on Mexican lime and stunting, seedling yellows and stem pitting on grapefruit and other citrus species (2). T-300, T-308, T-344, T-385, and T-388 have different dsRNA patterns, whereas T-362 and T-344 have the same dsRNA pattern (17).

Purification of coat protein. Bark was peeled from young shoots of healthy or CTV-infected sweet orange plants, pulverized in liquid nitrogen, and extracted (1:5, w/v) three times (15 min stirring each) in 0.1 M Tris-Cl buffer, pH 7.6. Extracts were centrifuged for 20 min at 10,000 *g*. The supernatant was adjusted to 4% polyethylene glycol (6,000 Da) and 1% NaCl, kept for 30 min after mixing and centrifuged at 17,000 *g* for 20 min. The pellet was resuspended overnight (3:1, fresh weight/v) in 0.04 M phosphate buffer, pH 8.0, and then centrifuged at 10,000 *g* for 20 min. Virions were pelleted by ultracentrifugation at 100,000

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g for 90 min. The whole process was carried out at 4 C. The pellet was resuspended (10 μl/g fresh weight) in 0.077 M Tris-Cl buffer, pH 8.0, containing 1.8% SDS, 4.4% (v/v) 2-mercaptoethanol, 8.8% (v/v) glycerol and heated for 4 min in boiling water. Proteins were separated by discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% acrylamide in the resolving gel, according to the method of Conejero and Semancik (7). To visualize the coat proteins, the central lane of each gel was sliced and stained with Coomassie blue. The major coat protein (CP) was separated from the unstained gel by slicing a 2-mm-wide band at the same migration distance as the visualized band. The protein was electroeluted from the gel band with a unidirectional electroelutor (IBI-UEA, New Haven, CT; two runs, 35 min each, at 125 V constant voltage in 0.1% SDS, 0.025 M Tris base, 0.192 M glycine buffer, pH 8.3) and trapped in 7.5 M ammonium acetate.

The CP preparation was desalted by ultrafiltration in an Amicon cell with a YM-10 membrane (Danvers, MA), collected in a small volume of distilled water, and freeze-dried after measuring optical density at 280 nm. The viral nature of purified protein was checked by a double antibody-sandwich enzyme-linked immunosorbent assay (ELISA-DAS) (23) and Western blots using monoclonal antibody 3DF1 (23) or polyclonal antisera 879, or 1053, to CTV, as described below.

Protease treatment of coat protein and peptide analysis. Freeze-dried CP preparations were rehydrated in distilled water adjusting the concentration to 1.4 absorbance units at 280 nm. Thirty-microliter aliquots of each CP preparation were partially digested for 15 or 30 min at 30 C with 1 μl of a 1 mg/ml preparation of the following proteases in distilled water: papain (EC 3.4.22.2), trypsin (EC 3.4.21.4), thermolysin (EC 3.4.24.4), chymotrypsin (EC 3.4.21.1) (Boehringer Mannheim), and V8 protease (EC 3.4.21.19) (Sigma Chemicals). The reactions were stopped by adding 30 μl of 0.02 M sodium phosphate buffer, pH 7.2, containing 30% ficoll, 4.0 M urea, 2% SDS, and 1 μl of 0.25 M dithioerythritol and then boiling for 1.5 min. Peptides were labeled with dansyl chloride by the method described by Falk and Tsai (9).

Dansyl-labeled peptides were separated by discontinuous SDS-PAGE (7), by using 17% acrylamide and 1 mm thickness in the resolving gel. Low molecular weight protein markers (14,400–97,400 Da) (Bio-Rad, Richmond, CA) and protease solutions at the concentrations used in the experiments were also labeled and electrophoresed under the same conditions. Peptides were visualized on a UV transilluminator at 302 nm and photographed using a Polaroid camera with an orange filter.

Western blots and serological detection of peptides. After electrophoresis, peptides were electro-blotted to a polyvinylidene difluoride membrane (PVDF) (Immobilon-PVDF, Millipore) by the method of Towbin et al (21), by using 0.025 M Tris base, 0.192 M glycine in 15% (v/v) methanol, pH 8.3, as a transfer buffer and a constant voltage of 70 V for 1 hr.

