Mechanics of biomolecules

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Abstract

Over the last few years molecular biomechanics has emerged as a new field in which theoretical and experimental studies of the mechanics of proteins and nucleic acids have become a focus, and the importance of mechanical forces and motions to the fundamentals of biology and biochemistry has begun to be recognized. In particular, single-molecule biomechanics of DNA extension, bending and twisting; protein domain motion, deformation and unfolding; and the generation of mechanical forces and motions by biomolecular motors has become a new frontier in life sciences. There is an increasing need for a more systematic study of the basic issues involved in molecular biomechanics, and a more active participation of researchers in applied mechanics. Here we review some of the advances in this field over the last few years, explore the connection between mechanics and biochemistry, and discuss the concepts, issues, approaches and challenges, aiming to stimulating a broader interest in developing molecular biomechanics.

Keywords: A. Chemo-mechanical processes; B. Biological material; B. Constitutive behavior; C. Mechanical testing; A. Mechano-chemical transduction

1. Introduction

It is well known that mechanical forces can influence the growth and form of plants and animals. Bone, for example, loses density without gravitational force. Cells that form the wall of a blood vessel behave abnormally without shear flow. Recent studies have confirmed that mechanical forces can affect cell growth, differentiation, locomotion, adhesion, signal transduction, and gene expression. It is not clear, however, how biological tissues and cells sense mechanical forces or deformations and convert them into biological responses. Nor is it well understood how chemical energy is converted...
into directed motions and mechanical forces in cells. In general, there is a lack of understanding of how mechanics and biochemistry are coupled together at the cellular and molecular levels. The key to addressing all these issues is to study the motion and force-induced conformational changes of biomolecules.

As deformable bodies, biomolecules such as proteins, nucleic acids and carbohydrates change their 3D configurations (conformations) under mechanical force. In general, a biomolecule can change its conformation with changes in temperature, solvent chemistry or molecular interactions. Here we use ‘deformation’ to refer to conformational changes due to mechanical or Brownian forces; we treat the unfolding and relative motion of globular domains of a protein as special cases of deformation. How, then, does a biomolecule deform under force? Why do we care? How do such deformations affect biochemical processes in living cells? How do molecular motors generate force and motion? In the following sections we review some of the progress made over the last decade or so in revealing the deformation and dynamics of biomolecules, explore the connection between mechanics and biochemistry, and discuss the concepts, issues, approaches and challenges, aiming to stimulating a broader interest. Emphasis is placed on the basic discoveries and new concepts in molecular biomechanics; more technical details may be found in the references.

2. The biological relevance of mechanical forces

Like all other living systems on earth, the human body is subject to gravitational force. Further, from embryonic development to maturation, various organs and tissues in a human body are subjected to shear flow or other mechanical forces or deformations. These forces are spread amongst cells, causing cellular deformation, altering cell–cell and cell–ECM (extracellular matrix) interactions, and inducing changes to cell behavior and functions.

As the basic unit of life, cells are complex biological systems. To perform their specialized functions, cells must express genetic information; synthesize, modify, sort, store and transport biomolecules; convert different forms of energy; transduce signals; maintain internal structures; and respond to external environments. All of these processes may involve one or more mechanical aspects. For example, in cell migration, contractile forces are generated within the cell which ‘pull’ the cell body forward (Fig. 1a). In cell division, the two copies of each chromosome in the mother cell must be pulled apart in order for the daughter cells to be born (Fig. 1b). In protein secretion, protein molecules are packaged in vesicles and transported to the cell membrane by molecular motor running along filaments in cells.

Conversely, mechanical forces and deformations can induce biological responses in cells. There is ample evidence to suggest that many normal and diseased conditions of cells are dependent upon or regulated by their mechanical environment. The effects of applied forces (or deformations) depend on the type of cells and how the forces are applied to, transmitted into, and distributed within cells. Before having more detailed discussions on the molecular issues, it may be helpful to review the structural basis of a typical animal cell. As shown schematically in Fig. 2a, in a rather simplistic view, a
cell is a ‘cage’ (cytoskeleton) wrapped by the plasma membrane and trapping inside it a nucleus surrounded by the ‘soup-like’ cytoplasm. The cytoskeleton is a system of protein filaments—mostly microtubules, actin filaments and intermediate filaments—that gives the cell its shape and the capability for directed movement (Fig. 2b). In a physiological environment many types of cells are attached to the extracellular matrix (ECM), a complex network of polysaccharides and proteins secreted by cells, which serves as a structural element in tissues. Proteins in the ECM, including collagen, fibronectin, vitronectin and laminin, also play a regulatory role of cellular function through binding to various receptor proteins, found on the cell surface (Fig. 2c).

For example, some of these receptors are members of the integrin family of transmembrane proteins. They are composed of two units, α and β (Fig. 2c) and are expressed on the membrane of a wide variety of cells. Attached to the cell cytoskeleton, integrins are critical to the mechanical stability of cell adhesion to the ECM and to other cells. They also serve as biochemical signaling molecules in normal and diseased states of cells, and are involved in regulating cytoskeletal organization. Recent experiments indicate that mechanical forces can influence the association of integrin with the cytoskeleton to form “focal adhesion complexes” (Chicurel et al., 1998a). Further, integrins are likely a major force transmitter, since they provide the mechanical linkage between the cell cytoskeleton and ECM. As such, cells may ‘sense’ mechanical forces or deformations through both integrin-mediated cell–ECM interactions (Chicurel et al., 1998b) and the subsequent force balance within the cytoskeleton. This balance may play a crucial role in regulating the shape, spreading, crawling, and polarity of cells. The question is again how mechanical force balance is being recognized by cells and transduced into biological responses.
Fig. 2. (a) With an average size of 10–30 μm, an animal cell consists of a cytoskeleton wrapped by the plasma membrane, and a nucleus surrounded by the cytoplasm containing mitochondria, Golgi apparatus, and other organelles. (b) The cell cytoskeleton consists of microtubules, actin filaments, intermediate filaments and other binding proteins. (c) Adherent cells attached to the extracellular matrix (ECM) through the focal adhesion complex consisting of integrin and other binding molecules.
Despite extensive research efforts over the last few years, the exact molecular mechanisms responsible for mechanochemical transduction in living cells remain unknown. However, a number of candidates have been proposed. For example, it is possible that ion transport through the cell membrane can be altered by mechanical forces, especially tension in the cell membrane (Hu and Sachs, 1997), thus changing the biochemical processes in cells. It is also possible that components of the cell cytoskeleton, such as actin filaments and microtubules, deform under mechanical forces (Arai et al., 1999), inducing conformational changes of other proteins attached to them (including motor molecules), and altering their functions. Further, ECM molecules such as fibronectin may deform under force, changing their interactions with cell surface receptors including integrins. It has been found that when the ECM is disrupted, fibronectin can contract to a fraction of its original length (Ohashi et al., 1999). This may serve as a mechanosensitive control of ligand recognition (Krammer et al., 1999). It is also likely that integrins change their conformations under force, altering their binding to the ligands and thus changing the downstream biochemical processes. In general, a ligand is any molecule that binds to a specific site on a protein or other molecules, and a receptor (intracellular or on a cell surface) refers to a protein that binds to a specific extracellular signaling molecule (ligand) and initiates a response in the cell. Existing experimental results suggest that the binding specificity and affinity between a receptor and a ligand can be changed by mechanical forces.

It has become clear over the last few years that the deformation of nucleic acids and protein molecules under mechanical force may be the key to understanding mechanochemical transduction in cells (Bao, 2000). In a broad sense, it may also play an essential role in understanding the connection between mechanics and biochemistry, which is a critical aspect in mechanics in general and biomechanics in particular. Deformation of biomolecules under force and mechanical forces or motions generated by motor proteins are just examples of this fundamental connection. In simple words, mechanics is the study of force, motion and deformation, while biochemistry involves the conformation, binding/reaction and transport of biomolecules. There is ample evidence suggesting the connections between the two; however, fundamental understanding of this connection needs to be further established (Zhu et al., 2000).

Theoretically, the deformation of DNA or protein molecules under applied forces $F_\alpha$ may be analyzed by determining the Gibbs free energy of a system

$$G = U - \sum F_\alpha \ell_\alpha - TS,$$

where $U$ is internal energy, $S$ is entropy, $T$ is temperature, $F_\alpha$ are generalized forces including pressure, and $\ell_\alpha$ represent generalized displacements (Reichl, 1980). The system considered here includes not only protein, DNA or RNA molecules under study, but also the surrounding solvent (water molecules, ions, and other small molecules such as sugars), and the internal energy $U$ includes chemical potentials. Under applied forces, the deformation of the biomolecules can be determined by the equilibrium condition $dG/dx_i = 0$, i.e., the conformational changes of the molecules are such that the Gibbs free energy attains its global minimum with respect to the variations in position $x_i$ of all the atoms.
The Gibbs free energy $G$ in Eq. (1) may include the contributions from intermolecular and surface forces such as covalent and Coulomb interactions, van der Waals forces, electrostatic forces, hydration and hydrophobic forces, and steric and Brownian forces, in addition to mechanical forces (Israelachvili, 1992). These forces represent physical and chemical interactions among atoms and molecules in the system. Thus, the interplay among internal energy, mechanical work and entropy as indicated in Eq. (1) underlies the fundamental connection between mechanics and biochemistry. Of particular importance is the entropy $S$ of the system which is determined by the total number of admissible conformations (or configurations) of the biomolecules, along with their interactions with the solvent (such as the formation of ‘caged’ water molecules). The second law of thermodynamics dictates that an isolated system with constant energy and volume attains its maximum value of entropy at equilibrium. For example, a polymer chain such as free DNA in solution has a random coil conformation which maximizes the entropy. Elongation of a random-coiled biomolecule under tension reduces the total number of conformational states it can assume, thus decreasing its entropy. When a DNA molecule is completely straightened, it can only have one conformational state (a straight line)—a state that requires an applied force. The resistance to elongation and the tendency to resume the random-coiled conformation in order to maximize entropy leads to the entropic elasticity of a polymer chain.

