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Simple models and simple analyses of virus capsid assembly

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Assembly of even a simple virus is a complex reaction. Yet, in many cases, the capsids of isometric viruses assemble spontaneously and with high fidelity *in vitro*. *In vitro* reactions can be used as the basis for interpreting assembly *in vivo*, searching for assembly-directed small molecules, or subverting normal assembly to generate novel structures. A model is required to interpret experimental observation of any complex reaction. To this end, we developed a thermodynamic–kinetic (master equation) model, in which assembly is described in terms of a cascade of low order reactions. The resulting model can readily be adjusted to match the specific features of a biological system. Simulations replicate experimental observations of assembly and lead to experimentally testable predictions. Analyses based on a basic model, in which only a single path from monomer to capsid was posited, are equally applicable to sparse and complete models that include selected intermediates and every possible intermediate, respectively.

Keywords: Virus assembly; Hepatitis B virus; Cowpea chlorotic mottle virus; Protein polymerization

1. Introduction

Viruses are parsimonious with their resources and profligate with those of their host. Symmetry is a convenient strategy by which viruses encode a relatively small protein that self-assembles to enclose the relatively large volume required to package the virus' genome. Structures with icosahedral symmetry make the most efficient use of the genome and the host [1] as they can be divided into 60 identical asymmetric units, while the symmetry groups of all other Platonic solids permit only a smaller number of asymmetric units. In icosahedral viruses, an asymmetric unit (asu) may contain several individual proteins. Usually, an asu contains T proteins in quasi-equivalent environments, so that proteins are either associated with icosahedral five-fold vertices or with quasi-six-fold vertices [2]. The geometric description of a virus does not necessarily reflect the process of assembly. For instance, pseudo-$T = 3$ poliovirus assembles from 12 to 15 mers, each comprised of five heterotrimers [3]; papovaviruses assemble from 72 pentamers [4]; many $T = 3$ plant viruses, such as cowpea chlorotic mottle virus (CCMV), assemble from 90 dimers [5]; $T = 4$ hepatitis B virus (HBV) also assembles from dimers [6]. These fundamental units of assembly are generally referred to as protomers [7]. In considering the process of capsid assembly, we should consider the reaction in terms of protomers.

What is to be gained from understanding assembly? Assembly of new virions is the goal of most infections. Understanding the nature of the infection and the regulatory elements are central to describing viral natural history, developing antiviral strategies and making use of viruses to build novel structures. Studies of HBV capsid protein suggest that its assembly may be allosterically regulated [8]. The assembly process itself may be a viable antiviral target [9–12]. In order to best attack this reaction we must be able to describe assembly quantitatively, using physically realistic terms.

Capsid assembly has been linked to crystallization [2,13]. However, this analogy is only "skin deep". Like crystals, icosahedral capsids are a geometric lattice. In an ideal crystallization experiment, a single crystal will form from a single nucleus after a prolonged lag phase (see http://menesyadev.Scripps.edu). Mathematical descriptions of crystallization (and formation of 1-D crystal, filaments) are well established [14,15]. However, *the analogy to crystallization is not accurate; analyses based on this comparison will not yield reliable results* [16]. Unlike crystals, capsids do not extend indefinitely. A typical protein crystal may be comprised of 10^{14} unit cells; a typical capsid has a few hundred. A typical *in vitro* assembly reaction will yield 10^{11}–10^{14} capsids from a similar number of nuclei in a biologically reasonable time frame [17–22].

A number of approaches have been taken to model assembly reactions. Coarse-grained dynamic simulations

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have been calculated for assembly of small numbers of capsids [23–25]. Dynamical studies allow examination of regulation of quasi-equivalence and the effects of capsid closure on diffusion controlled rates. However, to interpret assembly of a large population, a more appropriate model may be built by considering a master equation: a system of equations to provide a thermodynamic and kinetic description of the reaction [16, 26–28]. Master equations can be used to calculate simulations that are immediately comparable to experimental observations of assembly [8,21,29].

2. Model calculations

A model of a reaction must be physically reasonable and must match the behaviour it is intended to model. Ideally a model leads to testable predictions. Consider the assembly of a simple capsid: a dodecahedron constructed from 12 pentamers. This is the geometry of a picornavirus [3]. In the artificial case where there is only one possible path from monomer to 12-mer, assembly can be described in terms of a cascade of 12 simple differential equations, one for each species [27]. These equations have the form:

$$\begin{aligned}\frac{\mathrm{d}[n-\mathrm{mer}]}{\mathrm{d}t} &= k_{\mathrm{forward}}[\mathrm{predecessor}][\mathrm{subunit}] \\ &\quad - k_{\mathrm{forward}}[n\text{-mer}][\mathrm{subunit}] \\ &\quad - k_{\mathrm{back}}[n\text{-mer}] + k_{\mathrm{back}}[\mathrm{successor}]. \end{aligned} \quad (1)$$

Here, [ ] denotes concentrations. The individual forward rates are based on a microscopic rate, modified by statistical factors. The backward rates are based on the number of interactions to be broken, their stability, and the microscopic rate (remember, $K_{\mathrm{A}} = k_{\mathrm{forward}}/k_{\mathrm{backward}}$).

