Virus Research 157 (2011) 35–46

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Engineered *Tobacco mosaic virus* mutants with distinct physical characteristics *in planta* and enhanced metallization properties

Anan Kadri[a,*], Edgar Maiß[b], Nadja Amsharov[c], Alexander M. Bittner[c,1], Sinan Balci[c], Klaus Kern[c], Holger Jeske[a], Christina Wege[a,**]

[a] Universität Stuttgart, Institute of Biology, Department of Plant Molecular Biology and Plant Virology, Pfaffenwaldring 57, D-70550 Stuttgart, Germany
[b] Leibniz Universität Hannover, Institut für Pflanzenkrankheiten und Pflanzenschutz, Herrenhäuser Straße 2, D-30419 Hannover, Germany
[c] Max Planck Institute for Solid State Research, Heisenbergstraße 1, D-70569 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 22 July 2010
Received in revised form 25 January 2011
Accepted 29 January 2011
Available online 15 February 2011

The publication is dedicated to Karl-Wolfgang Mundry, founder of the Plant Virology Department at the University of Stuttgart, who deceased in 2009.

Keywords:
Tobacco mosaic virus (TMV)
Coat protein
Mutation
Nanotechnology
Metallization

ABSTRACT

Tobacco mosaic virus mutants were engineered to alter either the stability or surface chemistry of the virion: within the coat protein, glutamic acid was exchanged for glutamine in a buried portion to enhance the inter-subunit binding stability (E50Q), or a hexahistidine tract was fused to the surface-exposed carboxy terminus of the coat protein (6xHis). Both mutant viruses were expected to possess specific metal ion affinities. They accumulated to high titers in plants, induced distinct phenotypes, and their physical properties during purification differed from each other and from wild type (wt) virus. Whereas 6xHis and wt virions contained RNA, the majority of E50Q protein assembled essentially without RNA into rods which frequently exceeded 2 μm in length. Electroless deposition of nickel metallized the outer surface of 6xHis virions, but the central channel of E50Q rods, with significantly more nanowires of increased length in comparison to those formed in wtTMV.

1. Introduction

Tobacco mosaic virus (TMV) is the type member of the genus *Tobamovirus*. Its virion encapsidates a helical plus-sense single-stranded RNA (+ssRNA) genome of 6395 nucleotides in 2130 identical coat protein subunits of 17.5 kDa each, and forms a rigid helical tube with a length of 300 nm, a diameter of 18 nm, and a central channel 4 nm in width. As this structure exposes several distinct and repeated functional groups on the inner and outer surfaces (Clare and Orlova, 2010; Klug, 1999; Namba et al., 1989; Sachse et al., 2007) it is suitable for differential modifications such as metallization and has therefore been the subject of intensive studies in the field of nanotechnology as a versatile biotemplate, yielding an ever increasing number of close-to-application materials and devices (e.g. Balci et al., 2007, 2008, 2009; Dujardin et al., 2003; Gerasopoulos et al., 2010; Górzny et al., 2010; Knez et al., 2002, 2003, 2004a,b, 2006; Kobayashi et al., 2010; Lee et al., 2006; Lim et al., 2010; Miller et al., 2007; Rong et al., 2009; Royston et al., 2008, 2009; Schlick et al., 2005; Shenton et al., 1999; Smith et al., 2006; Tseng et al., 2006; Wu et al., 2010a; Yang et al., 2010; Yi et al., 2005).

TMV exists in a great number of different strains and mutants and accumulates to high levels in many host plants with variable symptom responses (reviewed in Culver, 2002; Zaitlin and Israel, 1975). Dependent on both the virus strain and the host genome, it may either spread systemically, or may be restricted to a local infection site, and induce yellowing, mosaic, severe systemic necrosis, or local necrotic lesions (Mundry et al., 1990; Zaitlin and Israel, 1975). Wild type (wt) virions are exceptionally stable under a broad range of chemical and physical conditions (Mutombo et al., 1992; Perham and Wilson, 1978; Zaitlin, 2000) and easily isolated from plants, but changes in the coat protein sequence may disrupt particle stability and impede purification.

