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Nanomechanics of biomolecules: focus on DNA †

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Abstract

Nano-mechanical measurements and manipulations at the single-cell and single-molecular levels using the atomic force microscope (AFM) and optical tweezers are presenting fascinating opportunities to the researchers in bioscience and biotechnology. Single molecule biophysics technologies, due to their capability to detect transient states of molecules and biomolecular complexes, are the methods of choice for studies in DNA structure and dynamics, DNA-DNA and DNA-protein interactions, and viral DNA packaging. The aim of this review is to describe the recent developments of scientific tools and the knowledge gained in single molecule DNA mechanics such as DNA elasticity, electrostatics, condensation and interactions of DNA with surrounding fluids during its hydrodynamic flow.

Keywords: Nanomechanics; DNA; Optical tweezers; Atomic force microscopy

1. Introduction

The engineering and science discipline of mechanics is the study of force, motion and deformation. Throughout history, mechanics has played a central role in the development of human civilization. Aside from more traditional fields such as automotive, civil, and aerospace engineering, mechanics reached out to other disciplines such as micro-electro-mechanical systems (MEMS), microelectronics, materials and geophysics [1, 2]. Another field where mechanical principles of solids and fluids can be usefully applied is in understanding the biological processes. There are recent findings that mechanical forces can influence cell growth, differentiation, locomotion, adhesion, signal transduction, gene expression and a host of significant biological processes that occur inside a cell [3]. On a micrometer scale, mechanics can be fruitfully applied in studying the motion of bacteria and other microorganisms. Using the variational principle of mechanics, the optimal shape of red blood cells can be determined [4]. In addition, with the recent developments in nanotechnology and nanofabrication, sophisticated nanomechanical tools such as optical tweezers and AFM now make it possible to manipulate a single biomolecule through application and measurement of piconewton forces. In particular, a variety of AFM approaches have been developed for investigating native cell surfaces with piconewton (nanonewton) sensitivity and nanometer lateral resolution, providing novel information on the nanomechanical properties of cell walls, on surface forces such as van der Waals and electrostatic forces, solvation and steric/bridging forces, and on the forces and localization of molecular recognition events [5-8]. Hence, many fascinating problems on the nanometer

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scale that involve the rich interplay between statistical and deterministic forces can be solved [9, 10]. This will allow better understanding of how biochemistry, which is a discipline that deals mostly with conformation, binding, reaction, and transport of biomolecules, couples together with mechanics in revealing how thermal, chemical, mechanical and electrical forces interact on the cellular and molecular level [3]. In achieving this goal, the mechanics of biomolecules differs from traditional engineering mechanics in that the molecular biomechanics needs to be integrated with thermodynamics, statistical mechanics, biochemistry and molecular biology. In this paper, a review is made on single-molecule DNA mechanics that forms the foundation for understanding more complicated biological processes including DNA elasticity, electrostatics of DNA, counterion-induced DNA condensation and DNA in hydrodynamic flow.

2. Tools of the trade

2.1 Optical tweezers

Optical tweezers, also known as laser-based gradient-force optical traps, have been around since the early 1970's and have enjoyed a wide range of applications. In particular, they played a crucial role in enabling researchers to manipulate single biomolecules [11]. A strongly focused laser beam has the ability to catch and hold dielectric particles of a size ranging from nanometer to micrometer (Fig. 1). By using light to trap microscopic objects such as beads that can be attached to a single biomolecule, optical tweezers can be an indispensable tool for ultra fine positioning, measurement and control. Forces up to 200 pN with sub-pN resolution can be applied on objects whose dimensions are of the same order as the wavelength of light [11]. Spatial resolution for positioning and detection can range from micrometers down to angstroms. Due to their extremely good controllable force-exerting properties, optical tweezers are useful for many nanomechanical measurements particularly in biological applications [12]. The apparatus ranges from simple, lens-based traps to complex integrated optical instrument. Biological objects such as microtubules, DNA molecules, lipid membranes, intact or fractionate cells and single biomolecule have been studied extensively with optical tweezers [12-15]. A study on the rate of DNA packaging by single bacteriophage φ29 portal motors under a fixed applied force was measured [13] by using an optical tweezers instrument. Mechanical folding and unfolding of proteins or nucleic acids, receptor-ligand binding interactions and nanoscale mechanics of biological motors are being successfully studied in the biophysics community [16].

