Published in final edited form as:

Nano Lett. 2008 December ; 8(12): 4365–4372.

Peeling Single Stranded DNA from Graphite Surface to Determine Oligonucleotide Binding Energy by Force Spectroscopy

Suresh Manohar^a, Amber R. Mantz^b, Kevin E. Bancroft^b, Chung-Yuen Hui^c, Anand Jagota^{a,*}, and Dmitri V. Vezenov^{b,*}

^aDepartment of Chemical Engineering, Lehigh University, Bethlehem, PA, 18015

^bDepartment of Chemistry, Lehigh University, Bethlehem, PA, 18015

^cDepartment of Theoretical and Applied Mechanics, Cornell University, Ithaca, NY, 14853.

Abstract

We measured the force required to peel single-stranded DNA molecules from single-crystal graphite using chemical force microscopy. Force traces during retraction of a tip chemically modified with oligonucleotides displayed characteristic plateaus with abrupt force jumps, which we interpreted as a steady state peeling process punctuated by complete detachment of one or more molecules. We were able to differentiate between bases in pyrimidine homopolymers – peeling forces were 85.3 ± 4.7 pN for polythymine and 60.8 ± 5.5 pN for polycytosine, substantially independent of salt concentration and the rate of detachment. We developed a model for peeling a freely jointed chain from the graphite surface and estimated the average binding energy per monomer to be 11.5 ± 0.6 k_BT and 8.3 ± 0.7 k_BT in the cases of thymine and cytosine nucleotides. The equilibrium free-energy profile simulated using molecular dynamics had a potential well of 18.9 k_BT for thymidine, showing that non-electrostatic interactions dominate the binding. The discrepancy between the experiment and theory indicates that not all bases are adsorbed on the surface or that there is a population of conformations in which they adsorb. Force spectroscopy using oligonucleotides covalently linked to AFM tips provides a flexible and unambiguous means to quantify the strength of interactions between DNA and a number of substrates, potentially including nanomaterials such as carbon nanotubes.

Interactions of polyelectrolytes with solid substrates have several important applications in materials science and engineering.^{1, 2} On complexation with neutral particles, polyelectrolytes convert these particles into charged species, enabling dispersions in aqueous media, and find use in detergents, cosmetics, gels, food additives, and oil recovery. In a biological context, our interest is in the interaction between single and double-stranded DNA (ssDNA and dsDNA) and substrates such as graphite and carbon-nanotubes (CNTs) that can potentially play a vital role in biomedicine,^{3, 4} nanotechnology,^{5, 6} and relevant for the understanding of the origin of life.⁷ Importantly, ssDNA has been used successfully for dispersion and solution-based manipulation of CNTs – ssDNA forms a stable hybrid with a nanotube by wrapping around the CNT in helical fashion.^{8, 9} The hybrid is useful for dispersion, sorting,^{8, 9} and patterned placement of nanotubes,⁶ for transportation of DNA into cells, and for killing cancer cells by thermal ablation.³ Strength of dispersion, ability to sort, and stability in the cellular environment all depend on the interaction between DNA and a CNT.¹⁰ Individual DNA bases also bind to a graphitic surface through non-covalent π - π interactions.^{11–14}

Very little is known quantitatively about the strength of binding between ssDNA and CNT's in spite of the fundamental importance of understanding such interactions.^{8, 15–17} As a first

model for ssDNA-CNT interactions, herein we present results on quantifying interactions of ssDNA with a surface of graphite using force spectroscopy. Studies of mechanical stretching of ssDNA and dsDNA have provided some of the best experimental verification of models for entropic elasticity.¹⁸ The ssDNA/graphite system, in a similar fashion, provides a good model system to understand the interactions of polyelectrolytes with hydrophobic substrates.^{19–25}

Single-molecule force spectroscopy has been used successfully to study the desorption process for polyelectrolytes (PEs) on charged substrates.^{19–27} Typically, when a long adsorbed PE chain is pulled from a surface, the required force reaches a plateau at distances greater than the Debye screening length of the electrolyte solution.²⁶ The force required to pull off the chain has been interpreted using an equilibrium process model, in which the work done by external force goes primarily to overcome the adsorption free energy.