The membrane was soaked overnight in 0.01 M Tris-Cl, pH 7.4, containing 0.9% NaCl and 0.5% (v/v) Tween 20 (TBST), incubated for 1.5 hr at 37 C in a 1 μg/ml solution of CTV monoclonal antibody 3DF1 in TBST and then washed three times (5 min each) with the same buffer. A 1 μg/ml solution of protein A (*Staphylococcus aureus*) conjugated with alkaline phosphatase (Sigma Chemicals) was then added to the membrane and incubated for 1.5 hr at 37 C. After washing three times (5 min each) with TBST and once with 10% diethanolamine buffer, pH 9.8, alkaline phosphatase activity was detected by the nitro blue tetrazolium method (12).

RESULTS

Purification of coat protein. Semipurified preparations from all CTV strains showed, upon SDS-PAGE, a major protein band (CP) of 28,000 Da (Fig. 1, lane a) and two minor bands of 27,500 and 26,000 Da, respectively, that were not present in similar preparations from healthy plants. The major band gave strong reaction with monoclonal or polyclonal antibodies specific to CTV when transblotted to a PVDF membrane (Fig. 1, lanes f and h), whereas the minor bands reacted with polyclonal antibodies (Fig. 1, lane f) but not with monoclonal antibody (Fig. 1, lane h). When CPs from Florida CTV strains T-3, T-4, and T-36 were prepared from highly purified virions, according to a procedure previously described by Lee et al (14,15), two bands designated CP1 and CP2 with electrophoretic mobilities similar to the minor bands described above, were obtained (Fig. 1, lane d). These two bands reacted with polyclonal antibodies but not with monoclonal antibody (Fig. 1, lanes e and g). When these given Florida isolates

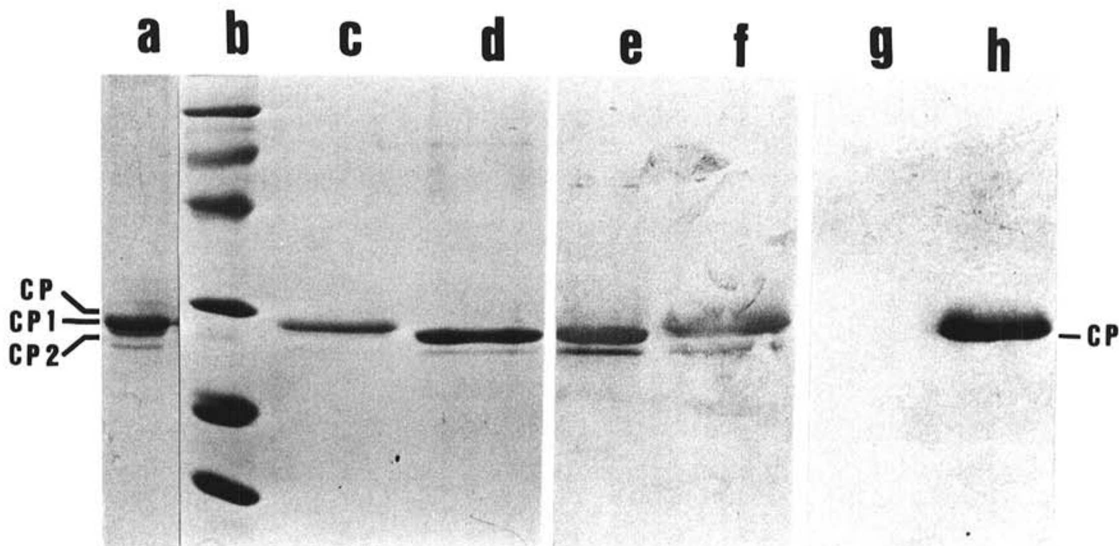

Fig. 1. Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (14% acrylamide in resolving gel) and Western blots of citrus tristeza virus (CTV) coat proteins (CPs) from Spain and Florida virus isolates obtained in the following conditions: Lane a, semipurified preparation of CTV T-308 (Spain). Lane b, molecular weight markers (rabbit muscle phosphorylase b, 97,400; bovine serum albumin, 66,200; hen egg white ovalbumin, 42,699; bovine carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; hen egg white lysozyme, 14,400). Lane c, CP from CTV T-308 electroeluted from a semipurified CTV preparation (as shown in lane a). Lane d, coat proteins obtained from ultrapurified virions of Florida CTV T-36. Lane e, coat proteins from CTV T-36 (as in lane d) transblotted to a PVDF membrane and reacted with polyclonal antibodies to CTV. Lane f, semipurified CTV T-308 preparation (as in lane a) transblotted to a PVDF membrane and reacted with polyclonal antibodies to CTV. Lanes g and h, same as lanes e and f, respectively, but reacted with monoclonal antibody to CTV 3DF1. Lanes a–d stained with Coomassie blue. Lanes e–h stained by the nitro blue tetrazolium (NBT) method.

were semipurified by the method described here, upon SDS-PAGE, they showed the same three bands as the Spanish isolates, and the major protein band (28,000 Da) reacted with 3DF1 monoclonal antibody (data not shown).