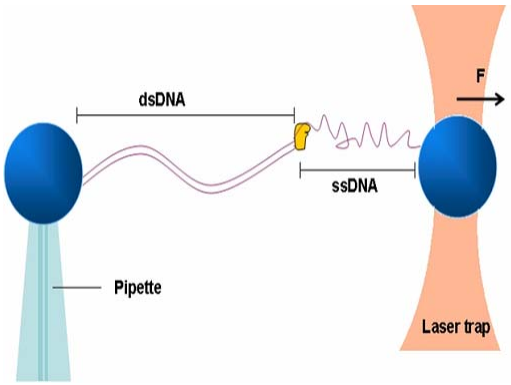

Fig. 1. Stretching of a DNA molecule between beads held in a micropipette and a force-measuring optical trap [38].

2.2 Atomic force microscopy (AFM)

AFM, a member of the family of scanning probe microscopes, was developed to image non-conducting samples which differ from its counterpart: the scanning tunneling microscope [17]. AFM uses a microscale cantilever with a fine tip to scan the surface of the samples, and the deflection of the cantilever is utilized to get information about the surface properties [18, 19]. Typically, these micro-cantilever systems are operated in three open-loop modes: non-contact mode, contact mode, and tapping mode. By a feedback control mechanism, the Z-position of the sample is adjusted such that the cantilever deflection (and hence the applied force) remains constant at an externally preset value. Recording this displacement along the Z-axis together with the lateral position of the tip allows generating a topographical image of the sample [20]. To probe electric, magnetic, and/or atomic forces of a selected sample, the non-contact mode is utilized by moving the cantilever slightly away from the sample surface and oscillating the cantilever at or near its natural resonance frequency. Alternatively, the contact mode acquires sample attributes by monitoring interaction forces while the cantilever tip remains in contact with the target sample. The tapping mode of operation combines qualities of both the contact and non-contact modes by

gleaning sample data and oscillating the cantilever tip at or near its natural resonance frequency while allowing the cantilever tip to impact the target sample for a minimal amount of time [21]. Over the last two decades AFM as a valuable biophysical tool has been established to probe the mechanics of different types of cells [22, 23], proteins [24, 25] and DNA [26, 27] on the nanometer size scale. A unique feature of this instrument is its capability to operate in two different modalities, topographic imaging and measuring of intermolecular interactions. The structure, dynamics, elastic properties or Young's modulus of DNA and drug-DNA interaction at the single molecule level have been investigated by using this powerful instrument [26-29]. AFM has also been most extensively employed in measuring the consequences of mechanical extension of protein domains [30].

3. Mechanics of single molecule DNA

3.1 DNA elasticity

The elasticity of DNA has important biological implications, in that its bending and twisting rigidities affect how it wraps around histones to form chromosomes, super coils during replication, bends upon interaction with proteins, and packs into the confined space of a virus [31, 32]. The DNA double helix has unique physical properties with unusually high stiffness compared to other natural or synthetic polymers. Double-stranded DNA (dsDNA) takes about 50 times more energy to form a circle than a single-stranded DNA (ssDNA). The phosphate backbone of DNA has a unit charge per base per strand, hence making it one of the most charged polymers [33]. Then, what is the energetic cost to package a negatively charged polymer with known elasticity into a small container when a fixed concentration of cations is available to screen the electrostatic repulsion? First, the elasticity of the DNA itself may depend on cation concentration. Second, the interaction energy between portions of the DNA molecule is not purely electrostatic. Additional energetic costs for condensing DNA into a small space arise from hydration changes, in which water is excluded from space occupied by the DNA, and from entropic effects due to changes in the number of available molecular configurations for a given condensed structure [12]. This makes it necessary to take into account the electrostatic charge interactions, in addition to the entropic and bending energies in studying the conformational dynamics of nucleic acids. These unique physical properties are exploited in the molecular level biological processes of copying, transcribing and packaging. An example of this protein machinery is the RNA polymerases which act as motors capable of moving along torsionally constrained DNA molecule. The RNA polymerase synthesizes RNA from a DNA template by unwinding the double helix to provide single-stranded template. Polymer's electrostatic potential guides DNA-binding proteins to cling to DNA while they move along the molecule in search of the target sequence. To avoid building up of torsional strain ahead of the replication fork due to the helical nature of DNA, topoisomerases break and rejoin the DNA during the transcription process. Basic study of DNA elasticity, therefore, is needed to lay the foundation in understanding the mechanism of single-molecule enzyme assays.