In a series of papers, Gaub and co-workers^{19–25} have studied the desorption process of anionic and cationic PE chains from different substrates (cationic, hydrophobic, and metallic). For PEs on charged substrates, the desorption force can be separated into a non-electrostatic component, an entropic contribution from the polymer chain, and electrostatic interactions. Electrostatic contribution can be decoupled from the overall force by measuring the effect of varying ionic strength of the solution. The contribution due to the conformational entropy of the adsorbed region of the PE is usually unknown and has so far been neglected.²⁵ Surprisingly, hydrophobic surfaces effectively act like negatively charged surfaces and, even for highly charged substrates, the non-electrostatic contribution ($\approx 3\text{--}6\text{ k}_\text{B}\text{T}$ as obtained in the limit of high salt concentration) dominates over the electrostatic contribution down to a salt concentration of 10 mM.²⁵

A different phenomenology has been observed when a strongly adsorbing moiety is small and tethered by a non-adsorbing chain to the AFM tip. In this case, the characteristic feature of the measured force-deflection curve was the stretching of a polymer chain followed by a single release event. For example, pyrene was tethered to the AFM tip via a poly(ethylene glycol) chain and the strength of the π - π interaction between a single pyrene molecule and graphite in aqueous media was found to be 55 pN.²⁸ The corresponding release force was interpreted as the strength of pyrene-graphite interaction.

The interaction of single DNA bases with graphite has been studied experimentally with Langmuir adsorption isotherms.^{7, 29} The binding strengths of the four DNA bases decreased in order as Guanine > Adenine > Thymine > Cytosine⁷ and the adsorption enthalpy for adenine was determined to be 8.1 $\text{k}_\text{B}\text{T}$.²⁹ Shi *et. al.*¹³ have studied the peeling of homopolymer ssDNA from a sheet of graphene using molecular dynamics (MD) simulations (CHARMM) and quote the binding energies for G/A/T/C to be 38/35/32/28 $\text{k}_\text{B}\text{T}$ for bases and 50/45/43/40 $\text{k}_\text{B}\text{T}$ for nucleosides, much higher than the experimental value for adenine. DNA-graphite interactions have so far not been accessible experimentally.

In this letter, we present results on single-molecule force spectroscopy of polythymine interacting with a graphitic surface. We used covalent attachment of ssDNA for AFM tip functionalization³⁰ and carried out our force spectroscopy experiments under varying ionic strength and loading rates. In our study, we have found two types of force-displacement curves – steady state peeling or polymer chain stretching – depending on the type of the substrate we used. For quantitative analysis of our experimental results, we developed a model that relates measured steady state peeling forces to adsorption free energy of an oligonucleotide.

Figure 1 shows schematically our experimental approach. UV-ozone³¹ or plasma cleaned gold-coated AFM tips were functionalized by covalent attachment of a mixed monolayer of thiol-modified ssDNA 50-mer and mercaptohexanoic acid (MHA).^{32–36} A mixed monolayer was used to reduce non-specific adhesion between the AFM tip and the graphitic surface, and to

reduce the surface density of ssDNA molecules to a level where detachment of individual molecules would be evident. The functionalized tips were brought into contact with the graphite surface and subsequently retracted. In control experiments using AFM tips having only an MHA monolayer, we measured purely repulsive forces between the tip and the surface at all tip-surface separations. In 10 mM phosphate buffer (pH 7.2), the forces decreased exponentially as a function of distance between the tip and substrate, with a characteristic decay distance of $\lambda=2.3$ nm, similar to the calculated Debye screening length of $\lambda_D=2.1$ nm, suggesting electrostatic repulsion (See supporting information (SI), Section 2).

When the gold-coated AFM tips functionalized with ssDNA and MHA were lowered into contact with the graphite surface in 10 mM phosphate buffer at pH 7.2 and then retracted, the force-distance curves were markedly different (Figure 2a) from those obtained in the experiments using tips functionalized with COOH groups alone (Figure S3). On approach, the interaction is first repulsive, as in the case of the control experiments with MHA tips, and likely also electrostatic in nature (same λ as for the MHA tips). Near the surface, presumably as dangling ssDNA strands find the surface, there is evidence of jump-to-contact at 6.5 ± 0.5 nm separation (mean $\pm$ standard deviation, $m=100$, where m is the number of force curves used). This jump-to-contact distance should reflect the size, R_z of the DNA molecule in the buffered solution. Assuming a Gaussian coil conformation for the ssDNA of contour length L , we can estimate the Kuhn length, b , using the relationship for the end-to-end distance $R=\sqrt{Lb}$. For a 50-mer ($L=27$ nm), the Kuhn length of the DNA under these conditions is then 1.6 ± 0.3 nm. We note that this value is comparable to the Kuhn length of 1.5 nm for ssDNA in 150 mM ionic strength solutions.^{37, 38} Due to the negative charge of the COOH-terminated tip surface, the similarly charged DNA should extend farther from the tip than the size of a Gaussian coil would imply. Indeed, our experiments on stretching DNA chains yield a value of Kuhn length considerably lower than that measured for long chains.³⁸

On retraction, we initially observe a large adhesive force likely due to the interaction between multiple DNA strands and graphite. There is a large drop in force after initial pull-off, but some tensile force persists over separations large compared to the Debye length and, in some experiments, up to the contour length of the fully extended 50-mer (27 nm). This tensile force reduces in characteristic discrete steps, which we interpret as resulting from the detachment of a small finite number of ssDNA strands. We found that, while there is considerable variability in the initial pull-off force (see Figure S8 for several examples of force-distance curves), the difference between plateau forces varies in a relatively narrow range. These observations are consistent with previous measurements of pull-off forces of polyelectrolytes with polyethylene backbone (usually with chain lengths of hundreds of nm – much longer than the oligomers studied here).^{22–25} The force jumps can be used to extract adhesion free energy between the ssDNA and the surface of graphite with the model we develop below for this system.

