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βC1 encoded by tomato yellow leaf curl China betasatellite forms multimeric complexes *in vitro* and *in vivo*

Xiaofei Cheng [a], Xiaoqiang Wang [b], Jianxiang Wu [a], Rob W. Briddon [c], Xueping Zhou [a,*]

[a] Institute of Biotechnology, Zhejiang University, Hangzhou 310029, Zhejiang, P.R. China
[b] Plant Biology Division, the Samuel Roberts Noble Foundation, Ardmore 73401, O.K., USA
[c] National Institute for Biotechnology and Genetic Engineering, Jhang Road, Faisalabad, Pakistan

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ABSTRACT

The βC1 protein encoded by betasatellites associated with begomoviruses is multi-functional. To investigate its properties, the βC1 protein encoded by tomato yellow leaf curl China betasatellite (TYLCCNB) was expressed in *Escherichia coli* and analyzed for its ability to self-interaction. The βC1 protein formed large soluble multimeric complexes *in vitro* and *in vivo*. Mutations that prevented formation of multimeric complexes *in vitro*, also prevented formation of granular bodies *in vivo*, suggesting that granular bodies resulted from βC1 oligomerization. Similarly, βC1 mutants unable to form complexes also did not induce typical symptoms in plants when expressed from a Potato virus X (PVX) vector, suggesting that βC1 self-interaction was required for symptom induction *in planta*. Deletion analysis revealed that amino acid sequences spanning two predicted α-helices at the C-terminal end of the protein were important in multimerization.

Introduction

The genus *Begomovirus* in the family *Geminiviridae* consists of a group of plant viruses which caused enormous economic losses to crops worldwide (Mansoor et al., 2006). The typical begomovirus genome consists of two molecules of circular ssDNA, known as DNA-A and DNA-B, and each is approximately 2.6–2.8 kb in length. However, the majority of begomoviruses in the Old World consist of only a single genomic component, homologous to DNA-A of bipartite begomoviruses (Stanley et al., 2005). Betasatellites are small circular ssDNA molecules (about 1.3 kb) associated with the majority of monopartite begomoviruses (Briddon and Stanley, 2006). Betasatellites are dependent on their helper begomoviruses for replication, encapsidation and movement within plants (Briddon et al., 2003; Briddon and Stanley, 2006) but are required, in many cases, by their helper begomoviruses for symptom induction in hosts from which they were isolated (Briddon et al., 2001; Jose and Usha, 2003; Li et al., 2005; Saunders et al., 2000). All betasatellite molecules encode an approx. 13.5 kDa protein, known as βC1, in the complementary-sense orientation, which is a pathogenicity (symptom) determinant (Cui et al., 2004; Guo et al., 2008; Saeed et al., 2005; Saunders et al., 2004). βC1 can also suppress host cell RNA silencing (Cui et al., 2005; Gopal et al., 2007; Sharma et al., 2010) and may complement movement of DNA-A component of bipartite begomoviruses in the absence of DNA-B (Saeed et al., 2007).

The βC1 encoded by tomato yellow leaf curl China betasatellite (TYLCCNB) is a symptom determinant, a suppressor of RNA silencing and it interacts with the protein Asymmetric Leaves 1 (AS1) from *Arabidopsis* to alter leaf development (Cui et al., 2004, 2005; Yang et al., 2008). Despite these findings, biochemical and structural properties of TYLCCNB βC1 have not been investigated. We report here that βC1 forms large multimeric complexes *in vitro* and granular bodies *in vivo*, and that multimerization is necessary for βC1 to induce typical disease symptoms in plants.

Results

Y10βC1 forms large multimeric complexes in vitro

A vector for expression of the βC1 gene of TYLCCNB isolate Y10 (Y10βC1) fused to an N-terminal His tag in *Escherichia coli* was produced (Fig. 1A, upper panel) and the protein was purified by affinity chromatography. During gel filtration, only a single peak at an elution volume of 48.6 mL (void volume = 45 mL) was detected (Fig. 1A, middle panel). The calculated MW of the peak is more than 1000 kDa. The peak elute was then subsequently analyzed by SDS-PAGE and western blot. A single band with MW of about 16 kDa, the size predicted for monomeric His-Y10βC1, was detected (Fig. 1A, bottom panel). The result indicated that Y10βC1 formed large multimeric complexes *in vitro*.

