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Mini Review

Nanomechanics of Biomolecules: A Review

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Abstract

Nowadays it is possible to manipulate biopolymers such as DNA and protein, in ways to observe at single molecule level, as well as to touch and move, stretch, and crush individual molecules. An increasing number of inter and intramolecular interactions can be probed using single molecule manipulation techniques. Apart from inter- and intra-molecular interactions, the charged molecules interaction with substrates plays a vital role in designing nanobiosensors. Furthermore, the nanomechanical response which is the bending motion via changes of the surface stress produced due to biomolecular interactions over the surface of cantilever has also been studied interestingly by researchers. This review article exemplifies nanomechanical approaches in the analysis of viral packaging, biomolecular interactions, protein folding and unfolding, and DNA sequencing, illustrating how biomolecules contributes to the potential of exploiting novel concepts in technological innovation.

Keywords: Viral packaging, Biomolecular interactions, Protein folding, Unfolding, DNA sequencing

Introduction

Mechanical techniques such as AFM are now able to investigate forces and interactions down to the molecular and atomic scale in a variety of environments. Biomolecular recognition is nature characteristics in DNA hybridization, DNA-protein interaction, protein-protein interaction, and cell-ligand binding. In the post-genomics era, proteins and their associated unique characteristics are of great interests that drive rapid development in fusion of bionanotechnology and microsystems. The technological integration is to fill the gap where current biotechnologies fail to cohesively meet the demand in efficient diagnostics, characterization and in-depth understanding of biomolecular interaction. Genetic recombination, packaging of DNA in cells and viruses, folding and unfolding of proteins and assembly of the organic matrix of bone are just a few of the many fundamental biological reactions that involve interactions between helical macromolecules[1,2]. The nanomechanical properties of hierarchical biological materials underwent an exciting development over the past several years, partly due to the emergence of physical science based approaches in the biological sciences, leading to cross-disciplinary investigations of materials, structures, diseases as well as the development of new treatment and diagnostic methods. DNA is the central object of the present review because, its analysis is more amenable to rigorous theory and because most of the empirical information was accumulated for it. We attempted to review some single molecule mechanics such as examination of protein conformational equilibrium and folding kinetics at a single-molecule level, forces that contribute to the cantilever bend due to DNA hybridization, force of DNA packaging within virus and of DNA sequencing.

Mechanics of DNA Packaging in Viruses

Experimental Aspects

A new generation of single-molecule experiments using optical tweezers has opened up many of the fundamental processes of biochemistry and molecular biology from quantitative perspectives. Interesting results have been reported in the use of optical tweezers in measuring the mechanical forces exerted by molecular motors during the key biological processes such as the transcription of DNA[3] and the packing of a viral genome into its capsid during the infectious life cycle of a virus. Once the viral DNA is inside the bacterium, it steals the protein production machinery of the bacterium to synthesize its own proteins that will make up the capsid. As part of the viral infectious cycle, viruses must package their newly replicated genome for delivery to other host cells. The Bacillus subtilis phage ϕ29, with its 19.3 kb genome and ter-

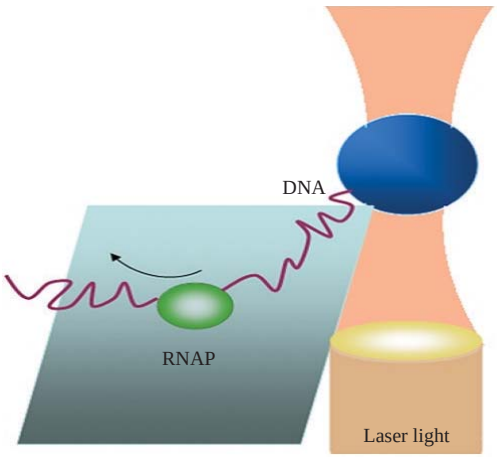

Figure 1. Force generation by DNA-dependent motors. A surface-bound RNA polymerase (RNAP) transcribes against a force exerted by an optical trap. RNAP generates about 25 pN of force (Ref. 39).

