

Theoretical studies of viral capsid proteins

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Recent results in structural biology and increases in computer power have prompted initial theoretical studies on capsids of nonenveloped icosahedral viruses. The macromolecular assembly of 60 to 180 protein copies into a protein shell results in a structure of considerable size for molecular dynamics simulations. Nonetheless, progress has been made in examining these capsid assemblies from molecular dynamics calculations and kinetic models. The goals of these studies are to understand capsid function and structural properties, including quarternary structural stability, effects of antiviral compounds that bind the capsid and the self-assembly process. The insight that can be gained from the detailed information provided by simulations is demonstrated in studies of human rhinovirus; an entropic basis for the antiviral activity of hydrophobic compounds, predicted from calculated compressibility values, has been corroborated by experimental measurements on poliovirus.

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Abbreviations

BBV	black beetle virus
FMDV	foot-and-mouth disease virus
HRV	human rhinovirus
KL	kinetically limiting
SBMV	southern bean mosaic virus
VP	viral protein

Introduction

Nonenveloped icosahedral viruses include a variety of animal and plant pathogens. The structures of the protein capsids from several of these viruses have been defined at atomic resolution (see [1] for a recent overview) and biochemical and virological investigations have provided detailed information on many physical aspects of the viral life cycle, including cell entry and assembly [2,3]. These combined advances provide a biophysical foundation that makes the examination of these systems by theoretical and computational methods an attractive and valuable endeavor.

The capsid of nonenveloped spherical viruses is built from one or a few types of polypeptide chain by icosahedral symmetry. An integral number of 60 copies of the assembly subunit enclose the viral genome. Understanding the properties and function of the capsid shell is necessary for delineating the events associated with cell entry, release of the genetic material for viral replication and assembly of

the progeny virus particles. Furthermore, detailed knowledge of the viral capsid is valuable for structure-based design of antiviral compounds and for understanding the mutational adaptations that viruses have evolved to evade the immunological defenses of the host. Theoretical work on virus capsids, reviewed in this article, has focused on capsid stability and features of quarternary structure, antiviral compounds and the assembly process of the picornaviruses, black beetle virus (BBV), and southern bean mosaic virus (SBMV).

Capsid stability

Many viral capsid proteins exhibit polymorphic quarternary structure, whereby the same protein subunit is found in different structural environments. Such variation of protein–protein contacts is necessary, as genetic economy limits the virus to relatively few gene products. This variation raises questions related to the specific recognition of different contact interfaces and the accuracy of assembling multiple protein copies into the proper capsid shell.

A first step in understanding the assembly process is to analyze the energetics of the different protein–protein associations. The nonbonding interactions for the different viral contact interfaces were calculated using molecular mechanics and continuum electrostatics [4••]. Interfaces associated with icosahedral symmetry or quasi-symmetry rotation axes were compared for viral capsids comprising either one or three types of capsid protein. With a single protein gene product, quasi-equivalent contacts are distinguished from icosahedral ones by polymorphic contacts, whereas capsids with three types of protein achieve quasi-equivalent packing through chemically distinct proteins. The calculated dimer association energies allowed predictions of possible assembly pathways, described below. Furthermore, in the case of human rhinovirus 14 (HRV14), in which the capsid comprises three protein types, interaction energies corresponding to protein–protein interactions within a unique protomeric unit were found to be substantially stronger than those between two symmetry related protomeric units. This feature was also revealed by time-averaged interaction energies calculated from molecular dynamics simulations of a reduced system of HRV14 [5].

The pH dependence of capsid structural stability has been implicated in cell entry, endocytosis and release of RNA by picornaviruses. Foot-and-mouth disease virus (FMDV) and mengovirus capsids dissociate to pentamers of the unique protomer unit at low pH, which is thought to be the mechanism by which FMDV delivers its RNA into the cytosol of the infected cell. Rhinovirus and poliovirus are structurally altered by acidic pH in a fashion that resembles the conformational changes induced by receptor binding.

The pH dependence of the stability of picornavirus capsids [6,7•] has been examined using a dielectric continuum model [8]. The interfaces of the pentameric units of assembly were modeled from two unique protomers related by the twofold rotation of icosahedral symmetry. All titratable groups were considered in the study of FMDV [7•]. Poisson–Boltzmann methods were applied to this large system (approximately 17,000 atoms in the dimer) in order to estimate the pH dependence of dimer association. Interfacial histidine residues within 5 Å of the interface can account for the pH dependence of stability. The calculation reproduced the observed decrease in stability at low pH, but not the differences between two type A viruses.

The functional significance of pH sensitivity on the viral life cycle has motivated theoretical studies to understand the structural and chemical basis of these effects. Certain aspects have been elucidated, but many factors, such as differences among the genera of picornavirus or among ligated states, remain poorly understood. Understanding the relationship between antiviral mechanisms and pH stability is also of interest to assist the development of new drugs. The effect of drug binding on pH stability has been reproduced using continuum methods (DK Phelps, CB Post, unpublished data) and the results should provide some insight into the molecular details of this effect.