To analyze the force data obtained from the poly(dT)-modified AFM tips, for each force curve, we measured the magnitude of average peel force from individual plateaus. We observed that peel force magnitudes varied over a large range, reflecting the changing number of ssDNA molecules interacting with the surface. However, there was a significant clustering of data in the range of 65 to 105 pN for the *difference* between adjacent force plateaus. The plateau force jumps were measured as the difference between the average forces estimated over a distance of 1 nm or more just before and after the jump. We interpret this collection of force jumps between neighboring plateaus as resulting from the detachment of a (usually) single ssDNA molecule from the surface of graphite. A Gaussian fit to the histogram (Figure 2b) of plateau force jumps, obtained from many such force-distance curves, yielded a mean force jump of 84.2 ± 8.4 pN ($\pm$ standard deviation, $m=75$).

The phenomenon of stepwise peeling was very reproducible in our experiments. For a given tip and oligonucleotide, repeated measurements at the same site, at different sites, and with different rates all resulted in similar forces. Mean force values converged quickly to the reported value, falling within 3 pN range for $m > 50$; this was tested by averaging 50, 100, 150, 200 and 250 force curves. We observed that the tip-to-tip variation in the average plateau force (95% confidence limit) was comparable to the width of the distribution of peeling forces for individual tips (~5% versus ~10% of the respective mean values). For poly(dT), the use of different tips produced *average* steady state forces in the range of 72 to 104 pN with 85 pN being the prevalent value (85.3 ± 4.7 pN, 14 experiments). The 5–10% variation in the absolute values of the force is consistent with the expected accuracy for cantilever spring constants determined by the thermal calibration method (about 20%). Here, we report results from the same AFM tip when changing some experimental parameter.

To show that the peeling of ssDNA bases from the graphitic surface occurs at equilibrium, we carried out experiments in 10 mM phosphate buffer with varying AFM tip velocities. The plateau force jump was found to be independent of tip velocity in the range of 200 nm/s to 1000 nm/s (Table S1). The mean and the standard deviation of the plateau force jump for poly(dT) obtained from the entire set of data for all tip velocities ($m=242$) were 83.8 pN and 9.1 pN, respectively.

In order to sort out the contributions of electrostatic and the non-electrostatic interactions to the plateau force, we studied the effect of salt concentration on measured forces. Using the same tip that produced results on the effect of the peeling rate, we recorded force-extension curves in 10 mM phosphate buffer where the total electrolyte concentration was varied by the addition of NaCl. The force jumps were independent of the salt concentration (Table S2); the mean value and the standard deviation of the peeling force, obtained from the entire set of force spectroscopy data on poly(dT) at varying salt concentration ($m=438$), were 81.4 pN and 5.6 pN, respectively. Therefore, for ssDNA, the contribution from electrostatic interactions to the binding free energy between nucleotides and graphite is much smaller than contributions of van der Waals forces (including π -stacking interactions) and hydrogen bonding (i.e. hydrophobic effect). This conclusion can be further substantiated by an order of magnitude calculation of electrostatic binding energy in the DNA-graphite system. Consider a neutral graphite surface and ssDNA backbone with charge density of one electronic charge, e , per phosphate group, located at a distance $r=0.5$ nm away from the surface in medium of dielectric constant ϵ . An upper bound on the interaction energy of this charge with the substrate can be obtained by ignoring counterion screening and computing image charge energy. In this case, the interaction between the negative charge of phosphate and its image³⁹ will be $e^2/(16\pi\epsilon\epsilon_0 r) = 0.35$ k_BT/nucleotide. We found that the binding energy per nucleotide estimated from our experiments is much larger than 0.35 k_BT, and, therefore, the electrostatic contribution is indeed expected to be small.