minal protein gp3, is an excellent model system for studying viral assembly. Fascinating experiments have been performed by Smith *et al*.[4] in measuring the DNA packaging force as a function of the fraction of viral genome that has been packed as shown in Figure 1. A simple theory of elasticity and an analytical model of charge and hydration forces are used to derive the force required to pack viral DNA into capsid[5,6]. Theoretical model showed good agreement with experimental observations. Bacteriophage ϕ29 packages all of its 6.6 μm long double-stranded DNA into a 42×54 nm capsid by means of portal complex that hydrolyzes ATP[4].

This is a remarkable process since entropic, electrostatic and bending energies must be overcome to pack the viral genome to nearly-crystalline density. By using force-measuring optical tweezers and pulling on single DNA molecule as they are packaged, Smith *et al*. obtained a force-velocity relationship and showed that the portal complex is a force generating motor[4]. This DNA packaging motor contains head-tail connector that couples rotation to DNA translocation. It shows that, on the average 57 pN force is generated indicating that the portal motor of the capsid is one of the strongest molecular motors known. A constant force feedback mode that maintained 5 pN tensions was used to measure DNA packaging which takes about 5.5 minutes on average. This translates to 20 bp/s packaging rate with 4 bp/s noise. There are 3.1 pauses per 1 μm of DNA packaged with mean duration of 4.0 seconds. More pauses are observed at higher capsid filling. During pauses the force stays constant indicating that the motor stays engaged. Apparently the motor loses grip on the DNA resulting in abrupt increase in their length, but can grasp the molecule and resumes packaging immediately. The length of a slip is about 44 bp and it occurs more often at a higher applied force. The packaging rate drops after 50% of genome is packaged. Initial packaging rate of ~100 bp/s drops to zero when fully packaged. The rate decrease is due to the internal pressure build up from the DNA confinement that exerts an opposing force. The force-velocity measurements suggest that an internal force of ~14 pN is acting on the motor. The force-velocity behavior also shows that the packaging rate decreases even for small forces indicating the rate-limiting mechanic-chemical cycle is force-dependent involving a mechanical displacement. The total force needed to stall the motor ranges between 40 and 70 pN with an average of ~45 pN. This makes ϕ29 one of the strongest molecular motors: eight times stronger than conventional kinetin or skeletal muscle myosin II motors, and two times stronger than RNA polymerase. The work done by the motor per ATP is estimated to be ~39 pN nm. This is obtained by multiplying the average stall force (57 pN) with the distance moved per ATP (~0.68 nm). With the assumption that the free energy of ATP hydrolysis is ~120 pN nm, the energy conversion efficiency is estimated to be ~30% which is comparable to those of myosin, kinetin and RNA polymerase motors. When the full genome has been packaged the internal force reaches a value of ~50 pN. One can obtain a rough estimate of a pressure of ~6 MPa inside the capsid by dividing the measured internal force by the hexagonal cell surface area. This is similar in order (~1 MPa) as the osmotic pressure necessary to condense DNA to a density similar to that of phage capsid. In order to withstand the pressure of ~6 MPa with the thickness of the capsid around ~1.5 nm, the tensile strength of the capsid must at least be in the order of several tens to ~100 MPa. This value is similar to that of the bulk tensile strength of a typical aluminum alloy. By integrating the force curve, an estimated total work done in packaging the ϕ29 genome is obtained to be $\sim 7.5 \times 10^{-17}$ J ($\sim 2 \times 10^4$ kT). A theoretical prediction for the equilibrium free energy change of DNA including electrostatic, bending and entropic penalties with no dissipative effects comes out to $\sim 5.6 \times 10^3$ kT. It is, therefore, possible that the bulk of the work done by the motor is reversible with little viscous dissipation. This experiment shows that, for ϕ29 the internal force provides the driving force for DNA injection into the host cell for the first half of the injection process.

