Direct Measurement of the Forces Between Complementary Strands of DNA

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Interaction forces between single strands of DNA were measured with the atomic force microscope by a procedure in which DNA oligonucleotides were covalently attached to a spherical probe and surface. Adhesive forces measured between complementary 20-base strands fell into three distinct distributions centered at 1.52, 1.11, and 0.83 nanonewtons, which are associated with the rupture of the interchain interaction between a single pair of molecules involving 20, 16, and 12 base pairs, respectively. When a third long DNA molecule was coupled between complementary surfaces, both intra- and interchain forces were observed. The intrachain interaction resulting from the molecule's elasticity manifested itself as a long-range cohesive force.

The intra- and intermolecular forces of the DNA double helix are central to understanding its structure and rich functional behavior (*1*). Until recently, our knowledge of these molecular forces was based on indirect physical and thermodynamic measurements such as x-ray crystallography, light scattering, and nuclear magnetic resonance spectroscopy (*2*). Direct measurement of interaction forces requires that the state of a system be monitored while an independent force is applied. The osmotic pressure technique has been applied to measure the nonspecific intermolecular forces between DNA helices (*3*). For more complex molecular interactions in which adhesive or orientation-specific forces are active, such as molecular recognition, a direct measurement between individual molecules is required. Techniques such as magnetic (*4*) and optical (*5*) trapping have been used to measure the mechanical properties of individual strands (≈10 μm long) of double-stranded DNA. The stiffness of the trapping potential generated by these techniques (≈10^{-5} N/m) limits the maximum applied force and the position accuracy to 10^{-12} N and 30 nm, respectively. The atomic force microscope (AFM) has recently been applied to the study of single intermolecular interactions of the biotin-streptavidin system (*6*, *7*). The microfabricated cantilever of the AFM, a force transducer of small yet variable stiffness (springs with stiffnesses of 10^{2} and 10^{-3} N/m have been fabricated) and high resonance frequency, produces force sensitivities and position accuracies as small as 10^{-15} N/Hz$^{1/2}$ and 0.01 nm, respectively (*8*, *9*). Moreover, spherical probes and surface analytical techniques can be used to characterize the orientation and physical chemistry of the surfaces (*6*). We report here the measurement of the interaction of a single pair of DNA strands. Two types of forces have been identified: interchain forces associated with Watson-Crick base pairing (*10*) between complementary strands of DNA and intrachain forces associated with the elasticity of single strands of DNA.

We measured interchain interaction forces by covalently immobilizing oligonucleotides 20 bases in length to a silica probe and surface (Fig. 1A). We chose to study the complementary oligonucleotides $(ACTG)_5$ and $(CAGT)_5$ because they do not contain self-complementary regions and the bond energy associated with the double-helix complex is not large enough to disrupt the attachment of the oligomer to the surface (*11*). Interaction force is plotted as a function of surface separation in Fig. 2A. As the surfaces are brought together, the force is negligible until a 5-nm separation is reached, at which point the surfaces jump into contact because of attractive nonspecific surface forces (*2*). Further approach of the surfaces produces a repulsive force. When the surfaces are separated, a significant hysteresis is observed that is the result of an adhesive force of 1.56 nN.

One hundred or more interchain interactions could be measured between a single pair of surfaces (*12*), verifying that the covalently immobilized oligonucleotides are not irreversibly modified by tensile stresses up to ≈1.7 nN. The magnitudes of the adhesive forces fall into four distinct populations centered at 1.52 ± 0.19, 1.11 ± 0.13, 0.83 ± 0.11, and 0.48 ± 0.10 nN, as shown in Fig. 2B. The adhesive force measured between noncomplementary oligonucleotides averaged 0.38 ± 0.33 nN; therefore, the adhesive rupture force distribution centered at 0.48 nN is attributed to nonspecific interactions. The sequence of the oligonucleotides restricts base pairing to complexes of 20, 16, 12, 8, and 4 base pairs, although only the first three interactions are expected to be thermodynamically stable (*13*). If multiple oligonucleotide interactions took place in a significant fraction of the force measurements, the rupture force distribution would form a single broad distribution. Thus, the distributions labeled a, b, and c in Fig. 2B are associated with the interchain interactions of single pairs of DNA oligonucleotides 12, 16, and 20 base pairs in length, respectively.