The major band (CP) was separated and concentrated as described under Materials and Methods. Variable yields were obtained depending on CTV strains, ranging from 0.4 to 1.8 A_{280nm} (1 ml of extract) per 100 g of fresh tissue. Desalted protein solutions reacted with monoclonal antibody 3DF1 in ELISA and Western blots. When purity of the protein was checked by SDS-PAGE, usually a single band was observed with the same migration as the major band (CP) from the semipurified preparations (Fig. 1, lane c). Occasionally, minor bands of high molecular weight were also observed. When transblotted to membranes these bands also reacted with antibodies specific to CTV; these were probably aggregates of the CP (data not shown).

When purified protein was dansylated and electrophoresed in SDS-PAGE (17% acrylamide) a major band of 30,000 Da was observed. Sometimes, a minor band of 18,000 Da could also be detected (data not shown). This minor band could not be identified after digestion with any of the enzymes. When a dansylated CP preparation was electroblotted to PVDF membranes only the upper band reacted with monoclonal antibody 3DF1 (data not shown).

Peptide maps. Preliminary assays with dansyl-labeling before or after digestion with endopeptidases showed that a better resolution and a higher number of defined bands was obtained when dansylation was carried out after digestion. It was also established that incubation at 30 C for 30 min was adequate for papain, trypsin, and V8 protease, whereas a 15-min incubation was enough

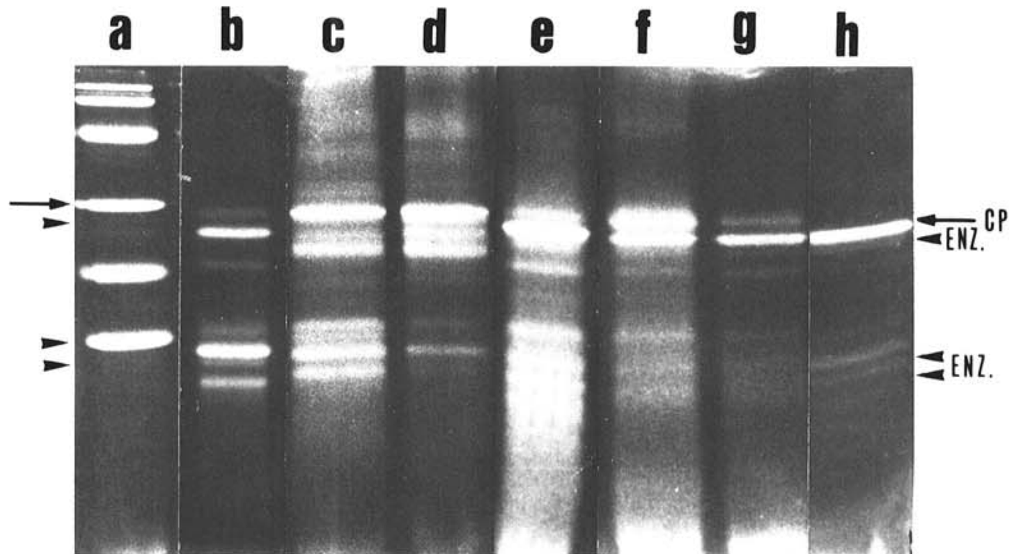

Fig. 2. Peptide maps obtained by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17% acrylamide in resolving gel) with coat protein (CP) preparations from different citrus tristeza virus (CTV) strains digested with papain. Lane a, molecular weight markers as in Figure 1 labeled with dansyl chloride. Lane b, CTV strain T-388; lane c, CTV T-308; lane d, CTV T-385; lane e, CTV T-300; lane f, CTV T-344; lane g, CTV T-362; and lane h, papain (1 μl of a 1 mg/ml aqueous solution). Thirty microliters of an aqueous solution (1.4 A_{280nm}) of each CP was digested with 1 μl of the enzyme solution (1 mg/ml), stained with dansyl chloride, electrophoresed, and visualized on a UV transilluminator. Arrows indicate CPs and arrowheads mark the position of enzyme bands.