Theoretical Aspects

The problem of DNA packaging in a viral capsid is

fascinating because it is recognized that the regions within which the DNA is packaged have linear dimensions that are comparable to the persistence length of the DNA, demanding a steep energy cost in accomplishing such packing. In order to characterize the mechanical forces that come into play during viral packaging, simple mechanical analysis of DNA elasticity was made[5,6]. Although many of the key viral capsids of interest are isooctohedral, we can approximate it to be a capped cylinder for the sake of simplicity. There are three factors that we need to take into account in characterizing the energetic of viral packaging: (i) the entropic effect that makes the DNA to take more spread out configuration than that in the viral capsid, (ii) energetics of elastic bending of DNA, and (iii) charge interaction of DNA with neighboring DNA and with surrounding solution. It is shown that the entropic effect is about one tenth of elastic and charge interaction energies; therefore, it is neglected in this analysis for the calculation of internal forces within the capsid. Simplifying the model with the DNA as a linear elastic beam with no torsion and twist, the bending energy can be written as

$$E_{el}(L)=\frac{\xi_p k_B T}{2}\int_0^L \frac{ds}{R(s)^2} \tag{1}$$

where R(s) is the radius of curvature of the DNA at arc length s. Neglecting the helical pitch and assuming that the DNA is packed in a hexagonal array, the stored elastic energy can be expressed as

$$E_{el}(L)=\frac{\pi\xi_p k_B T}{\sqrt{3}\,d_s/2}\int_0^{R_{out}} \frac{N(R')dR'}{R'} \tag{2}$$

where d_s is the spacing between adjacent loops, R_{out} is the radius of the capsid and R is the radius of the innermost set of loops, and $N(R')$ is the number of loops that are packed at the radius R'. This expression gives the elastic energy in terms of the inner radius R of the packed DNA. In order to compare with the experiments conducted by[4], we need to express the energy and force in terms of the length of the genome packed which can be given as

$$L=\frac{2}{\sqrt{3}\,d_s}\int_R^{R_{out}} 2\pi R' N(R')dR' \tag{3}$$

Simplifying the geometry of φ29 virus to a simple cylinder without the caps, the number of loops at radius R can be expressed by $N(R')=z/d_s$. The packed length for this configuration can be given by

$$L(R)=\frac{2\pi z}{\sqrt{3}\,d_s^2}(R_{out}^2-R^2) \tag{4}$$

The elastic energy as a function of packed length can now be expressed as

$$E_{el}(L)=\frac{\pi\xi_p k_B T}{\sqrt{3}\,d_s^2}\log\left(1-\frac{\sqrt{3}\,d_s^2 L}{2\pi z R_{out}^2}\right) \tag{5}$$

This energy expression can be used to find the elastic contribution to the packing force as a function of length packed by $F_{el}(L)=dE_{el}/dL$ yielding

$$F_{el}(L)=\frac{(\xi_p k_B T/2R_{out}^2)}{1-\sqrt{3}\,d_s^2 L/2\pi z R_{out}^2}. \tag{6}$$

The elastic effects must now be supplemented by interaction terms related to the presence of charges both on the DNA and in surrounding solution. In the purely repulsive regime, the force per unit length between adjacent DNA strands with spacing d_s is given by

$$f(d_s)=\frac{F_0}{\sqrt{3}}d_s\exp\left(\frac{-d_s}{c}\right), \tag{7}$$

where F_0 and c are constants that respectively characterize the strength and decay length of the interaction. The values of $c=0.27$ nm and various multiples of $F_0=55{,}000$ pN/nm^2 give the best fit to the experimental data[4]. The interaction energy per unit length needed to bring the strands together separated by d_s is

$$e(d_s)=3\int_\infty^{d_s} f(x)dx=\sqrt{3}\,F_0(c^2+cd_s)\exp\left(\frac{-d_s}{c}\right). \tag{8}$$