Antiviral effects

The picornaviruses HRV and polio have an internal ligand-binding pocket in viral protein 1 (VP1), near the icosahedral fivefold rotation axis. This pocket is deep inside the protein and located at one end of the β sandwich. The binding of long, hydrophobic antiviral compounds in this pocket inhibits the life cycle of the virus either by disrupting binding of the virus to the host cell receptor or by stabilizing the virus capsid, thereby preventing the release of RNA. The source of stabilization in the latter case was suggested to be the loss of conformational flexibility required for release. This possibility was examined by using molecular dynamics [9,10••] to estimate the thermodynamic compressibility from the fluctuations in local density at the drug-binding pocket. In contrast to expectation, the compressibility was found to increase when an antiviral compound is bound within the internal pocket of HRV14, compared with the unligated state of HRV. This counterintuitive result can be understood from the empirical observation that greater compressibility correlates with an increase in the entropy of the folded protein. It was argued that the binding of the antiviral compound increased the entropy of the HRV capsid and reduced the free energy driving force for uncoating, thus inhibiting disassembly. Time-averaged interaction energies calculated between the viral protein and the antiviral compound are less favorable than those between the protein and the water molecules in the pocket [5], consistent with an entropic basis for the observed binding stabilization of the viral capsid. The proposal of entropic stabilization as a mechanism for antiviral activity

that resulted from the simulation studies is now supported by recent experimental measurements. Hogle and co-workers [11] found that drug binding lowers the entropy of activation associated with the heat-induced conformational transition from a native particle to a particle form believed to mediate cell entry [12].

The increase in compressibility is thought to be associated with the system becoming more nonpolar in nature as a result of binding the antiviral compound. Such a proposal predicts a certain temperature dependence of the atomic positional fluctuations and this effect was tested and supported by results calculated from simulations equilibrated at different temperatures [10••]. In addition, drug binding to HRV14 was found, using stochastic-boundary molecular dynamics simulations, to affect large-scale collective properties, while leaving local atomic properties unperturbed [5]. Thus, long-range correlations in atomic motions were weakened by drug binding, while neither the fast-timescale atomic fluctuations nor the time development of the fluctuations show a consistent distinction between the drug-bound and drug-free virus.

Another mechanism for the activity of antiviral compounds has been proposed. Based on the assumption that RNA is released through the center of the VP1 pentamer, antiviral compounds are suggested to prevent the expansion of the opening required for exit of RNA [13]. An imaginary state to test the resistance of the opening to expansion was imposed in a computer ‘experiment’. The resistance was calculated using a string of van der Waals spheres that had increasing radii and were positioned in the opening. A correlation between the ‘stiffness’ of the opening and the antiviral efficacy was found for HRV1A, but not HRV14. Although an interesting computer exercise, this simplified approach neglects conformational flexibility and changes known to occur upon receptor binding.

The internal pocket of poliovirus has been used in the development of a structure-based computational method for the *de novo* design of antiviral compounds [14]. The approach, known as multiple-copy simultaneous search (MCSS), locates energetically favorable positions in the pocket for binding small fragments that can be joined to generate a larger compound with antiviral activity. A number of novel and unusual compounds were designed.

Simulations of whole viral capsids

The fantastically rapid increase in computing power, coupled with advances in simulation methods and potentials, means that simulations of systems as large as a virus capsid are reasonable and can provide information useful to the structural and molecular virologist, as well as to the theoretician. The initial direction of these studies centers on a detailed understanding of the mechanism of antiviral activity and assembly. Algorithmic developments that improve the computational speed of the long-range electrostatic terms include the use of particle mesh Ewald summation

[15,16] and cell multipole methods [17,18]. Nonetheless, the size of a complete viral capsid, approximately 750,000 protein atoms, remains beyond practical limitations. A more reasonable approach is to take advantage of the icosahedral rotational symmetry to effect simulation of the entire capsid. The explicit interactions determined for the primary unit are reproduced in the neighboring symmetry related image units, as in the method of periodic boundary conditions commonly applied to space-filling symmetries. The rotational symmetry operations described in detail [19] have been demonstrated in short 60 ps trial simulations [20].

The icosahedral symmetry method is also successful in longer simulations (B Speelman, CB Post, unpublished data), for which more statistically reliable analysis can provide useful information on properties of interest. Simulations have been calculated on the asymmetric unit of HRV14, which comprises four fully solvated capsid proteins (VP1, VP2, VP3 and VP4), as shown in Figure 1. Water molecules fill the center of the 300 Å diameter capsid and surround the outside of the virus to simulate the average environment of bulk water. The entire 'wedge' system is subject to the icosahedral symmetry conditions, while a spherical quartic potential is used to constrain the outer water molecules. The asymmetric unit has approximately 12,400 protein atoms, 20,000 water atoms and one calcium ion on each threefold and fivefold axis. Molecular dynamic simulations were performed with and without the antiviral compound WIN 52084 for a period of 1 ns and were found to be stable according to the conservation of the total energy of the system and a constant temperature. Density fluctuations, interactions of protein subunits and protomers, and positional fluctuations in regions of the protein that affect temperature sensitivity, drug resistance and host cell recognition are being compared for the capsid with and without bound WIN 52084. The results could provide a basis for rational strategies to devise novel antiviral compounds.

