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The epitope structure of *Citrus tristeza virus* coat protein mapped by recombinant proteins and monoclonal antibodies

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ABSTRACT

It has been known that there exists serological differentiation among *Citrus tristeza virus* (CTV) isolates. The present study reports three linear epitopes (aa 48–63, 97–104, and 114–125) identified by using bacterially expressed truncated coat proteins and ten monoclonal antibodies against the native virions of CTV-S4. Site-directed mutagenesis analysis demonstrated that the mutation D98G within the newly identified epitope ^{97}DDDSTGIT^{104} abolished its reaction to MAbs 1, 4, and 10, and the presence of G98 in HB1-CP also resulted in its failure to recognize the three MAbs. Our results suggest that the conformational differences in the epitope I ^{48}LGTQQNAALNRDLFLT^{63} between the CPs of isolates S4 and HB1 might contribute to the different reactions of two isolates to MAbs 5 and 6. This study provides new information for the antigenic structures of CTV, and will extend the understanding of the processes required for antibody binding and aid the development of epitope-based diagnostic tools.

Introduction

Citrus tristeza virus (CTV) is a member of the genus *Closterovirus* within the family *Closteroviridae* (Martelli et al., 2002). Tristeza disease which is caused by the virus has seriously affected the development of the citrus industry in some countries (Moreno et al., 2008). The CTV virion contains a large single-stranded, positive-sense genomic RNA (gRNA) of ~19.3 kb, consisting of twelve open reading frames (ORFs). The viral particle has a unique bipolar architecture coated by two coat proteins (CP and CPm) (Febres et al., 1996). CP coats most of the gRNA (genomic RNA), and CPm coats only ~630 nt at the 5′ terminus (Satyanarayana et al., 2004). Thus, serological diagnosis of CTV is mainly based on the detection of its CP with specific antibodies.

So far, at least twenty complete genomic sequences of CTV isolates have been determined. Nucleotide sequence analysis showed that CTV isolates were highly variable, and could be grouped into six genotypes, namely VT, T3, T30, T36, B165, and RB based on the phylogenetic analysis of the 5′ proximal half (about 11 kb) of the genome (Harper et al., 2010; Hilf et al., 1999, 2005; Roy and Brlansky, 2010). Four genotypes were identified in the CTV population in China by analyzing the sequences of multiple molecular markers (MMMs) and restriction fragment length polymorphism patterns (RFLP) of the CP gene of CTV isolates (Jiang et al., 2008; Wu et al., 2013). The biological indexing on a set of indicator plants has revealed the pathogenicity differentiation of CTV isolates from different citrus-growing areas (Ballester-Olmos et al., 1993). Many CTV isolates, namely severe strains, are aggressive and are associated with the symptoms of decline and death of citrus trees propagated on sour orange (*Citrus aurantium* L.) rootstock or stem pitting (SP) of the scion irrespective of the rootstocks. Only a few reported isolates, namely mild strains, induce slight leaf chlorosis or are symptomless on Mexican lime (Moreno et al., 2008). The discrimination between mild and severe strains can provide valuable information for the effective control of the viral disease, and the CP gene sequences have been used extensively to discriminate CTV strains, but other regions can also be used (Cevik et al., 1996; Herrera-Isidron et al., 2009; Sambade et al., 2003).

Serological technique is one of the most widely used tools for the reliable and high-throughput identification of plant viruses, and also for the discrimination of strains or serotypes of some

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viruses. Epitopes play a pivotal role in antigen recognition. The precise localization of an epitope can be essential in the development of serological diagnostic kits for the specific viral strains or variants. The antigenic structures of many plant viruses have been investigated through the identification of epitopes recognized by monoclonal antibodies (MAbs) and polyclonal antisera, which have greatly improved the development of highly specific serological reagents. The serological differentiation has been observed among CTV strains with different biological characteristics. Nikolaeva et al. (1998) described an IDAS-ELISA system developed to distinguish among a wide range of isolates which cause stem pitting in sweet orange indicator plants from those that do not cause sweet orange stem pitting. The development of MAbs significantly improved the differentiation of CTV strains. A previous study identified a monoclonal antibody, MCA-13, which reacted selectively with the majority of CTV severe isolates (Permar et al., 1990). Several monoclonal and polyclonal antibodies have been developed against various CTV isolates (Rocha-Peña and Lee, 1991; Vela et al., 1986; Wang et al., 2006), which have made it possible to map the epitopes for CTV. Pappu et al. (1993, 1995) found that the amino acids at positions 2 and 124 played crucial roles in the binding of the MAbs 3DF1 and MCA-13, respectively. Nikolaeva et al. (1996) screened 30 CTV-specific MAbs and assigned them into five groups based on epitope specificity. Albiach-Martí et al. (2000) developed a serological analysis procedure which was utilized to produce peptide maps by using protease digestion with MAbs and polyclonal antibodies (PAbs) to detect and discriminate CTV isolates. Recently, Peroni et al. (2009) developed four specific MAbs against the recombinant protein of the most virulent Brazilian CTV genotype "Capão Bonito" (CB) and identified three epitope regions (aa 32–40, 50–61, and 120–131) by ELISA screening of the overlapping recombinant peptides. However, information on the types and distribution of epitopes on the CP of CTV is currently limited as compared with those of other economically important plant viruses, such as *Plum pox virus* (Candresse et al., 2010; Croft et al., 2008), potyviruses (Desbiez et al., 1997; Shukla et al., 1989), and *Tobacco mosaic virus* (Dore et al., 1987; Holzem et al., 2001).