In addition to measuring the interchain forces, we also used the AFM to study intrachain forces within long strands of DNA. We observed intrachain interactions using polydispersed homopolymers of inosine (I) (*14*, *15*) averaging 160 bases in length and complementary cytosine-functionalized surfaces (Fig. 1B). A force versus distance

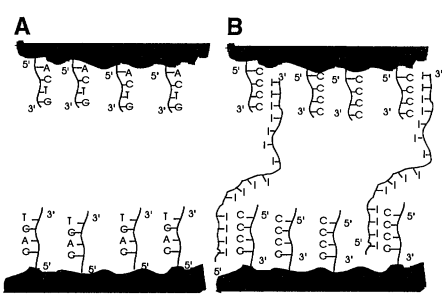

Fig. 1. Surface chemistry used to measure the (**A**) interchain and (**B**) intrachain forces between and within single strands of DNA. For the measurement of the interchain interaction, the complementary oligomers 5′-HS–ACTG–ACTG–ACTG–ACTG–ACTG-3′ [abbreviated $(ACTG)_5$] and 5′-HS–CAGT–CAGT–CAGT–CAGT–CAGT-3′ [abbreviated $(CAGT)_5$] were covalently attached to opposing surfaces. We synthesized the deoxyoligonucleotides by using standard phosphoramidite chemistry (*18*) and thiol-labeled them at the 5′ and 3′ ends (*19*, *20*) (Glen Research, Sterling, Virginia). We covalently attached the thiolated oligonucleotides to self-assembled monolayers of γ-aminopropylaminoethyltrimethoxysilane (Huls, Piscataway, New Jersey) on silica surfaces by using succinimidyl 4-(*p*-maleimidophenyl)butyrate (*21*) (Pierce, Rockford, Illinois). For measurements of intrachain forces, 5′-CCCC–CCCC–CCCC–CCCC–CCCC–SH-3′ and 5′-HS–CCCC–CCCC–CCCC–CCCC–CCCC-3′ (abbreviated as C_{20}) oligomers were immobilized on opposing surfaces, and a homopolymer of inosine [poly(I)] was hybridized to one of the surfaces in 1.0 N NaCl, pH 7.0, for 1 hour. The poly(I) was produced commercially (Pharmacia Biotech, Piscataway, New Jersey) with terminal transferase (*22*). The homopolymer has an average length of 160 bases, but polyacrylamide gel electrophoresis revealed that the poly(I) was composed of a wide distribution of molecular weights. Studies of the immobilized oligonucleotides with ^{32}P radiolabeling (*23*) revealed that the surface densities of covalently bound $(ACTG)_5$ and C_{20} were one molecule per 3.5 ± 0.7 nm^2 and one molecule per 4.5 ± 1.7 nm^2, respectively. However, only 3.3 ± 0.1% and 7.7 ± 0.8% of the immobilized $(ACTG)_5$ and C_{20}, respectively, are available for hybridization with oligonucleotides in 1.0 N NaCl, pH 7.0, at 25°C.

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curve measured between a poly(I)–C_{20} surface and a surface functionalized with C_{20} is shown in Fig. 3. As the surfaces were brought together and separated, the typical jump toward or away from contact (as observed in Fig. 2A) was not observed. We attribute this pattern of behavior to the repulsive steric interaction between the homopolymer and the oligonucleotide surface (*2*). However, as the surfaces of the homopolymers continue to separate, there is little interaction until the surface-probe separation reaches ≈220 nm, at which point a rapidly increasing force must be applied to the probe in order to increase the surface separation. The forces between the surface and probe increase until 1.51 nN at ≈240 nm, at which point the probe abruptly jumps away from the surface. The large adhesive force is indicative of the formation of a double-helix conformation (an interchain interaction), whereas the extended rupture length and elongation force are the result of the length of poly(I) (an intrachain interaction). Adhesive force curves of this form were observed in ≈20% of the 210 measurements made with three surface-probe pairs. The magnitude of the rupture forces fell in a single population averaging 0.46 ± 0.25 nN, but the distribution of rupture lengths varied significantly with the specific surface-probe pair studied. We attribute the variation in rupture length to the wide variation in poly(I) length and the limited number of poly(I) molecules in the probe-surface contact area.