* Corresponding author. Fax: +86 571 86971498.
E-mail address: zzhou@zju.edu.cn (X. Zhou).

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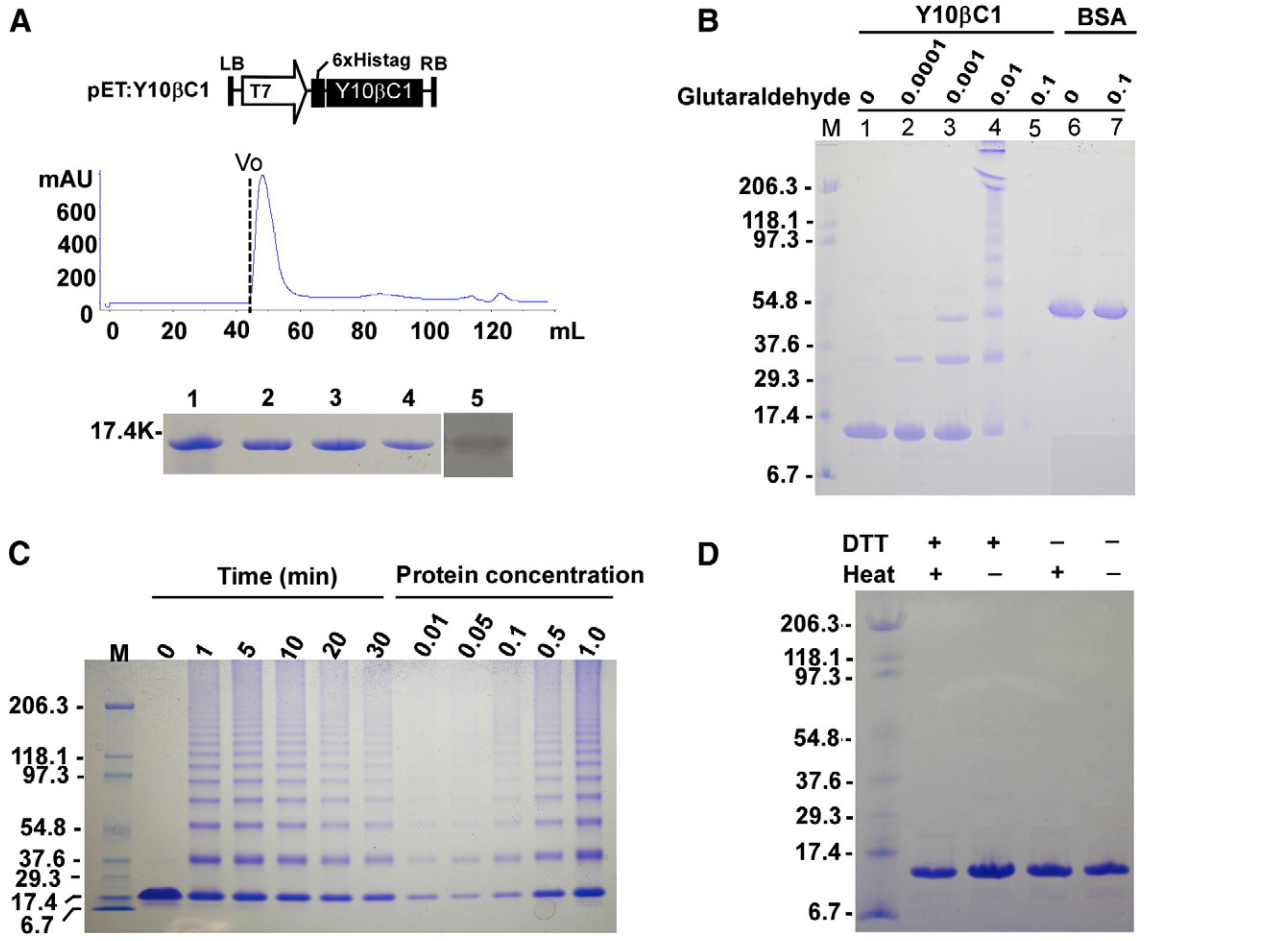