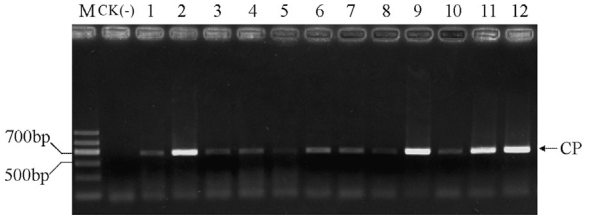

Fig. 1. The detection of CTV virions captured by MAbs and PAb by IC-RT-PCR. M: DNA marker from Tiangen (Beijing, China); lane CK(−): Normal mouse serum; lanes 1–10: MAbs 1–10, respectively; lane 11: PAb-L5; lane 12: Mixed MAb. CP indicates the CP product with a size of 672 bp.

CTV infection occurs widely in citrus plants in central and southern China, and the most prevalent CTV isolates are associated with the stem pitting syndrome (Jiang et al., 2008; Zhou et al., 2007). Previously, our group raised a polyclonal antiserum and a set of monoclonal antibodies against virions of two CTV isolates (Wang et al., 2006), respectively. In the course of a serological survey of Chinese CTV strains, a CTV isolate from pummelo (HB1), which differed serologically from all previously studied isolates, was identified. The HB1 isolate was unable to be recognized by some MAbs, and bioinformatics analysis indicated that three amino acid sites (S84, G98, and G190) specifically present in the CP of HB1 might affect its recognition by those MAbs (Wang et al., 2007). Comprehensive knowledge of the epitope structures as well as the characterization of new epitope-specific MAbs is necessary for the development of novel epitope-based diagnostic tests. In this study, we initiated a survey of the epitopes of the CTV CP by using ten CTV-specific MAbs raised against a CTV stem-pitting isolate S4, and three epitope regions were identified. Site-directed mutagenesis analysis demonstrated that one important amino acid at the 98 (G98) position of the CP was involved in the antibody binding with HB1-CP. The data obtained contributes to a better understanding of the antigenic structure of the virus and to the improvement of epitope-based diagnostic tools.

Results

Reactivity of monoclonal antibodies with the CTV virions and the full-length and truncated CPs of CTV expressed in Escherichia coli strain BL21 (DE3)

The reactivity of ten MAbs with CTV-S4 virions was tested by immune capture (IC) RT-PCR, and compared with that of mixed MAbs and the polyclonal antibody PAb-L5. The results showed that all of the antibodies were able to capture CTV virions, and gave positive results in the RT-PCR tests (Fig. 1).

Meanwhile, the full length CP and truncated CPs, named CΔ-1 to -9, were successfully expressed in *E. coli* strain BL21 (DE3) and visualized by SDS-PAGE analysis (Fig. 2A). However, the expressed amount of full-length CP and two amino-terminal deleted fragments CΔ-4 and CΔ-5 were less than that of the other truncated CPs. The purified extracts of the CP and CΔ-1 to -9 with the expected molecular weights of 29.0 kDa, 24.4 kDa, 21.9 kDa, 14.8 kDa, 15.5 kDa, 20.2 kDa, 16.2 kDa, 14.4 kDa, 14.8 kDa, and 16.2 kDa, respectively, were obtained by using the high-affinity Ni-NTA agarose (Fig. 2A). The CP, CΔ-4, and CΔ-5 were purified under native conditions, while the other seven truncated CPs were

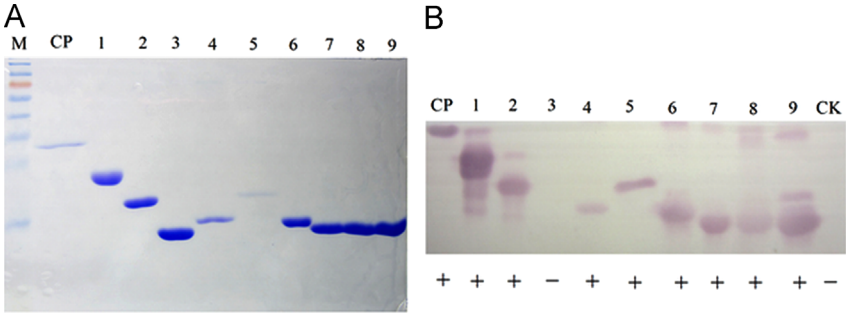

Fig. 2. Detection of the CTV coat protein and its truncated fragments expressed in *E. coli* BL21 (DE3). The purified fusion proteins (A) was stained with Coomassie blue after 12% SDS-PAGE analysis. The reactivity of those proteins with mixed MAbs was detected by Western blotting (B). Molecular weight markers (Fermentas) are shown in lane M. Lanes CP and 1–9: CP and nine truncated CPs CΔ-1 to -9, respectively. Cell lysates of *E. coli* transformed with the empty pET28a vector were used for the cell control (Lane CK). '+' and '−' indicate the positive and negative reactions, respectively.