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Distinguishing reversible and irreversible virus capsid assembly

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Abstract

Summary—Capsids of spherical viruses may be constructed from hundreds or thousands of copies of the major capsid protein(s). These assembly reactions are poorly understood. In this communication we consider the predicted behavior for assembly where the component reactions have weak association energy and are reversible and compare them to essentially irreversible reactions. The comparisons are based on mass action calculations and the behavior predicted from kinetic simulations where assembly is described as a cascade of low order reactions. Reversible reactions are characterized by a pseudo-critical concentration whereas irreversible reactions consumed all free subunits. Irreversible reactions are more susceptible to kinetic traps comprised of numerous small intermediates. In the case where only the ultimate step is irreversible, very low concentrations of intermediates slow the completion of the reaction so that overall it closely matches the predictions for the reversible reactions that make up the majority of the cascade. Data in the literature strongly support the hypothesis that most viruses are held together by many weak interactions.

Keywords

virus assembly; protein polymerization; kinetics; energy surface

The capsid of a spherical virus is a protein complex. It may have roles in intra- and extra-cellular trafficking, protecting and delivering the virus' nucleic acid, and even nucleic acid metabolism. Generally, but not always, spherical capsids are constructed with icosahedral symmetry, which requires that they be constructed from multiples of 60 subunits. Analytical descriptions of virus assembly run the gamut from coarse-grained simulations,^{1; 2} to defining preferred assembly paths,^{3; 4; 5; 6} to master equation/kinetic simulations of essentially infinite populations.^{7; 8; 9; 10} Relating such models to real viruses requires formulation of constraints based on experimental studies. Here we examine how to distinguish between reversible and irreversible assembly reactions, and how to estimate association energy where the reactions are reversible.

The case where all reactions are reversible is well established but necessary background.¹¹ Assuming the assembly reaction equilibrates, the law of mass action dictates a relationship between free capsid protein (Cp) and assembled Capsid comprised of N copies of Cp:

$$K_{capsid} = [\text{Capsid}] / [\text{Cp}]^N \quad (1)$$

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With the further assumptions that interactions between proteins in the capsid are approximately equivalent and that their association energies are additive,¹² the value of K_{capsid} can be decomposed with respect to the geometry of Cp and Capsid.^{7; 13} Specifically, it is a function of the number of contacts per Cp (c), the number of Cp per capsid (N), the average free energy of association per contact (ΔG°_c), and subunit degeneracy (d). A slightly more complex scenario is required when interactions are not equivalent¹⁴ and for heteropolymers.¹⁵

$$K_{\text{capsid}} = \frac{d^{N-1}}{N} e^{-(Nc/2)\Delta G^\circ_c / RT} \quad (2)$$

where $(Nc/2)$ is the number of intersubunit contacts holding the capsid together and (d^{N-1}/N) is reaction degeneracy. RT is the universal gas constant multiplied by temperature. For the system investigated in this communication, an icosahedron assembled from 30 tetravalent “dimers” (strictly speaking, a rhombic triacontahedron, described in reference 6), the expression for K_{capsid} is:

$$K_{\text{capsid}} = \frac{2^{29}}{30} e^{-60\Delta G^\circ_c / RT} \quad (3)$$

The extent of assembly (i.e. the amount of Capsid formed and Cp remaining) can be directly calculated from equations 1 and 3. However, kinetic simulations can identify kinetic traps or barriers that may be encountered. In the simplest simulations, assembly is assumed to follow a single path of highly stable intermediates by addition of one subunit at a time.¹¹ For these simulations, a single microscopic forward rate is used for all reactions, modified by the degeneracy of that reaction. To minimize trapping, a kinetic governor is applied by creating a slow nucleation rate. Since the association constant for a specified reaction is the ratio of forward and backward rates, the backward rate is simply the quotient of k_{forward} and K_{eq} for the specified intermediate.⁷ Simulations of large populations are calculated using a master equation form that accounts for association and dissociation reactions. The time dependent concentration of each intermediate M can be related to the concentrations of its predecessor (s), $M-1$, and its immediate scion(s), $M+1$. For the simplest case where assembly proceeds by the addition of one Cp at a time:

$$d[M] / dt = k_{\text{forward},M} [M-1][\text{Cp}] - k_{\text{backward},M} [M] - k_{\text{forward},M+1} [M][\text{Cp}] - k_{\text{backward},M+1} [M+1] \quad (4)$$

The reverse rate is calculated from $K_{\text{equilibrium}} = k_{\text{forward}}/k_{\text{reverse}}$, by considering the degeneracy of the reaction and the number of bonds to be broken.⁷ In these simulations, a nucleation rate for the earliest steps is defined by the coefficient λ , $\square\square\square\square\square$ is the ratio $k_{\text{forward}}/k_{\text{nucleation}}$. (This kinetic nucleation mechanism is distinct from classical nucleation where the nucleating interactions are much less stable subsequent interactions.¹⁶) In the pre-nuclear steps of the reaction, the forward rate in equation 4 is thus $k_{\text{nucleation}} = \lambda k_{\text{forward}}$.