The model is very simple, but the behaviour is not. Kinetics are sigmoidal as is the extent of assembly [27]. The lag phase in kinetics is *not* due to nucleation. The lag arises because there are many intermediates preceding the capsid—there must be a significant concentration of dimers before trimers can form, and so on for tetramers and the other succeeding species. The concentrations of each species rapidly approaches its equilibrium concentration, so that through most of the course of the reaction, intermediates approximate a Briggs-Haldane steady state. This steady state allows evaluation of fundamental constants describing the reaction: nucleus size and nucleation rate [16]. In contrast to crystallization (or filament formation), where the crystals are effectively infinite and the lag is a function of nucleation [14], capsids are finite and the lag is due to the build-up of an assembly line of intermediates.

The "lag" in the extent of assembly arises because assembly follows the law of mass action [27,30]. For a capsid of N subunits:

$$K_{\mathrm{capsid}} = \frac{[\mathrm{Capsid}]}{[\mathrm{subunit}]^N} \quad (2)$$

Thus, at low [subunit], there will be negligible capsid concentration. Above a threshold concentration, nearly all additional subunits will be assembled. We refer to this pseudo-critical concentration as $K_D$apparent [30]. K_D apparent is not a true critical concentration because subunits cannot freely transfer between free and bound phases, and because the free [subunit] is not constant for protein concentrations above $K_D$apparent [31].

The simple models used to develop kinetic analyses are based on a single path. However, there are 71 possible intermediates to be found in the assembly of a dodecahedron [28]. The number of possible intermediates increases exponentially with the number of subunits [32]. However, it turns out that the more sophisticated models have little effect on the conclusions reached with the simple ones. Equilibrium behaviour only depends on end states. The kinetic analyses for nucleus size and nucleation rate depend on the reaction achieving a steady state, which also remains unchanged [16,28].

3. Experimental models

HBV has been an extremely powerful system for studying assembly. HBV capsids, the protein shell of the virus' core, are formed from 120 dimers ($T = 4$) and to a lesser extent, 90 dimers ($T = 3$) where the C-terminus of the assembly domain is an important switch between forms [33]. Kinetic analysis indicates assembly is nucleated by a trimer of dimers [20]. The pairwise association energy between dimers is weak, -3 to -4 kcal/mol, and entropically driven [34]. These data indicated that assembly was dominated by hydrophobic forces [35]. More recently, we have accumulated data indicating that HBV capsid protein exists in assembly-active and -inactive states, based on mutants [36], small molecule inhibitors [10], and molecules that enhance assembly [8,12]. This determination has important implications for understanding the biology of assembly regulation and for developing novel approaches to antivirals [10,12].

CCMV was the first spherical virus assembled *in vitro* [17,37], demonstrating that all the information needed for assembly was included in the subunits. Understanding its assembly has proven to be more complicated than with HBV. Assembly of empty particles, driven by low pH, begins with the rapid accumulation of pentamers of dimers (PODs) [21] followed by slow addition of a dimer to an existing POD [38] as the nucleating step. Subsequent assembly involves rapid addition of dimers and PODs [38,39]. Assembly in the presence of RNA is similarly complicated. Given about 10 dimers per 3000 nucleotide RNA, the protein binds to RNA with low cooperativity and folds the RNA into a more compact complex; protein binds to this complex with high cooperativity [40]. This

suggests a mechanism for ensuring that only viral RNA is packaged that requires a few high-affinity interactions between RNA and protein.

4. Model predictions recapitulate experimental observation

Simulations are prone to kinetic traps, where assembly has so many starts that there is insufficient free subunit to complete the nascent capsids [20]. Trapped intermediates have been observed with HBV [8] and CCMV [38,41]. Kinetic traps can be avoided by incorporating a nucleation step [20]. But experimentally and in simulations, this regulation can be overcome by forcing the reaction with high protein concentration and extreme assembly conditions. The pattern of trapping is consistent with model predictions and can lead to formation of novel structure [38].

Most viruses must persist as stable structures in less than ideal environments. At first this would seem inconsistent with the thermodynamic–kinetic model, which predicts weak intersubunit interactions [27,30]. In simulations, even though the individual reactions comprising a given model are microscopically reversible, we found that dissociation required a catastrophic weakening of association energy [42]. It turns out that removing one subunit from a capsid creates a high-affinity site, which is filled rapidly compared to the loss of a second subunit. This hysteresis between association and dissociation has been observed in HBV [42] and other viruses [43].

5. Conclusions and future directions

As more virus assembly systems are investigated, a wealth of biological, chemical and physical constraints on assembly are being discovered (this volume describes some). Quantitative analysis of assembly reactions will enable development of more accurate models, which will enable discovery of the regulatory steps *in vivo*. Conversely, investigating alternative assembly models *in silico* (or on the chalk board) will give biologists a preview of the diversity of behaviours that they may expect.

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