The total packing energy for a cylindrical geometry can be expressed as a sum of the elastic and the electrostatic interaction energies;

$$E(R,d_s)=L\sqrt{3}\,F_0(c^2+cd_s)\exp\left(\frac{-d_s}{c}\right)+\frac{\pi\xi_p k_B T}{\sqrt{3}\,d_s^2}\log\left(1-\frac{\sqrt{3}\,d_s^2 L}{2\pi z R_{out}^2}\right) \tag{9}$$

The equilibrium inter-strand spacing, d_s can be obtained by $\partial E/\partial d_s=0$ while holding L constant, while the packing force as a function of the percent of genome packed can be obtained by

$$F(L)=dE/dL;$$

$$F(R(L),d_s(L))=L\sqrt{3}\,F_0(c^2+cd_s)\exp\left(\frac{-d_s}{c}\right)+\frac{\xi_p k_B T}{2R_t^2} \tag{10}$$

It is of great interest to study the structural integrity of the capsid, when it is subjected to an internal pressure arising from the packaging of DNA. It is speculated[4] and theoretically estimated[7] that capsid can be subjected to pressure of ~60 atm. The expression for the pressure inside the capsid can be obtained by $p_i = -\partial E/\partial V$. For the spherical capsid with a volume, $V = 4\pi R_{out}^3/3$, we arrive at

$$p_i = -\frac{1}{4\pi R_{out}^2}\frac{dE}{dR_{out}} = -\frac{1}{4\pi R_{out}^2}\frac{\partial E_{el}}{\partial R_{out}} \quad (11)$$

Using expression (2) for a sphere, the elastic energy can be found to be

$$E_{el}(R) = -\frac{4\pi\xi_p k_B T}{\sqrt{3}\,d_s^2}\left(\sqrt{R_{out}^2 - R^2} + R_{out}\log\left(\frac{R_{out} - \sqrt{R_{out}^2 - R^2}}{R}\right)\right) \quad (12)$$

Substituting in relevant numbers for φ29, $R_{out} = 22.03$ nm, $d_s = 2.792$ nm, and $L = 6.584$ μm, we obtain $p_i = 60.3$ atm. Assuming that the capsid is a hollow sphere with the inner and outer radii of $R_i = 12.3$ nm and $R_o = 13.8$ nm, respectively, loaded by a pressure p_i from inside and a pressure p_o from outside, we can estimate the maximum stress sustained by the capsid wall using a result from theory of elasticity;

$$\sigma_T^{max} = \frac{3p_o R_o^3 - p_i(2R_i^3 + R_o^3)}{2(R_i^3 - R_o^3)} \quad (13)$$

This expression yields $\sigma_T^{max} \approx 25$ Mpa, which is nearly one forth of the bulk tensile strength of a typical aluminum alloy.

So far we have dealt with mechanics involving only a single biomolecule; however, in the presence of many molecules or a single biomolecule packed into a small space such as in a capsid, it may be helpful to develop a continuum theory taking into account the discontinuities that may influence the force interactions. To this end Klug and Ortiz have applied the concept of material force to biological systems[8]. This is reminiscent of the material momentum tensor concept developed by Eshelby[9], which were applied to defect mechanics of materials[10,11]. This demonstrates that the nanomechanics of biological systems can be a fertile ground for further research utilizing well-developed mechanics concepts. Finally, further experiments measuring DNA ejection forces as a function of solution conditions as well as DNA packaging forces will shed significant new light on the important biophysical problem of DNA packaging.

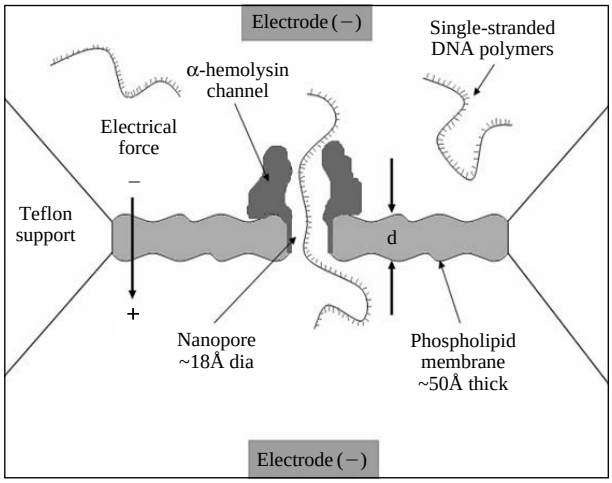

Figure 2. Negatively charged single-stranded DNA molecules and salt ions are electrically driven through a single α-hemolysin protein pore in phospholipid membrane. Most of the ionic current through the pore is blocked during DNA passage (Ref. 40).

