Langevin dynamics simulation of DNA ejection from a phage

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Received: 18 February 2013 / Accepted: 22 March 2013 / Published online: 23 April 2013
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Abstract We have performed Langevin dynamics simulations of a coarse-grained model of ejection of dsDNA from $\Phi 29$ phage. Our simulation results show significant variations in the local ejection speed, consistent with experimental observations reported in the literature for both in vivo and in vitro systems. In efforts to understand the origin of such variations in the local speed of ejection, we have investigated the correlations between the local ejection kinetics and the packaged structures created at various motor forces and chain flexibility. At lower motor forces, the packaged DNA length is shorter with better organization. On the other hand, at higher motor forces typical of realistic situations, the DNA organization inside the capsid suffers from significant orientational disorder, but yet with long orientational correlation times. This in turn leads to lack of registry between the direction of the DNA segments just to be ejected and the direction of exit. As a result, a significant amount of momentum transfer is required locally for successful exit. Consequently, the DNA ejection temporarily slows down exhibiting pauses. This slowing down occurs at random times during the ejection process, completely determined by the particular starting conformation created by prescribed motor forces. In order to augment our inference, we have additionally investigated the ejection of chains with deliberately changed persistence length. For less inflexible chains, the demand on the occurrence of large momentum transfer for successful ejection is weaker, resulting in more uniform ejection kinetics. While being consistent with experimental observations, our results show the nonergodic nature of the ejection kinetics and call for better theoretical models to portray the kinetics of genome ejection from phages.

Keywords Genome ejection from phages

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1 Introduction

The precise molecular mechanism of transfer of a dsDNA molecule from bacteriophages to the host cells is still not fully understood [1, 2]. The major steps comprised of recognition of proteins of the host by the phage, activation of the release of DNA from the lumen of the phage, and the eventual translocation of DNA into the host cell have been well recognized in the literature [3–6]. Each of these steps involves biochemical processes associated with protein–protein and protein–DNA interactions. In addition, there are physical forces mainly arising from the release of pressure initially generated by a motor protein in encapsidating the DNA inside the phage capsid. The release of pressure, concomitant with the slippage of DNA from its packaged conformation, is known to be the driving force for the DNA translocation. The release of DNA can also be enzyme-driven. In addition to this push by the internal pressure of the capsid, there can be pulling forces from the host cell.

There has been substantial progress towards a fundamental understanding of this process based on both *in vivo* and *in vitro* studies. Despite this progress, the mechanism of DNA ejection from phages is still not understood. One of the major puzzles is the occurrence of pauses during DNA ejection. In the recent *in vivo* single-molecule Hershey–Chase experiment, Van Valen et al. [7] have shown that there are significant variations in the ejection time, although it is the same molecule that is being ejected. The ejection trajectories exhibit abundant, but sporadic, pauses. The instantaneous velocity of ejection changes from an essentially constant value in the initial stage to essentially to zero at the end of the process. Remarkably, the instantaneous velocity depends mainly on the ejected DNA length instead of what remains inside the phage.

Complementary to the *in vivo* studies, there have been many *in vitro* studies [8–21]. By monitoring DNA ejection from a phage against a solution with controllable osmotic pressure, the ejection can be stopped midway by a threshold value of the external osmotic pressure [8, 10, 13]. The ejection rate of DNA in the *in vitro* experiments is an order of magnitude faster than that in the *in vivo* experiments [7]. The roles of electrostatic interactions and multivalent ion binding to DNA have also been investigated [17, 19]. Based on light scattering measurements, the DNA ejection is shown to require a crossing of an activation barrier of tens of thermal energy [12, 16]. As in the *in vivo* studies, the *in vitro* studies show the occurrence of pauses during DNA ejection. Chiaruttini et al. [21] have shown that the DNA ejection kinetics proceeds by rapid transient bursts with substantial occurrence of pauses. They have established that these pauses are not correlated with any specific genome sequences or defects along the DNA. They have also ruled out the possibility of any gating mechanism of ejection complex channel contributing to the observed pauses. They have conjectured that the transient pauses are due to local phase transitions of DNA inside the capsid.

