Energy Landscape for DNA Rotation and Sliding through a Phage Portal

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ABSTRACT Molecular motors involved in the packaging of DNA in tailed viruses are among the strongest known. The mechanism by which the motors operate has long been speculated to involve a coupling between rotation of the portal pore (the gate through which DNA passes upon its packaging or ejection), and translation of DNA. Recent experimental evidence rules out portal rotation with a substantial degree of certainty. We have created an atomistic model for the interaction between DNA and the portal of the bacteriophage SPP1, on the basis of cryo-electron microscopy images and of a recently solved crystal structure. A free energy surface describing the interaction is calculated using molecular dynamics simulations, and found to be inconsistent with a mechanism in which portal rotation drives DNA import. The low-energy pathways on the surface are used to advance a hypothesis on DNA import compatible with all available experiments. Additionally, temperature-dependent kinetic data are used to validate computed barriers to DNA ejection.

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Many-tailed double-stranded DNA bacteriophages and herpes viruses, including ϕ29 and SPP1, package their genetic material inside preformed procapsids before they efficiently deliver DNA into the cell they infect (1,2). The translocation machinery that pushes the DNA in the viral capsid is one of the most powerful molecular motors known. Single molecule experiments on DNA import into the capsids of ϕ29 (3,4) and T4 viruses (5) have indicated that, in both cases, forces in excess of 50 pN are exerted, and that the viral DNA is packed to pressures of 60 atm within the capsid (3). Despite its importance, little is known about the precise structural mechanism and energetics of DNA packaging or ejection. In the cases of both ϕ29 and SPP1, crystal structures and cryo-electron microscopy (cryo-EM) images have identified a homo-12-mer protein pore as forming the portal through which DNA is imported during compaction or ejected during viral infection (6). This portal sits embedded in a particular fivefold symmetric vertex of the icosahedral capsid and has a turbinelike shape (see Fig. 1). In the case of SPP1—chosen herein because of the higher resolution of available structural data (7)—the protein gp6 is the subunit of which a translocationally active 12-mer is composed.

Although ATPases located outside the capsid and around the portal provide the energy necessary for DNA import (8), it has long been speculated that the portal itself plays a role in exerting force on DNA, and that portal rotation is a key feature of DNA import (6,7,9,10). This speculation is largely due to the symmetry mismatch between the 12-fold symmetry of the portal, and the fivefold symmetry of the icosahedral capsid vertex in which it rests. The ambiguous nature of any specific interactions between the portal and the capsid, and the spiral motif in the structure of the portal have led to proposals that the portal rotates during DNA import, and that this rotation might drive the import through a mechanism in which rotational motion of the portal is coupled to translational motion of DNA (6,7). Recent experimental evidence from a combination of magnetic tweezers with single-molecule fluorescence shows that rotation of the portal during DNA import is highly unlikely (11). However, mutations in the portal protein affect import efficiency (12,13), indicating that the protein plays a more significant mechanistic role than that of a passive portal.

To study the mechanistic role of the protein-DNA interaction, we have developed an atomistic model of the portal of SPP1 with a 48-basepair helix of DNA inserted, including explicit water and counterions. The model was based on the recently solved crystal structure of a 13-mer of gp6 (7), and on a cryo-EM image of the connector particle for SPP1, which contains the portal protein as a 12-mer (14). Model building consisted of three steps (detailed in Supporting Material). First, the structure of the gp6 13-mer was closed up after deleting one monomer to form a 12-mer in a guided molecular dynamics simulation that brought together the ends of the ring and relaxed steric clashes at the interface of the newly-jointed portal monomers. The resulting 12-mer structure was then fit to a cryo-EM image of the portal-containing SPP1 connector particle (14); subsequently, we applied molecular dynamics (MD) and normal-mode flexible fitting (15) to refine the portal. Finally, double-stranded DNA (48 basepairs) was placed in the portal structure obtained by MD and normal-mode flexible fitting. The DNA and surrounding protein were solvated with explicit water in a cylinder 210 Å long and 50 Å in diameter, Na^+ ions were added to neutralize the DNA charge, and we equilibrated with constraints on the periphery protein atoms where the ATPases (of unknown structure for SPP1) would bind.