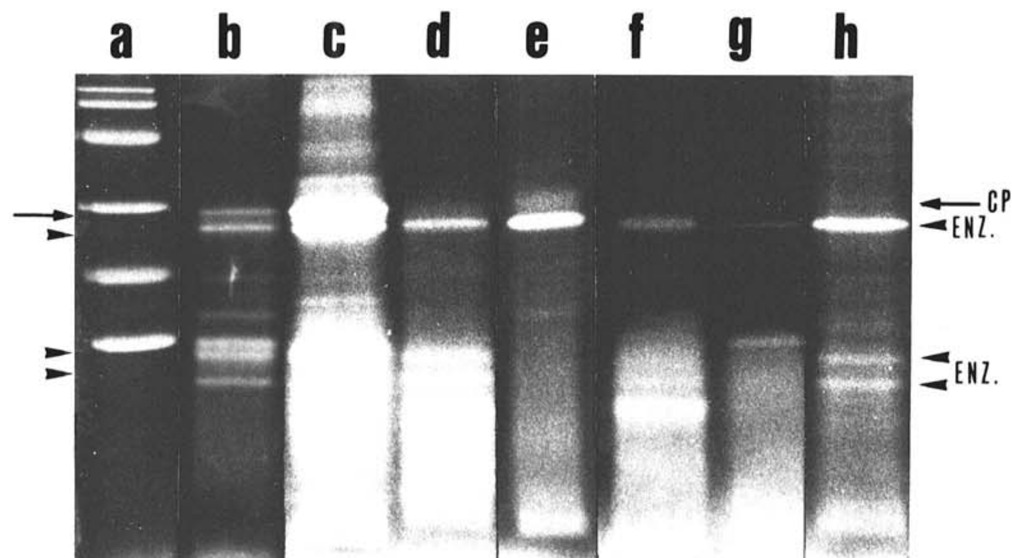

Fig. 3. Peptide maps obtained by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17% acrylamide in resolving gel) with coat protein (CP) preparations from different citrus tristeza virus (CTV) strains digested with trypsin. Lane a, molecular weight markers as in Figure 1 labeled with dansyl chloride. Lane b, CTV strain T-388; lane c, CTV T-308; lane d, CTV T-385; lane e, CTV T-300; lane f, CTV T-344; lane g, CTV T-362; and lane h, trypsin (1 μl of a 1 mg/ml aqueous solution). Thirty microliters of an aqueous solution (1.4 A_{280nm}) of each CP was digested with 1 μl of the enzyme solution (1 mg/ml), stained with dansyl chloride, electrophoresed, and visualized on a UV transilluminator. Arrows indicate CPs and arrowheads mark the position of enzyme bands.

for thermolysin and chymotrypsin. Longer incubation periods (up to 1 hr) did not enable detection of additional peptide bands.

Figure 2 shows the peptide maps obtained when 30 μl of aqueous solution of CP (1.4 A_{280nm}) from the different CTV strains were digested with papain. Generally, CP from strains T-388 and T-362 were more digested by papain (less undigested CP remaining in the gel) than the CP from other strains. Peptide maps of strains T-388 and T-308 showed four low molecular weight bands in the range of 14,400 Da differing in mobility and intensity between both strains. The position of some of these bands was coincident with that of minor bands of the enzyme (Fig. 2, lanes b, c, and h), but their high intensity and the reaction with monoclonal antibody in Western blots (see Fig. 7, below, lanes a and b) indicated that they contained CP peptides. Peptide maps of the other strains showed only one to three peptide bands in the same range. Variability among strains was also observed in the zone between 14,400 and 31,000 Da. A major peptide band of about 25,000 Da was present in T-308 and T-385, whereas a minor band of 22,000 Da was observed in T-388, T-300, T-344, and T-362.

Peptide maps obtained after digestion with trypsin are shown in Figure 3. The CP from strains T-388 and T-308 was less hydrolyzed than protein from the other four strains. In the zone of molecular weights below 14,400, two bands corresponding to the enzyme were observed in some peparations. Above this zone, additional peptide bands were observed in some strains, i.e., a 14,400-Da peptide band was visible in T-388, T-308, and T-362; a 20,000-Da band was visible in T-388 and very weak in T-300; a 20,500-Da band was present only in T-308 and a weak band of 21,000 Da was obtained in T-388 and T-308.