3. Conformational dynamics of nucleic acids

Nucleic acids including deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) are essential to life. A double-stranded helical DNA consists of two long chains held together by hydrogen bonding within the complementary Watson–Crick base pairs A–T (adenine–thymine) and G–C (cytosine–guanine). This base pairing gives rise to informational redundancy and allows for chemical fidelity in replication. The base pairing can be used to assemble new strands of DNA, allowing for information transmission to offspring, and can be used to assemble strands of RNA, letting the DNA control which proteins are manufactured at what times (Calladine and Drew, 1997). These functions are realized as well as controlled by gene regulatory proteins and other DNA associating proteins through their interactions with DNA, which often result in DNA twisting, stretching, and bending. An RNA molecule, on the other hand, typically exists as a single strand rather than a double-stranded helix, though it can also form double-stranded helices with the complementary DNA. It contains the base uracil (U) instead of thymine (T). The elasticity of DNA and RNA has very important biological implications. For example, the bending and twisting rigidities of DNA affect how it wraps around histones to form chromosomes, supercoils during replication, bends upon interactions with proteins, and packs into the confined space within a virus (Strauss and Maher, 1994; Cluzel et al., 1996; Geiselmann, 1997; Strick et al., 1998). In what follows, attention will be focused on the deformation of DNA due to the extensive studies it has received; much less work has been done on the deformation of RNA.
Fig. 3. (a) A double-helical DNA chain is about 2 nm in diameter and has ten base pairs (bp) per helical turn. With a short length, it is rodlike, but over long contours it is flexible. (b) DNA under stretching has at least four deformation regimes: (I) entropic-elasticity dominant, (II) worm-like chain, (III) contour elongation and (IV) B-DNA to S-DNA phase transition. The comparison between experiment and models (FJC and WLC) are given.

3.1. DNA under tension

As shown schematically in Fig. 3a, a DNA molecule is typically in the form of a right-handed double helix (the B form, or B-DNA) with about 10 base pairs (bp) per helical turn (3.4 nm pitch). Over short lengths a double-helical DNA chain is rodlike, but over long contours the chain gradually curves and bends in arbitrary directions,
forming a random coil. Upon stretching by an applied force \( f \) the coiled DNA elongates, as illustrated by the experimental result shown in Fig. 3b (Strick et al., 2000b; Baumann et al., 2000). There are at least four different regimes of DNA deformation under stretching with, respectively, (i) \( f < 0.08 \) pN, (ii) \( 0.08 \leq f < 12 \) pN, (iii) \( 12 \leq f < 65 \) pN and (iv) \( f \geq 65 \) pN. With low applied forces (\( f < 0.08 \) pN, see Fig. 3b), the deformability of DNA molecules is largely controlled by the entropic elasticity (Bustamante et al., 1994, 2000), i.e., resistance to deformation to maximize entropy. The simplest model of entropic elasticity of DNA or any polymer chain is the Gaussian chain model that treats the polymer as a chain of statistically independent segments whose orientations are uncorrelated in the absence of an external force. It can be demonstrated (de Gennes, 1979) that the resistance to deformation of such a polymer chain under stretch is entirely due to the changes in entropy. Under small forces, the Gaussian chain model leads to a linear relationship between force \( f \) and the mean extension \( x \) of the chain

\[
\frac{f A}{k_B T} = \frac{3}{2} \frac{x}{L} \tag{2}
\]

where \( k_B \) is the Boltzmann constant and \( T \) the absolute temperature (1 \( k_B T = 4.3 \) pN nm at 37°C); \( A \) and \( L \) are, respectively, the persistence length (the distance over which two segments of the chain remain directionally correlated) and contour length of the DNA molecule (the length of DNA when it is fully extended). The persistence length \( A \) is an intrinsic parameter of a polymer chain; it is a measure of resistance to shape changes of the chain due to thermal fluctuations (Howard, 2001). For double-stranded DNA, \( A \approx 50 \) nm (Taylor and Hagerman, 1990).

Another model of entropic elasticity of DNA is the freely joined chain (FJC) model (Doi and Edwards, 1986), which also treats the polymer as a chain of rotationally uncorrelated segments but imposes inextensibility. It assumes that the chain consists of \( N \) segments of length \( b = 2A \) (the Kuhn length, \( b = L/N \)) and has a configuration \( \Gamma_x \) determined by the ensemble of the segments with different orientations represented by angles \( \theta_i \), as shown in the insert of Fig. 3b. Under applied force \( f \), the potential energy of the chain with any admissible configuration \( \Gamma_x \) is

\[
\varepsilon_{\text{FJC}} = -fb \sum_{i=1}^{N} \cos \theta_i. \tag{3}
\]

The partition function \( \Psi \) of the chain is given by

\[
\Psi = \sum_{\Gamma_x} e^{-\varepsilon_{\text{FJC}}/k_B T} = \sum_{\Gamma_x} e^{fb \sum \cos \theta_i/k_B T} = \sum_{\Gamma_x} \prod_{i=1}^{N} e^{fb \cos \theta_i/k_B T} \tag{4a}
\]

\[
= \left[ \int_{-\pi}^{\pi} dQ e^{fb \cos \theta/k_B T} \right]^{N}, \tag{4b}
\]

where \( dQ = \beta 2\pi \sin \theta d\theta \) is the number of segments lying in between \( \theta \) and \( \theta + d\theta \) and \( \beta \) is a proportional constant (Cantor and Schimmel, 1980). In deriving Eq. (4b), one uses the fact that the angle \( \theta_i \) of each segment is arbitrary and summing over all possible configurations should cover all possible angles at each segment. It can be shown that
the Helmholtz free energy of the chain can be given by $H = U - ST = -k_B T \ln \Psi$ (e.g., Howard, 2001) from which the mean extension $x$ of the chain can be calculated to give

$$x = \frac{\delta H}{\delta f} = k_B T \frac{\delta}{\delta f} \ln \left[ \frac{4\pi \beta k_B T}{fb} \sinh \frac{fb}{k_B T} \right] = L \left[ \coth \frac{fb}{k_B T} - \frac{k_B T}{fb} \right]. \quad (5)$$

It is readily seen that with small forces $f \lesssim k_B T/b$, Eq. (5) recovers the Gaussian chain model given in Eq. (2); when $f < k_B T/A (b = 2A)$ the FJC model is fairly accurate compared with the experimental measurements as shown in Fig. 3b. However, at large forces the FJC model is inaccurate due to the omission of the thin-rod elasticity of DNA under bending.

With moderate applied loads, $0.08 \leq f < 12 \text{ pN}$, the bending rigidity of DNA becomes important, and the worm-like chain (WLC) model represents more accurately the DNA elasticity based on the Lagrangian function (Fixman and Kovac, 1973; Marko and Siggia, 1995a)

$$E = k_B T \int_0^L A \kappa^2 \, ds - fx \quad (6)$$

where $\kappa$ is the curvature, and the force $f$ and the extension $x$ are assumed to be along the same direction (see insert of Fig. 3b). Clearly, the first term in (3) is the elastic bending energy and the second term is the work done by the applied force. The partition function $\Psi$ and the free energy $H$ of the DNA as a flexible polymer chain can be calculated rather elegantly using an analogy with the quantum mechanics problem of a dipole in an electrical field, as shown by Marko and Siggia (1995a). Although the exact force-extension relationship of the WLC model is complicated, it can be approximated accurately by an interpolation formula (Marko and Siggia, 1995a)

$$\frac{fA}{k_B T} = \frac{x}{L} + \frac{1}{4(1-x/L)^2} - \frac{1}{4}. \quad (7)$$

As revealed by Smith et al. (1992, 1996), for a single $\lambda$-phage DNA molecule with an applied force $f \leq 12 \text{ pN}$, the force versus extension relationship can be represented accurately by the WLC model under uniaxial stretch conditions, with $A = 53.4 \text{ nm}$ and $L = 32.8 \mu\text{m}$, as illustrated in Fig. 3. It follows from Eq. (7) that when extension $x$ is small, the DNA molecule behaves like a linear spring (as in the FJC model); however, the behavior is nonlinear as $x$ becomes large. As illustrated in Fig. 3c, a force of 2–3 pN is able to stretch the DNA to 90% of its contour length, with the force rising sharply when $x$ approaches $L$.