A double-helical DNA chain (B form) is about 2 nm in diameter and has ten base pairs (bp) per helical turn. A DNA chain behaves as a rod over short lengths, but over long contours it behaves as a flexible chain that can bend in arbitrary directions forming a random coil. The Gaussian chain model that treats the polymer as a chain of statistically independent segments is the simple model for this behavior. Under small forces, the Gaussian chain model shows a linear relationship between force f and the mean extension x,

$$\frac{f\xi_p}{k_B T} = \frac{3}{2}\frac{x}{L}, \tag{1}$$

where k_B is the Boltzmann constant, T is the absolute temperature ($1\ k_B T = 4.3$ pN nm at 37° C), ξ_p is the persistence length and L is the contour length (fully extended DNA length). The persistence length is an intrinsic parameter of a polymer chain and can be interpreted as the measure of resistance to shape change of the chain due to thermal fluctuations. For double-stranded DNA the persistence length is ~50 nm [34]. The worm-like chain (WLC) model describes the DNA elasticity more accurately through the Lagrangian function [35]:

$$E = \frac{k_B T}{2}\int_0^L \xi_p \kappa^2 ds - fx \tag{2}$$

where E is the elastic bending energy and κ is the curvature. The last term is the work done by the applied force. This can further be approximated by an interpolation formula,

$$\frac{f\xi_p}{k_BT} = \frac{x}{L} + \frac{1}{4(1-x/L)^2} - \frac{1}{4} \tag{3}$$

When the applied force is less than 12 pN, the force versus extension relationship can be accurately modeled by WLC model with ξ_p =53.4 and L =32.8 nm. Eq. (3) shows linear spring behavior for small extension, but for large *x* the behavior is nonlinear. Hence, the model needs to be modified with an assumption that the extension of DNA backbone depends linearly on the applied force [36] yielding

$$E = \frac{k_BT}{2}\int_0^L \xi_p \kappa^2 ds - \frac{1}{2}\int_0^L K\left(\frac{s}{s_0}-1\right)^2 ds - fx \tag{4}$$

where K is the stretch modulus and s_0 is a reference length (0.34 nm/bp). The local strain can be expressed as $\Delta s/s_0 = (s-s_0)/s_0$. Further approximating DNA as a thin cylindrical rod made of homogeneous linear elastic material with Young's modulus Y and radius $R \approx 1$ nm, the stretch modulus and the persistence length can be expressed as

$$K = \pi R^2 Y \quad \text{and,} \tag{5}$$

$$\xi_p = \frac{YI}{k_BT} = \frac{\pi R^4 ED}{4k_BT} \tag{6}$$

respectively.

For λ-phage DNA in a solution containing 150 nM Na+, it was found that $Y \approx 300$ MPa and $K \approx 1000$ pN [32]. Solving Eq. (5) for end-to-end distances longer than the contour length gives the force-extension relationship that is valid for $12 \le f < 65pN$

$$\frac{x}{L} = 1 - \frac{1}{2}\sqrt{\frac{k_BT}{f\xi_p}} + \frac{f}{K} \tag{7}$$

A double-stranded DNA may undergo a phase transition from B-form DNA (0.34 nm per bp) to S-form DNA (0.58 nm per bp) when the applied force reaches near 65 pN with a dramatic increase in extension similar to a yielding behavior in metals [37]. With the further increase in the applied load, the DNA may be overstretched to a point of unwinding and unstacking of molecules or even a breakage of covalent bonds in the backbone structure can occur [38]. It has been reported that the force-extension behavior of λ-phage DNA is not sensitive to the loading rate; however, the transition from double-stranded DNA into two single-stranded DNA can occur at higher loading rate [39].

AFM-based single molecule force spectroscopy (AFM-SMFS) has become an important tool to study the elasticity and conformations of nucleic acids, proteins, and polysaccharides [40]. In AFM-SMFS, two ends of a molecule are anchored to the substrate and the AFM cantilever tip, respectively. The molecule is mechanically stretched and its length and tension are measured with a subnanometer and piconewton resolution. By systematically varying the position of the attachment point on the substrate relative to the AFM tip, Ke et al. [41] investigate empirically and theoretically the effect of the pulling geometry on force extension characteristics of double-stranded DNA. Similarly, Wenner and coworkers [42] have measured the elasticity and overstretching transition as a function of monovalent salt concentration by stretching single DNA molecules in an optical tweezers apparatus.