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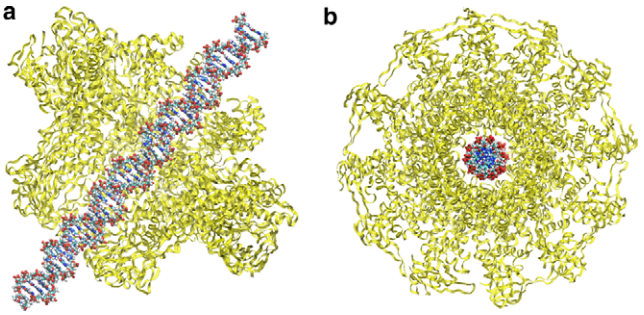

FIGURE 1 (*a*) Cut-out side view, and (*b*) top view of 12-mer portal-DNA complex after MD equilibration. Portal in yellow. Water and ions excluded for clarity. Capsid (not shown) would be in upper-right corner of panel *a* and inwards in panel *b*.

Umbrella sampling in combination with a multidimensional weighted histogram analysis method (details in Supporting Material) were then used to construct a two-dimensional free energy surface describing translocation and rotation of DNA relative to the portal, providing insight into the probable pathways for import and ejection in relation to DNA-portal interaction. The two coordinates selected were $q = 1/n\Sigma_i(z_i - z_{0i})$, where n is the number of heavy atoms in the DNA, z_i is the z coordinate of the ith heavy atom, and z_{0i} is the z coordinate of the ith heavy atom of an idealized DNA helix centered in the portal pore, and $\phi = 1/n\Sigma_i(\theta_i - \theta_{0i})$, where θ_i is the angle made with the x axis in the xy plane containing it by the ith heavy atom. The portal pore was centered so that translocation inside the capsid occurs along the z axis in the positive direction. The coordinate q thus summarizes translation of the DNA through the portal, while ϕ summarizes rotation of the DNA relative to the portal. Harmonic restraining potentials were used for umbrella sampling windows that covered (q, ϕ) grid points. The system is periodic in q after the import of a basepair followed by a rotation of 6° (neglecting effects due to inhomogeneity in the DNA sequence), and in ϕ after a rotation of 30° at constant q. These symmetry considerations were imposed in the calculation of the energy surface.

The calculated rotational and translational free energy surface—our central result—is depicted in Fig. 2 *a* (a contour plot of several periods in both directions) and Fig. 2 *b* (a three-dimensional representation of a single period of the surface along the two coordinates). Minima repeat periodically after a rotation of 30° with no translation, or after a translation of 3.4 Å accompanied by a rotation of 6°. A preferred orientation of DNA with respect to the portal exists and is unique: a single free energy minimum occurs in each two-dimensional ''tile''. The lowest energy path joining neighboring minima occurs for the concerted translocation of one basepair, corresponding to a change in q of 3.4 Å, accompanied by a rotation of 6°. The barrier for this transition is ~8 kcal/mol. Two other pathways, corresponding to a rotation by 30°, and a translocation of 3.4 Å accompanied by a rotation of −24° also occur, both with potential energy barriers of ~11 kcal/mol. If rotation of

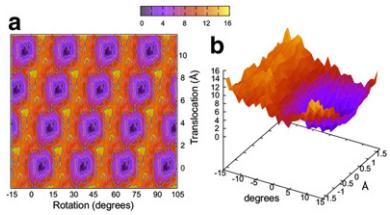

FIGURE 2 Periodic two-dimensional, rotation, and sliding free energy surface for DNA-portal complex.(*a*) Free energy contour plot for four periods of DNA translation through the portal, and four of DNA rotation within the portal. (*b*) A single period (a "tile") of the free energy surface along the two coordinates; 120 MD simulations were run at 12 × 10 grid points on this tile.

the portal relative to the capsid is ruled out (11), our lowest free energy path segment involves rotation of the DNA with respect to the portal and the capsid in which it is embedded. This would likely result in supercoiling on either side of the portal on which the full DNA strand is not free to rotate along with the segment passing through the portal. However, the barrier to rotation of the DNA 30° back, to recenter itself with respect to the portal, is only a few kcal/mol higher than the lowest energy barrier to translocation. The buildup of a potential gradient due to supercoiling could easily result in a compensation for the 3 kcal/mol difference in barrier heights, allowing for supercoil relaxation. Such compensatory slip-back rotation, with or without accompanying DNA translocation, is the newly proposed ingredient that can give rise to an import mechanism consistent with the observation that the portal does not rotate.