* Corresponding author. Tel.: +49 711 685 65072; fax: +49 711 685 65096.
** Corresponding author. Tel.: +49 711 685 65073; fax: +49 711 685 65096.
E-mail addresses: anan.kadri@bio.uni-stuttgart.de (A. Kadri), christina.wege@bio.uni-stuttgart.de (C. Wege).
[1] Present address: CIC nanoGUNE Consolider, Tolosa Hiribidea 76, E-20018 Donostia - San Sebastian, and IKERBASQUE, Basque Foundation for Science, E-48011 Bilbao, Spain.

0168-1702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2011.01.014

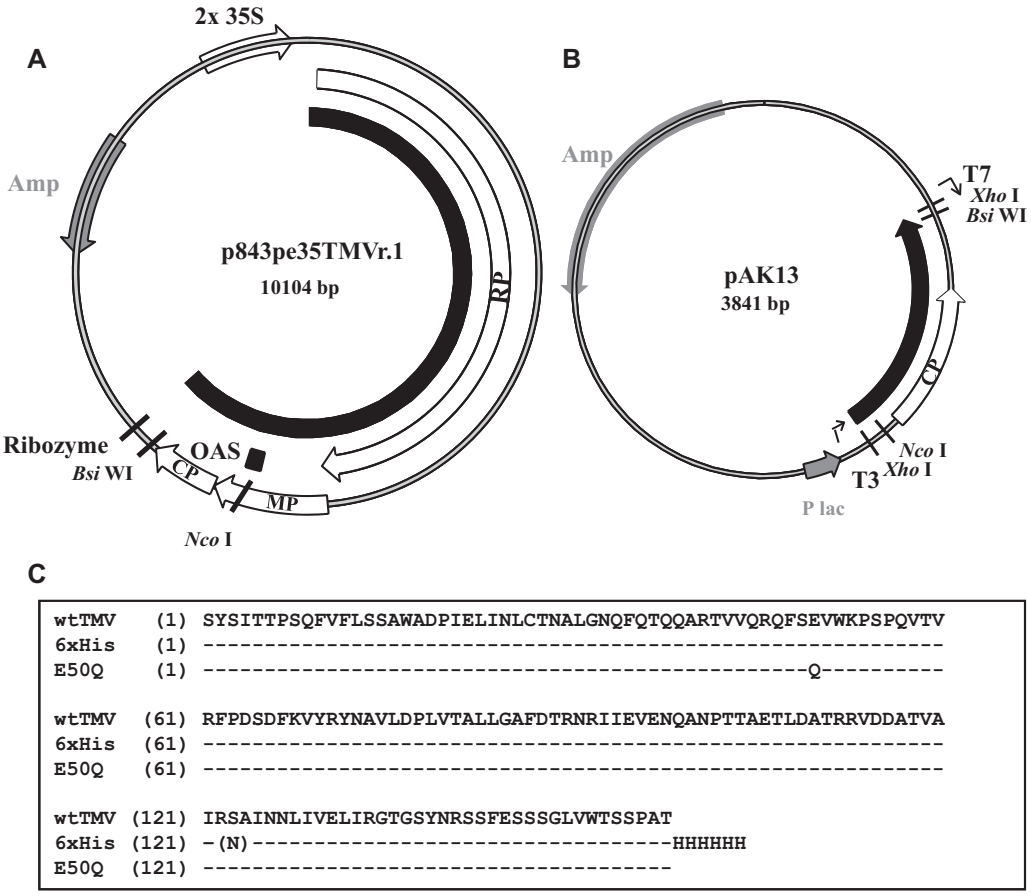

Fig. 1. Constructs and sequences used. Genetic maps at different scales for the infectious wtTMV plasmid construct p843pe35TMVr.1 (A) or the mutagenesis sub-clone pAK13 (B), and alignment of the TMV CP amino acid sequences (C). Genetic elements and restriction sites used in cloning strategies, mutagenesis and to generate TMV CP-specific probes are indicated: RP, RNA-dependent RNA polymerase; MP, movement protein; CP, coat protein; OAS, origin of assembly. The resulting transcripts for the full-length TMV genome and the TMV fragment (nts 5433-6306; NC 001367) are indicated by black arcs. Alignment of the CP amino acid sequences (C) deduced from the nucleotide sequences of the indicated constructs.

The virion structure (Clare and Orlova, 2010; Namba et al., 1989; Sachse et al., 2007) provides some explanation for its stability. Carboxyl–carboxylate interactions tighten adjacent coat protein subunits, and carboxylate–phosphate pairs bind protein subunits to RNA (reviewed in Culver, 2002). Upon infection, virions face physiological conditions with lower calcium concentrations and higher pH values than in the extracellular environment, and protons as well as calcium ions are removed from the carboxyl–carboxylate and carboxylate–phosphate pairs. The resulting repulsive negative electrostatic charges destabilize the subunits' interactions (Caspar, 1963; Caspar and Namba, 1990), and permit disassembly necessary for translation and replication. Hence, one of the so-called "Caspar-carboxylate" groups (glutamic acid at position 50) was changed to glutamine in this study, in order to stabilize the rod structure and prevent its disassembly as described (Bendahmane et al., 2007; Culver et al., 1995; Lu et al., 1996), to improve the robustness of the biotemplate for metallization purposes.