3.2 Electrostatics of DNA

All biological helices produce strong electric fields near their surfaces, whether they are highly charged like DNA or zwitterionic like α-helices or collagen. These fields are important for biological function. In simplified models the helical structure is neglected and the molecule is approximated by a charged line or cylinder. All-atom computer simulations account for the structure, but they are usually applied to quantify local events, for example, binding of counterions or ligands. Both of these approaches have already been well reviewed [43]. The electrostatics of highly charged molecules in an electrolyte solution is more complicated. Even for homogeneously charged cylinders, pertinent models of charge screening are still debated in the literature. The most conceptually simple and therefore popular approach used to calculate the electrostatic potential inside an electrolyte solution is based on the Poisson-Boltzmann (PB) theory. Theoretical arguments suggest that the PB theory works so well because counterion-surface correlations, in the case of ions with low valence, are more important than correlations between electrolyte ions [44, 45]. However, practical applications of the PB theory usually rely on the electrostatic potential outside rather than inside the dense layer of water and ions at the surface. PB theory can be reduced to the simpler Debye-Hueckel (DH) theory, since it can be applied everywhere outside the dense layer of water and ions

at the surface. With corrections for ion size, the DH theory gives accurate predictions for ion solvation energies and activity coefficients up to physiological concentrations [46]. Furthermore, the DNA charge in cells is neutralized primarily by polycations, including polyamines (e.g., spermine and spermidine are present in cells in millimolar concentrations) and basic polypeptides (e.g., protamine and tails of histone proteins). An important feature of their interaction with DNA is that hydrogen bonding exists between their amine groups and oxygen atoms on the phosphates and bases, potentially resulting in a large chemical contribution to the binding energy [47]. The helical harmonics in the potential of DNA can be neglected only at very large distances. When the DNA charge is completely neutralized (e.g., due to chemisorption of such counterions as spermine or spermidine), the helical harmonics entirely determine the potential at any distance. The electrostatic potential created by DNA is significantly different from that of a charged cylinder at almost all distances relevant for the biology or the physics of DNA aggregates.

The Donnan model provides a simple illustration of how to calculate the electrostatic potential within a dilute polyelectrolyte assembly and an estimate of when to expect a nonlinear potential. But it cannot be used for calculating the energy of an aggregate of parallel charged rods because it oversimplifies the charge distribution. Instead, a combination of the nonlinear PB theory with a cylindrical cell model is commonly used as a better approximation [48, 49]. An alternative approach for calculating the electrostatic energy builds on the idea of counterion condensation is linearized PB theory with renormalized charge. The benefit of this model as compared to the nonlinear PB description is its flexibility in handling the degree of counterion condensation. The main limitation is that this model requires the thickness of the layer of condensed and adsorbed counterions to be small compared to the rod or cylinder radius [50, 51]. Interactions between DNA molecules have been studied at different ionic strength, composition of bulk electrolyte, temperature, and so on [52-58]. In many cases, DNA forms cholesteric and line hexatic rather than columnar assemblies. The short-range repulsive and midrange attractive forces between DNA in the presence of several polyamines and cobalt hexamine have been carefully mapped by combination of osmotic stress and magnetic tweezers measurements [59]. The amplitude of the attraction was dependent on the counterion. The authors interpreted the attraction and the short-range repulsion as hydration forces. Alternatively, these forces can be interpreted as the sum of electrostatic zipper attraction and image repulsion (the net charge of DNA under conditions of these experiments was small, $\theta \approx 0.9–1$, as indicated by the absence of longer-range electrostatic repulsion). It was therefore concluded by Rhodes and. Klug [60] that "in …fibers of DNA, the molecules still interact, however weakly, through several layers of water and influence each other to produce an integral screw." This appeared to be the property of fibers from long, natural DNA. The structure of synthetic DNA oligomers in crystals was determined to be closer to the nonideal helical structure in solution than to the ideal structure in fibers [61]. Some of the experimental observations can be rationalized in terms of the existing models, providing some confidence of theories being on the right track. However, few ideas have been rigorously proven and many experimental observations still seek explanation [16].

3.3 Counterion-induced DNA condensation

When added to DNA solution, some counterions induce aggregation of DNA double helices. On addition of these condensing agents, a single (or many) long DNA molecules will condense primarily into dense toroidal structures and, to a lesser extent, rod like particles [62-69]. The only reasonable point like cations that condense B-DNA without significantly affecting its structure (as confirmed by x-ray diffraction) are divalent Mn_{2+} and Cd_{2+}[57, 70]. Interestingly, unlike Sp, Spd, and Co-hex, Mn_{2+} condenses DNA much more efficiently at elevated temperatures, e.g., $MnCl_2$ condenses DNA only above 40-45 °C. In 150 mM Mn $(ClO_4)_2$, DNA is condensed already at 5 °C [57], but the strength of the attraction between DNA molecules still increases with increasing temperature, as indicated by decreasing interaxial spacing [49] and measured intermolecular forces [71]. Notably, the alkaline earth ions Ca_{2+} and Mg_{2+} do not cause DNA condensation at the same conditions [57, 70, 72]. Counterion-correlation forces are likely to be a factor only in DNA condensation by cobalt amines. But, even in this case, they are not likely to be the only or the dominant condensation mechanism. For homogeneously charged rods the larger and more hydrophobic Co-sep_{3+} ion would be expected to have a lower binding constant, in comparison to Co-hex_{3+}. There-