The interchain interactions may be understood in terms of the theory of elasticity of polymeric molecules (*16*). For the freely jointed chain model, it has been shown that the force (F) applied to the molecules is proportional to the inverse Langevin function (L^*) for the elongation of the molecule (x):

$$F = \frac{kT}{a} L^*\left(\frac{x}{Na}\right)$$

where T is absolute temperature, k is the Boltzmann constant, a is the unit length, and N is the number of units in the chain (*17*). Comparison of the theoretical and experimental behavior (Fig. 3 inset) shows that the model correctly predicts that the force necessary to elongate the molecule will increase rapidly near the full length of the molecule. Deviation from theory and measured elongation force may result from the fact that the highly simplified model does not take into account molecule-dependent conformational changes such as rotation about the C–O and P–O bonds of the polynucleotide backbone (*1*).

The probe-surface contact area is large enough that multiple interchain bridges formed for ≈10% of the force measurement,

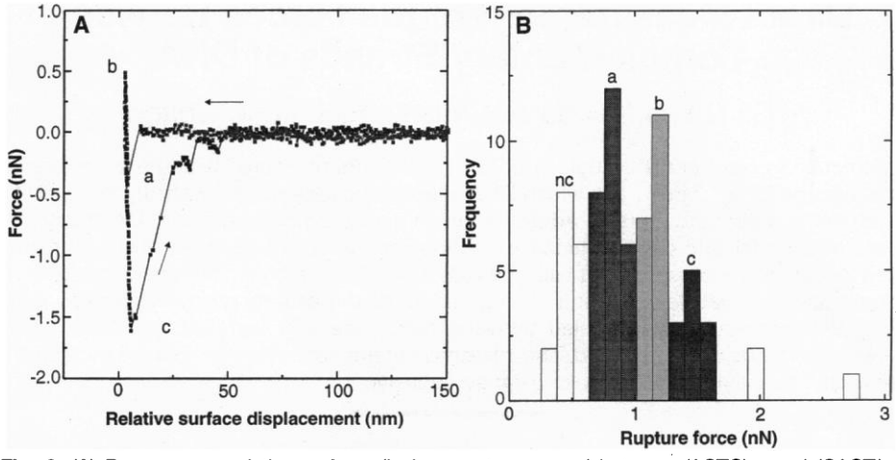

Fig. 2. (**A**) Force versus relative surface displacement measured between $(ACTG)_5$- and $(CAGT)_5$-functionalized surfaces in 0.1 N NaCl, pH 7.0, at 25°C (standard conditions). Points a, b, and c indicate the jump into contact, a region of repulsive force, and the jump away from contact, respectively. The rupture force, period, and length are 1.56 nN, 1.4 s, and 3 nm, respectively. Each data point (filled squares) on the curve represents 12 measurements taken for a ≈10-ms period. The AFM used in this study was designed specifically for force measurements in liquid; an optical detection scheme (*9*) was used to measure both the deflection of the cantilever and the position of the surface. Although the theoretical limits of force detection imposed by the optical detector (*24*) and by thermal noise (*25*) are ±0.002 nN and ±0.001 nN, respectively, variations in the optical properties limit the precision of force measurements to ±0.01 nN in practice. The instrument was operated in a variable-force mode in which the surface was ramped toward the probe at velocities of 10 to 0.1 nm/s until a repulsive force of ≈1 nN was sensed. Silica spheres, 60 to 120 μm in diameter, were attached to silicone oxynitride–microfabricated cantilevers (*8*) with a chemically inert epoxy, and the spring constant of each cantilever was measured at the point of probe contact (*6*). (**B**) Histogram of the rupture forces measured for a single pair of surfaces over a 2-hour period. The distribution of rupture forces labeled nc is attributed to nonspecific surface forces. The distributions of rupture forces labeled a, b, and c are attributed to a single pair of oligonucleotides involving 12, 16, and 20 base pairs, respectively. The observation of adhesive forces greater than 2 nN is attributed to multiple molecular interruptions.