Nanopores for Individual DNA Sequencing

Single DNA molecule can be examined using atomic force microscopy[12], video fluorescence microscopy, and force-measuring laser tweezers. One of the most simple and powerful applications of nanotechnology to biology is in measuring changes of ionic conductivity caused by threading an RNA or DNA molecule through a membrane ion channels also know as nanopores[13]. Nanopore systems are outstanding nano-scale biosensors that can detect biomolecules such as DNA and protein through label-free detection and an extremely simple structure[14,15]. The basic idea behind nanopores is that it can act as the molecular amplifier to detect single molecule by simply measuring the ionic current through an electrolyte-filled nanopore that spans an insulating membrane. When the nanopore is filled only with electrolyte, a voltage bias induces ions to flow through the nanopore by a tiny amount (~picoampere) that can be readily measured. When single or double stranded oligonucleotides are drawn into the nanopore by the voltage bias, the oligonucleotides partially obstruct the nanopore and reduce its ionic conductivity. Furthermore, if a DNA molecule could be induced to slowly translocate through a suitable nanopore, the atomic-scale changes in the chemical and electrostatic environment within the nanopore might be sufficient to directly reveal the sequence or the single nucleotide difference of the

translocating DNA as a series of ionic conductivity signature[16]. Such techniques allow direct microsecond-time scale nucleic acid characterization without the need for amplification, chemical modification, surface absorption, or binding of probes or intercalators that are currently being employed in DNA chips for sequencing and molecular diagnostics. It has also been suggested that an engineered nanopore[17] could be used for sequencing at higher rates. If we can harness this technique for the development of fast and low cost (less than $1,000) sequencing method, it can have an enormous commercial impact on genomic sequencing as the current technology takes months and costs several millions of dollars to sequence, for example, the human genome. It was shown in Figure 2 that[18] an electric field can drive single-stranded RNA and DNA molecule through a 2.6 nm diameter ion channel that was formed by a α-hemolysin channel inserted in a lipid bilayer. Such blockage should make it possible to use single channel recordings to characterize the length and possibly other characteristics such as individual sequence bases in single molecule of DNA or RNA[19]. The routine DNA analysis and sequencing will require a robust nanopore. Solid state nanopores perhaps with integrated nanosensors would be necessary, but today's fabrication methods will have to be substantially improved to develop an electrically addressable array of pores with reproducible diameters in the required nanometer range. Better physical models of the molecular interactions and ion current flow within the nanopore will have to be developed so that much more information could be obtained from the electrical signals[20]. Nanometer sized sensors on or around the pore should also be developed along with a nanofluidics platform that can induce electrophoretic translocation of DNA strands through a nanopore.

