

Relationship between Stiffness and Force in Single Molecule Pulling Experiments

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Introduction

The atomic force microscope (AFM) is finding increasing use as a mechanical device for pulling apart single molecules^{1–4} as well as receptor–ligand pairs.^{5,6} In these measurements the deflection of a cantilever is recorded as it is pulled away from the surface after sticking to the target molecule. Images are not usually obtained because of the requirement that the tip stick to the target molecule. Operated at high enough amplitude, the dynamic force microscope (DFM) can overcome adhesion and thereby image sticky surfaces.^{7,8} This is possible even at lower amplitudes if the microscope is operated in a noncontact mode.^{9,10} It is of interest to consider what additional information might be obtained from a molecule-pulling experiment in which the tip is oscillated at the same time as the molecule is pulled. High-frequency modulation of the tip has been used in the past to probe the stiffness of surfaces.^{11–16} In this Note, we explore the value of recording DFM oscillation amplitude alongside traditional force curves.

In the absence of dispersion or dissipation, the DFM operates by sensing the change in interfacial stiffness, $S(z)$. If the tip motion is small enough in amplitude so that $S(z)$ remains constant over a swing¹⁷

$$S(z) = k \left(\frac{A_0}{A(z)} - 1 \right) \quad (1)$$

where A_0 is the oscillation amplitude far from the surface, $A(z)$ is the amplitude a distance z from the surface, and

k is the force constant of the cantilever. If the oscillation amplitude is large, the surface stiffness can be extracted from¹⁰

$$S(z) = \frac{k}{\left(\frac{dz}{dA}(z) - 1 \right)} \quad (2)$$

where $S(z)$ and dz/dA are measured at the low point of the swing. Equation 2 becomes unreliable as the tip makes hard contact with the surface, because dz/dA becomes unity from that point on. It is possible to analyze the signal obtained from a dissipative interface using a damped harmonic oscillator model provided that the tip motion is small compared to distances over which properties of the interface change.^{18,19} In this Note we present force curves obtained by pulling on chromatin constructs stuck to a glass substrate in buffer solution, restricting our study to a range of frequencies over which the response appears to be predominantly elastic. Stiffness data were obtained simultaneously by monitoring the amplitude of a small oscillatory motion applied directly to a magnetized tip by means of a solenoid. A similar study of polylysine was recently carried out by Lantz et al. (M. A. Lantz, personal communication).

Experimental Section

We used a magnetically oscillated DFM (MAC Mode AFM from Molecular Imaging, Phoenix, AZ) supplied with magnetized force-sensing levers of spring constant 0.5 ± 0.1 N/m. This value was obtained from tests on several similar levers using a calibrated glass fiber.²⁰ The instrument was modified to extract the ac component of the cantilever deflection using an RC filter inserted immediately prior to the lockin which demodulates the amplitude signal. This signal is similar to a conventional force curve, save that it is an average over the position of the oscillated tip. This modified instrument was used to study a reconstituted chromatin molecule, the 172-12 construct.²¹ This consists of double helical DNA composed of 12 repeats of a 172 base-pair sequence known to align nucleosomes. The construct, reconstituted only with core histones, gave repeating sawtooth signals in the force curve when the tip was retracted, similar to those observed for titin.¹ The repeat occurred up to 11 times for a 12 nucleosome construct, suggesting the peaks are associated with the pulling of individual nucleosomes from the surface (S. H. Leuba et al., unpublished data). Chromatin constructs were adsorbed onto flame-annealed glass from a solution of 0.1 mg/mL in 5 mM triethanolamine HCl, pH 7.0, 0.1 mM EDTA by leaving the solution in place for 10 min. The samples were then rinsed in the same buffer and imaged in buffer using the magnetically driven DFM. Images (unpublished data) showed that the constructs were well separated and spread onto the surface so that attachment of the tip to more than one complex was unlikely.

Results

Typical force and amplitude curves are shown for a retraction experiment in Figure 1a. For these data the oscillation frequency was 25 kHz with a 5 nm peak to