fore, one would predict a higher concentration necessary for the onset of DNA condensation. The experimental observations are exactly opposite. The preferential condensation by one of the Co-en_{3+} stereoisomer is equally difficult to explain within this mechanism. It comes into conflict with the basic idea of ion correlations based on the ability of condensed counterions to move freely along the DNA surface. Instead, such stereoisomer specificity suggests the importance of preferential binding at some specific sites, which can be described only if one incorporates the DNA structure into the theory [16].

The electrostatic zipper mechanism of DNA condensation requires only the following. (i) A sufficient fraction of the DNA charge should be neutralized. Hence the condensation is not induced by monovalent metal ions. (ii) A sufficient fraction of condensed counterions should be located in the major groove. Hence the condensation is not induced by Mg_{2+} and Ca_{2+} which have very high affinity for phosphates and preferentially adsorb onto phosphate strands and/or the minor groove [73-75]. A distinct set of predictions for structure-dependent electrostatic interactions between DNA has been formulated within the electrostatic zipper model. In particular, this model predicts that counterion adsorption in the major groove of DNA will promote a zipper-like alignment between the negatively charged strands and positively charged grooves on opposing helices, resulting in intermolecular attraction and aggregation [16].

3.4 DNA in hydrodynamic flow

Future development of lab-on-a-chip for molecular diagnostics will require single-molecule detection [76] that involves hydrodynamic flow of DNA in nanometer size channels (Fig. 2). To understand the interaction between DNA molecules and the surrounding fluids during hydrodynamic flow, controlled experiments were performed to measure relaxation time [77], hydrodynamic drag [78] and conformational dynamics [79]. Study was made on the passage of DNA molecules driven by an electric field through a microfabricated channel with 90 nm size constriction to characterize the motion of long DNA polymer in an artificial channel with entropic traps [80]. To understand the behavior of dilute polymers in an elongation flow, stretching of individual polymers in a spatially homogeneous velocity gradient was observed through the use of fluorescently labeled DNA molecules [81]. The relaxation time is defined as the time needed to find equilibrium configuration by the Brownian forces after an initial perturbation from equilibrium is made. This was measured by attaching a bead to one end of a DNA molecule while exposing it to a uniform flow and recording the time it take for fully extended molecule to recoil after the flow is stopped. Dependence of DNA extension on the contour length L, flow velocity and solution viscosity can also be measured by using a similar experiment [82]. DNA molecules in a plane Couette flow can be subjected to rotational and elongation flow, but unlike a tumbling motion of rigid ellipsoidal particles, the dynamic behaviors of DNA molecules can be more complex due to their deformation.

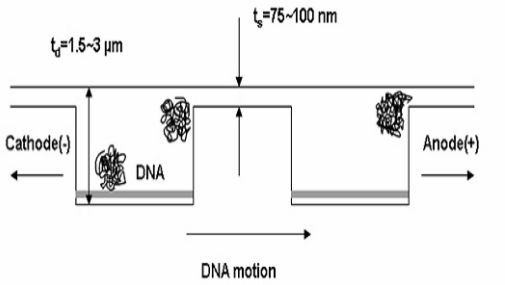

Fig. 2. Cross sectional view of the nanofluidics separation device with many entropic traps. Electrophoresed DNA molecules are trapped in thin region because their radius of gyration is much larger than the thin region depth [83].

4. Conclusion

We have shown in this review article that mechanics can play an important role in advancing biotechnology and biological science. Molecular biomechanics issues in functional genomics, proteomics and nanobiotechnology have only begun to attract attention of mechanics researchers. By working with biologists they can contribute significantly in gaining understanding of protein and DNA conformational dynamics, diffusion, reaction, transport of biomolecules, and the structure-function relationship of molecular motors and machines. With the rapid development of advanced tools and technologies such as nanofabrication and single molecule nanomechanics, systematic and quantitative studies of biological processes at the molecular level can further be made. This presents great opportunities for engineers and biological scientists to work together synergistically in carrying out breakthrough research that can have

significant scientific and commercial impact.

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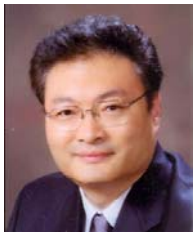

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