The primary focus of the present paper is to explore the molecular origin of the variations in the instantaneous speed of DNA ejection. We use Langevin dynamics simulation of a coarse-grained model of the DNA ejection process under the simplest condition of only the DNA confinement force inside the capsid driving the ejection. The present work is complementary to other simulation and theoretical works already known in the literature [22–33]. In particular, the roles of chain flexibility, capsid shape, solvent quality, salt concentration, and presence of knots on the ejection kinetics have been investigated in addition to the structure of packaged DNA under a motor force. The theoretical models inevitably use simple models for capsid pressure, based on equilibrium arguments as input for describing the kinetics of ejection [32].

We report here that the instantaneous speed of DNA ejection from a capsid is uniquely correlated with the angle of approach of the polymer segment at the pore mouth for ejection. If the angle of approach is not in registry with the pore axis, then substantial momentum transfer is required for the exit, which causes the temporary pause. The occurrence of such pauses is inevitable for stiff chains such as dsDNA as we demonstrate below. Further, the ejection trajectory of DNA is unique to the particular assembled structure of DNA under the motor force before the initiation of the ejection. This leads to the occurrence of highly stochastic variations in the ejection kinetics. The assembled structures of DNA under the motor forces of experimental relevance are not in equilibrium and as a result the stochasticity in the ejection kinetics is a reflection of the extent of the nonequilibrium conformations of the packaged DNA inside the capsid. Our simulation results are entirely consistent with the *in vitro* results of Chiaruttini et al. [21] and the *in vivo* results of Van Valen et al. [7].

The rest of the paper is organized as follows. The simulation model, method, parameterization, and data collection are described in Section 2, followed by results on packaging and ejection in Section 3. The final section gives a summary of conclusions.

2 Coarse-grained model and simulation method

In this paper, we model the ejection of dsDNA from $\Phi 29$ phage. The details of the coarse-grained models of the various components of the problem, namely, the capsid, motor, and dsDNA are described first, followed by the details of the Langevin dynamics modeling of packaging and ejection. The methods of data collection and structural characterization are also presented.

2.1 Capsid

The pseudo-atomic coordinates of the protein capsid of the $\Phi 29$ phage are obtained from Protein Data Bank [34] and each residue is represented as a united atom bead of diameter 3 Å with the center at the C- α position. Since the coordinates represent a matured capsid without any opening for DNA packaging/ejection, we created an artificial hole at the topmost location on the capsid along the z-axis. The hole has a diameter of 27.5 Å, which is slightly larger than the diameter of the coarse-grained DNA beads (25 Å) for packing and ejection.

2.2 Motor protein

The motor protein is very complex in reality. However, for the present enquiry of generic features of ejection kinetics, we take the motor to be simply a force generator at the pore mouth of the capsid. In view of this, we construct an assembly of a cuboid, consisting of a small hollow cylinder on top of the artificial hole created on the capsid. The internal diameter of the cylinder is 27.5 Å and its length is 12 Å. The cylinder is constructed of beads of diameter 3 Å. The cuboid has a length of 12.5 Å along the axis of the cylinder and a width of 27.5 Å with its axis coinciding with that of the hollow cylinder. The capsid along with the motor protein is shown in Fig. 1.

Fig. 1 Coarse-grained models of capsid, motor, and dsDNA. **a** The cuboid enclosing the cylinder represents the motor protein and the capsid wall is united-atom representation. **b** The initial state of equilibrated dsDNA at the pore mouth of capsid for packaging. **c** Close-up of initial configuration of DNA near the capsid mouth

2.3 Double-stranded DNA

Following our earlier work [25], a coarse-grained bead-spring model with bending rigidity is used to represent dsDNA. The diameter and the bond length of the DNA beads is 25 Å (hydrated diameter of DNA) and 12.5 Å, respectively, to represent roughly the cylindrical geometry for the DNA helix [25]. One coarse-grained DNA bead has roughly eight base pairs. The dsDNA is modeled to have a persistence length of roughly 60 nm. The electrostatic interaction between the DNA beads was found to affect only the amount of DNA packed without any effect on the qualitative nature of packaging dynamics [25], especially due to short Debye length in comparison with the diameter of dsDNA. Furthermore, our present focus is not related to the roles played by multivalent ions. As a result, we consider only the excluded-volume interactions among the DNA beads and DNA-capsid beads. Furthermore, in an effort to gain insight into the ejection mechanism, we have deliberately varied the persistence length of the polymer model by appropriately changing the intrachain bending force constant, as described below.