With applied forces $12 \leq f \leq 65 \text{ pN}$, the contour length of DNA increases. Because of the inextensible assumption in the WLC model, Eq. (7) implies that as $x$ equals $L$ the force $f$ becomes infinitely large which is, of course, not realistic. Assuming that the extension of the DNA backbone depends linearly on the applied force, Eq. (6) can be modified to give (Odijk, 1995)

$$E = k_B T \int_0^L A \kappa^2 \, ds - \frac{1}{2} \int_0^L K \left( \frac{s}{s_0} - 1 \right)^2 \, ds - fx, \quad (8)$$
where $K$ is the stretch modulus and $s_0$ is a reference length (e.g., 0.34 nm/bp). In fact, $\Delta s/s_0 = (s - s_0)/s_0$ is the local strain. If one approximates DNA as a thin cylindrical rod of homogeneous isotropic linear elastic material with Young’s modulus $Y$ and radius $R \approx 1$ nm (Voet and Voet, 1995) then

$$K = \pi R^2 Y \quad A = \frac{Y I}{k_B T} = \frac{\pi R^4 E D}{4 k_B T}.$$  

For λ-phage DNA in a solution containing 150 nM Na⁺, $Y \approx 300$ MPa and $K \approx 1000$ pN (Bustamante et al., 2000). Solving Eq. (8) for end-to-end distances longer than the contour length yields (Odijk, 1995)

$$\frac{x}{L} = 1 - \frac{1}{2} \sqrt{\frac{k_B T}{f A}} + \frac{f}{K}$$

which is valid for applied forces $f$ between 12 and 65 pN.

Finally, when the applied load $f$ reaches about 65 pN, 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), with a dramatic increase in length similar to metal yielding, as shown in Fig. 3b. When $f$ is further increased, the DNA may be overstretched, resulting in unwinding and unstacking of the molecule (Smith et al., 1992), or even the breakage of covalent bonds in the DNA backbone. It has been found that the force-extension behavior of λ-phage DNA is not very sensitive to different pulling rates, although the transition from a double helical DNA into two single strands occurs at a higher force when the pulling rate is larger (Clausen-Schaumann et al., 2000).

### 3.2. DNA in hydrodynamic flow

To understand the interactions between DNA molecules and the surrounding fluid during hydrodynamic flow, controlled flow fields were applied to DNA to quantify relaxation time (Perkins et al., 1994), hydrodynamic drag (Perkins et al., 1995; Larson et al., 1997), and conformational dynamics (LeDuc et al., 1999; Smith and Chu, 1998; Smith et al., 1999a). The relaxation time of DNA, defined as the time needed for Brownian forces to globally rearrange its configuration (Smith and Chu, 1998), was obtained by attaching a bead to one end of a DNA molecule, exposing it to a uniform flow, and recording the time necessary for a fully extended molecule to recoil after the flow was stopped. A similar experimental set-up was used to evaluate the dependence of DNA extension on its contour length $L$, flow velocity $v$, and the solution viscosity, $\mu$ (Perkins et al., 1995). The conformational dynamics of DNA molecules in a shear flow (plane Couette flow) was studied experimentally (LeDuc et al., 1999; Smith et al., 1999a). A plane Couette flow with shear rate $\dot{\gamma}$ can be decomposed into a rotational flow with vorticity $\omega = -\dot{\gamma}/2$ and an elongational flow with strain rate $\dot{\varepsilon} = \dot{\gamma}/2$. Thus, DNA molecules in a plane Couette flow exhibit both extension and tumbling, as observed by LeDuc et al. (1999) and Smith et al. (1999a). Unlike the tumbling motion of rigid ellipsoidal particles in a shear flow, which has been analyzed by Jeffery (1922), the dynamic behavior of DNA molecules in a shear flow is more complex due to their deformations.
3.3. DNA under torsion

The topology of, and the degree of supercoiling in, DNA plays an important role in gene replication, transcription, and recombination. The supercoiling of a DNA molecule can be described by two parameters, twist $T_w$, the number of helical turns along the molecule, and writhe $W_r$, a measure of the coiling of the DNA axis about itself (Voet and Voet, 1995). For example, a relaxed, linear DNA molecule has a twist $T_w$ equal to the number of base pairs divided by 10.4 (the number of base pairs per turn for B-form DNA) and a writhe $W_r$ of zero. The sum of $T_w$ and $W_r$, $L_k = T_w + W_r$, known as the linking number (or link), does not change under continuous deformations when the two ends of a DNA molecule are torsionally constrained, and is therefore a topological quantity (White, 1969; Fuller, 1971). The degree of supercoiling (excess linking number) is characterized by $m_{DCA} = \frac{\Delta L_k}{L_k}$, i.e., the change in linking number $\Delta L_k = L_k - L_{k0}$ normalized by the linking number $L_{k0}$ of torsionally relaxed DNA. A DNA molecule with a positive value of $m_{DCA}$ is overwound, while a DNA with a negative value of $m_{DCA}$ is unwound. Most living organisms are thought to have partially unwound DNA with a value of $m_{DCA} \approx -0.06$ (Strick et al., 1998).

DNA supercoiling and the long-range transmission of torsional strain may play a crucial role in gene expression (Travers and Muskhelishvili, 1998). For example, it has been found that at the initiation of transcription, the formation of the RNA polymerase-promoter complex unwinds the DNA, resulting in negative supercoils (Ansari et al., 1992; Su and McClure, 1994). The rate of gene expression has been found to be directly dependent on the degree of supercoiling (Condee and Summers, 1992): increased negative supercoiling leads to higher gene expression rates and decreased supercoiling results in the converse.

As shown schematically in Fig. 4a, a relaxed circular or linear DNA can become supercoiled upon the action of topoisomerases. During the elongation phase of transcription, there is positive supercoiling in front of the transcription ensemble and negative supercoiling behind the ensemble (Wu et al., 1988). Experimental evidence suggests that this transcription-induced negative supercoiling can exert a regulatory effect over a distance of 4000 bases or more. It has been shown that when linear (relaxed) DNA templates are introduced into cultured cells, no transcripts can be detected, whereas supercoiled circular templates are found to be active (Dunaway and Ostrander, 1993). Similar to transcription, the initiation of replication also requires negative supercoiling of the DNA template (Walter and Newport, 2000)—a lack of unwinding prevents the DNA polymerase from binding.

To better understand the mechanics issues involved in the supercoiling of DNA, quantitative studies of single linear DNA molecules under twist have been carried out in which DNA molecules are attached to a treated glass coverslip at one end and bound to a small magnetic bead at the other end, as shown in Fig. 4b. By applying a magnetic field to the bead, the DNA molecule can be mechanically overwound and underwound as well as stretched. Two types of experiments were conducted: force versus extension studies at constant supercoiling, and extension versus supercoiling at constant force (Strick et al., 1996; Allemand et al., 1998). These experiments have revealed three different regimes of supercoiled $\lambda$-phage DNA in the presence of
(a) A relaxed circular DNA is supercoiled. (b) The effect of tension on the supercoiling of DNA is studied with controlled stretch and twist using a magnetic device. (c) Extension as a function of $\eta$ of supercoiled DNA. With a low tensile force (e.g., $f = 0.2 \text{ pN}$), DNA contraction is the same under overwound ($\eta > 0$) and underwound ($\eta < 0$). With an intermediate force (e.g., $f = 1.2 \text{ pN}$), DNA contracts only with overwinding. With a high force regime (e.g., $f = 8 \text{ pN}$), DNA does not contract under both underwound and overwound.
tension and with moderate ionic conditions. As illustrated in Fig. 4c, at a low tensile force \((f < 0.5 \text{ pN})\), overwound and underwound DNA molecules behave essentially the same. Like a phone cord, the DNA molecule shortens \(\sim 0.08 \mu \text{m per turn}\), as observed experimentally (Boles et al., 1990; Bednar et al., 1994). At an intermediate force regime \((0.5 < f < 3 \text{ pN})\), DNA behaves very differently in overwinding and underwinding. The overwound DNA continues to contract, while the extension of an underwound DNA does not change with \(\eta\). In the high force regime \((f > 3 \text{ pN})\), the behavior of DNA becomes independent of torsion for both under- and overwound molecules and assumes a force versus extension curve of a torsion-free DNA.

Models for stretching supercoiled DNA have been developed over the last few years (Marko and Siggia, 1994, 1995b; Marko, 1997; Moroz and Nelson, 1997; Vologodskii, and Marko, 1997). For example, by extending the WLC model to include torsional energy, Moroz and Nelson (1997) was able to successfully model DNA undergoing small degrees of twist, \(-0.01 < \eta < 0.03\), using the Lagrangian function

\[
E = \frac{k_B T}{2} \int_0^L [Ak^2 + C(\Omega - \omega_0)^2] \, ds - fx - \frac{2\pi k_B T \tau L_k}{\gamma}
\]

where \(C\) is the torsional persistence length, \(\Omega\) is the twist strain (twist angle per unit length), \(\omega_0 = 1.85 \text{ nm}^{-1}\), and \(\tau\) is a nondimensional torque corresponding to the required twist. Solving Eq. (11) gives (Moroz and Nelson, 1997)

\[
\frac{x}{L} = 1 - \frac{1}{2} \left( \frac{Af}{k_B T} - \left( \frac{\tau}{2} \right)^2 - \frac{1}{32} \right)^{-1/2} + \frac{f - k_B T \Omega_0 \omega_0 \eta}{\gamma} + \frac{A}{L \lambda},
\]

where \(\tau = \omega_0 \eta / [1/C + 1/(4A \sqrt{Af/k_B T})]\), \(\lambda = Af/k_B T - \tau^2\), \(D\) and \(\gamma\) are, respectively, a characteristic length and force to be determined. Fitting this formula to the experimental data yields values of \(A = 49 \text{ nm}\), \(C = 120 \text{ nm}\) and \(D = 50 \text{ nm}\). This value of \(C\) is somewhat larger than the experimental result \((C = 75 \text{ nm})\) (Crothers et al., 1992).