To determine whether ssDNA-graphite interactions are sensitive to the chemical nature of the base, we carried these force spectroscopy experiments using a ssDNA polymer having another pyrimidine base – cytosine. As with polythymine, we observed stepwise detachment of individual strands occurring under steady state forces of the type shown in Figure 2. While steric contribution is expected to be similar for the two bases, we observed distinctly different peeling forces for poly(dT) and poly(dC) 50-mers. In the case of cytosine, the average peeling force of DNA homopolymer was 60.8 ± 5.5 pN substantially below 85.3 ± 4.7 pN for thymine (14 experiments in both cases). Stronger binding with graphite observed for thymine versus cytosine is consistent with ordering of experimental enthalpies for individual bases⁷ and calculated interaction energies for DNA polymers.¹³

Using DNA/MHA modified gold coated tips, we repeated force spectroscopy experiments on amine terminated substrates. The substrates were prepared by modifying silicon wafers with 3-aminopropyltriethoxysilane (APTES) in anhydrous toluene.^{28, 40} In solution, the amine monolayer is expected to be positively charged, whereas the ssDNA molecule is negatively charged; thus, we expect significantly higher adsorption and diffusion barrier energies for the APTES surface than for the neutral graphite surface. Indeed, instead of the force plateaus, we observed a response characteristic of single molecule stretching (Figure 3) with the pull-offs at distances between 8 and 16 nm from the surface. Fitting of our force spectroscopy data for polythymine/APTES to an extended model of stretching a freely jointed chain (eFJC)⁴¹ gave a mean Kuhn length, b , of 0.51–1.00 nm and a segment elasticity, κ , of 2.4–6.2 nN. The b values are lower than expected for the ssDNA from the previous optical tweezers^{18, 37, 38} and diffusion measurements.⁴² We note, however, that the eFJC fits with the Kuhn length fixed at the appropriate value (2.5 nm for 10 mM ionic strength) produces a result of reasonable quality (black line in Figure 3). (See SI, Section 1.5 for details)

Here, we use a simple equilibrium model for the system that relates the measured peeling force to the binding free energy, therefore, leading to an estimate of the binding energy per nucleotide from the measurement of the plateau force. By equilibrium we mean that the time required for the adsorbed and desorbed parts of the ssDNA chain to sample their conformational space is much smaller than the time over which the molecule is peeled off. This assumption is supported experimentally by the finding that peeling forces are independent of peeling rate (See SI for additional arguments). We specifically do not assume equilibrium between the partially adsorbed and completely detached states of the molecule.

Figure 1 depicts an AFM tip pulling a polymer chain partially adsorbed on the surface. If the lateral mobility of the chains on the surface is high, peeling of the molecule under external force will occur while maintaining a fixed right angle with respect to the substrate. Experiments and theory both suggest that the frictional barrier for lateral movement of DNA bases on graphite is small ($<2 k_B T$)^{16, 17, 43} consistent with our observation of different force-distance profiles for graphite and amino-functionalized substrates (peeling versus stretching). We, therefore, assume that desorption of the polymer chain from graphite occurs in equilibrium; this assumption is further supported by insensitivity of measured peeling forces to pulling rate. That is, the ssDNA on this surface cannot support a net horizontal force and must peel off at an average angle of 90° with respect to substrate.

Our system consists of the ssDNA chain, which is modeled as a freely jointed chain^{37, 38, 44} with a contour length Nb , where N is the number of Kuhn links. A force f is applied at one end (via the AFM tip, Figure 1), while the other end is adsorbed on the surface due to the action of an adhesion free energy per unit length, γ , which is the property we wish to determine. We assume that the free energy of the system, G_{total} , under these conditions has contributions due to (i) the free energy of the adsorbed ssDNA, G_{adh} , and (ii) the entropic and enthalpic free energy stored in the desorbed regions of ssDNA, which has n links, G_{FJC} . The free energy of $(N-n)$ ssDNA links adsorbed on the graphite is given by:

$$G_{adh} = -b(N-n)\gamma = -k_B T(N-n)\Gamma, \quad (1)$$

where $\Gamma = \gamma b / (k_B T)$ is the dimensionless free energy of adhesion per Kuhn segment. The value of γ incorporates various physical contributions, *e.g.* γ includes the van der Waals and electrostatic interactions between ssDNA and the graphite, hydrogen bonding rearrangement in the solvent, solvation of the base and graphite surface, and the entropic contribution from the adsorbed DNA.²⁵

Under fixed load, the internal energy of all conformations of the desorbed part of the FJC chain is the same and set to zero. Only the potential of the external load distinguishes them. The corresponding free energy, following Rubinstein and Colby⁴⁴ is

$$G_{\text{FJC}} = -k_B T n \ln \left[\frac{4\pi \sinh F}{F} \right], \quad (2)$$

where

$$F = \frac{fb}{k_B T} \quad (3)$$

is a dimensionless force. The equilibrium condition is obtained by minimization of the total free energy $G_{\text{total}} = G_{\text{adh}} + G_{\text{FJC}}$ under fixed force and binding free energy, *i.e.*,

$$\left. \frac{\partial G_{\text{total}}}{\partial n} \right|_{f, \gamma, T} = 0. \quad (4)$$

