Studies on the disassembly of cowpea chlorotic mottle virus
by Na Li

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Plant Sciences
Montana State University

Abstract:
The mechanism of the spherical virus disassembly has been under investigation for understanding the early events during virus infection, and eventually for helping to design reagents to block the process for releasing genetic information into host cells, thus preventing viral infection. The virus has to be stable enough to protect the genetic materials inside the virion, yet dynamic enough to release the nucleic acids to establish infection. Cowpea chlorotic mottle virus provides genetic and biochemical advantages for this purpose. The role of virus swelling for disassembly was studied based on characterization of a salt stable mutant of CCMV in vivo and in vitro. The salt stable mutant is as infectious as the wild type, swells like the wild type but shows negative signal for translation, thus swelling is not required for CCMV disassembly. The N-terminus of the coat protein on the virion is not ordered under X-ray crystallography, it is proposed to be involved in the virus disassembly. Biochemical and immunological analysis of the N-terminus shows that the N-terminus is dynamic, undergoes structural transition to the exterior of the virion presumably to form a channel on the five-fold axis during disassembly. This work will lead more biochemical studies in detail on CCMV disassembly process.

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APPROVAL

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Na Li

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistence, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The mechanism of the spherical virus disassembly has been under investigation for understanding the early events during virus infection, and eventually for helping to design reagents to block the process for releasing genetic information into host cells, thus preventing viral infection. The virus has to be stable enough to protect the genetic materials inside the virion, yet dynamic enough to release the nucleic acids to establish infection. Cowpea chlorotic mottle virus provides genetic and biochemical advantages for this purpose. The role of virus swelling for disassembly was studied based on characterization of a salt stable mutant of CCMV *in vivo* and *in vitro*. The salt stable mutant is as infectious as the wild type, swells like the wild type but shows negative signal for translation, thus swelling is not required for CCMV disassembly. The N-terminus of the coat protein on the virion is not ordered under X-ray crystallography, it is proposed to be involved in the virus disassembly. Biochemical and immunological analysis of the N-terminus shows that the N-terminus is dynamic, undergoes structural transition to the exterior of the virion presumably to form a channel on the five-fold axis during disassembly. This work will lead more biochemical studies in detail on CCMV disassembly process.

CHAPTER 1

INTRODUCTION

During the infection cycle of a virus, the virus has to be stable enough to protect the genetic information located inside of the virion, yet dynamic enough to disassemble in order to deliver the viral RNA inside the cell to get access to the host translational machinery. Understanding the mechanisms of viral disassembly would help to discover the chemical basis for genetic material release, and eventually help to design reagents to block the process for releasing genetic information into host cells, thus preventing viral infection.

Cowpea chlorotic mottle virus, CCMV, is a small spherical virus with T=3 icosahedral quasi-symmetry. CCMV belongs to the Bromoviridae family (alpha-virus like superfamily) of the plant viruses, along with broad bean mottle virus (BBMV) and brome mosaic virus (BMV). CCMV and BMV have been model systems for studying the spherical virus assembly and disassembly mechanisms (Bancroft *et al.*, 1974, 1975, 1976; Fox *et al.*, 1996; Zhao *et al.*, 1994; Albert *et al.*, 1997). CCMV was the first spherical virus that had been shown to be reassembled *in vitro* from its protein and RNA components (Bancroft *et al.*, 1967). In laboratory studies, *E.coli* and yeast systems for heterologous expression of CCMV particles have been developed (Zhao *et al.*, 1994; Young laboratory, unpublished data). CCMV infects members of the leguminosae family, such as cowpea. CCMV can accumulate to high levels in host plants; normally from one kilogram of infected plant tissue yield one gram virus. The CCMV virus is stable under a

wide range of salt and pH conditions. Infectious clones are available for *in vitro* studies (Ahlquist *et al.*, 1984; Allison, *et al.*, 1988). This provides useful tools to examine CCMV mutants *in vivo*, such as assembled empty virus particles and engineered CCMV coat proteins. Altogether, the availability of genetic and biochemical studies have made CCMV a model system for studying virus structure, assembly and disassembly.

Genome Organization

There are four single stranded positive sense RNAs in the CCMV genome (Fig. 1). RNA 1 and RNA 2 encode for RNA-dependent-RNA replicases, which along with certain host factors, perform viral RNA replication and transcription. RNA 3 is di-cistronic, which encodes a 32 kD movement protein and a 20 kD coat protein. The coat protein is expressed from RNA 4 via a sub-genomic promoter (sgp) on RNA 3. RNA 1 and 2 are encapsidated into separate particles, RNA 3 and RNA 4 are copackaged together into a third particle, with 1:1 molar ratio of RNA 1 and 2 particles (Loesch *et al.*, 1980). All three particles are required to establish successful infection in host cells.

CCMV Structure

The structure of the wild type CCMV has been solved to 3.2 Å resolution (Fig. 2; Speir *et al.*, 1995). The structure of a salt stable mutant of CCMV has been also solved to 2.7 Å resolution (Dr. Qu, the Scripps Research Institute, unpublished data). The high resolution of the salt stable structure provides detailed information on the chemical basis for the salt stable phenotype. The CCMV virus particle is composed of 180 identical coat

protein subunits, arranged on a T=3 icosahedral lattice. There are 20 hexameric and 12 pentameric capsomers in the virion. X-ray crystallography structure of the CCMV coat protein has revealed a striking feature. It has an eight-stranded, anti-parallel ß-barrel core, with C-terminus and N-terminus arms that extend in different directions from the core structure (Fig. 3). The N-terminus of the coat protein is not ordered in the X-ray

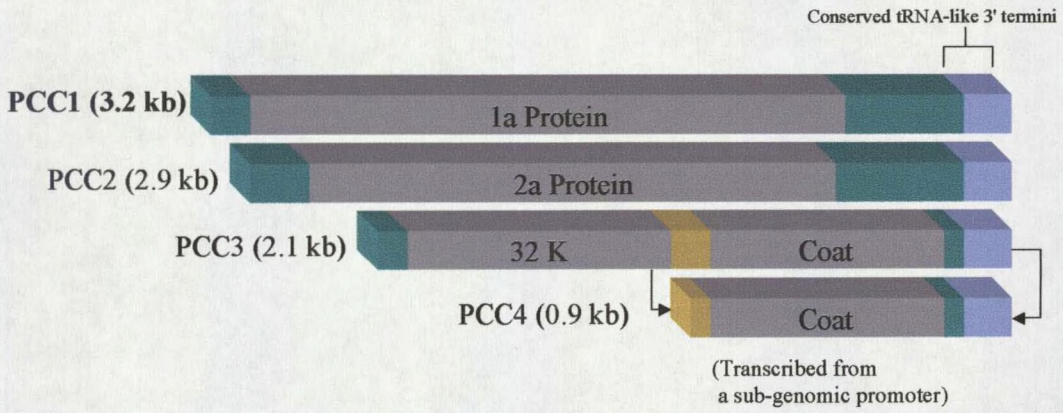

Fig. 1. Genome organization of CCMV (adapted from Arnold, M.S thesis).

The 5' and 3' end untranslated regions are shown in green, and the 3' t-RNA like region is shown in blue. Yellow shows the subgenomic promoter (sgp) on RNA3 and RNA4.