2.4 Simulation technique

The force fields on the individual beads are computed by Langevin dynamics simulation in LAMMPS (<http://lammps.sandia.gov>):

$$m_i \frac{d^2 r_{ij}}{dt^2} = -\zeta_i \frac{dr_{ij}}{dt} - \nabla_j U_i + f_{ij} + f_{i*j}^{motor} \quad (1)$$

where r_{ij} is the j th component of the position vector of the i th bead, and t is the time. m_i and ζ_i are the mass and friction coefficient of the i th bead, respectively. U_i is the net potential acting on the i th bead, as given below. f_{ij} is the j th component of the random force acting on the i th bead obeying the fluctuation-dissipation theorem with its magnitude given by $\sqrt{2k_B T \zeta_i \delta t}$ ($k_B T$ is the Boltzmann constant times the absolute temperature, and δt is the simulation time step). f_{i*j}^{motor} represents the motor force on the i^* bead/s with its center located in the motor protein region. The net potential acting on the i th bead is the sum of all the non-bonded and bonded potentials:

$$U = U_{LJ} + U_{bond} + U_{angle} \quad (2)$$

Excluded-volume interaction between the DNA–DNA beads and DNA–capsid beads is modeled as

$$U_{LJ} = 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right] + \epsilon_c \quad r < r_c, \quad 0 \text{ otherwise} \quad (3)$$

where

$$\epsilon_c = -4\epsilon \left[\left(\frac{\sigma}{r_c} \right)^{12} - \left(\frac{\sigma}{r_c} \right)^6 \right] \quad (4)$$

and σ and ϵ are the parameters and r_c is set to 1.12σ . A Hookean bead spring model is used to represent the connectivity between adjacent beads in the DNA:

$$U_{bond} = K_{bond}(r' - r_0)^2 \quad (5)$$

where r' is the bond length, r_0 is the equilibrium bond length and K_{bond} is the force constant for the bond. A three-body interaction potential is used to set the stiffness of the chain:

$$U_{angle} = K_{angle}(\cos\theta - \cos\theta_0)^2 \quad (6)$$

where θ is the angle between three consecutive beads along the chain, θ_0 is the equilibrium value of bond angle, and K_{angle} is the force constant for the angular stiffness.

The capsid and the motor protein are kept fixed throughout the simulation. The velocity and position of the DNA beads are updated at every timestep by the velocity Verlet algorithm.

All variables are expressed in dimensionless Lennard-Jones (LJ) units, fully consistent with LAMMPS. In order to non-dimensionalize the various parameters, the fundamental quantities of mass, length, and energy are m_0 (taken to be 5.2 kg/mole corresponding to the average molar mass of eight base pairs), σ (taken to be 25 Å, the diameter of the coarse grained DNA bead), and $1 k_B T$, respectively. The other important quantities for non-dimensionalization are expressed in terms of the fundamental quantities such as time ($\sqrt{\sigma^2 m_0 / \epsilon}$), force (ϵ / σ), temperature (ϵ / k_B) and pressure (ϵ / σ^3). The fundamental quantities that are based on the ϵ are estimates based on the approximation of $1 k_B T$. In reality, they can be different, but they are of the same order of magnitude based on this approximation. m_0 / ζ is chosen to be 300 LJ time units. ϵ is chosen to be 1 unit, σ and is chosen to be 1 unit for the DNA beads and 0.12 units for capsid beads, which are equivalent to 25 Å and 3 Å, respectively, in real units. A K_{bond} value of 1,500 units is used and is sufficient to keep the bond length within 1% of its original r_0 value (12.5 Å) of 0.5 LJ units. θ_0 is set to 180° and the K_{angle} is tuned to set the stiffness of the chain as described below. The LJ interaction between three consecutive beads on the DNA/polymer strand were switched off to avoid any kind of interference from the LJ interaction on the bonded

and angular interactions. The LJ interaction between the capsid beads was switched off for computational efficiency. Temperature is set to 1 unit and the time step is chosen to be 0.0003 units for packing and 0.003 for ejection simulations, respectively.

2.5 Initial configuration

There are three major steps in our simulation protocol. First, we equilibrate a chain with the desired chain stiffness as a function of K_{angle} . In the second step, a polymer chain of prescribed chain stiffness is packed into the capsid under the force from the motor. In the last step, the overhanging part of the polymer is removed and we monitor the ejection of the DNA without any interference from the motor protein.