Equation 4 gives the relationship between the dimensionless energy Γ and the dimensionless force F ,

$$\Gamma = \ln \left[\frac{4\pi \sinh F}{F} \right]. \quad (5)$$

Thus, for a given adhesion free energy, peeling of the chain is predicted to occur at fixed force. For small F ,

$$\Gamma \approx \ln(4\pi) + \frac{F^2}{6}, \quad (6)$$

while, for large F ,

$$\ln \left[\frac{4\pi \sinh F}{F} \right] \approx \ln(4\pi) + F - \ln(F) \approx F; \quad \gamma = f, \quad (7)$$

which is independent of the Kuhn length. The small force limit (Equation 6) can be derived directly by approximating the FJC as a Gaussian chain. The large force limit has also been obtained by a work balance approach by explicitly ignoring contribution from stretching of the desorbed segment,²³ analogous to peel analyses in macroscopic materials.¹³ A further correction to the estimated adhesion energy can be made by using a Kuhn length elongated due to enthalpic stretching to $b(1+f/\kappa)$, which reproduces the extended Langevin force-displacement relationship:³⁸

$$F = \frac{fb(1+f/\kappa)}{k_B T}. \quad (8)$$

The elasticity κ of each link of ssDNA as cited in the literature is around 0.80 nN;³⁸ our fitting of the ssDNA stretching yielded a range of 2.4–6.5 nN.

The analysis presented above, while conceptually simple, remains approximate, because the free energy has been written in terms of contributions that are strictly valid under fixed n whereas the system is actually under fixed γ . Below, by deriving the partition function for a FJC adsorbed to a surface with fixed γ and subjected to fixed force f ,^{38, 45} we demonstrate that under typical experimental conditions, this approximation does *not* cause a significant error. We show that, except for very short chains, a plateau force develops during peeling and that its value is given accurately by Equation 5. One can also show that extension of the chain under either displacement or force control yields identical results for the relationship between plateau force and adhesion free energy (see SI, Section 8).

Any configuration of the system (Figure 1) is defined by the position, R_z , of the applied force, f , and the number of desorbed links, n . The energy of the system in terms of these two parameters is

$$U = -fR_z - (N - n)\gamma b. \quad (9)$$

End-to-end distance of the desorbed links, R_z , can be represented as the sum of the projections of n links along z direction (Figure 1, inset).

$$R_z = \sum_{i=1}^n b \cos \theta_i \quad (10)$$

We assume that n varies from 0 to N , θ varies from 0 to π and ϕ varies from 0 to 2π . By not restricting the range of allowed angles, we incur an error for small n due to allowing conformations where parts of the chain penetrate the graphite surface. However, these conformations are of little consequence except for very small forces, as we have confirmed numerically.

The partition function is, therefore:

$$Z = \sum_{\text{states}} \exp \frac{-U}{k_B T} = \sum_{n=0}^N \left[\int_0^{\pi} \int_0^{2\pi} \exp \left(\frac{fR_z + (N - n)\gamma b}{k_B T} \right) \left(\prod_{i=1}^n \sin \theta_i d\phi_i d\theta_i \right) \right] \quad (11)$$

The inner integrals over angles can be evaluated, yielding

$$Z = \exp(\Gamma N) \sum_{n=0}^N \exp\{n(X - \Gamma)\} = \exp(\Gamma N) \frac{1 - \exp[(X - \Gamma)(N + 1)]}{1 - \exp(X - \Gamma)} \quad (12)$$

where $X = \ln \left(\frac{4\pi \sinh F}{F} \right)$. Since we are in P, T, γ, f ensemble:

$$dG = VdP - SdT - (N - \langle n \rangle)b d\gamma - \langle R_z \rangle df, \quad (13)$$

so that $\langle R_z \rangle = - \frac{\partial G}{\partial f} \Big|_{P,T,\gamma}$ and $N - \langle n \rangle = - \frac{1}{b} \frac{\partial G}{\partial \gamma} \Big|_{P,T,f}$, which, with the free energy $G = -k_B T \ln Z$ yields the results for mean values of n and R_z :

$$\begin{aligned} \langle n \rangle &= \left(\frac{1}{\exp(\Gamma - X) - 1} - \frac{(N+1)}{\exp((N+1)(\Gamma - X) - 1)} \right) \\ \langle R_z \rangle &= b \left(\coth F - \frac{1}{F} \right) \langle n \rangle \end{aligned} \quad (14)$$

It can be shown that when $\langle n \rangle = N/2$

$$X = \Gamma, \quad (15)$$

which is identical to Equation 5.