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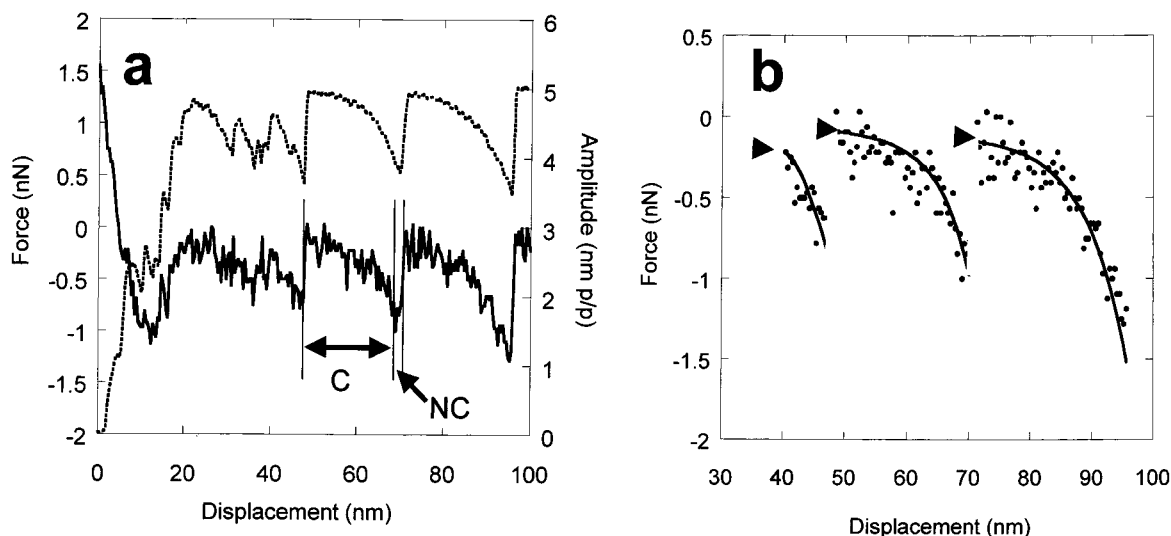


Figure 1. (a) Signal amplitude (dotted line) and dc deflection signal (solid line, calibrated in nN) obtained simultaneously as a synthetic chromatin molecule was pulled from a glass substrate. Region labeled “C” corresponds to a conservative (reversible) interaction, region labeled “NC” corresponds to nonconservative (nonreversible) interaction. (b) Force as calculated from amplitude data (solid lines) with integration restarted at the onset of each region of conservative interaction as labeled by arrowheads. Points are measured force data corresponding to parts of the solid line in (a).

peak amplitude although other amplitudes and frequencies were tried. The tip was pushed into a chromatin molecule with a force of 30–100 nN for approximately 0.5 s and pulled back at a speed of 100 nm/s. The force curves display a series of peaks of approximately 0.5 nN amplitude separated by about 30 nm. The amplitude curves show similar features where the amplitude changes abruptly by a little over 1 nm. The modulation is not negligibly small, but because both force and amplitude curves are averaged over the same swing, we expect that eq 1 will still hold in regions of elastic behavior. The higher modulation levels might be expected to distort the force data very close to the peaks, but we saw no obvious effect on reducing the level down to zero, although the sensitivity of our measurements was limited by the noise on the force signal.

Neglecting the difference between true extension and tip displacement (for the relatively stiff cantilevers used in this work the correction is less than a nanometer at the peak values of force) and within the approximations discussed above, the force curves should be reproduced by integrating eq 1

$$F(z) = -k \int \left(\frac{A_0}{A(z)} - 1 \right) dz + C \quad (3)$$

Here, C is the force at the point where integration is started. It is immediately apparent that eq 3 does not apply to all the data. The slope of the force-displacement signal is clearly *negative* in places, while the amplitude signal is always positive. Equation 3 is predicated on a conservative force, and in regions where force increases with displacement (labeled “C” in Figure 1a) the force signal is generally reversible on reversing the displacement, i.e., conservative. This is not the case in regions where force decreases with displacement (one region of negative slope is labeled “NC” in Figure 1a). Thus, we expect that eq 3 will hold only in the “reversible” regions, and this is illustrated in Figure 1b. Here, C was set equal to the measured force at each of the points marked by arrowheads and numerical integration used to calculate $F(z)$. The solid lines in Figure 1b show the results of these calculations. They are in excellent agreement with the

measured force data, the only fitting parameter being the initial value of force, C .

This finding implies that dissipation does not play a major role outside the regions where the force “collapses”. We see little change in the form of these data with the modulation frequency changed over the range 1–25 kHz, suggesting that there are no dissipative processes at these frequencies that change over these distances. The cantilever is heavily damped, of course, but the squeeze viscosity does not change significantly at nanometer tip heights.¹⁸

Discussion and Conclusions

The force and stiffness data are found to be in excellent agreement in regions of conservative behavior suggesting that elastic behavior dominates this region of the pulling curve at the frequency we used for measurement. This shows how simultaneous acquisition of static and oscillatory data serves as a check of nondissipative, nondispersive behavior. This information is not trivial, for it implies that no irreversible processes contribute to the force curve in the region where the stiffness and force data agree. Thus, these portions of the curve must correspond to an elastic distortion of the chromatin (as opposed to an unraveling of the structure). In addition, Figure 1b shows how the ac signal offers greatly enhanced signal to noise when force curves can be obtained from it by integration. This presumably results from rejection of the $1/f$ noise that dominates the displacement signal at low frequencies.²² We have illustrated the relationship between these curves with a simple example, but more complex behavior could be studied by recording both the amplitude and phase of the cantilever displacement over a wide range of frequencies. In this way the complex modulus of the molecule could be extracted with a sufficiently sensitive cantilever.

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