



Fig. 1. Tobacco mosaic virus structure and genome organization. The positions of ORFs in the genomic RNA are indicated by boxes. The sizes of the proteins in kDa produced from these ORFs are indicated in brackets. The 17.5-kDa CP is encoded by ORF 4. Additional features of the genomic and subgenomic RNAs are indicated above and below.

the virus (7). The protein has been shown to increase the plasmodesmatal size exclusion limit (8) and to have nucleic acid-binding activity (9). The fourth ORF encodes the coat protein (CP), which is required for virion formation and systemic movement of the virus (10). The CP is produced from a second 3' coterminal subgenomic RNA that is capped at its 5' end, and starts nine nucleotides upstream of the initiating methionine of the CP. The origin of assembly site (OAS) (11), from which particle formation is initiated, is located within the third ORF. Some other tobamoviruses have an OAS in the CP gene. Hence, depending on the tobamovirus, one or both subgenomic RNAs can be encapsidated. The TMV CP gene is followed by an untranslated region that contains several pseudoknot sequences, which are involved in translational enhancement (12). At the 3' end of the genomic RNA is a tRNA-like structure that can be aminoacylated.

TMV was the first virus to be purified, in 1935 (13), and since then many methods have been developed for its purification. Tobamoviruses have proven easy to purify because of the high accumulation of viral particles in many host plants, and because the particles are stable under a wide range of chemical and physical conditions. The purification method described in **Subheading 3.1** has been chosen because of its simplicity, and because it does not involve ultracentrifugation. The method presented is based on that described by Gooding and Hebert (14). Purification is dependent on the process of virion precipitation in the presence of the hydrophilic polymer polyethylene glycol (PEG), described by Leberman (15).