Figure 4 shows the peptide maps obtained after digestion with V8 protease. The CP from strains T-362 and T-385 was more hydrolyzed than protein from the other strains. A protein band, of approximately 27,000 Da, was evident with variable intensity in some strains. Up to three bands of less than 14,000 Da were resolved. Migration of these bands was slightly different from similar bands produced by papain or trypsin. The CTV strains assayed differed by the number and intensity of bands.

Chymotrypsin completely digested the CP from most CTV strains and only the CP of strain T-308 showed some resistance to the enzyme (Fig. 5). Only T-308, T-385, and T-388 showed one or two low molecular weight bands.

Thermolysin completely digested the CP from all CTV strains (Fig. 6). The CP of T-308 produced a strong band around 14,000 Da, that was also present in T-388, but with lower intensity. In addition, T-308 showed several minor bands. The other strains did not yield any resolved bands.

CP from each CTV strain was purified two to five times and each batch digested two or three times with all five enzymes. Reproducible patterns for the major peptides were obtained for each strain and enzyme.

Western blots. Figure 7 shows Western blots obtained from CPs of strains T-388, T-308, and T-385 digested with the five enzymes and reacted with monoclonal antibody 3DF1.

After digestion with papain, no reaction of T-388 CP could be detected in Western blots, whereas T-308 and T-385 CPs gave strong reactions (Fig. 7, lanes a–c). Two of the low molecular weight bands produced in T-388 CP and three of the four observed in T-308 CP (see Fig. 2, lanes b and c) reacted with 3DF1. The CPs of strain T-385 (Fig. 7, lane c) and strains T-300, T-344, and T-362 (data not shown) had single stained bands coinciding with CP migration, their intensity being proportional to the resistance to the enzyme (see Fig. 2).

The CPs of strains T-388 and T-308 digested with trypsin (Fig. 7, lanes d and e) yielded two major stained bands, the larger one corresponding to the CP. T-388 also had two minor bands. No band was stained in the other strains, though low molecular weight peptides could be visualized in some of them (see Fig. 3).

Western blots of T-388 and T-308 CPs treated with V8 protease (Fig. 7, lanes g and h) showed two stained bands, the major one corresponded to the CP. The other four strains gave a single heavily stained band, corresponding to CP, this reaction being stronger in T-344 (data not shown) than in T-300, T-385, and T-362.

Western blots of chymotrypsin digests of T-388 and T-308 CPs yielded two stained bands (Fig. 7, lanes j and k). The upper band, which corresponded to CP, was more stained in T-308, which coincides with its higher resistance to the enzyme. The other strains did not yield any stained band.

When CP from all strains was treated with thermolysin, only T-308 CP yielded a heavily stained band (Fig. 7, lane n) corresponding to the 14,000 Da peptide observed in gels (see Figure 6, lane c).

Aggregated CP strongly reacted with 3DF1 and gave stained spots above the coat protein band.