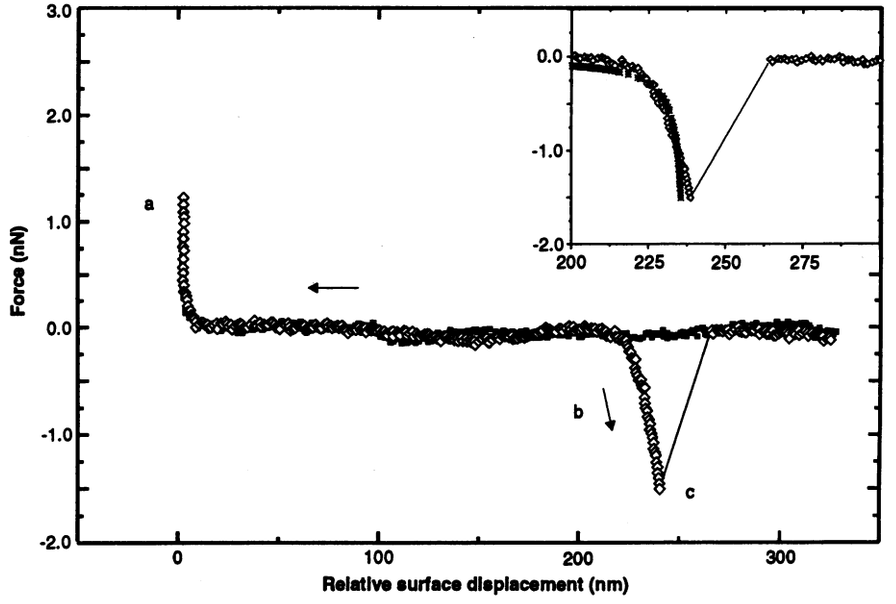

Fig. 3. Force versus relative surface displacement measured between a C_{20}–poly(I) surface and a C_{20}-functionalized probe under standard conditions. Points a, b, and c indicate repulsive contact, a region of rapidly increasing force necessary to elongate the polymer, and the jump away from contact, respectively. Rupture force, period, and length are 1.51 nN, 1.6 s, and 241 nm, respectively. The advancing and receding force curves are plotted with square and diamond symbols, respectively. (Inset) The measured and theoretical forces necessary to elongate a freely jointed chain of 883 units (N) with a unit length of 0.3 nm (a) were plotted in diamond and asterisk symbols, respectively. The value of a obtained from this force curve is slightly smaller than the unit length of common polymers such as polystyrene (a = 0.53 nm) but is significantly larger than the values measured for cellulose (*26*) or double-stranded DNA (*4*).

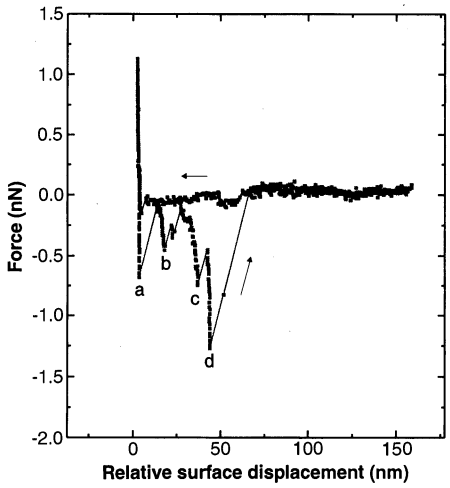

Fig. 4. Force versus relative surface displacement measured between a C_{20} surface functionalized with poly(I) and a C_{20} probe under standard conditions. Rupture forces, periods, and lengths for the individual polymers are as follows: a, 0.6 nN, 0.63 s, 0 nm; b, 0.4 nN, 0.57 s, 14 nm; c, 0.7 nN, 0.86 s, 33 nm; and d, 1.2 nN, 0.92 s, 40 nm.

as illustrated by the force curve shown in Fig. 4. Striking features of this force curve are the multiple rupture points with varying rupture forces. The differing rupture lengths are the result of the wide distribution length of poly(I) and the curvature of the spherical probe. The fact that poly(I)–C_{20} base pairing is not unique but varies from 20 base pairs down to the minimum number of bases that are thermodynamically stable is responsible for the variation in rupture force. The multiple discrete rupture points within a given force curve illustrate the monomolecular nature of these measurements.