There have been extensive theoretical studies on the supercoiling of DNA (Schlick, 1995; Moroz and Nelson, 1997). The elastic rod model treats the DNA molecule as a long, thin and initially straight elastic isotropic rod with a circular cross section. Although a first-order approximation, this model works remarkably well in predicting the supercoiling of DNA under torsion, including the onset of supercoiling, the abrupt nonplanar buckling, and the DNA configurations as determined by \(\eta\) (Yang et al., 1998). However, as pointed out by Marko and Siggia (1994), thermal fluctuations and entropic effects, which have been taken into account in the modified elastic thin-rod model (e.g., Moroz and Nelson, 1997), play important roles in stabilizing the interwound superhelical DNA.

It has been well established that DNA replication, condensation and transcription rely on the conformational matches between DNA and the associated proteins (Erie et al., 1994). However, deformation of DNA can alter this conformational match, hence the structural rigidity of DNA as well as related protein enzymes comes into play (Bustamante et al., 2000). Single-molecule biomechanics studies of DNA over the last few years have revealed many intriguing features of the elasticity of DNA under stretching,
twisting, and in hydrodynamic flow. The constitutive modeling efforts, such as the development of the worm-like chain model, have helped capture the essence of the DNA deformation under force. However, many important issues remain to be addressed. For example, we still do not have a clear picture of how mechanical forces are applied to DNA in cells, be they through DNA–protein interactions or due to hydrodynamic flow. We still lack a good understanding of how mechanical forces on DNA contribute to the regulation and control of gene replication and transcription in cells. For example, if a DNA molecule deforms under force, how does such deformation affect the onset and rate of gene transcription? What is the distribution of torsional deformation along DNA? What is the force required to open the transcription complex as determined by the tension and torsion in DNA? Answering these questions will not only advance our understanding of DNA mechanics, but also help understand the functions of protein enzymes involved in gene replication and transcription.

4. Motion and deformation of proteins

Proteins are cellular machines and constitute about 60% of a cell’s dry weight. They perform most of the cellular functions such as transducing signals, providing structural support, transporting biomolecules, regulating biological responses, and catalyzing biochemical reactions for metabolism. Proteins are formed by an assortment of 20 different amino acids arranged in a specific sequence encoded in the corresponding gene. Typically consisting of hundreds to thousands of amino acids, proteins can have complex structures that are maintained by hydrophobic and electrostatic interactions, and hydrogen bonding. There are four different levels of structural organization in proteins. The primary structure of a protein is its amino acid sequence. As shown in Fig. 5, the α helices and β sheets formed due to regular hydrogen-bond interactions are protein’s secondary structure. Protein domains consisting of α-helices and β-sheets compacted into folded globular units are called the tertiary structure. Protein quaternary structure refers to multiple domains linked by polypeptides (loops) or secondary structures. A native, folded protein is a solid-like polymeric material since its domains are closely compacted. Specifically, many proteins or protein domains have an approximately rounded shape, refer to as globular proteins.

An essential feature of proteins is that the functions of a protein are determined by its 3D conformation, i.e., the spatial arrangement of the atoms in its folded structure. As illustrated in Fig. 5d, the recognition and binding of two proteins is largely dictated by the geometric and chemical complementarity of the interacting surfaces, ensuring binding specificity in a ‘lock-and-key’ fashion. Such biomolecular recognition underlies almost all cellular processes. In other words, although nonspecific binding between proteins may occur, it is the specific, noncovalent binding of two proteins in the intra- and extra-cellular environment that enables cells to survive and function. However, as described in more detail below, protein molecules are deformable, thus their conformations can be altered by mechanical forces. Such alterations can affect protein–protein and protein–DNA recognition, binding and unbinding, leading to changes in downstream biochemical processes that control cellular behavior and function. Protein
Fig. 5. The structure of a protein includes: (a) α helices, (b) β sheets, (c) domains. (d) The complicated 3D structure of proteins dictates their recognition and binding in a ‘lock-and-key’ fashion.

definition, therefore, is an important concept in molecular biomechanics (Bao, 2000; Zhu et al., 2000).

Proteins are not static rigid structures. Rather, they are dynamic and undergo constant motions and structural changes in cells under normal physiological conditions. These changes include large-scale (∼5–50 Å) movements of domains as well as small-scale (∼0.5 Å) random movements of secondary structures or domains, or ‘breathing’. The timescales for protein motion and deformation in biological processes may span many orders of magnitude, ranging from one femtosecond (10^{-15} s) to a few seconds. For example, hinge motion of a protein may take only one nanosecond, whereas local denaturing may take a few seconds (McCammon and Harvey, 1987). To illustrate, consider a globular protein immobilized on a surface through a lever-arm (e.g., an α-helix), as shown schematically in Fig. 6a. Under an applied force $F$ the small motion of the protein in the vertical direction can be analyzed based on a mass-spring-dashpot system shown in Fig. 6b. The corresponding governing equation for displacement $y$ is

$$m\ddot{y} + \eta\dot{y} + ky = F; \quad (13a)$$

where $m$ is the mass and $\eta$ the viscous drag coefficient of the protein, and $k$ is the elastic constant of the lever arm. Neglecting the inertial effect and assuming that the
Fig. 6. The motion of a protein under applied force $F$. (a) A globular protein immobilized on a surface through an $\alpha$-helix. (b) The mass-spring-dashpot system as a model for protein motion.

protein is stationary at $t = 0$, we have

$$y(t) = \frac{F}{\eta} (1 - e^{-\left(\frac{k}{\eta}\right)}).$$  \hspace{1cm} (13b)

If the globular protein has a mass of 100 kDa (1 Dalton = $1.66 \times 10^{-24}$ g) and a drag coefficient of 60 pN s/m (Howard, 2001), and the lever arm has $k = 6$ pN/nm, the relaxation time of the protein motion is $\tau = \eta/k = 10$ ns. Note that the above analysis is valid only if $F \gg k_B T/a$, where $a$ is a characteristic length of the protein.

Energetically, proteins are not very stable in that small changes in temperature or pH of the solution can convert the proteins from a biologically active (native) state to a biologically inactive (denatured) state (Voet and Voet, 1995). Specifically, although the changes in enthalpy and entropy between these two states can both be large ($\sim 100$ kcal/mol), the corresponding free energy change is only about 5–15 kcal/mol, which is just a few times the energy of a single hydrogen bond in water, 1 kcal/mol (6.92 pN nm per bond). This may be a fundamental reason why a small force acting on a protein could generate a large conformational change.

The motion and deformation of protein molecules can have various modes under different forces. These modes include domain motion (Subbiah, 1996), domain deformation and unfolding (Erickson, 1997; Rief et al., 1997; Kellermayer et al., 1997; Tskhovrebova et al., 1997; Oberhauser et al., 1998), and denaturing of secondary structures (Idiris et al., 2000), as shown schematically in Fig. 7. In general, in domain hinge motion (Fig. 7a), the individual domains have very limited deformation; the motion largely consists of rotations of domains around a flexible hinge (e.g., loops, $\alpha$-helices or $\beta$-sheets that join the domains together), or relative (in-plane and out-of-plane) ‘shear’ motions between domains. Mechanistically, these three modes of protein motion resemble the three fracture modes (Mode I, II, III). It may also have mixed mode motions, such as a combination of rotation about a hinge and twist about an axis of the domain. Such motions are typically driven by Brownian forces or moments, which may involve forces of 0.1–1 pN. When force becomes large, e.g. 1–100 pN, the domains may begin to deform (Fig. 7b). For protein molecules such as titin and tenascin, domain unfolding occurs when the applied force is $\sim 100$ pN (Tskhovrebova et al., 1997; Oberhauser
et al., 1998). The denaturing (i.e. melting) of $\alpha$-helices and $\beta$-sheets (Fig. 7c) may require an even larger force. Although very rough, the above order-of-magnitude estimates for the force scales may provide some guidance to the analysis of protein deformation.

Protein molecules are viscoelastic in nature, similar to all polymeric materials, especially those in aqueous environment. As such, their deformations are rate-dependent. In what follows, we discuss some basic features of protein hinge motion, domain deformation and unfolding, and the implications of protein deformation to receptor–ligand binding. Since the study of protein deformation is still in its infancy, many basic issues remain to be addressed. For example, how does the deformation of a protein relates to its 3D structure? What are the deformation mechanisms? How protein deformations are coupled to protein function? What is the dynamic behavior of proteins? In addition to single-molecule studies of protein deformation and dynamics, it is also necessary to study the deformation of a cluster of two or more proteins binding together, and protein assemblies.

4.1. Protein hinge motion

Polypeptide chains of more than $\sim 200$ amino acids usually fold into two or more globular clusters known as domains. These structurally independent domains have an average size of $\sim 2.5$ nm and possess unique 3D geometries. Most protein domains have specific functions such as the binding of small molecules (ligands) and other protein domains. The ligand-binding sites in multidomain proteins are often located in the clefts between domains, making the interactions with different ligands flexible.

