In vitro assembly of bacteriophages: folding, kinetic control and intermediates

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Bacteriophage P22 serves as a model for the assembly and maturation of other icosahedral double-stranded DNA viruses. P22 coat and scaffolding proteins assemble into a procapsid which subsequently expands during DNA packaging (maturation). Assembly and maturation is accompanied by coat protein folding [1]. In the presented experiments *in vitro* assembly was initiated by a rapid mixing of monomeric coat protein with different engineered scaffolding proteins. Assembly was followed by time-resolved small angle X-ray scattering. The scattering curves revealed that a slower nucleation phase was followed by a rapid assembly of closed shells when the wild type scaffolding protein was used. On the other hand, an engineered covalent dimer of the scaffolding protein accelerated the nucleation step and resulted in a rapid polymerization that yielded a polydisperse mixture of incomplete shells. Thus, the outcome of polymerization is determined by the ratio of the nucleation and elongation rates. The nucleation and elongation rates can be modulated by the oligomeric state of the scaffolding protein (e.g. dimerization equilibrium). These experiments indicated that P22 assembly is under kinetic control.

Double-stranded RNA viruses infecting bacterial hosts belong to the *Cystoviridae* family (bacteriophages $\phi 6$ – $\phi 14$) and share structural as well as functional similarities with dsRNA viruses infecting eukaryotic hosts (e.g. L-A, rotavirus, bluetongue virus, and reovirus) [2]. The first step on the assembly pathway is the procapsid formation, for which *in vitro* systems have been developed [3, 4]. The procapsid is composed of major structural protein P1 (120 copies), RNA-dependent RNA polymerase P2 (12 copies), 12 hexamers of the packaging ATPase P4, and 30 dimers of assembly factor P7 [2]. The *in vitro* assembly systems

have been instrumental in revealing assembly intermediates and conformational changes [3–5]. The two enzymes that are associated with the procapsid (P2 and P4) play essential role in nucleation and stabilization of early assembly intermediates [3,4]. The nucleation complex contains one or more P1 tetramers and is further stabilized by P4 hexamers. Interaction of P1 and P4 during assembly is accompanied by a folding event [5], a feature common with the P22 assembly. P4 protein also stabilizes on-pathway intermediates and limits off-pathway aggregation [4], a feature similar to the P22 scaffolding protein.

In summary, coat protein assembly in more complex bacteriophage proceeds via a nucleation step, which is controlled by other viral proteins. The polymerization reaction is coupled to capsid protein folding and cannot be considered as a simple sequence of reversible associations. The final outcome of assembly seems to depend on the intermediates rather than preference for icosahedral symmetry or stability of the final structure.

References

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