These results, the quantitative measurement of the force necessary to rupture the DNA double helix conformation and the elasticity of single-stranded DNA, demonstrate that the AFM can be used to study both the inter- and the intramolecular interactions of complex biological and synthetic macromolecules with atomic force and displacement resolution. Furthermore, this study demonstrates that the AFM has the analytical capacity to detect the presence and relative position of specific base sequences with angstrom resolution.

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11. Using the approach of K. J. Breslauer, R. Frank, H. Bocker, and L. A. Marky [*Proc. Natl. Acad. Sci. U.S.A.* **83**, 3746 (1986)] and data obtained by H. H. Klump [in *Biochemical Thermodynamics*, M. N. Jones, Ed. (Elsevier, Amsterdam, ed. 2, 1988), pp. 105–144], we calculate that the $(ACTG)_5$ and $(CAGT)_5$ double helix standard free energy [$\Delta G^{\circ}_{(ACTG)_5-(CAGT)_5}$] is 115 kJ/mol in 0.1 N NaCl. This bond energy is approximately half that of the covalent bonds anchoring the oligonucleotides to the silica surfaces.
12. A slow loss of activity over several hours was observed and is attributed to noncovalently adsorbed oligonucleotides that are transferred from surface to probe [L. A. Chrisey *et al.*, *Mater. Res. Soc. Symp. Proc.* **330**, 179 (1994)].
13. The melting temperature (T_m) of the oligonucleotides may be estimated with the approach of L. A. Marky and K. J. Breslauer [*Biopolymers* **26**, 1601 (1987)]. The T_m of the 12- and 8-mer in 0.1 N NaCl at an oligonucleotide concentration of 10^{-4} M is 48° and 21°C, respectively. Stable double helices will rapidly start to form at temperatures below T_m; therefore, we expect to observe the 12-mer but not the 8-mer interchain interaction under the experimental conditions (27°C). The oligonucleotide concentration was estimated to be $\approx 10^{-4}$ M assuming that (i) 10 active molecules are immobilized within the ≈900-nm² probe contact area (*6*) and (ii) a 100-nm distance should be used to calculate the volume element of the active molecules.
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Use of Taylor-Aris Dispersion for Measurement of a Solute Diffusion Coefficient in Thin Capillaries

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A method for the fast measurement of the diffusion coefficients of both small and large molecules in thin capillaries is reported. The method relies on Taylor-Aris dispersion theory and uses standard instrumentation for capillary zone electrophoresis. With this equipment, which consists of thin capillaries (50 to 100 micrometers in inner diameter), an injection system, detector ports, and computer data acquisition, a sample plug is pumped through the capillary at known velocity and the peak dispersion coefficient (D^*) is measured. With the experimentally measured values of D^* and flow velocity, and knowledge of the inner diameter of the capillary, the molecular diffusion coefficient (D) can be rapidly derived. For example, for ovalbumin a D value of 0.759×10^{-6} square centimeter per second is found versus a tabulated value of 0.776×10^{-6} square centimeter per second (error, 2 percent). For hemoglobin a D value of 0.676×10^{-6} square centimeter per second is obtained versus a literature value of 0.690×10^{-6} square centimeter per second (error, 1.5 percent).

The idea of using the dispersion of a solute plug in a laminar Poiseuille flow for measurements of the diffusion coefficient of solute molecules was presented in a classic paper of Taylor (*1*). This method is attractive as it offers a possibility for a fast evaluation of diffusion coefficients and thus estimation of the effective dimensions of the particles in a solution (the Stokes radius). However, this method has not been brought into everyday practice. This report concentrates on a practical application of Taylor's method for measuring diffusion coefficients in a wide range of soluble substances, from ionic solutions to proteins.

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