Many protein molecules undergo hinge motion in cells, which has many biological implications (Subbiah, 1996; see also http://bioinfo.mbb.yale.edu/MolMovDB/). In
these proteins, one or more domains rotate about a hinge due to Brownian force or molecular interactions such as ligand binding. For example, the motor molecule myosin ‘moves’ on an actin filament by generating hinge motion of its head upon ATP binding and hydrolysis. While some motor molecules rely on hinge motion to convert chemical energy into mechanical force, other proteins may utilize hinge motion to transduce signals, regulate interactions, and facilitate enzymatic activities. As an example, the structure of *E. coli* periplasmic dipeptide binding protein is displayed in Fig. 8a. It has two domains linked by a hinge consisting of two β-sheets; its hinge motion has been well documented (http://bioinfo.mbb.yale.edu/MolMovDB/).

Biologically, both the magnitude and rate of the rotational hinge motion are important. Some insight can be gained by performing a Langevin dynamics analysis of protein hinge motion in which proteins having two domains linked by a hinge is considered (Fig. 8b). The angle of rotation $\theta$ of a domain about the center of the hinge is assumed to obey the stochastic differential equation (McCammon et al., 1976)

$$I_0 \ddot{\theta}(t) + \eta \dot{\theta}(t) + k\theta(t) = R(t)$$

or

$$I_0 \ddot{\theta}(t) + \int_0^t \alpha(t-s)\dot{\theta}(s)\,ds + k\theta(t) = R(t),$$

where $I_0$ is the moment of inertia of the domain, $\eta$ is the rotational viscous drag coefficient, $\alpha(t)$ is a dissipation function, and $k$ the elastic constant of the hinge. The Brownian torque $R(t)$ in Eq. (14) is a random function of time and represents the collective effect of the random collisions of the surrounding molecules in the solvent with the protein. The ensemble average of $R(t)$ is zero. Similar to almost all the studies...
of stochastic processes, Boltzmann’s ergodic hypothesis is adopted (Coffey, 1985) and the time average of a parameter \( \bar{a} \) is assumed to be identical to its ensemble average \( \langle a \rangle \).

In most of Langevin dynamics studies of Brownian motion it is assumed that the values of the Brownian torque at different times are completely uncorrelated, i.e., \( R(t) \) is a ‘white noise’ random process with the autocorrelation function

\[
\langle R(t)R(t+s) \rangle = 2k_B \eta \delta(s), \tag{15}
\]

where the noise strength \( 2k_B \eta \) comes from the equipartition theory of classical thermodynamics (van Kamper, 1981; Risken, 1984). In this case Eq. (14a) is employed. Although this ‘white noise’ random process model is usually adequate, it may not always be accurate if the solvent is very viscous. In fact, in living cells the viscosity of cytoplasm can be three orders of magnitude higher compared with water (Crick and Hughes, 1950). As an alternative, colored noise may be considered and the autocorrelation function of \( R(t) \) may obey an exponential-decay function with time

\[
\langle R(t)R(t+s) \rangle = k_B \eta \beta \exp(-\beta |s|), \tag{16}
\]

where \( 1/\beta \) is the correlation time of the random process, which is an intrinsic property of the solvent. Consequently, Eq. (14b) should be used in this case according to the fluctuation–dissipation relation (Uhlenbeck and Ornstein, 1930; Wang and Uhlenbeck, 1945; Fox, 1978). It is possible to analyze protein hinge motion by solving Eqs. (14) –(16), or to simulate the protein hinge motion using molecular dynamics calculations. However, it remains to be very challenging to experimentally observe or quantify protein hinge motion due to its characteristic time scale (1–100 ns).

4.2. Protein domain deformation and unfolding

Protein domains can sustain deformation or even unfolding under normal physiological forces. It has been revealed that, under stretching with AFM or optical tweezers, the immunoglobulin domains of muscle molecule titin may deform and unfold, with a characteristic sawtooth pattern (Rief et al., 1997; Kellermayer et al., 1997), as shown schematically in Fig. 9. Clearly, a force of \( \sim 100 \) pN is required for domain unfolding of titin. The FN-III domains of the ECM protein tenascin unfold upon stretch in a similar fashion (Oberhauser et al., 1998). Single-molecule biomechanics studies, both experimental and theoretical, have been performed on the forced unfolding of ECM molecule fibronectin, which regulates many cellular functions through its binding to integrin (Krammer et al., 1999; Ohashi et al., 1999; Craig et al., 2001). Experimental evidence suggests that, during cell adhesion and locomotion, the FN-III domains of fibronectin may unfold and refold due to the tensile and contractile forces the cells apply to the ECM (Ohashi et al., 1999). More important, this unfolding and refolding may have significant implications to cell signaling, since the resulting structural changes of the RGD loop seems to regulate the binding between fibronectin and integrin, as revealed by molecular dynamics simulations (Craig et al., 2001; Vogel et al., 2001).
It is possible that cells utilize force-induced domain deformation and unfolding in proteins to alter molecular recognition, thus coupling mechanical force with biochemical reactions.

Although proteins are structurally far more complex than nucleic acids, their deformations share certain common features with that of DNA. For example, analysis of the deformation and unfolding of individual domains in titin has revealed that the force-extension relationship of titin can be described by the WLC model (Eq. (7)), with $A = 0.4 \text{ nm}$, and $L = \lambda_0 + n\lambda$ ($n = 0, 1, 2, \ldots$) where $\lambda_0 = 58 \text{ nm}$, $\lambda$ ranges from 28 to 29 nm. A similar expression was found to fit very well the measured force-extension curve for tenascin (Oberhauser et al., 1998). It is intriguing that the WLC model seems to work well for domain deformation of protein molecules containing multiple, individually folded domains with $\beta$-sandwich structures.

Protein domain deformation can be a result of protein–protein and protein–DNA interactions, since it typically only requires forces of 1–10 pN. But what is the extent of protein unfolding in cells under physiological conditions? It is likely that only proteins associated with the cell cytoskeleton and ECM may sustain mechanical forces large enough to cause domain unfolding, which requires $\sim 100$ pN. The contractile machinery of the actin filament system in a cell can generate forces of 10–100 nN (Galbraith and Sheetz, 1997), resulting in significant deformation of the ECM. Further, cells under stretch and shear due to injury, motion, growth and blood flow may deform, leading to large forces in the cytoskeleton. It will be very valuable if direct observations of domain unfolding of proteins in cells can be made.

The unfolding of biomolecules may also provide insight into the toughness of natural materials such as abalone shell. It has been revealed through AFM studies that molecules in the $\sim 30$ nm thick organic adhesive layer in the abalone shell exhibit saw-tooth force-extension curves similar to that of titin and tenascin (Smith et al., 1999b). The successive unfolding of individual modules in a long molecule seems to be more fracture resistant, leading to both high strength and toughness. Therefore, the understanding of the deformation and unfolding of protein molecules may lead to novel engineering design concepts of nanostructured materials and composites.
4.3. Receptor–ligand binding

Perhaps one of the most important aspects of protein deformation under force is its effect on receptor–ligand binding, an essential process in cells (Lauffenburger and Linderman, 1993), because specific molecular recognition and interactions rely on conformational matches and charge complementarity between the receptor and the ligand. In general, protein–protein and protein–DNA interactions are determined by many factors, including electrostatic double-layer force, van der Waals force, and ‘steric’ repulsion forces, hydrogen-bonding, and hydrophobic contacts. Most of these factors operate within short ranges (Israelachvili, 1992). Therefore, the 3D geometry local to the binding pocket of the receptor and ligand contributes significantly to the characteristics of their binding. Good conformational matches usually lead to strong and long-lasting bound states, whereas poor conformational matches do the converse. The underlying reason is that receptor–ligand binding is realized largely through noncovalent bonds such as hydrogen bonds, which are rather weak individually but, in sufficient number, can be strong collectively. However, hydrogen bonding operates only within narrow geometric ranges; thus, to form a large number of hydrogen bonds, a good conformational match between the receptor and ligand at the binding pocket is necessary.

Both domain hinge motion and domain deformation of proteins can affect receptor–ligand binding. As mentioned above, many proteins have the binding pocket for a ligand formed between the domains and located near the hinge. Therefore, depending on the magnitude and rate of domain hinge motion, receptor–ligand binding may be altered or even blocked. Specifically, if the rate of hinge motion is much faster than the time required for certain ligands to diffuse into the binding pocket, it is likely that binding of these ligands is prohibited. This may serve as a ‘selection’ mechanism for specific receptor–ligand binding.