2.5.1 Persistence length determination

A polymer chain of 5,000 beads (with a particular K_{angle} value) is equilibrated in a cubical simulation box of length 200 LJ units with periodic boundary conditions. Following equilibration, the instantaneous average bond angle is monitored to calculate the persistence length [25]. The persistence length is given by:

$$L_p = r_0 / (1 + \langle \cos\theta \rangle) \quad (7)$$

where r_0 is the equilibrium bond length and $\langle \cos\theta \rangle$ is the instantaneous average of the cosine of the bond angle. Persistence length is thus obtained as a function of the K_{angle} . The double-stranded DNA is reported to have a persistence length close to 50–60 nm, hence K_{angle} value of 750 is used to simulate a chain with stiffness similar to DNA. The effect of packing a polymer chain of smaller persistence length is also investigated by choosing smaller values of K_{angle} , in accordance with our earlier report [25].

2.5.2 DNA packing

The capsid with the motor protein is placed in a cubical simulation box of length 200 LJ units with the center of the capsid coinciding with the center of the simulation box. The outward normal to the hollow cylinder points in the positive z-axis. A chain (with chosen stiffness) of 5,000 beads is placed in the simulation box, with three beads from one end of the chain inside the mouth of the pore as shown in Fig. 1c. The center of the third bead and the center of the outer face of the cylinder coincide, with the other two beads lying within the cylinder. The chain is equilibrated keeping the three polymer beads in the mouth immobile. After equilibration, the effect of the motor protein is simulated by applying a downward normal force in the mouth of the pore. Any bead with its center lying within the motor protein region experiences a downward force of appropriate magnitude. Six different motor forces were used: 5 pN, 10 pN, 20 pN, 30 pN, 40 pN, and 55 pN. The average magnitude of the applied motor force [25] is known to be 55 pN for $\Phi 29$. The magnitude of the motor force determines the amount and rate of packaging. The goal is to investigate the effect of the packaging rate on the morphological ordering and the internal pressure. The simulation is executed for approximately 3,000 time units. The position of the DNA beads, pressure and energy are collected at regular intervals for further analysis. Ten independent simulation runs are conducted and the average values are reported.

2.5.3 DNA ejection

After packaging, the DNA remaining outside of the capsid is removed, followed by simulation without the action of the motor protein. Again, the position of the DNA beads, pressure and energy are collected at regular intervals for further analysis. Twenty independent simulations are conducted and we report individual kinetics.

2.6 Data collection

2.6.1 Packed length

The packaged length and the ejected length at any point of time are evaluated by determining the bead index at the mouth. For the ejection case, when the distance between the center of the mouth of the hole to any polymer bead exceeds 1 LJ unit, then the chain is considered as fully ejected. The total packed length is determined at the end of 2,750 LJ time units.

2.6.2 Pressure

The pressure of the whole system is calculated by LAMMPS using the formula:

$$P = \frac{Nk_B T}{V} + \frac{\sum_i \vec{r}_i \cdot \vec{F}_i}{dV} \quad (8)$$

where N is the number of beads in the system, $k_B T$ is the Boltzmann constant times temperature, d is the dimensionality of the system (3 in this case), V is the system volume, and the second term is the virial computed for all pairwise (LJ in this case) as well as two-body (bond stretching interaction), and three-body interactions (angular interaction potential).

$$\sum_i \vec{r}_i \cdot \vec{F}_i = \sum_i \sum_{j>i} \vec{r}_{ij} \cdot \vec{F}_{ij} \quad (9)$$

where $\vec{r}_{ij}$ is the vector distance between i th and j th beads, $\vec{F}_{ij}(= \nabla_{\vec{r}_{ij}} U(\vec{r}_{ij}))$ is the force between i th and j th beads, and $U(\vec{r}_{ij})$ is the sum total of potential between the i th and j th beads. The reported value of pressure from LAMMPS is for the whole simulation box, but the contribution to pressure from the beads outside the capsid is negligible; hence the reported pressure value is multiplied by a factor $V_{\text{Simulationbox}}/V_{\text{capsid}}$ to estimate the pressure inside the capsid in LJ units, with V being the volume. The capsid is approximated as a sphere of inner diameter 41 Å, which is about 16.4 LJ units and the simulation box is a cube of edge 200 LJ units. The obtained pressure is then multiplied by ϵ/σ^3 to estimate the pressure in real units. As mentioned earlier, ϵ is assumed to be 1 $k_B T$ and the value for σ is 25 Å in real units. The average value of the pressure as a function of packed fraction is presented in the following section.

2.6.3 Ordering

The morphology of the DNA inside the capsid is followed as a function of time. We consider both the radial and axial order.