Simulations (Fig. 1) show that with input values of $\Delta G^\circ_c = -3.4$ kcal/mol and $\lambda = 10^4$, the kinetic simulations achieve the equilibrium concentrations predicted by mass action. This simulation recapitulates similar equilibrated simulations^{6; 7} and experimental observations for hepatitis B virus,¹⁷ cowpea chlorotic mottle virus,¹⁴ and bacteriophage P22.^{18; 19} In this simulation, the ΔG°_c was a value for hepatitis B virus.¹⁷ Using calculated data, we also observe the logarithmic dependence of Cp on Capsid, implicit in equation 1 ($\log[\text{Capsid}] = \log(K_{\text{capsid}}) - \log[\text{Cp}]^N$). Experimentally, this plot is unlikely to be well-defined for large values of N . Given the typical experimental data of assembled capsid and remaining Cp, K_{capsid} can be calculated using equation 1; ΔG°_c can be extracted from K_{capsid} using equation 2 if capsid geometry is defined.

Given the preceding background, the question to be addressed in this paper is irreversibility. Practically speaking, irreversibility in association reactions occurs when the backward reaction is too slow to be relevant to observable kinetics. To a first approximation this can be simulated

by using a very strong ΔG° (Fig. 2). With a constant initial Cp concentration, at low values of ΔG° , there is little assembled capsid. At moderate values, the amount of Capsid increases proportionally with ΔG° . With very strong ΔG° , the yield of capsid plummets. This is due to trapping of metastable intermediates that rearrange very slowly on the time course of the reaction. At strong ΔG° , there are two distinct regimes of attenuated assembly: at $\Delta G^\circ = -18$ kcal/mol for each Cp-Cp interaction, dimers (which have only a single pairwise contact) are so stable that they persist throughout the 100,000 s simulation; at $\Delta G^\circ = -10$ kcal/mol dimers are labile enough that they are consumed for the sake of completing larger intermediates. Kinetic trapping can also be simulated with high initial Cp. For simulations with varying initial Cp, when a labile but biologically reasonable ΔG° of -3.4 kcal/mol¹⁷ is used, the trapping effect due to high Cp concentration is not evident at 10^5 seconds because trapped intermediates resolve in the course of the simulation; such reactions are notably slower (not shown).

In the trapped reactions shown in figure 2, intermediates accumulate because assembly starts at many loci and there are not enough free Cp molecules available to complete the capsids. This effect has been experimentally demonstrated with bacteriophage P22²⁰ and with hepatitis B virus²¹. This type of kinetic trap can be ameliorated by controlling nucleation of the reaction. At high values of $\lambda \geq 10^3$ (Fig. 3), reactions are robust over a broad range of concentrations; sensitivity to concentration is a diagnostic for lower values of λ ⁸. However, even at high λ , reactions get trapped at very high values of ΔG° . The traps due to high association energy that is strong enough to overcome any nucleation effect are characterized by the absence of free Cp and the accumulation of very small intermediates.

Very high ΔG° approximate the case where all component reactions are irreversible; another case of interest is where only the last reaction in the assembly cascade is irreversible. Biologically this is observed with post-assembly maturation steps, which are usually evident as chemical or morphological changes in bacteriophages, picornaviruses, papovaviruses, herpesviruses, and others. A slow irreversible step following assembly will obviously have no immediate effect on the assembly reaction. Here we investigate the effect of an irreversible step that occurs on the same time scale as assembly by incorporating a high-energy ultimate step in a simulation (Fig. 4).

In the case where the last step is irreversible, the amounts of free Cp and assembled Capsid are nearly the same as predicted by the ΔG° for the component reactions excepting the last step (Fig. 4). Under a range of energies, λ values, and initial [Cp], the amount of capsid was only slightly elevated with a compensating decrease in [Cp].