When mechanical forces are applied to a receptor (and ligand), the receptor may deform, thereby altering the conformational match between the receptor and ligand, as illustrated schematically in Fig. 10. Most likely it does not take much deformation to significantly reduce the binding strength because, for hydrogen bonds, increasing the distance between the hydrogen-binding atoms by 1 Å can reduce binding strength (in

![Fig. 10. Receptor–ligand binding can be affected by protein deformation. (a) A good conformational match between the receptor and ligand leads to strong binding and reaction. (b) When the receptor deforms under force, the binding affinity decreases due to poor conformational match.](image-url)
terms of the free energy) from 1 kcal/mole (∼7 pN nm) to a much lower value. This suggests that small deformations of proteins could have large influences on receptor–ligand binding. It is important to point out that the specific effect of mechanical forces on receptor–ligand binding may depend on the mode and the magnitude of the deformation of a receptor or a ligand (or both), which are in turn determined by the structure of the molecules, the solvent surrounding, and loading history (including rate). It is possible that deformation only alters the kinetic rates (Evans and Ritchie, 1997; Merkel et al., 1999). However, in certain cases, only deformation can expose the binding site, thus switching between the ‘on’ and ‘off’ states of the receptor. It is also possible that deformation can change the specificity of a receptor/ligand pair, i.e. binding to ligand B instead of ligand A upon deformation. The latter two effects are both well known for molecules such as integrins that can be activated biochemically. It is likely that force-induced protein deformation may yield similar results.

While protein deformation can affect receptor–ligand binding, the converse is also true, i.e. ligand binding can induce protein conformational change (Alberts et al., 1994). Such conformational changes can serve as a means to transduce biochemical signals, or to facilitate an enzymatic reaction. For example, when a ligand is bound to the receptor, the rotational motion of the domains about the hinge can be altered significantly. In fact, during the receptor–ligand binding processes, both the receptor and the ligand may change their conformations in order to have the ‘best’ fit, i.e. to realize the most energetically favorable state. Such ‘induced fit’ may be driven by entropic effects (Bongrand, 1999), hydrophobic interactions, and the formation of hydrogen bonds, e.g. ‘caged’ water molecules trapped in the binding pocket. Here again, mechanical properties of a protein such as its rigidity distribution come into play, since the conformational changes are ultimately determined by the structure of the protein.

5. The mechanics of molecular motors

Motor molecules, a special class of proteins, play an essential role in many cellular processes, including muscle contraction, cell movement, cell division, vesicle transport, signal transduction, and DNA replication, condensation and transcription (Alberts et al., 1994). Specific motor molecules include the kinesin and dynein superfamily (Hirokawa, 1998; Okada and Hirokawa, 1999), the myosin superfamily (Mermall et al., 1998), and numerous proteins involved in the interactions with DNA. These motor molecules directly convert chemical energy into mechanical work via conformational changes induced by ATP hydrolysis. As more detailed 3D structures of motor molecules (e.g. Cramer et al., 2000) and images of their movements (e.g. Walker et al., 2000) become available, the structure-function relationship of molecular motors has begun to emerge. It is highly likely that molecular motors utilize protein conformational changes to store energy, generate motion, and control/regulate motor function (Vale and Milligan, 2000; Kikkawa et al. 2001). However, for most of the motor proteins, their mechanics, i.e. how they move, how their conformational changes are related to ATP hydrolysis, how the force generation is related to their structural rigidity, is still largely unknown.
Fig. 11. Kinesin and myosin motors. (a) Upon ATP hydrolysis, myosin molecules generate sliding motion between actin and myosin thick filament through the swing of the lever arm. (b) Kinesin molecules ‘walk’ along microtubules by alternating the binding and unbinding of its two heavy chains to the microtubule.

5.1. Kinesin and myosin motors

To date the most extensively studied motor molecules are muscle myosin and conventional kinesin. Myosin II (skeletal and smooth muscle myosin) is a two-headed linear motor that generates sliding between actin and myosin filaments; only one head attaches to actin at a given time. As shown in Fig. 11a, each myosin head consists of a globular motor domain and a long lever-arm domain (Rayment et al., 1993). ATP hydrolysis results in the swing (rotational hinge motion) of the lever arm, converting chemical energy into mechanical motion. Although early measurements of myosin mechanics revealed an average step size of 11 nm and a force of \( \sim 4 \) pN (Finer et al., 1994), it was suggested that the step size is only \( \sim 4 \) nm for skeletal muscle myosin and \( \sim 7 \) nm for smooth muscle myosin (Molloy et al., 1995; Howard, 1997). The 4–11 nm step size due to the ‘working stroke’ of the myosin head and the 36 nm separation between two consecutive binding sites of myosin on actin filament implies that multiple myosin molecules must work together to move continuously along actin, which is indeed the case in muscle contraction and cell movement. It is still debatable, however, as to how many steps per each hydrolyzed ATP, how large is the force generated by myosin, and how conformational changes of myosin are related to ATP hydrolysis and actin binding.

Kinesin is also a two-headed linear motor, transporting vesicles along microtubules. As illustrated in Fig. 11b, each head consists of a heavy chain with an ATP binding
domain and a light chain on which a cargo is attached. The two light chains form a coiled-coil neck domain, as can be seen from Fig. 11b. Unlike myosin, a single kinesin molecule can move along a microtubule for several micrometers before dissociating, with a step size of 8 nm (Schnitzer and Block, 1997), the distance between two subunits of tubulin. With very low applied mechanical load, kinesin advances one step per each ATP hydrolysis; however, it remains to be seen if this one-to-one correspondence also holds true at high load. It has been uncovered that the force required to stop a single kinesin molecule is about 5–7 pN (Meyhofer and Howard, 1995; Kojima et al., 1997), and the rate of kinesin movement decreases linearly with applied load (Svoboda and Block, 1994).

The 3D structure of kinesin indicates that the two putative tubulin-binding regions are separated by \( \sim 5 \) nm, implying that there must be conformational changes in kinesin in order to separate the two heads by 8 nm. Indeed, the work by Rice et al. (1999) suggests that the neck linker of kinesin undergoes a large conformational change upon ATP binding, assisting the attachment of one head to microtubule. The other head then moves 16 nm forward driven by ATP hydrolysis and the re-zippering of the neck linker.

Despite extensive studies on kinesin, it is not well understood how the 8 nm mechanical step is generated, and how it is related to the conformational changes of the neck linker, and ATP hydrolysis. The hand-over-hand model hypothesizes that the two kinesin heads are symmetric and, with each step of kinesin, the coiled-coil neck domain rotates 180°. However, as revealed by recent experimental evidence, such rotation does not occur during kinesin movement (Hua et al., 2002). As an alternative model, it was suggested that the two kinesin heads are not symmetric in that one is always ahead of the other during each successive cycle, i.e., the two heads move in an inchworm fashion, consisting with the experimental observations. A better understanding of the mechanochemical coupling in conventional kinesin, which involves its structural features, conformational changes, binding to microtubule, ATP hydrolysis, and Brownian motion, will undoubtedly lead to great advances of the study of the kinesin superfamily, as well as the myosin superfamily, since the two motor families share certain similarities.

5.2. ATP synthase

ATP synthase is a special motor enzyme, which can either pump protons across an insulating membrane against the electrochemical gradient using ATP hydrolysis or manufacture ATP from ADP and phosphate using the energy derived from a transmembrane proton motive gradient (Boyer, 1993). The general structure of ATP synthase is shown schematically in Fig. 12 in which the \( \varepsilon \) and \( \delta \) subunits are omitted for clarity. It consists of a transmembrane component, \( F_0 \), comprising the proton channel, and a soluble component, \( F_1 \)-ATPase, containing the catalytic sites. It has been observed that, when ATP is added, the \( F_1 \)-ATPase rotates 120° step-wise, with one ATP molecule hydrolyzed per step (Noji et al., 1997). As perhaps the world’s smallest rotary engine, ATP synthase carries out both its synthetic and hydrolytic cycles through conformational changes of its \( \beta \) domains in the \( F_1 \) component (Fig. 12a). For example, it has
been suggested that in pumping protons, the rotation of the \( \gamma \)-subunit in the \( F_1 \) component, which contains catalytic sites located at the interfaces, is due to the hinge motion of the \( \beta \) domain driven by ATP hydrolysis (Fig. 12b). In analyzing energy transduction in ATP synthase, it is critical to understand how the protein enzyme converts the free energy of nucleotide binding into elastic energy. Here, protein deformation serves as the key mechanism for mechanochemical coupling, generating mechanical torque from the free energy of ATP binding, or producing ATP using torques generated by the protonmotive force (Elston et al., 1998; Wang and Oster, 1998).

5.3. Motors involved in DNA–protein interactions

In order to conduct gene transcription, replication and DNA condensation, cells rely on many motor proteins, including RNA polymerase, DNA polymerase, and topoisomerases, to name only a few. \textit{E. coli} RNA polymerase, for example, carries out the synthesis of an RNA copy of the template DNA by progressing along it at speeds more than 10 nucleotides per second. T7 DNA polymerase, on the other hand, catalyses DNA replication at rates of more than 100 bases per second. It has been uncovered over the last few years that forces applied to enzymes can affect DNA replication and transcription in cells. To reveal the effect of mechanical force, single-molecule studies of RNA and DNA polymerases have been conducted using optical tweezers, as shown schematically in Fig. 13. Specifically, a polymerase is immobilized on a surface...
and a tensile force is applied to it through the transcribing DNA, which is attached to a bead trapped by the optical tweezers. Transcription of DNA is then proceeded against the applied force. It was found that at saturating nucleotide triphosphate (NTP) concentrations, *E. coli* RNA polymerases stall at applied forces of 14–25 pN (Yin et al., 1995; Wang et al., 1998), and the transcription velocity decreases with applied force (Wang et al., 1998, see the insert of Fig. 13). The RNA polymerases have different intrinsic transcription rates and different propensities to pause and stop (Davenport et al., 2000). It was also found that the velocity of T7 DNA polymerase along their single stranded DNA templates is sensitive to tension in the templates (Wuite et al., 2000), suggesting that deformation of DNA under tensile forces may affect DNA–polymerase interactions. Further, torsion in DNA molecules appears to influence the relaxation of DNA supercoils by topoisomerase molecules (Strick et al., 1996, 2000a), indicating again that force in DNA can alter DNA–protein interactions during gene expression and transmission. Although the exact roles of mechanical force in these interactions remain elusive, it is possible that deformation of DNA may change the energy barriers the motor molecules need to overcome or change the conformation (and therefore functionality) of the motor proteins.