Radial Order: The bead density at any instant is projected onto the plane perpendicular to the packaging axis (axis of the hollow cylinder), by following our earlier procedure [25]. Projected densities are then averaged azimuthally to obtain a radial distribution function.

Axial Order: The axial order parameter defines the degree to which the packaged DNA forms a toroid-like assemblage of stacked hoops aligned with the packaging axis. Fifteen consecutive beads are considered along the chain. A unit normal vector for each arc is defined by normalizing the cross-product of the two vectors connecting the middle bead to each of the end beads ($\vec{v}_1 = \vec{r}_1 - \vec{r}_8$ and $\vec{v}_2 = \vec{r}_{15} - \vec{r}_8$). For each arc along the chain contour, a unit arc normal is computed and projected its length along the packing axis ($\vec{v} = \vec{v}_1 \times \vec{v}_2 / |\vec{v}_1 \times \vec{v}_2|$). The axial order parameter is determined by dividing the sum of the magnitude of projected arc normals by the number of arcs. The order parameter [25] approaches unity for a perfect stack of hoops, whereas a group of randomly oriented arc normals gives a value of 0.5.

$$\text{Axial Order} = \frac{1}{N_{\text{Arcs}}} \sum_i |v_z| \quad (10)$$

where N_{Arcs} is the number of arcs and v_z is the projection of the arc normal along the z-axis.

2.6.4 Trajectory and displacement of labeled beads

In an effort to understand the ejection kinetics at different stages of the process, we have labeled three beads and followed their trajectories for illustrative purposes. The position of the beads is chosen randomly along the length of the polymer to be 375 nm, 1,875 nm, and 2,750 nm. Although they are randomly selected, they are far apart along the polymer contour. The goal is to investigate the path followed by beads at different packed fractions. The three-dimensional trajectory followed by any bead is tracked by plotting a vector from the starting point to the destination point during a time step. The trajectory gives an idea of the kind of path followed during the ejection: zig-zag or projectile. The displacement (ΔR) is the magnitude of the positional vector from the initial time ($t = 0$) to any time (t). The displacement is plotted against time to determine the nature of pathway followed by a stiff chain relative to less stiff chains. The trajectory and displacement both describe the nature of the pathway followed by a stiff chain and a less stiff chain at different packing fractions.

3 Results and discussion

As we show below, the process of DNA ejection from the capsid has a unique one-to-one correspondence with the packaged conformation. The tremendous stochasticity in the ejection kinetics arises from varying conformations in the starting point. In view of this, we first present a brief data set on the dynamics of packing under the motor force. This discussion is then followed by ejection kinetics.

3.1 Packing dynamics

We have monitored the packaging of the polymer by varying the motor force and the chain stiffness. The effect of motor force is given in Fig. 2a, where the packed fraction is plotted against time for different motor forces (5 pN, 10 pN, 20 pN, 30 pN, 40 pN, and 55 pN). The time dependence is qualitatively similar for all these forces. After a uniform rate in

Fig. 2 Time evolution of packed fraction. **a** Effect of motor force; *inset*: dependence of maximum packed length. **b** Effect of persistence length of polymer; *inset*: dependence of maximum packed length

the initial stages, the packed fraction reaches a saturation limit. For a given motor force, the loaded length of the polymer reaches a plateau value. The dependence of the maximum loaded length on the motor force is given in the inset of Fig. 2a. Two parameters were used: (a) motor force determining the rate and amount of packaging, and (b) persistence length of the polymer providing insight into the role of orientational correlation of the chain backbone at the pore mouth. The packaging is qualitatively similar for all the cases (Fig. 2) of motor force (5 pN, 10 pN, 20 pN, 30 pN, 40 pN, 55 pN). The packaging proceeds at approximately a constant rate until the motor protein reaches its limit. Beyond a certain loaded length of the dsDNA, the packaging attains a plateau, similar to the trends previously observed by us [25]. The effect of the persistence length on the packing dynamics is given in Fig. 2b. As seen from this figure, the time evolution of the packing fraction is insensitive to the persistence length for a given motor force.