Mechanics of Formation of DNA Self-assembled Monolayers and Hybridization

Nanomechanical biosensors are commonly based on the beam bending method, which measures differences in the surface stress between the opposite sides of a microcantilever that are different in composition. Hybridization of ssDNA attached to the cantilever with complementary DNA produced a measurable deflection of the cantilever whose magnitude and sign have varied in different reports[21-25]. To determine the forces that contribute to the cantilever bending, the nanomechanical signal during the attachment of the ssDNA probes to the microcantilever was measured and compared to the hybridization signals[26-29]. The nanomechanical response is not strictly governed by the number of adsorbed DNA molecules, and it critically depends on the nature of the interaction between the cantilever surface and the DNA[30,31]. The forces intervening in the surface stress can be split into (i) the reduction of conformational entropy of the DNA when it is anchored to the surface and neighboring molecules limit the thermal motion, (ii) the electrostatic double layer repulsion arising from the osmotic pressure between the counterions that surround the negatively charged DNA molecules, and (iii) the repulsive hydration forces resulting from the perturbation of the hydrogen network in water by the DNA molecules[24,32,33]. Finally, as occurs in the immobilized single-stranded DNA, a significant contribution of the hydration force is not expected during hybridization. The main source of surface stress during the immobilization is the covalent bond between the surface atoms and the atoms of the DNA probes. In contrast, the only contribution to the surface stress during hybridization is the intermolecular forces between neighboring DNA molecules. The detection of nucleic acid hybridization with nanomechanical sensors needs reference cantilevers sensitized with noncomplementary DNA to decouple the molecular recognition signal from nonspecific signals[31].

Mechanics of Protein Unfolding and Refolding

In the last decade, single-molecule manipulation techniques, based on optical tweezers and atomic force microscopy have been developed[6]. These make it possible to characterize the physical properties of individual protein molecules, one at a time. AFM has been used to study how an external force unfolds and drives a protein molecule towards non equilibrium conformations, and how its chain relaxes when the tension is afterwards gradually released[34]. Different modes of application of the force have been developed for this kind of single-molecule manipulation experiment. The molecules have been pulled while clamping either the pulling velocity or the applied force. In velocity-clamp modes, a protein molecule can be mechanically unfolded by binding its ends to an AFM cantilever tip and to a flat surface that are moving apart at constant velocity by the piezoelectric actuator. The force applied to the molecule is constantly monitored by the deflection of the AFM cantilever and reported as a function of the molecular extension. Whereas in force clamp modes, AFM instrument that unfold protein mechanically either keeping

the applied force constant at a set value or increasing it linearly over time[35]. The function thus obtained by holding the pulling force constant seemed to suggest that the mechanical unfolding of this module could be described by a simple two-state (folded and unfolded) Markovian process[36]. The ramp mode exposes the protein to increasingly higher forces, causing all of the modules to unfold within a much shorter time. When the force-ramp mode was applied to the study of the unfolding of ubiquitin, Fernandez and co-workers also observed unfolding events that followed a path through multiple steps that could not be described by a two-state Markovian process. Individual molecules followed different trajectories[37]. This is further clear evidence of the importance of performing experiments at the single bio molecular level. Finally, unfolding and refolding at controlled force can be performed using force-step mode. After having stretched a molecule of this protein at a constant high force, the force was quenched to a lower value and kept constant, letting the molecule refold. Afterwards, they raised the force again in order to control whether the refolding was successful[38]. The AFM-based setups ensure a better positional resolution and less limitation in time resolution compared to those based on optical tweezers, but this is accompanied by a higher force noise. The choice of optical tweezers instruments is still restricted for mechanical measurements on processes that involve forces on the scale of a few pN. On the other hand, the gap in the force sensitivity between these two kinds of apparatuses will be drastically reduced by the advent on the market of smaller AFM cantilevers and instruments that can take full advantage of them.

Conclusions

These examples illustrates the increasing prominence of mechanical measurements as powerful tools for obtaining unique information that is central to understanding biomolecules like DNA or protein function. We presented significant new information on a biologically important model system that will allow further testing of biophysical theories that describe DNA bending and DNA interaction. The possibility of singling out and characterizing folding trajectories of individual molecules with a spatial resolution on the order of a few fractions of a nanometer can help to discover how the secondary structure elements can form and collide during folding. The needs for a major understanding of the mechanisms responsible for surface stress due to the biomolecular interactions have also been highlighted. This knowledge is crucial for the development of immobilization procedures in which the geometry of the receptor molecules is addressed to generate high interaction forces between neighboring molecules during molecular recognition. More generally, the resulting discovery of physical principles relating chemistry to nanomechanics continues to open up new approaches to investigating fundamental principles of biomolecule structure and function.

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