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Short Communication

Thermal transition of native tobacco mosaic virus and RNA-free viral proteins into spherical nanoparticles

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Spherical nanoparticles (SNPs) were generated by two-step thermal remodelling of native tobacco mosaic virus (TMV) at 94 °C. Particles of irregular shape and varying size were generated by TMV at 90 °C. They could be converted into SNPs by heating at 94 °C and were considered to be intermediate precursors of SNPs. In addition to SNP monomers (53 nm diameter), generated by individual TMV virions, large SNPs (100–800 nm diameter) were assembled. The size of the SNPs depended on the TMV concentration. The SNPs could be generated by distinct forms of RNA-free TMV coat protein (CP) aggregates and individual CP subunits. A one-step SNP assembly appeared to occur in these cases. These results show that SNPs represent a new type of particle nanoplatform for producing compositions of SNPs with foreign protein molecules bound to their surface.

The rod-like particles of tobacco mosaic virus (TMV) of 18 nm diameter and 300 nm modal length consist of 2130 identical 17.5 kDa protein subunits arranged helically into a rigid tube. The viral RNA is intercalated between the protein turns (Zaitlin & Israel, 1975; Butler, 1999; Klug, 1999). TMV can be disassembled into protein subunits with subsequent reassembly (reconstitution) of viral particles *in vitro* from the nucleic acid and coat protein (CP) (Butler & Klug, 1978; Fraenkel-Conrat & Singer, 1999; Klug, 1999). In the absence of nucleic acid, the viral CP may be assembled into several types of aggregate. It has been established that polymerization of TMV CP is an endothermic, concentration-dependent and reversible process. TMV protein polymerizes when the concentration and/or temperature is increased and depolymerizes when they are decreased (Lauffer & Stevens, 1968). At a pH of approximately 6.5, TMV CP can be repolymerized into virus-like particles that are structurally similar to native virions (Anderer, 1963; Caspar, 1963; Butler & Klug, 1978; Namba *et al.*, 1989; Butler, 1999). At a pH near 8.0 and at low ionic strength, a mixture of monomers and two-layer trimers, called A-protein, is formed (Schramm & Zillig, 1955; Lauffer & Stevens, 1968; Butler & Klug, 1978; Butler, 1999). The predominant aggregate at neutral pH and low ionic strength is a 20S two-layer polar disc made of 34 subunits (Díaz-Avalos & Caspar, 1998).

Details of Methods and supplementary figures are available with the online version of this paper.

Lauffer & Price (1940) found that heat inactivation of TMV is closely associated with CP denaturation. Hart (1956) used electron microscopy to analyse the morphological changes induced in TMV by heating and reported that heating in the range 80–98 °C for 10 s resulted in a swelling of TMV particles at one or both ends. Eventually, the rods were converted into 'ball-like particles' with the approximate volume of the original rod.

Here, we found that the size of the spherical nanoparticles (SNPs) generated by heating TMV did not necessarily correlate with that of the original rod, but varied in a wide range from approximately 50 to 800 nm. The SNPs were not only generated by the native TMV rods, but were also readily produced by different forms of RNA-free TMV CP. The evidence implied that, upon thermal denaturation, the CP subunits acquired a specific conformation favourable for assembly into SNPs of widely varying size. An additional point to emphasize is that SNPs represent a new type of particle nanoplatform capable of producing compositions of SNPs with foreign protein molecules bound to their surface.

Transmission electron microscopy (TEM) examination of the swellings revealed that the heated TMV particles underwent at least two structural transitions in the course of SNP formation. In the first stage, heating of TMV up to 90 °C was manifested as a swelling at one or both ends of the rod. Significantly, the majority of particles produced were not spherical, but represented irregular particles (IPs)