The packing occurs in three stages: motor driven, motor driven against crowding, and saturation. In the first stage, the motor protein is pushing the DNA without essentially any resistance as the packed pressure is not sufficient to exert an opposing force. In the second and third stages, the capsid is already crowded and the motor is still pushing the DNA, leading to creation of inner layer of DNA and also addition of beads to the existing layers. This leads to a sharp increase in the bending energy as well as the LJ interaction energy towards the end (around 0.9 fraction packed) of the packing (as shown in Fig. 4 given below) with a reduced rate of packing. In the last stage, the system is trying to reach the mechanical equilibrium by packing as much DNA as possible to balance the applied pressure from the motor force. The total packed length as shown in Fig. 2a saturates with an increase in motor force rather than increasing linearly. Increase in packing rate introduces more randomness in the system and hence the packing is not very efficient leading to earlier saturation in packed length. A more ordered system would pack more length for a given motor force.

In an effort to estimate the role of chain flexibility, the packing dynamics is followed for polymer with 20-nm persistence length as shown in Fig. 2b. The dynamics seems to be independent of the persistence length and is only dependent on the motor force, although the total packed length is higher for a less stiff polymer as expected (see inset Fig. 2b). The first stage lasts until at least 0.5 fraction is packed (can go as high as 0.8 fraction for 55 pN motor force) and the saturation stage starts at about 0.9 fraction packed. The intermediate stage can start from somewhere between 0.5 fraction packed to 0.8 fraction packed. The interesting point to be noted about the packing trajectory is that the packing time is about the same for

Fig. 3 Dependence of pressure on packing fraction for different motor forces with $\epsilon = 1k_B T$

all the cases of motor force. The higher motor force is able to push DNA/polymer inside the capsid at a faster rate initially, but the rate of packing suddenly drops down at later stages. In order to gain more insight, the dependencies of pressure and different components of energy on the packed fraction are given in Figs. 3 and 4, respectively. The sudden drop in packing rate is due to sudden increase in the resistance at high packing fraction to the entry arising from the sudden rise in the pressure inside the phage as shown in Fig. 3. The rate of packing with lower motor force decreases smoothly to saturation. As shown in Fig. 3, the pressure increases steadily with increase in packing fraction.

The pressure builds up to tens of atmospheres as expected (Fig. 3) due to the increase in both bending energy and the repulsion within the system (Fig. 4). The morphology of the dsDNA inside the capsid is followed as a function of time. As pointed out in the previous section, we are concerned with two different aspects of the dsDNA arrangement inside the capsid: (a) radial order, and (b) axial order. For radial order, the bead density from simulation (averaged for 50 equilibrated runs after packaging is ceased) is projected onto the plane perpendicular to the packing axis (at the center of the capsid). Projected densities are averaged azimuthally to obtain a radial distribution function (Fig. 5). The radial order is the same as our previous result [25]. The radial order plot shows that the dsDNA is arranged radially in layers with a layer thickness of approximately 2.5 nm and the dsDNA is packaged more densely near the capsid wall. The number of peaks decreases with a decrease in motor force and the peaks are shifted more towards the center. To understand these characteristics, we have investigated the axial ordering inside the phage (Fig. 6). The axial order parameter defines the degree to which the packaged dsDNA forms a toroid-like

Fig. 4 Dependencies of Lennard-Jones (Vdwl) and bending (angle) energies on motor force (F20 = 20 pN and F55 = 55 pN) and persistence length (PL) of 20 nm and 60 nm

Fig. 5 Dependence of the radial density of the packaged dsDNA on the motor force

assemblage of stacked hoops aligned with the packing axis. The order parameter approaches unity for a perfect stack of hoops, whereas a group of randomly oriented arc normals gives a value of 0.5. As shown in Fig. 6, the axial order always decreases with increase in the loading length and the slow rate of packaging (low motor force) gives rise to more axial ordering because the dsDNA gets more time to order before another segment is pushed into the crowded environment. For the motor force corresponding to $\Phi 29$ (with a motor force of approximately 55 pN) there is some axial ordering in the beginning but it becomes more random as time progresses. Hence, the dsDNA is more concentric than coaxial, as shown in the literature [25], unlike many simplifying theoretical assumptions. The axial order of dsDNA packed with 5 pN motor force and 55 pN is shown in Fig. 7 as an illustration. As shown in Fig. 7a, the DNA is ordered better but it is not perfectly stacked hoops, and hence the value of axial order is about 0.75. Figure 7b shows that the packing is completely random with an order parameter approaching 0.5.