Fig. 1. Y10βC1 forms large soluble multimeric complexes *in vitro*. (A) Estimation of the apparent molecular weight of His-Y10βC1 by gel filtration. The construct containing the full coding sequence of Y10βC1 (pET:Y10βC1) is shown in the upper panel. Left and right borders of the binary vector are indicated as LB and RB, respectively. The positions of the T7 promoter and Histag are shown. The elution profile of His-Y10βC1 is shown in the middle panel. Absorption at 280 nm is indicated on the left, elution volume is indicated at the bottom and the void volume (Vo) is shown by a dashed line. SDS-PAGE and Western blot of peak elutes from gel filtration are given in the bottom panel. Lane 1 to 4, elution of 46, 47, 48 and 49 mL, respectively. Lane 5, Western blot of peak elution (48 mL) using monoclonal antibodies against the Y10βC1 protein. (B) Cross-linking of Y10βC1 protein (lanes 1 to 5) or bovine serum albumin (BSA, lanes 6 and 7) with glutaraldehyde. Final concentration of glutaraldehyde in each reaction is indicated at the top. The products of cross-linking were electrophoresed on 8–25% PhastGel. (C) Effects of incubation time and protein concentration on cross-linking. Reaction time (minute) and protein concentration (mg/mL) are shown at the top of the gel. (D) Effect of DTT and heat on the multimerization of Y10βC1. Purified Y10βC1 protein was either untreated (–) or treated (+) with DTT and/or heating to 100 °C for 5 min prior to electrophoresis.

Cross-linking is a widely used method for characterizing protein self-interaction (Nadeau and Carlson, 2002). Purified Y10βC1 was incubated with various concentrations of glutaraldehyde. After cross-linking, products were analyzed on SDS-PAGE gels. After incubation with low concentrations of glutaraldehyde (0.0001% and 0.001%), two higher molecular mass forms were present, their MWs corresponded to dimeric and trimeric Y10βC1. Following treatment with 0.01% glutaraldehyde, a ladder-like pattern of bands with a regular spacing was observed. At an even higher concentration of glutaraldehyde (0.1%), Y10βC1 failed to enter into the gel during electrophoresis, remaining in the wells, suggesting that cross-linked protein complex was too large to enter the gel matrix. In contrast, BSA migrated at a constant position on SDS-PAGE gels, even after treatment with 0.1% glutaraldehyde (Fig. 1B).

Incubating time and protein concentration have been reported to affect cross-linking results (Rudolph et al., 1997). To avoid this possibility, purified Y10βC1 protein was diluted approximately 100 times (to about 0.01 mg/mL protein) and then treated with 0.01% glutaraldehyde for various time periods and a series of protein dilutions were treated with glutaraldehyde for a set time period (20 min). The same ladder-like pattern was observed for all treatments (Fig. 1C), indicating that cross-linking products were not resulted from high protein concentrations or extended incubation times.

The predicted sequence of Y10βC1 contains a cysteine residue at amino acid position 68. To rule out that multimerization is mediated by formation of intermolecular disulfide bonds, purified Y10βC1 was analyzed by SDS-PAGE under reducing and non-reducing conditions. Thus, the protein samples were prepared in sample loading buffer with or without reducing agent DTT and with or without heat treatment at 100 °C for 5 min prior to electrophoresis. The results showed that a single band of approx. 16 kDa, representing Y10βC1 monomer, was present in protein samples following all treatments (Fig. 1D). This indicated that βC1 multimerization was not due to intermolecular disulfide bonds.

Y10βC1 forms granular bodies in vivo

To eliminate that the observed multimeric complexes represent aggregations of misfolded Y10βC1, bimolecular fluorescence complementation (BiFC) assay was used to evaluate the ability of Y10βC1 to self-interact *in vivo*. No YFP signal was observed in leaves co-infiltrated with empty vectors (pYN1 and p2YC), nor in leaves inoculated with pYN:Y10βC1 and p2YC or pYN1 and p2YC:10βC1