6. The connection between mechanics and biochemistry

As illustrated above, mechanics and biochemistry are tightly coupled together in the studies of DNA elasticity, protein conformational dynamics, receptor–ligand binding,
and motor molecules. Specifically, forces and motions (including deformations) are involved in conformational dynamics, binding, reaction and transport of biomolecules. For example, bending, twisting and stretching of DNA are clearly mechanical processes; however, they are crucial to the expression and transmission of genetic information, which is an important subject of biochemistry. Force-induced protein conformational changes can alter many biochemical processes, including reaction kinetics, biomolecular recognition, and protein–DNA interactions. In addition, as cellular machines, motor molecules convert chemical energy directly into mechanical motion. Mechanical forces may also affect protein trafficking and secretion, ion transport through membranes, and enzymatic activities in living cells. It is clear that in performing mechanics analysis of the deformation and dynamics of biomolecules, it is necessary to consider the biochemical aspects such as the sequences of DNA and RNA, the conformations of proteins, the chemistry of the solvent, polymerization and depolymerization of polymers, and the reaction kinetics.

6.1. Mechanochemical transduction

Mechanochemical transduction in cells involves two different aspects. The first is how mechanical forces and motions are being ‘sensed’ by cells and transduced into biochemical and biological responses, the second is how chemical reactions generate mechanical forces or motions through molecular motors and machines, both are still largely unknown. A possible mechanism for mechanochemical transduction in cells is the induced conformational changes of proteins, as suggested in Bao (2000) and Zhu et al. (2000). For example, the receptor-mediated signaling processes in cells can be regulated by deformation of ECM molecules such as fibronectin, resulting in the exposure of an RGD (arginine-glycine-aspartate) sequence for binding to integrin (Sanchez-Mateos et al., 1996), whose functions include both adhesion and signal transduction. As discussed earlier, integrins are likely a major force transmitter, because they provide the mechanical linkage between ECM and cytoskeleton. As such, these receptors may also serve as a force sensor. In addition to the ability of switching between resting and active states of proteins upon ligand occupancy, it is conceivable that cells utilize more continuous conformational changes in response to various forces to produce more gradual exposure of the functional domain or affect the binding rate for the downstream signaling molecules, thereby transducing mechanical forces and deformations into biochemical responses. Taking together, it is essential to study protein deformation in order to understanding mechanochemical transduction in cells.

6.2. Reaction kinetics

Altered kinetics of biochemical reaction by mechanical forces is another major aspect of mechanochemical coupling (Khan and Sheetz, 1997). As shown in Fig. 14, the applied forces can lower the energy barrier of molecular unbinding, thus influencing the reaction kinetic rates (Evans, 2001). To illustrate, consider the binding between two proteins, a receptor and a ligand, under an applied force \( f \) in the direction of pulling them apart. Based on the fracture analysis of solids, Bell (1978) predicted that
the applied force $f$ will increase the unbinding rate and suggested that the equilibrium association constant $K_{eq}$ of receptor–ligand binding is dependent on the applied force $f$ according to (Bell et al., 1984)

$$K_{eq}(f) = K_{eq}^0 \exp\left(-\frac{fx}{2k_B T}\right),$$

(17)

where $K_{eq}^0$ is the value of $K_{eq}$ at $f = 0$, and $x$ is a characteristic length. More recently, Evans and Ritchie (1997) developed a theoretical model for bond failure under force based on Kramers’ rate theory (Kramers, 1940). This model predicts that the bond strength varies with loading rate, which was experimentally verified by measuring the unbinding force of biotin–avidin and biotin–streptavidin pairs (Merkel et al., 1999).

The energy-based approach discussed above is simple and elegant. However, it is often necessary to quantify the deformation of proteins in order to add the mechanical work to the binding energy. This is true whether the applied force is in the unbinding direction or not. For example, even if the applied force on the receptor is perpendicular to the unbinding direction of ligand, as shown in Fig. 10, the conformational changes of receptor may still alter the binding strength. In addition, depending on the loading history, protein deformation can be local as well as global, which affects not only the strength but also the number of contributing hydrogen bonds. For example, when the loading rate is low, bonds may dissociate nearly spontaneously without inducing global deformation of the receptor–ligand complexes, thereby only involving interactions local to the binding pocket. In contrast, high loading rate may result in global deformation, which may alter the dissociation path by involving interactions with structures away from the binding pocket. Thus, protein deformation may play an essential role in determining the rate of bond dissociation; its effects on the changes of energy landscape should be included for a complete description of the relation between force and kinetic rates.
6.3. Effect of solvent

A basic feature of the deformation of proteins and DNA is that they are always in an aqueous environment in order to function. Solvent chemistry, including the polar character of water and ionic strength, plays an essential role in the conformational dynamics of biomolecules. For example, one of the major determinants of protein folding is to bury the nonpolar side chains in the core of the domain. The 3D structure of a protein is formed and stabilized largely by establishing hydrogen bonds among its amino acids, and with water molecules (Creighton, 1993). However, the concentrations of ions in the solvent can significantly change the structural stability of a protein or DNA molecule, altering its deformability. For example, the elasticity of lambda-phage DNA molecules in different buffer solutions was found to change with the ionic strength $I$ (Baumann et al., 1997)

$$I = \frac{1}{2} \sum_i c_i Z_i^2,$$

(18)

where $c_i$ is the molar concentration of the $i$th ionic species and $Z_i$ is its ionic charge (Voet and Voet, 1995). Specifically, the persistence length $A$ was found to decrease as $I$ increases (Baumann et al., 1997), obeying a nonlinear Poisson–Boltzmann theory

$$A = A_0 + 0.0324/I, \text{ nm}$$

(19)

where $A_0 = 50$ nm and $I$ is in molar units. The stretch modulus $K$ (Eqs. (8)–(9)), however, was found to increase with increasing $I$, contradicting with the classical elasticity theory in which the bending and stretching rigidities are related by $A = KR^2/4k_B T$ for a circular rod as given by Eq. (9).

6.4. Mechanochemical equivalence?

There is ample experimental evidence to suggest that there might be some equivalence between mechanical and biochemical effects as far as cells and biomolecules are concerned. For example, mechanical forces can induce neural cell growth, so could biochemical stimuli (Smith et al., 2001). Mechanical forces can denature proteins, so could ions in solution (and temperature, see Paci and Karplus, 2000). Mechanical forces can alter receptor–ligand binding, so could other ligands that bind at different sites (allostery). It is possible that in cells mechanical forces or motions function the same as biochemical agents, such as ligands, ions, growth factors and hormones. This might be the fundamental reason why living cells can alter their behavior and functions in response to changes in the mechanical environment. Although the effect of mechanical forces can be understood theoretically from a thermodynamics viewpoint as indicated by Eq. (1), it is still necessary to elucidate the basic features of the possible mechanochemical equivalence, especially when nonequilibrium thermodynamical processes are involved. Needless to say, not all biochemical effects can be induced mechanically, nor can all mechanical effects on cells be reproduced biochemically. This possible mechanochemical equivalence is again related to the most critical aspect of the
connection between mechanics and biochemistry, i.e., the 3D conformations of proteins determine their functions, and these conformations can be altered by mechanical forces.

7. Major mechanics issues and challenges

The development of molecular biomechanics as an emerging field inevitably involves new concepts in mechanics, new theoretical and experimental approaches, and new challenges. Given below is a brief discussion of these aspects.

7.1. New features in mechanics

In addition to the direct mechanochemical coupling discussed in Section 6, entropic elasticity and Brownian forces play essential roles in the mechanics of proteins and nucleic acids. As mentioned before, the tendency of maximizing entropy resists deformation that imposes restrictions on the accessible conformational states. Brownian forces, on the other hand, act in many processes even when the entropy of a system is constant. Although the entropic contribution to free energy is important for polymeric materials such as rubber, entropic elasticity may become dominant in the deformation of single biomolecules. Indeed, as illustrated by de Gennes (1979), under forces \( < 0.08 \text{ pN} \), the elasticity of DNA is almost entirely due to changes in entropy, leading to a linear force-extension relationship. The derivation of the FJC model for DNA under tension given in Section 3.1 is clearly different from the continuum mechanics approach in that: (1) statistical distribution of the orientation of the chain segments is considered and (2) the entropy of the chain under tension is calculated. The use of free energy in calculating extension, however, is analogous to using strain energy function to calculate strain. Note that if temperature \( T = 0^\circ \text{F} \), the elastic spring constant in Eq. (2) (and in FJC and WLC models) becomes zero, indicating that there is no resistance to deformation. These features may also have engineering implications. Since a DNA molecule has a specific sequence, is very stable and easy to synthesize chemically, it may become an important material for nanotechnology (Quake and Scherer, 2000). Thus, understanding the entropic elasticity of DNA may help design functional nanodevices.

