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Purification and biochemical characterization of a monomeric form of papaya mosaic potexvirus coat protein

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Abstract

Papaya mosaic virus (PapMV) is a flexuous rod shape virus made of 1400 subunits that assemble around a plus sense genomic RNA. The structure determination of PapMV and of flexuous viruses in general is a major challenge for both NMR and X-ray crystallography. In this report, we present the characterization of a truncated version of the PapMV coat protein (CP) that is suitable for NMR study. The deletion of the N-terminal 26 amino acids of the PapMV CP ($CP_{27–215}$) generates a monomer that can be expressed to high level and easily purified for production of an adequate NMR sample. The RNA gel shift assay showed that $CP_{27–215}$ lost its ability to bind RNA in vitro, suggesting that the multimerization of the subunit is important for this function. The fusion of a 6× His tag at the C-terminus improved the solubility of the monomer and allowed its concentration to 0.2 mM. The CD spectra of the truncated and the wild-type proteins were similar, suggesting that both proteins are well ordered and have a similar secondary structure. $CP_{27–215}$ was ^{15}N labeled for NMR studies and a 2D $^{1}H–^{15}N$-HSQC spectrum confirmed the presence of a well-ordered structure and the monomeric form of the protein. These results show that $CP_{27–215}$ is amenable to a complete and exhaustive NMR study that should lead to the first three-dimensional structure determination of a flexuous rod shape virus.

Keywords: Papaya mosaic virus; Coat protein; Monomer; Potexviruses; NMR

Papaya mosaic virus (PapMV)[1] is a member of the large family of the *Flexiviridea* [1] and the gender potexvirus. The virus harbors a flexuous rod shape of 500 nm in length and 14 nm in diameter. There are 1400 subunits of the viral coat protein (CP) in each rod assembled around a plus strand genomic RNA of 6656 nucleotides [2]. PapMV CP is a protein of 215 amino acids having a molecular weight of 23 kDa. Many studies about the in vitro reconstitution of PapMV nucleocapsid-like particles (NLPs) were done using CP extracted from purified virus treated with the acetic acid degradation method [2–7]. We have recently shown that PapMV CP can be expressed to high level in *Escherichia coli* and form NLPs that are similar to the WT virus [8]. Twenty percent of the protein was found as NLPs while the remaining was found as a multimer of 450 kDa (20 subunits) that forms a disk [8]. The disks were shown to be the basic building block of the NLPs in vitro. The assembly process occurs probably by a similar mechanism in planta.

Alignment of the amino acid sequences of filamentous viruses showed two distinct groups: the flexuous group made of the potex-, carla-, clostero-, poty-, and bymoviruses and

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[1] *Abbreviations used:* PapMV, papaya mosaic virus; CP, coat protein; NLPs, nucleocapsid-like particles; PVX, potexvirus X; NMV, narcissus mosaic potexvirus; TMV, tobacco mosaic virus; EM, electron microscopy; DSS, 2,2-dimethyl-2-silapentane-5-sulfonic acid; EMSA, electrophoresis mobility shift assay; PVBV, pepper vein banding virus.

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the group of rigid rod made of the tobamo-, tobra-, hordei-, and furoviruses [9]. A comparison between the Raman spectra of two flexuous rods (potato potexvirus X (PVX) and narcissus mosaic potexvirus (NMV)) and a rigid rod (tobacco mosaic virus (TMV; tobamovirus)) revealed that proteins of both types are rich in α-helices but that potexviruses have longer loops between the helices [10]. Potexviruses coat proteins are also more hydrated which probably contribute to their flexibility [10]. A similar study on PVX also revealed that the N- and C-terminal ends of the coat protein are located close to the surface of the virus [11,12]. An X-ray diffraction study of PVX showed 8.75 CP per turn of helix of the virus particle on which 4–5 nucleotides are associated [13]. Finally, the crystallization and preliminary X-ray analysis of PapMV did not lead to the resolution of the structure of the virus due to the heterogeneity of the sample used for crystal growth and the small size of the crystals [14].

PapMV CP exists in *E. coli* as disks and NLPs, two multimeric states that are not amenable to NMR structure determination. The three-dimensional structure determination approach by NMR typically requires a total molecular weight of less than 60 kDa and concentrated samples. It is therefore necessary to produce an uniform and low molecular weight sample. In this report, we present a monomeric form of the PapMV CP, $CP_{27–215}$ that has a similar secondary structure than the wild-type protein. Preliminary biochemical and NMR data confirmed that the protein is amenable under this form to an extensive 3D NMR structural study.