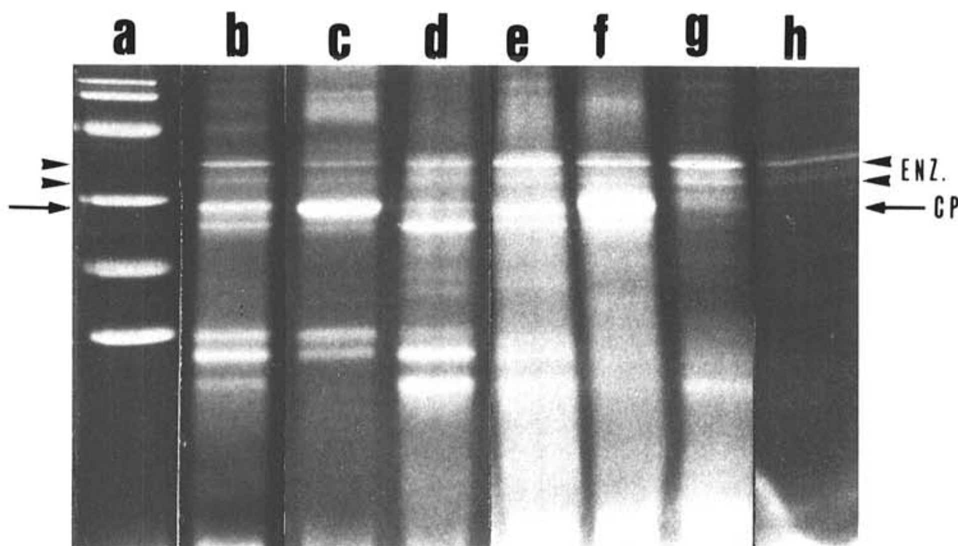

Fig. 4. Peptide maps obtained by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17% acrylamide in resolving gel) with coat protein (CP) preparations from different citrus tristeza virus (CTV) strains digested with V8 protease. Lane a, molecular weight markers as in Figure 1 labeled with dansyl chloride. Lane b, CTV strain T-388; lane c, CTV T-308; lane d, CTV T-385; lane e, CTV T-300; lane f, CTV T-344; lane g, CTV T-362; and lane h, V8 protease (1 μl of a 1 mg/ml aqueous solution). Thirty microliters of an aqueous solution (1.4 A_{280nm}) of each CP was digested with 1 μl of the enzyme solution (1 mg/ml), stained with dansyl chloride, electrophoresed, and visualized on a UV transilluminator. Arrows indicate CPs and arrowheads mark the position of enzyme bands.

DISCUSSION

Semipurified preparations of different CTV strains from Spain and Florida (data not shown) had a major protein band (CP) of 28,000 Da (estimated in SDS-PAGE) that was not present in healthy extracts or in coat protein preparations obtained from ultrapurified CTV virions. This band reacted in Western blots and ELISA with monoclonal and polyclonal antibodies to CTV. Semipurified preparations showed, in addition, two minor bands with the same migration as CP1 and CP2 obtained from CTV virions ultrapurified by the method of Lee et al (14,15) (Fig. 1). CP1 was the major band obtained by the last procedure (15). Monoclonal antibody 3DF1 reacted with crude extracts of plants infected with Florida CTV strains in ELISA-DAS (unpublished data). The absence of CP and high ratio of CP1 in ultrapurified preparations suggest that CP1 could be a degradation product of CP. Lack of reaction of CP1 with monoclonal antibody 3DF1 supports the idea that a fragment of CP containing the epitope recognized by 3DF1 was lost during the long purification process. CP2 was present as a minor band in both purification processes. This band reacted with polyclonal but not with monoclonal antibodies. It could be a degradation product of CP. Molecular weight estimated for CP is similar to that reported by Flores et al (10) and slightly higher than that estimated by other authors (3,15).

After electroelution and desalting, purified CP maintained its electrophoretic mobility and antigenic properties. Antigenicity of CP remained unchanged when dansylated, but a slightly higher molecular weight was estimated when compared with dansylated molecular weight markers in SDS-PAGE (17% acrylamide). This difference may be explained by differential labeling of coat protein