In addition, amino acids may be replaced or inserted at the C-termini, the N-termini, or within the exposed loop in order to functionalize the outer surface of the virion specifically (Lee et al., 2005, 2006; Smith et al., 2006; Yi et al., 2005). Thus, in order to exploit the well-known binding affinity of histidine to nickel, cobalt or zinc, a hexahistidine oligopeptide was fused to the C-terminus of the coat protein. The stabilities and metal-binding properties of both mutants compared to those of wtTMV virions are discussed.

2. Materials and methods

2.1. Construction of infectious clones for TMV coat protein mutants

cDNA was generated from the RNA of wt full-length TMV vulgare strain (PV0107; DSMZ, Germany), inserted into the bacterial plasmid pT7T3 19U (Pharmacia) between a duplicated *Cauliflower mosaic virus* 35S promoter (Restrepo et al., 1990) and a hammerhead ribozyme (Shintaku et al., 1996) using standard cloning procedures (Sambrook and Russell, 2001), resulting in the plant-infectious plasmid p843pe35TMVr.1 (Fig. 1A).

To generate mutants, a sub-clone (pAK13, Fig. 1B) comprising nucleotides 5433 to 6306 of TMV cDNA was raised from p843pe35TMVr.1 using pBluescript-SKII + (Stratagene, Heidelberg, Germany) as vector: the corresponding 874 bp cDNA fragment was amplified by PCR [95 °C, 5 min; 25 cycles 95 °C, 30 s, 72 °C, 30 s, 72 °C, 1 min; 72 °C, 10 min] using Taq DNA polymerase (Qiagen, Hilden, Germany), forward primer #1 and reverse primer #2, each adding an *Xho* I restriction site (Table 1), and inserted into the same restriction site of the vector following standard protocols (Sambrook and Russell, 2001). Mutants were raised by PCR amplification of the entire sub-clone pAK13 in the presence of mutant primers and blunt-end re-ligation, for pAK-6xHis using Taq DNA polymerase (Qiagen) with primers #3 and #4 [95 °C, 5 min; 25 cycles 95 °C, 30 s, 72 °C, 30 s, 72 °C, 3 min; 72 °C, 7 min], or for pAK-E50Q using proofreading DNA polymerase (Qiagen) and primers #5