In Figure 4, we plot the average length, $\langle R_z \rangle/b$, and average number of desorbed links, $\langle n \rangle$, for a chain with Kuhn length $b=1.5$ nm at different forces for $N=18$ and Γ chosen such that the peeling force $f_{\text{peel}}=85$ pN. The main striking feature is that, except for an initial transient build-up in force, and a terminal region near full extension of the chain, peeling of the molecule occurs at fixed force, consistent with the simple model (Equation 5). With increasing applied force, the transition to the plateau force is nearly complete by the time five links have desorbed. Because in our model we do not let the chain desorb completely (n cannot exceed N) as n approaches N , the model predicts an increase in force. Repeating the calculation with different values of N , we find that for chains with 10 Kuhn links or more, a clear plateau force regime is predicted to occur.

Equation 5, Equation 6, and Equation 7 are plotted in Figure 5. From the measured force, f , and fitted value of Kuhn length, b , we compute the normalized force, F . Peeling forces are in a range where the low force (Gaussian chain) limit is inaccurate. Equation 5 provides the resulting value of Γ , from which we obtain the value of γ . The average steady state force for desorbing ssDNA from the graphite obtained from several ($m=14$) different experiments is 85.3 ± 4.7 pN for poly(dT) and 60.8 ± 5.5 pN for poly(dC). Taking the distance between phosphate groups to be 0.56 nm, we obtain a value of the *effective* binding free energy of 11.5 ± 0.6 $k_B T$ for single thymine nucleotide and 8.3 ± 0.7 $k_B T$ for single cytosine nucleotide using $b=0.51$ nm and $\kappa=2.4$ nN. Since b appears in both dimensionless parameters, and we are approaching the high force limit, the estimated value of γ is practically insensitive to the choice of Kuhn length (see Equation 7).

Experiments under varying salt concentration suggest that binding free energy is dominated by non-electrostatic interactions. To understand the relationship between measured binding energy and the structure, we conducted molecular dynamics simulations of the interaction between thymidine and a graphitic surface using the CHARMM force-field.^{46, 47} Using thermodynamic integration,⁴⁸ we computed the binding free energy between thymidine and a graphitic surface to be 18.9 $k_B T$ (See SI, Section 7), which is significantly larger than the binding energy extracted from force measurements. Within the limitations of the force-field used, these simulation results support the notion that non-electrostatic contributions form the major component of the binding free energy between nucleotides and graphite. Note that the experiment measures an effective binding energy per unit length of the ssDNA backbone. For example, as suggested by Manohar *et. al.*,¹⁶ the effective binding energy can be reduced significantly compared to that of an isolated nucleotide if some fraction of bases remain unbound due to steric hindrance from neighbors.

Estimation of the energy for base pairing in solvated nucleic acids is usually available only from theoretical calculations because of the difficulty in resolving contributions of various components to the net interaction (hydrogen bonding, electrostatic forces, and π stacking). The single molecule force spectroscopy experiments on the DNA-graphite system discussed here can be viewed as a convenient model system to measure the contribution to base pairing energy from purely base stacking interactions and possibly to rank the four bases in this respect. It has been demonstrated theoretically that electrostatic interactions destabilize complexes of base dimers in solution.⁴⁹ The stability of thymine-containing dsDNA is only 1.5–3.2 k_BT per base in term of the free energy change on melting a duplex DNA, where electrostatic contributions should be large.⁵⁰ These values are much lower than our experimental or theoretical values for thymine-graphite interaction in aqueous solutions. Therefore, hydrophobic interaction appears to be the main contributor to observed nucleotide-graphite binding energy, as was also argued theoretically for base stacking interactions.⁴⁹ This interpretation is consistent with higher binding energy observed for thymine versus cytosine, since thymine, while similar in size to cytosine, has an additional methyl group. Comparison of accepted calculated values for stacking energy with our experimental and theoretical values for binding energy between polythymine and graphite confirms the notion that the non-electrostatic part of stacking energy is a major contributor to stability of the DNA duplex and DNA-CNT constructs.

Using chemical force spectroscopy, we have measured the force required to peel pyrimidine DNA homopolymers from graphite. From this measurement, using a new equilibrium peeling model, we estimated an effective binding energy of 11.5 k_BT per nucleotide for interaction between polythymine and graphite. This experimental result is in reasonable agreement with the calculated free energy profile of thymidine-graphite system. Results from previous MD simulations on peeling DNA homopolymers overestimated binding energy, probably due to the non-equilibrium nature of the modeled process. Force spectroscopy was sensitive to the chemical nature of the base in ssDNA and could differentiate between polythymine and polycytosine in their interactions with graphite. Interaction of graphite with polycytosine was less favorable than with polythymine by 3 k_BT per nucleotide.