Intuitively, by Le Chatelier's principle, one would expect an irreversible step to pull the reaction forward, leaving no free reactant. In simulations this effect is limited because the model is based on a cascade of reactions in which a steady state of intermediates, naturally generated during the reaction, sustains production of capsids. The equilibrium concentrations of intermediates can be calculated directly from the concentration of free Cp.⁷ The extremely slow rate for the latter stages of this reaction is attributable to the extremely low concentrations of intermediates exacerbated by the irreversible last step. Such starved reactions would be very difficult to discern from equilibrating or kinetically trapped reactions. A slowly resolving trap will also show slow changes in the relative concentrations of Cp and capsids and traps (that may actually resemble capsids).¹⁴

The differences between a reaction that has an ultimate irreversible step and one that is at equilibrium are subtle. In the irreversible reaction, the concentration of free Cp does continue to fall over time, but so slowly that it may be difficult to discern it from experimental noise. Capsids are not dissociable in the irreversible reaction; however, dissociation will be inhibited in reversible reactions due to hysteresis.²² Experimentally, reversibility can be indicated by

exchange with free subunit.²³ The extent of assembly in the irreversible reaction does not show the correct concentration dependence (unlike the case shown in Fig. 1B), but when there are hundreds of subunits per capsid the slope may be experimentally inaccessible. On the other hand, it is difficult to rationalize an irreversible last step without chemical, spectroscopic, or structural evidence of conformational change; consider that in a large capsid, most subunits are completely surrounded by other subunits long before the capsid is complete.

In conclusion, simulations show that there are distinct differences between capsid assembly where the component reactions are stochastic and bidirectional on the one hand, and unidirectional reactions on the other. Under appropriate conditions, both types of reactions can yield intact particles.

Reactions where every step is irreversible are characterized by an absence of free subunits. In the simulations described here, a well-defined nucleation step eliminated most trapping. At high association energies, the most frequently trapped intermediates were small oligomers which, when allowed to participate in assembly, can lead to efficient capsid production.⁹ The absence of error correction in unidirectional assembly can lead to incorporation of defects²⁴ and heterogeneity in the assembly products.²⁵ Thus, completely irreversible reactions are easily distinguished from reversible reactions.

Reversible assembly reactions are necessarily characterized by relatively weak association energies. This results in an experimentally observable pseudo-critical concentration of unassembled subunits⁷. Defects in particles can be minimized due to thermodynamic editing. Even under extremes of assembly conditions, kinetic traps can resolve by dissociation of a fraction of oligomers (usually the smallest and weakest) so that their components can be consumed to complete others (usually the larger and more stable oligomers). The analysis described in this paper supports the weak interaction energies reported for hepatitis B virus,¹⁷ cowpea chlorotic mottle virus,^{14; 26} and bacteriophage P22,¹⁵ based on in vitro assembly studies. Free coat protein exchanges with bound in P22 procapsids, providing convincing evidence of reversibility.²³ It is interesting to note that using alternative assays, weak interaction energies have also been reported for retroviruses,^{27; 28; 29} alphaviruses,³⁰ and bacteriophage HK97³¹. Even though the local interactions are weak, the multivalent nature of the subunits, as well as hysteresis to dissociation,²² result in a persistent and globally stable structure. However, even reversible reactions can attain the appearance of irreversibility due to hysteresis.²²

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Figure 1.

(A) Equilibrium concentrations of Capsid and free capsid protein (Cp). Capsid and Cp concentrations calculated assuming equilibrium are shown as filled and open squares, respectively. Values from kinetic simulations are shown with solid (Capsid) and dashed (Cp) lines. For both calculations, ΔG°_c was -3.4 kcal/mol. For kinetic simulations, the nucleation rate was $10^4 \text{ M}^{-1}\text{s}^{-1}$ and the elongation rate was $10^8 \text{ M}^{-1}\text{s}^{-1}$ (i.e. $\lambda = 10^4$), and equilibrated for 100,000 s. (B) Logarithmic dependence of Capsid on Cp.

Figure 2.

Capsid assembly is inhibited at high association energy. Assembly simulations were carried out for 10M Cp with other constants as in Figure 1. Kinetic traps cannot be predicted from mass action calculations. The amount Cp present as free Cp (dashed line) and assembled capsid (solid line) are shown as a function of the pairwise contact energy.

Figure 3. Capsid assembly as a function of association energy and nucleation. A logarithmic scale is used for λ ($k_{\text{elcongaion}}/k_{\text{nucleation}}$); simulations were calculated for ratios of 1, 10, 100, and 1000. Pairwise association energy, ΔG° , varied from a weak, biologically reasonable value of -3 to a very strong value of -12 kcal/mol.

Figure 4.

Comparison of assembly at equilibrium (heavy dashed line) to assembly with an effectively irreversible last step (thin line). Kinetics and extent of assembly are nearly identical. Kinetic simulations were run as in figure 1 showing capsid and dimer concentrations. These traces nearly perfectly overlap kinetics where the final step is effectively irreversible (a -68 kcal barrier).