DISCUSSION

The pattern of the contour plot in Fig. 2 *a* suggests such a model for the passage of DNA through the portal that consists of visiting the minima in zigzag pathways on the surface. During compaction, forces on DNA tilt the surface down along the positive q direction, which lowers the activation barrier for translation into the capsid; this corresponds to a pathway segment up and to the right on the two-dimensional plane. As the DNA advances in that direction, the rotation angle ϕ of the DNA relative to the portal (hence capsid) starts to increase, which can build up torque onto DNA. This torque, in turn, progressively tilts the landscape toward the negative ϕ-direction, lowering the activation barrier between the minima down that direction, yielding a slip to the left (or left and up). After the slip-back rotation, torque is relaxed and a new segment of diagonal motion on the surface up and to the right occurs, etc. Because slipping back cancels the accumulated overall rotation, our zigzag model is consistent with the single molecule data. Moreover, our surface is also in accord with an energy map hypothesized by Lebedev et al. (7) based on structural periodicity grounds. Furthermore, our lowest energy pathway on the surface (also corresponding to the shortest path between minima) is along the coordinate proposed by Simpson et al. (10), involving a rotation of 6° per basepair imported. This can be contrasted with the

nut and bolt mechanism proposed by Hendrix (6), which involves a 36° rotation per imported basepair, and with the ''peristaltic pump'' mechanism proposed by Dube et al. (9), involving a rotation of −9° per imported basepair.

We now turn to comment on kinetics. Structural rearrangements within the portal are essential for import (but not for ejection); point mutations in the gp6 monomer (12), and mutations which constrain inter-monomer motion (13) slow down import. It is difficult for us to say more about import because large-scale portal motions are limited by our restraints on the outer periphery where the ATPases are located. Moreover, it is the ATPase motor, and not the portal-DNA interaction, that is rate-limiting for DNA import. However, interesting connections arise when comparing dynamics on our two-dimensional surface to measured DNA ejection kinetics. An accurate study by Raspaud et al. (16) on SPP1 used light-scattering measured exponential DNA ejection, and rates were reported at seven temperatures in the interval 10–41°C. When we plotted those data points using a simple transition state estimate of the T-dependence of the rate, $k = \frac{k_B T}{h} e^{-\Delta G^\dagger / k_B T}$, we obtained an activation free energy barrier of $\Delta G^\dagger = 11.5$ kcal/mol, in good agreement with the barriers on our two-dimensional surface.

DNA ejection occurs through the portal and continues through the phage tail. Because the inner diameter of the portal is smaller, the agreement between the barrier values suggests that DNA-portal interactions can be the rate-limiting factor for ejection. Although our computed barriers are consistent with the T-dependent data, the entire viral machinery involves complex kinetic steps. Additionally, there are uncertainties in our calculations involving, e.g., the additional electrostatic screening and the hydration change should divalent cations be present, the effect of barrier recrossing on the prefactor of the transition state rate estimate, and the structure fitting used in model building. As such, alternative models for the origin of the ejection barrier cannot be ruled out.

In closing, we note that, although the minimum energy pathway for DNA import indicated by our energy landscape involves a rotation of the portal relative to the DNA, the surface is not consistent with a translocational force arising due to coupling between rotation of the portal, and translation of the DNA. Such a mechanism would require significantly higher barriers along the ϕ-direction for pure rotation compared to the barrier for the coupled motion. An overall model for import compatible with all available experimental data and our calculations involves conformational changes of the ring of ATPases surrounding the portal that push DNA into the capsid (8), and conformational changes in the portal itself, akin to a ''Chinese finger trap'', which allows inward, but prevents outward, DNA translocation (11). Future simulations may address the effect of such conformational changes on the energy landscape mapped herein. Experimental tests of the features of the landscape would be ejection kinetics studies with mutations in the loops that are closest to DNA and in varying Mg^{2+} concentrations. Additionally, the surface we computed can serve as a component of a larger-scale model for encompassing the entirety of the important aspect of DNA import or ejection.

SUPPORTING MATERIAL

Computational methods and references are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)00008-3.

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