3.2 Ejection dynamics

The ejection trajectories of DNA from the packaged state are found to be wildly stochastic. As illustrative examples, 20 independent traces of DNA ejection for the model $\Phi 29$ capsid are given in Fig. 8a. The plot shows the length of DNA remaining inside the phage as a function of time. There are two classes of ejection, one that involves intermediate slow ejection kinetics (85% of total runs) and another that ejects without slow intermediate state (15% of total runs). The ejection starts at a fast rate, but most of the time (85% of total runs) the ejection is slowed down for a certain period of time, followed by a sudden increase in

Fig. 6 Dependence of the axial density of the packaged dsDNA on the motor force

Fig. 7 Dependence of DNA organization after complete packaging on motor force. **a** Better axial order for lower motor forces (5 pN) and **b** loss of axial order for higher motor forces (55 pN)

the rate of ejection and ultimately ending with a slow exit. The intermediate slow rate starts somewhere between 2% to 60% and the last stage starts when the length of DNA remaining inside the phage is of the order of the phage diameter (50 nm). The intermediate slow rate is

Fig. 8 Large fluctuations in the ejection kinetics. 20 time evolutions of packed length are illustrated for different motor force F during packaging and persistence length (PL) **a** $F = 55$ pN, $PL = 60$ nm **b** $F = 55$ pN, $PL = 20$ nm **c** $F = 20$ pN, $PL = 60$ nm **d** $F = 5$ pN, $PL = 60$ nm

observed for a time period of 1,000 LJ units to 10,000 LJ time units. These are in qualitative agreement with experimentally observed trends [7, 21].

In an effort to gain further insight, we have investigated the ejection kinetics by varying the motor force and the persistence length of the polymer. The case of low motor force is considered for determining the kinetics of ejection from other phages in general and a low persistence length polymer is considered for determining the role of chain stiffness in the ejection kinetics. Higher motor force was not considered because the packing kinetics saturates around 55 pN motor force ($\Phi 29$) as mentioned earlier. The ejection kinetics of the system with lower persistence length (20 nm) does not show any pause. For flexible chains, the ejection occurs in two successive stages, pressure-driven drift followed by diffusion. The pressure-driven stage lasts until about 60% of the polymer is ejected. The crowding slowly vanishes and hence the diffusion-like behavior slowly creeps into the kinetics, replacing the pressure-driven drift. Coming back to the effect of motor force on stiff chains, with decrease in the motor force, the number of events with intermediate slow kinetics decreases. For 5 pN motor force, there are only a few traces that show an intermediate slow kinetics, but the distinction between the traces is not very apparent. For all practical purposes, the intermediate slow kinetics can be considered to be absent for such small motor forces.

In order to understand the reason for the slow intermediate ejection kinetics, the trajectory (Fig. 9) and the displacement versus time (Fig. 10) of three labeled beads along the DNA/polymer are tracked during the ejection. As can be seen in Figs. 9 and 10, the rigid DNA chain takes circular trajectory and it cannot take a sharp turn to escape the high-pressure situation, unlike the polymer of small persistence length. However, at low packing fraction, the behavior of the less stiff chain and the stiff chain are similar. This can be

Fig. 9 Trajectories (in x,y,z coordinate system) of three labeled beads (at 375, 1,875, and 2,750 nm from the end of the chain packaged first) as a function of time (motor force is 55 pN during packaging). **a** Persistence length is 60 nm. At higher persistence length, the trajectories exhibit very long orientational correlation times leading to wrong orientational approach to the pore mouth, which in turn leads to pauses. **b** Persistence length is 20 nm. For flexible chains, the trajectories are zig-zag with lowered probability of pauses

Fig. 10 Displacement of three labeled beads (at 375, 1,875, and 2,750 nm from the end of the chain packaged first) as a function of time (motor force is 55 pN during packaging). **a** Persistence length = 60 nm. **b** Persistence length = 20 nm. In **a**, the beads undergo circular motion due to the orientational correlation of their trajectories. As a result, the beads approach the pore mouth without alignment with the direction of exit from the capsid. The necessary requirement of momentum transfer at the pore mouth for the exit of such bead conformations leads to temporary pause. In **b**, the potential for causing pauses is weaker due to small persistence length of the polymer. Oscillations are observed in both **a** and **b** at later times, but in the initial stages oscillations occur only for dsDNA and not for flexible polymer

explained by the availability of the space allowing both kinds of chain to avoid sharp turns. Only at high packing fraction, the less stiff chains are able to take a zig-zag trajectory to escape from a high-pressure environment, while the stiff chains do not have the freedom to take zig-zag paths.