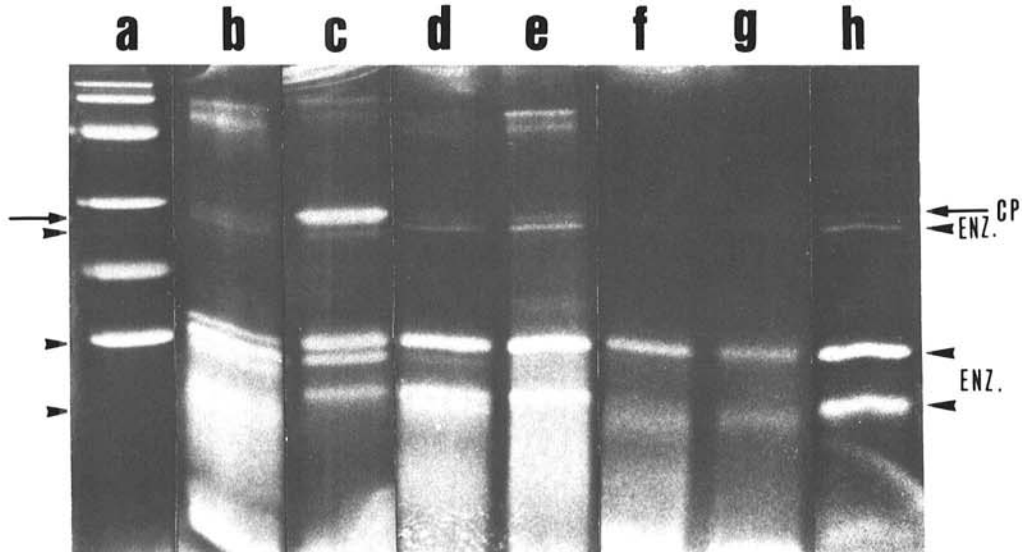

Fig. 5. Peptide maps obtained by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17% acrylamide in resolving gel) with coat protein (CP) preparations from different citrus tristeza virus (CTV) strains digested with chymotrypsin. Lane a, molecular weight markers as in Figure 1 labeled with dansyl chloride. Lane b, CTV strain T-388; lane c, CTV T-308; lane d, CTV T-385; lane e, CTV T-300; lane f, CTV T-344; lane g, CTV T-362; and lane h, chymotrypsin (1 μl of a 1 mg/ml aqueous solution). Thirty microliters of an aqueous solution (1.4 A_{280nm}) of each CP was digested with 1 μl of the enzyme solution (1 mg/ml), stained with dansyl chloride, electrophoresed, and visualized on a UV transilluminator. Arrows indicate CPs and arrowheads mark the position of enzyme bands.

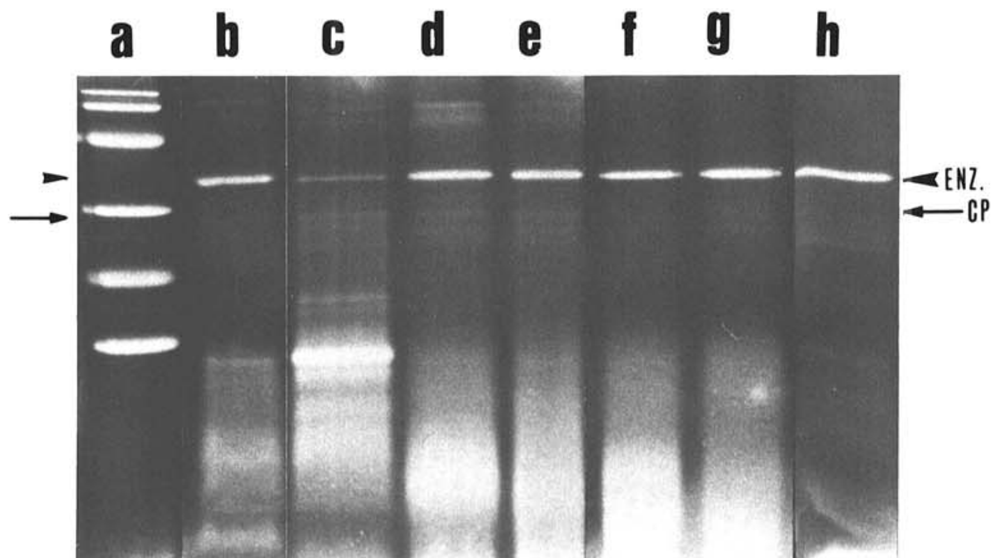

Fig. 6. Peptide maps obtained by discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17% acrylamide in resolving gel) with coat protein (CP) preparations from different citrus tristeza virus (CTV) strains digested with thermolysin. Lane a, molecular weight markers as in Figure 1 labeled with dansyl chloride. Lane b, CTV strain T-388; lane c, CTV T-308; lane d, CTV T-385; lane e, CTV T-300; lane f, CTV T-344; lane g, CTV T-362; and lane h, thermolysin (1 μl of a 1 mg/ml aqueous solution). Thirty microliters of an aqueous solution (1.4 A_{280nm}) of each CP was digested with 1 μl of the enzyme solution (1 mg/ml), stained with dansyl chloride, electrophoresed, and visualized on a UV transilluminator. Arrows indicate CPs and arrowheads mark the position of enzyme bands.

