

Comovirus Isolation and RNA Extraction

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1. Introduction

Comoviruses are small, icosahedral viruses with a diameter of approx 28 nm (**Fig. 1**). Presently the genus *Comovirus* is comprised of 15 different viruses, of which the type species, cowpea mosaic virus (CPMV), is the most thoroughly studied (*1,2*). All viruses are transmitted by beetles and have a rather narrow host range. Most comoviruses have legumes as their natural hosts and usually cause mosaic or mottling symptoms. Comoviruses are mechanically transmissible, and can replicate to high levels in infected cells. Purified preparations of comoviruses consist of two, sometimes three, distinguishable particles, which can be separated by centrifugation on sucrose density gradients. These particles are designated as bottom (B), middle (M), and top (T) component, corresponding to their position in the centrifuge tube. The B- and M-component are nucleoprotein particles, each containing a segment of the single-stranded, bipartite RNA genome (denoted RNA-1 and RNA-2, respectively); T-component consists of empty protein shells. Both B- and M-components, or their RNAs, are necessary for infectivity (*3*).

The protein capsids of B-, M-, and T-component are identical, consisting of 60 copies each of a large (L) and small (S) coat protein; the observed differences in sedimentation coefficient and density are exclusively caused by differences in RNA content. Top components do not seem to have a specific function in virus infectivity and may be regarded as a side product of the viral assembly process. The amount of T-component produced varies greatly among different comoviruses and even among different isolates of the same virus, and seems to be dependent on the condition for growth.

Molecular weights of RNA-1 and RNA-2 reported for different comoviruses are all in the range of $2.0\text{--}2.4 \times 10^6$ and $1.2\text{--}1.45 \times 10^6$, respectively. The