The energy involved in Brownian motion scales with \( k_B T \) which, at room temperature, equals 4.1 pN nm. For large-scale materials and structures, Brownian forces and motions are negligible. However, the motion and deformation of DNA and proteins can be driven by Brownian forces. The reason is simple: these biomolecules are of nanometer size, and their deformations require only piconewton forces. Therefore, the energy of \( 1k_B T \) is enough to cause significant conformational fluctuations. In fact, Brownian forces due to the fluctuation of water molecules can assist the breakage of hydrogen bonds in a protein domain (Lu and Schulten, 2000). It is clear that Brownian motion induced diffusion is essential for the encountering, rotation, binding and reaction of proteins in the cytoplasm, and for protein–DNA interactions in the cell nuclei (Kramers, 1940; Hanggi et al., 1990). It is likely that cells utilize ‘biased’ or ‘rectified’ Brownian motion to facilitate specific cellular processes including binding,
transport, signaling, and energy conversion (Simon et al., 1992; Astumian, 1997; Fox and Choi, 2001), i.e., generate order from disorder, although its thermodynamic basis is not well understood (Feynman et al., 1963). The interplay between Brownian forces, viscous drag, and chemical energy due to ATP hydrolysis may provide the basis for a rich class of phenomena in living cells and is an exciting research topic in molecular biomechanics.

Similar to the studies of engineering materials, it is important to determine the constitutive behavior of proteins and nucleic acids in order to predict their deformations under force. This involves both the formulation of the constitutive equations, and the determination of the material constants. Such equations may be in the form of force-extension, torsion-twist, or stress-strain relations. However, in performing mechanics analysis of the deformation of proteins, the continuum assumption may not be valid. Further, it may not always be necessary nor desirable to define local stresses and strains, which usually depend on the specific amino acid sequence and the 3D globular structure unique to the protein molecule under study. Therefore, the constitutive behavior for one protein defined in terms of stresses and strains may not be applicable to other proteins. The global deformation, such as overall elongation, unfolding or twisting, however, may be insensitive to the amino acid sequence and thus easier to use in making predictions. Most of the biomechanics studies of proteins and nucleic acids concerns mainly their deformations, or conformational changes under force, not their mechanical failure strength. Therefore, it may be more convenient to use forces (or moments) and extensions (or rotations) in the deformation analysis, although in some cases stresses need be defined in order to satisfy stress boundary conditions such as shear stress and pressure.

7.2. Major challenges

Like any emerging field, the development of molecular biomechanics faces many challenges. For example, to understand how forces regulate cell function it is necessary to know how the applied forces are transmitted through cell–ECM and cell–cell contacts and distributed within the cells. Contractile forces generated by cells during locomotion (Dembo et al., 1996; Dembo and Wang, 1999) and mitosis (Burton and Taylor, 1997) have been measured with a deformable-substrate method or microelectromechanical systems (MEMS) technology (Galbraith and Sheetz, 1997). However, to date it still lacks good experimental and theoretical methods to quantify how these forces are distributed among various subcellular structures inside a cell. The reason is that a significant portion of forces is supported as well as generated by the cell cytoskeleton; however, cells are active and the cytoskeleton structures are dynamic, with simultaneous polymerization and depolymerization of the major cytoskeletal components including microtubules, actin filaments, and intermediate filaments. This poses a major challenge to both modeling (possibly finite element) and experimental efforts.

Structural (static and dynamic) analysis and constitutive modeling of the cell cytoskeleton, major subcellular components such microtubules, actin filaments and stress fibers, membrane systems, cell–ECM linkages, and the interactions among them is another challenging task. This includes the viscoelastic behavior and reorganization of
cytoskeletal networks, bending and buckling of various filaments, docking, fusion and budding of vesicles, the formation and strength of the cell adhesion complexes, and the strength and structural principles of elastin, collagen, and other large protein assemblies. Since the diameter of these filaments is on the order of 10 nm, their deformation (and constitutive models) may exhibit interesting features not found in large scale structures. Further, one may use the constitutive equations of these filaments as building blocks for the development of a structural model for the cell cytoskeleton. Analyzing these subcellular structures will not only provide a better understanding of the biological issues involved, but may also lead to new engineering design principles and approaches for developing advanced materials and composites.

It is very critical and challenging to quantify the deformation of DNA, RNA and protein molecules under applied mechanical forces and moments. Experimental techniques for force measurement in single-molecule biomechanics studies have been developed over the last decade or so including atomic force microscope (AFM, with a force range of 0.01–100 nN), optical tweezers (0.01–150 pN), micropipette/microneedle (0.01–1000 pN), surface force apparatus (10 nN–1 µN) and magnetic beads (0.01–100 pN) (Mehta et al., 1999; Leckband, 2000; Strick et al., 2000b). Quantifying protein deformation, however, is more difficult because a typical protein is about 5–50 nm in size; its deformation is usually a few nanometers or even just a few angstroms (Alberts et al., 1994; Creighton, 1993), which cannot be observed directly using optical microscopy. As a potential ‘deformation gauge’, the fluorescence resonance energy transfer (FRET) between donor and acceptor dye molecules is extremely sensitive to their relative distance (<10 nm); it has been used for studying the conformational changes during RNA folding (Zhuang et al., 2000), and coexisting conformations of fibronectin in cell culture (Baneyx et al., 2001). When properly labeled with FRET dye molecule pairs, proteins undergoing deformations of > 1 nm may be detected. It may also be possible to study conformational dynamics of proteins such as domain motion using FRET in fluorescence correlation spectroscopy (FCS). Another challenge is to generate controlled deformation in a single-molecule experiment. Unlike large-scale structures, proteins are so small that it is hard to anchor them, grab them and deform them mechanically without causing alterations in their conformation. It may be necessary to genetically engineer ‘handles’ or ‘tethers’ on proteins to facilitate the experimental studies. Despite all these difficulties, perhaps systematically classifying and quantifying the deformation of proteins and DNA is one of the most important research topics in molecular biomechanics. For example, we need to understand how different modes of deformation and dynamics of proteins are related to their structural rigidities (or sequences), quantify the time scales of protein motions and deformations as determined by the structural features, and explore whether protein function can be predicted directly from its mechanical properties.

Molecular dynamics (MD) simulations are based upon numerical solutions of the classical Newtonian equations of motion in which the force exerted on each atom is given by the negative gradient of the potential energy function with respect to the position of the atom (McCammon and Harvey, 1987; Wang et al., 2001). These simulations have become the ‘workhorse’ for exploration of structure dynamics of biomolecules, protein/DNA interaction, and the effect of solvent. Examples of MD
simulations include the predictions of protein folding, unfolding, motion, unbinding, and more recently its elasticity. For example, the forced unfolding of the FN-III domains of fibronectin can be simulated (Krammer et al., 1999; Vogel et al., 2001). The unbinding between biotin and streptavidin molecules has also been analyzed using MD simulations (Grubmuller et al., 1996). However, currently MD simulations can be performed only for a short time period, typically a few nanoseconds. The results of these simulations are often qualitative rather than quantitative due to the limitations of the force fields, i.e., the potential functions used to characterize the atomic and molecular interactions such as covalent and Coulomb interactions, electrostatic and van der Waals forces, and hydrogen bonding. These force fields are usually obtained using direct quantum mechanics calculations, or based on experimental measurements, of the interaction of limited pairs of atoms. Since many biomolecular processes in cells occur on a time scale ranging from a few microsecond to a few second, it is necessary to seek a new numerical approach to simplify the simulations while still preserve the basic features of the molecular interactions. It is unclear if a continuum-based approach similar to a finite element method based on the minimization of free energy is valid for studying molecular biomechanics issues.

Theoretical modeling of the deformation and dynamics of proteins and nucleic acids is a very rich area, and some of the basic approaches in applied mechanics are still valid. For example, the dynamics of proteins can be modeled at different levels: for example as a rigid body, a mass-spring-dashpot system, an Euler beam, or a full 3D structure. This is analogous to the study of large-scale engineering systems such as the motion of a tall building. It is worth mentioning, however, that the mechanics of biomolecules is quite different from conventional engineering mechanics in that mechanics needs to be integrated with thermodynamics, statistical physics, biochemistry and molecular biology.

8. Concluding remarks

As a basic discipline in engineering and science, mechanics has made tremendous progress over the last century. There is little doubt that mechanics can play an important role in advancing life sciences and medicine. Cellular and molecular biomechanics, addressing issues in cell locomotion, cell adhesion, cell spreading, cell–ECM interactions, and the dynamics of cell cytoskeleton, is important to wound healing, immunology, cell and tissue engineering, and cancer studies. Molecular biomechanics issues in functional genomics, proteomics and nanotechnology have also begun to attract attention, including those in protein and DNA conformational dynamics, diffusion, reaction, secretion and transport of biomolecules, and the structure-function relationship of molecular motors and machines. The rapid accumulation of sequence and structural information about proteins and nucleic acids, and the fast development of advanced technologies over the last few decades has provided a basis for systematic and quantitative studies of the mechanics of biomolecules. This presents great challenges and opportunities for researchers in solid mechanics.
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