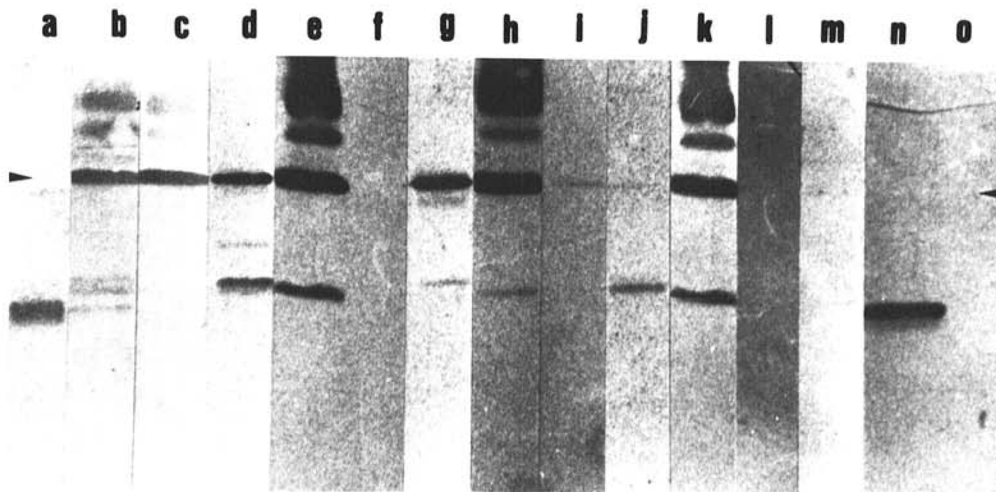

Fig. 7. Western blots obtained digesting coat protein (CP) from citrus tristeza virus (CTV) strains T-388 (lanes a, d, g, j, m), T-308 (lanes b, e, h, k, n), and T-385 (lanes c, f, i, l, o), with papain (lanes a–c), trypsin (lanes d–f), V8 protease (lanes g–i), chymotrypsin (lanes j–l) and thermolysin (lanes m–o) and reacting with monoclonal antibody 3DF1. Arrowheads indicate coat protein.

and molecular weight markers due to a different content in tyrosine and lysine, the amino acids that bind dansyl chloride (9).

The purification procedure followed in this work is a convenient method to obtain CTV coat protein. It eliminates several virus purification steps, and the protein preparation obtained usually contains no detectable contaminant. In addition, a less degraded CP preparation is obtained by the present procedure than by ultrapurification of CTV virions.

Peptide maps obtained by partial digestion with specific proteases enabled differentiation of all the CTV strains assayed. Two types of differences were observed: sensitivity of coat protein to enzyme digestion and number and size of peptides obtained. Differential sensitivity to enzyme digestion (a different ratio of CP remaining undigested) could be due to conformational variations among CPs in aqueous solution making them more or less susceptible to the enzyme. The number and size of peptides obtained depends on the primary structure of CPs. Strains differing by biological characteristics and dsRNA pattern were easily distinguished by peptide mapping (i.e., T-308 and T-344 showed a different pattern with all the enzymes), whereas more similar strains like T-362 and T-344 showed minor differences with only three of the enzymes tested (Figs. 2–4). Lee et al (15) found only minor differences among peptide maps of three Florida CTV strains using trypsin, thermolysin, and V8 protease. Several factors could account for the improved ability to differentiate CTV strains in this work: 1) The higher size of CP obtained by the present purification procedure could give raise to different or additional peptides. 2) Lee et al (15) dansyl-labeled CP before digestion. Some enzymes might be unable to reach specific points in dansylated proteins. In fact, we found a higher number of bands when dansylation was done after enzyme digestion (data not shown). 3) Separation of peptides was done by using a discontinuous SDS-PAGE with a resolving gel 1 mm thick and 17% acrylamide, whereas Lee et al (15) used preparative gels with 15% acrylamide.

Western blot analysis with monoclonal antibody 3DF1 showed fewer differences among strains than peptide maps. Many of the bands that stained with dansyl chloride did not react with the monoclonal antibody specific to CTV and consequently, did not appear in Western blots. Nevertheless, this analysis provided additional information on the peptides obtained by enzyme treatment. For example, papain digestion produced four defined low molecular weight bands in T-388 and T-308, three in T-300, and one in T-385 (Fig. 2), whereas in Western blot analysis only two peptides of T-388, three of T-308, and none of T-300 or T-385, could be visualized (Fig. 7). The two peptides of T-388 were different from those stained in T-308. This is an indication that peptides separated by SDS-PAGE were antigenically different.

Peptide maps and Western blot analysis enabled us to distinguish CTV strains having a similar biological behavior and the same dsRNA pattern. This approach to characterizing virus strains takes longer than dsRNA analysis and may not be adequate as a routine method for strain diagnosis. Nevertheless, it is a powerful tool of research that can be used to supplement information obtained from biological tests or dsRNA analysis.

A combination of peptide maps and Western blot analysis with different monoclonal and polyclonal antibodies would widen the possibilities of strain differentiation and would help better characterize CTV coat protein.

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