Peeling forces in our system turned out to be independent of ionic strength. Therefore, graphite can serve as an ideal substrate to study non-polar contributions to polyelectrolyte adsorption on solid surfaces and help to decouple various components of the base stacking energy in DNA. Using the imaging capability of the AFM, it should be possible to apply force spectroscopy in a highly localized manner to probe interaction between macromolecules and nanomaterials, such as forces responsible for the formation of stable DNA-CNT complexes. Potential differences in binding energies between nucleotides and single CNTs could be important for applications aiming to exploit chemical sensitivity of these interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by NIH grant R21 HG004141 (D.V.) and NSF grant CMS-0609050 (A.J.). We have used XPS and SEM facilities supported by the Center for Advanced Materials and Nanotechnology of Lehigh University.

References

1. Fleer, G.J.; Cohen Stuart, M.A.; Scheutjens, J.M.H.M.; Cosgrove, T.; Vincent, B. *Polymers at Interfaces*. London: Chapman&Hall; 1993. p. 1-40.

2. Dautzenberg, H.; Jaeger, W.; Kötz, J.; Philipp, B.; Seidel, C.; Stscherbina, D. Polyelectrolytes: Formation, Characterization and Application. Cincinnati: Hanser/Gardner; 1994. p. 1-9.
3. Kam NWS, O'Connell M, Wisdom JA, Dai HJ. P Natl Acad Sci USA 2005;102:11600–11605.
4. Kam NWS, Liu ZA, Dai HJ. Angew Chem Int Edit 2006;45:577–581.
5. Staii C, Johnson AT. Nano Lett 2005;5:1774–1778. [PubMed: 16159222]
6. McLean RS, Huang XY, Khripin C, Jagota A, Zheng M. Nano Lett 2006;6:55–60. [PubMed: 16402787]
7. Sowerby SJ, Cohn CA, Heckl WM, Holm NG. P Natl Acad Sci USA 2001;98:820–822.
8. Zheng M, Jagota A, Semke ED, Diner BA, Mclean RS, Lustig SR, Richardson RE, Tassi NG. Nat Mater 2003;2:338–342. [PubMed: 12692536]
9. Zheng M, Jagota A, Strano MS, Santos AP, Barone P, Chou SG, Diner BA, Dresselhaus MS, McLean RS, Onoa GB, Samsonidze GG, Semke ED, Usrey M, Walls DJ. Science 2003;302:1545–1548. [PubMed: 14645843]
10. Lustig SR, Jagota A, Khripin C, Zheng M. J Phys Chem B 2005;109:2559–2566. [PubMed: 16851257]
11. Sowerby SJ, Edelwirth M, Heckl WM. J Phys Chem B 1998;102:5914–5922.
12. Edelwirth M, Freund J, Sowerby SJ, Heckl WM. Surf Sci 1998;417:201–209.
13. Shi XH, Yong K, Zhao YP, Gao HJ. Acta Mech Sinica 2005;21:249–256.
14. Freund JE, Edelwirth M, Krobelt P, Heckl WM. Phys Rev B 1997;55:5394–5397.
15. Ikeda A, Hamano T, Hayashi K, Kikuchi J. Org Lett 2006;8:1153–1156. [PubMed: 16524291]
16. Manohar S, Tang T, Jagota A. J Phys Chem C 2007;111:17835–17845.
17. Johnson RR, Johnson ATC, Klein ML. Nano Lett 2008;8:69–75. [PubMed: 18069867]
18. Bustamante C, Bryant Z, Smith SB. Nature 2003;421:423–427. [PubMed: 12540915]
19. Florin EL, Moy VT, Gaub HE. Science 1994;264:415–417. [PubMed: 8153628]
20. Rief M, Oesterhelt F, Heymann B, Gaub HE. Science 1997;275:1295–1297. [PubMed: 9036852]
21. Clausen-Schaumann H, Seitz M, Krautbauer R, Gaub HE. Curr Opin Chem Biol 2000;4:524–530. [PubMed: 11006539]
22. Friedsam C, Becares AD, Jonas U, Seitz M, Gaub HE. New J Phys 2004;6
23. Sonnenberg L, Parvole J, Borisov O, Billon L, Gaub HE, Seitz M. Macromolecules 2006;39:281–288.
24. Sonnenberg L, Parvole J, Kuhner F, Billon L, Gaub HE. Langmuir 2007;23:6660–6666. [PubMed: 17489614]
25. Friedsam C, Gaub HE, Netz RR. Europhys Lett 2005;72:844–850.
26. Chatellier X, Senden TJ, Joanny JF, di Meglio JM. Europhys Lett 1998;41:303–308.
27. Janshoff A, Neitzert M, Oberdorfer Y, Fuchs H. Angew Chem Int Edit 2000;39:3213–3237.
28. Zhang YH, Liu CJ, Shi WQ, Wang ZQ, Dai LM, Zhang X. Langmuir 2007;23:7911–7915. [PubMed: 17590031]
29. Sowerby SJ, Mörtz C-M, Holm NG. Astrobiology 2001;1:481–487. [PubMed: 12448981]
30. Vezenov DV, Noy A, Ashby P. J Adhes Sci Technol 2005;19:313–364.
31. Fujihira M, Okabe Y, Tani Y, Furugori M, Akiba U. Ultramicroscopy 2000;82:181–191. [PubMed: 10741669]
32. Herne TM, Tarlov MJ. J Am Chem Soc 1997;119:8916–8920.
33. Lee CY, Gong P, Harbers GM, Grainger DW, Castner DG, Gamble LJ. Anal Chem 2006;78:3316–3325. [PubMed: 16689532]
34. Mourougou-Candoni N, Naud C, Thibaudau F. Langmuir 2003;19:682–686.
35. Castner DG, Hinds K, Grainger DW. Langmuir 1996;12:5083–5086.
36. Noy A, Vezenov DV, Kayyem JF, Meade TJ, Lieber CM. Chemistry & Biology 1997;4:519–527. [PubMed: 9263640]
37. Smith SB, Finzi L, Bustamante C. Science 1992;258:1122–1126. [PubMed: 1439819]
38. Smith SB, Cui YJ, Bustamante C. Science 1996;271:795–799. [PubMed: 8628994]
39. Griffiths, DJ. Introduction to Electrodynamics. Prentice-Hall Inc.; 1981.
40. Kudera M, Eschbaumer C, Gaub HE, Schubert US. Adv Funct Mater 2003;13:615–620.