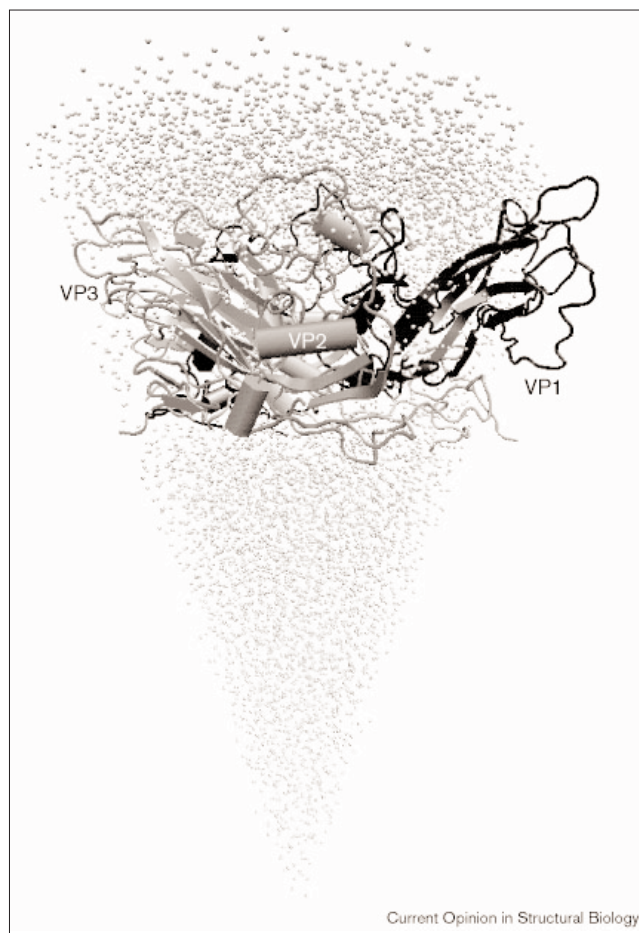
Assembly energetics

Energetics calculated for protein–protein interfaces of an all-atom model has been used in conjunction with a combinatorial approach to evaluate possible assembly pathways [4••]. The combinatorial approach to assembly [21] depends on the ratios of dimer association energies for different interfaces. Dimerization energies estimated for HRV14, comprising three major coat proteins, and for BBV and SBMV, with only one type of coat protein, indicated interesting distinctions in the assembly of these viruses. In the case of HRV14, each interface is formed by a unique set of interacting residues and the pathway is highly directed. In contrast, in the case of BBV and SBMV assembly, which requires conformational switching to achieve polymorphic contacts between protein subunits, the pathway is branched as a result of isoenergetic states of different assembly intermediates.

Kinetic models

A general analytical kinetic model for the self-assembly of virus capsids has been shown to identify key growth features

Figure 1



Ribbon diagram of the protomeric unit of HRV14, comprising VP1, VP2, VP3 (labeled) and VP4 (unlabeled), included in a molecular dynamics simulation calculation (B Speelman, CB Post, unpublished data) for a 1 ns period. Water molecules, shown as a point for each oxygen atom, fill the interior of the capsid, as well as solvating the exterior. The effect of the full virus capsid is achieved using icosahedral periodic images of the asymmetric unit (32,400 atoms) shown here.

in the assembly of icosahedral viruses. The assembly process, described as a cascade of low-order associations, was recently modified to include a kinetically limiting (KL) nucleation event [22••]. In contrast to the less robust equilibrium model [23], which describes assembly using a single weak association energy for all subunit interactions, the KL model prevents too many initiation events and an insufficient subunit concentration, leading to nonproductive particle formation. An interesting outcome of this work is the observation that antiviral agents targeting assembly should only be effective against viruses that assemble through a KL nucleation process.

A molecular dynamics simulation approach based on simple assembly units has also been proposed for examining the kinetics of self-assembly. The use of a probabilistic simulation method has the potential to analyze intermediate distributions and the fidelity of assembly [24•]. Such computationally

feasible simulations of the assembly of a full virus capsid allow one to explore questions concerning conformational switching, the formation of irregular particles and nucleation events, but lack the atomic-level detail needed to connect directly with experimental structural information.

Conclusions

Understanding the self-assembly and stabilization of a regular macromolecular assembly at a molecular level is a considerable challenge to the theoretician. Assembly of the regular nonenveloped icosahedral shell is merely the first level of challenge; more daunting is the delineation of the assembly of enveloped viruses with multiple core structures and more elaborate arrays of several protein types and lipid. A major goal of future work will be to decipher basic principles related to the recognition and accuracy of assembly, and the conformational changes associated with particle maturation during the virus life cycle. Furthermore, insights into assembly, stability and maturation have the potential to assist the development of antiviral compounds that target each of these properties.

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