Materials and methods

Cloning and expression of the recombinant proteins

The PapMV CP gene $CP_{6–215}$ was described elsewhere [8]. The truncated version of PapMV CP: $CP_{27–215}$, $CP_{46–215}$, and $CP_{60–215}$ were amplified by PCR from the clone $CP_{6–215}$. The following forward primers were used: $CP_{27–215}$, 5′-agt cccatggatccaacgtccaatcttctg-3′, $CP_{46–215}$ 5′-agctccatggtagct gctaaggttccagcagcc-3′, and $CP_{60–215}$, 5′-agctccatggcattggag ttggttaacttctgc-3′. The same reverse oligonucleotide primer 3′-gaaggtgggggcttgtggtagtggtagtggtaatcattcctaggcgta-5′, was used for all the truncated versions of PapMV CP. The PCR products were digested with *Nco*I and *Bam*HI and inserted into the vector pET-3d to generate the truncated clones $CP_{27–215}$, $CP_{46–215}$, and $CP_{60–215}$.

Media and growth conditions for synthesis of PapMV $CP_{27–215}$ in E. coli

Two different growth media were used: a rich medium that permits large production of proteins for the biochemical characterization of the protein and one minimal medium for ^{15}N labeling. To overexpress in the rich media 2× YT (1.6% peptone, 1% yeast extract, and 0.5% NaCl), a 25 ml overnight preculture of *E. coli* strain BL21 (DE3) RIL (Invitrogen) transformed with the pET-3d plasmid was prepared from a single colony. A 5 ml of this preculture was inoculated to 500 ml of the same media containing 50 μg/ml ampicillin. The protein expression was induced at $OD_{600} = 0.6 \pm 0.1$ overnight at 22 °C, 250 rpm, using 1 mM isopropyl-β-D-galactopyranoside IPTG (Promega) and 25 μg/ml ampicillin.

For labeling with ^{15}N, 5 ml of $OD_{600} = 1.0 \pm 0.1$ preculture in 2× YT^{amp} medium was inoculated to 500 ml of the modified M9 minimal medium [2.5 mM Betaine, 0.2 M ($Na_2HPO_4 \cdot 7\ H_2O$), 0.1 M KH_2PO_4, 0.09 M $^{15}NH_4Cl$ (Cambridge Isotope Labs), 0.04 M NaCl, 3.5 g/L glucose, 2 mM $MgSO_4$, 2 mM $FeCl_3$, 0.1 mM $CaCl_2$, 50 mM $ZnSO_4$, and 0.5% w/v thiamine] containing 50 μg/ml ampicillin. The protein expression was induced at $OD_{600} = 0.7 \pm 0.1$ overnight at 22 °C, 250 rpm, using 1 mM IPTG and 25 μg/ml ampicillin.

Purification of PapMV $CP_{27–215}$

(a) Ni–NTA–agarose column

The whole purification procedure was performed at 4 °C. The *E. coli* expression strain BL21 (DE3) RIL (Stratagene) was transformed with the plasmid pET-3d containing either of the different constructs pET3d-$CP_{6–215}$, pET3d-$CP_{27–215}$, pET3d-$CP_{46–215}$, and pET3d-$CP_{60–215}$. The bacteria were cultured as described in the previous section. The pellet was resuspended in ice-cold lysis buffer (50 mM Na H_2PO_4 [pH 8.0], 300 mM NaCl, 10 mM imidazole, 40 μM PMSF, and 0.1 mg/ml lysozyme) and bacteria were lysed by sonication. The lysate was centrifuged twice for 30 min at 13,000 rpm to eliminate cellular debris. The supernatant was mixed, for 4–16 h, with 1 ml of Ni–NTA–agarose matrix (Qiagen D40724). The purification was made by gravity-flow on a polypropylene chromatography column (Econo-Pac Columns, Bio-Rad Laboratories). The resin was washed with 10 bed volumes once with a first wash buffer (lysis buffer supplemented with 20 mM imidazole), once with the second wash buffer (lysis buffer supplemented with 50 mM imidazole) and once with the third wash buffer (10 mM Tris–HCl (pH 8.0) and 50 mM imidazole). The protein was incubated 30 min minimum with 2.5 bed volumes of the elution buffer (third wash buffer supplemented with 1 M imidazole) before elution. The concentration of the sample or the changes of buffers was done a stirred ultrafiltration cell (Amicon, Millipore) of 3 ml with a YM10 membrane (Amicon, Millipore). $CP_{27–215}$ was stored in presence of 10 mM DTT to improve protein stability and render its concentration to 0.2 mM possible.

(b) Cation exchange and gel filtration

The whole purification procedure was made at 4 °C. After overnight induction, cells were then harvested and resuspended in the cation exchange start buffer (25 mM MES, pH 6.2) containing 20 μM PMSF, 10 mM DTT, and 1 mg/ml lysozyme (Sigma). Cells were then ruptured by at least three passages through a French press and centrifuged twice at 14,000 rmp for 30 min at 4 °C. The supernatant was filtrated