41. Noy, A. Handbook of Molecular Force Spectroscopy. New York: Springer; 2007.
42. Doose S, Barsch H, Sauer M. Biophys J 2007;93:1224–1234. [PubMed: 17513377]
43. Gowtham S, Scheicher RH, Ahuja R, Pandey R, Karna SP. Phys Rev B 2007;76
44. Rubinstein, M.; Colby, RH. Polymer Physics. New York: Oxford Univeristy Press; 2003. p. 74-76.
45. Klushin LI, Skvortsov AM, Gorbunov AA. Usp Fis Nauk+ 1998;168:719–730.
46. Reddy SY, Leclerc F, Karplus M. Biophys J 2003;84:1421–1449. [PubMed: 12609851]
47. Foloppe N, MacKerell AD. J Comput Chem 2000;21:86–104.
48. Leach, AR. Molecular Modeling: Principles and Applications. New York: Prentice Hall; 1996. p. 561-568.
49. Friedman RA, Honig B. Biophys J 1995;69:1528–1535. [PubMed: 8534823]
50. Breslauer KJ, Frank R, Blocker H, Marky LA. P Natl Acad Sci USA 1986;83:3746–3750.

Figure 1.

Experimental scheme for force spectroscopy experiments. Gold coated AFM tip with covalently bound ssDNA and mercaptohexanoic acid pulls away from the graphite surface in phosphate buffer under ambient conditions. Inset: each Kuhn link of length b is represented by a vector $\mathbf{r}_i$ with orientation given by θ_i and ϕ_i angles. All possible conformations are obtained by varying θ_i from 0 to π and ϕ_i from $-\pi$ to π .

Figure 2.

Typical results from force spectroscopy experiments in 10 mM phosphate buffer solution at a tip velocity of 200 nm/s using gold coated tips modified with a mixed monolayer of polythymine and MHA. (a) Peeling of multiple DNA strands occurs at a steady force in multiple steps. Inset shows multiple force jumps during retraction. (b) Typical histogram of plateau force jumps with a mean of 84.2 pN and a standard deviation of 8.4 pN.

Figure 3.

Interaction between ssDNA linked to gold coated tips and APTES modified silicon wafers. Instead of force plateaus we observe a single molecule stretching event. Two fits using the extended FJC model are also shown, assuming placement of the DNA strand on the AFM tip apex (i.e. separation represents true extension) and either adjustable (blue line) or fixed, 2.5 nm, (black line) Kuhn length. The contour length and elastic modulus of the ssDNA were chosen as fitting parameters. Typically, up to three stretching events with different pull-off distances could be observed in each force-distance curve, only the last stretching event was used in fitting.

Figure 4.

Plots of average length, $\langle R_z \rangle/b$, and average number of desorbed links, $\langle n \rangle$ at different forces for $N=18$, $b=1.5$ nm and peeling force $f_{\text{peel}} = 85$ pN.

Figure 5.

Dependence of dimensionless adhesion free energy, $\Gamma = \gamma b / k_B T$ on dimensionless force, $F = fb(1 + f/\kappa) / k_B T$ based on Equation 5, Equation 6, and Equation 7. The shaded regions correspond to the range of measured steady state forces for polythymine (blue region, 85.3 ± 4.7 pN) and polycytosine (pink region, 60.8 ± 5.5 pN), with $b = 0.51$ nm and $\kappa = 2.4$ nN.