After spending considerable effort in investigating the behavior of many different parts of the chain and the labeled beads, we have eventually found that the orientation of the beads in the neighborhood of the pore mouth is critical in dictating the local velocity of ejection. The angle between the beads near the pore mouth and the axis along the pore mouth indicates how bent the DNA is at the pore mouth. The angle was measured between the z-axis and the vector from the three adjacent beads (about to exit the pore) just below the cylindrical pore mouth. For most of the trajectories, the DNA at high packed fraction remains bent, and waits for an opportunity to become less bent when the DNA packed fraction becomes low. This results in the intermediate slow kinetics. However, sometimes the DNA gets an opportunity to become less bent at the pore mouth, leading to smooth exit as shown in Fig. 11, where four typical trajectories of DNA ejection are illustrated. The transition from highly bent to less bent near the pore mouth is purely stochastic. With a decrease in motor force, the packed DNA inside the capsid is less and, as a result, it has a higher probability to transition itself to a less bent form. Therefore, the percentage of trajectories with slow intermediate kinetics reduces with decrease in the motor force. Moreover,

Fig. 11 The approach angle of the beads to the pore mouth dictates the local ejection speed. **a** The angle between the chain near the mouth with the pore axis versus time. The corresponding ejection trajectories (slopes give the local ejection speed) are given in **b**

the system with low packing force is highly ordered, hence the stiff chain is mostly not bent near the mouth (not shown here), resulting in smoother exit. The lower persistence length polymer is able to exit smoothly, since the bending restriction is not so high.

In summary, we find that the jamming near the pore mouth to be the cause of pauses and intermediate slow rates in dsDNA ejection. The higher the ordering and the lower the amount of chain packed, the higher the probability of transition of the conformation of the DNA near the mouth from a bent to a less bent state.

4 Conclusions

We have investigated the packing and ejection of dsDNA (persistence length of 60 nm) in $\Phi 29$ (internal diameter of about 41 nm) virus using Langevin dynamics simulation. In order to understand the molecular mechanism behind the experimentally observed pauses and significant variations in the instantaneous speed of DNA ejection, we have simulated the packing and ejection by varying the motor force and chain stiffness. The packing process is qualitatively the same for different motor forces and chain stiffness. In general, it occurs in three stages: motor driven, motor driven against resistance, and saturation. If the packing is slow enough, then the chain packed inside the phage is able to equilibrate until the next

batch of chain gets inside. Hence, the low motor force leads to slow and better ordered packing, whereas the packing with a high motor force leads to more beads packed but with less ordering. The pressure generated during the packing process and the particular structure of the packaged chain play important roles during the ejection process. The ejection of the less stiff chain is qualitatively different from that of the stiff chain. The less stiff chain ejects in two stages, a mainly drift-dominant regime at higher packing fraction and a diffusion-dominant regime at low packing fraction irrespective of the motor force. The less stiff chain is randomly ordered at the end of packing, irrespective of motor force. On the other hand, the qualitative nature of the ejection of dsDNA is dependent on the motor force. With lower motor force, the DNA is highly ordered and the amount of DNA packed is low, hence it ejects smoothly. With an increase in motor force, the ordering of DNA at the end of the packing cycle suffers from nonequilibrium conformations.

The ejection process is uniquely related to the final structure of the packaged polymer at the end of the packaging cycle. The key observation is that as a segment of dsDNA (at higher packing fractions) approaches the pore mouth it subtends an angle that is not in alignment with the pore axis, due to orientational correlation of the chain backbone. This therefore necessitates a momentum transfer, which in turn requires a certain amount of time for the local jammed state to relax. This effect is manifested as a pause. Since the very early stage of ejection starts with the correct orientation of the chain end and since the undesirable orientations are rare at very late stages, the ejection kinetics is slower in the intermediate stage. The consequences of the vivid details from our simulations are consistent with the observations in both in vivo and in vitro experiments. Although there are no phase transitions seen in our simulations, our conclusions fully support the speculations made by Chiaruttini et al. [21].

Finally, our results suggest that new theoretical attempts need to be made by accounting for the temporal correlation between the DNA ejection and jammed nonequilibrium DNA conformations at the pore mouth inside the capsid.

Acknowledgements Acknowledgment is made to the National Science Foundation (NSF Grant No. 1105029), National Institutes of Health (NIH Grant No. R01HG002776-07), Air Force Office of Scientific Research (AFOSR Grant No. FA9550-10-1-0159) and the Materials Research Science and Engineering Center at the University of Massachusetts, Amherst.

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