Elastically Coupled Two-Level Systems as a Model for Biopolymer Extensibility

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We present Monte Carlo simulations for the elasticity of biopolymers consisting of segments that can undergo conformational transitions. Based on the thermodynamics of an elastically coupled two-level system, the probability for a transition and a related change in length of each segment was calculated. Good agreement between this model description and measured data was found for both the polysaccharide dextran where the conformational changes are fast and the muscle protein titin where the marked rate dependence of the transition forces could be explained by nonequilibrium processes. [S0031-9007(98)07735-7]

New techniques combining high force sensitivity (piconewtons) with accurate positioning (angstroms) enable us to perform mechanical experiments with single molecules [1–7]. Such experiments are uniquely suited to test theoretical predictions on polymer elasticity. In the low force regime, measurements with magnetic beads on single DNA molecules have shown good agreement with the standard theories on the entropy elasticity of polymers [8]. Only minor refinements were necessary. In the medium force regime (starting at several tens of pN), which is accessible with optical tweezers, deviations from the ideal behavior due to elastic deformation of the polymer backbone became apparent [9]. These were even stronger expressed in the force regime beyond 300 pN, which is reached with atomic force microscope (AFM) related techniques [1]. It turned out that at high forces the majority of the investigated biopolymers show marked deviations [1] even from those polymer elasticity models which include elastic deformations of the backbone [9,10]. At such forces the subunits of these biopolymers undergo conformational transitions, resulting in an additional length increase. Such molecule-specific effects lead to a variety of molecular "fingerprints" in the extensibility which depend on the increase in length during the transition.

For DNA overstretching, where a highly cooperative transition of B-DNA into an elongated S form had been observed experimentally [9,11], theoretical models based on equilibrium thermodynamics [11–13] and molecular dynamics simulations [14] have been put forward. Here we present a description which includes the kinetic aspects of the internal transitions giving rise to a marked rate dependence of the extensibility, particularly when the experimental time scale becomes comparable to the molecular kinetics. We show that this model is capable of explaining the visco-elastic properties of very different biopolymers.

A typical experiment in which the elasticity of single polymer molecules is probed with an AFM is depicted in Fig. 1. The force exerted on the stretched polymer is measured via the deflection d of the cantilever spring on which the tip is mounted (for details, see [1] and [2]).

Figure 2(a) shows several such force curves recorded on various single molecules of the polysaccharide dextran. All of the curves exhibit the same characteristic elastic behavior. At around 700 pN the curves deviate from a simple shape and show a kink. Using molecular dynamics simulations it was shown that this kink is due to a conformational transition within each dextran monomer where the C5-C6 bond of the sugar ring flips into a new conformation, thus elongating the monomer by 0.65 Å ($\sim$10% of its length) (cf. [1]). The first two traces show an experiment where a single dextran strand was stretched and relaxed again. No hysteresis can be observed between the cycles. Also, the force at which the transitions occur is not speed dependent. This means that the bond flips occur on a faster time scale than the experiment, and therefore stretching is an equilibrium process.

Force curves of an at first sight completely different kind of polymer are shown in Fig. 2(b). These curves were taken from a recombinant construct consisting of eight immunoglobulin (Ig) domains of the muscle protein titin at an extension speed of $\sim$1 μm/s. The 89 aminoacid residues of each Ig domain are folded into a compact β-sheet structure of 4 nm in diameter. Under the influence of an external force, the domains unfold in an all or none process. Upon unraveling each domain gains
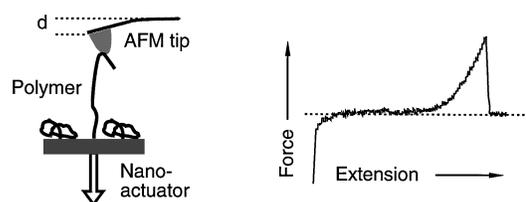

FIG. 1. A typical experiment in which a single molecule is stretched by an AFM tip. The tip is brought into contact with the sample, which is covered by a layer of polymer molecules. If a molecule has bound to the tip it can be stretched and the force is measured via the deflection of the cantilever spring as a function of the extension. When the maximum binding force is exceeded, the molecule ruptures from the tip and the tip is free again.
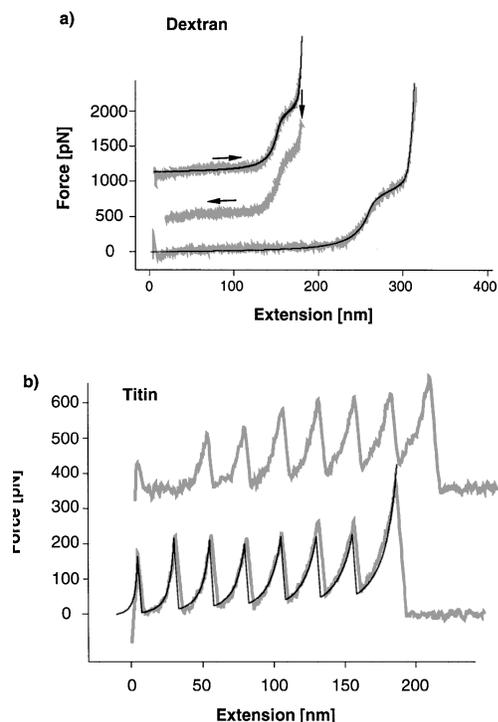

FIG. 2. Characteristic shapes of the force versus extension traces of different polymers. (a) Three curves taken on 2 dextran strands of different length. All curves show a kink at around 700 pN where a bond angle within each monomer flips into a new position. For clarity the traces are offset from each other. The first trace shows the extension and the second trace the relaxation of the same dextran strand. The black solid lines are fits with the Monte Carlo simulation (see Fig. 4). (b) Two force curves taken on a recombinant construct comprising 8 immunoglobulin domains of the muscle protein titin. The peaks reflect the unfolding of the individual domains. The last peak in each force curve is typically much higher and is due to the desorption of the polypeptide strand from the tip. The curves were recorded in PBS buffer (phosphate buffered saline, pH 7.4, 150 mM NaCl). The black curve is the result of the Monte Carlo simulation (for parameters see Fig. 4).

28 nm in length. The pronounced sawtooth pattern in the force curves reflects the subsequent unfolding of domains. The unfolding forces rise from the first throughout the last peak. This is due to the fact that the eight Ig domains in the construct are not identical but just structurally similar. Thus, the weakest domains break first, the strongest last. For details of the experiment see [2].

Despite the apparent difference in structure and force versus extension curves between these two biopolymers, the underlying physical principle is the same: In a simple model both polymers consist of modules that can undergo a transition between two energetically different states in which the modules have different length (see Fig. 3). The length in the folded state is I_f in the unfolded state I_u. In the case of dextran the modules are the sugar rings. The compact low energy state and the more extended high energy state are depicted in Fig. 5(a). For dextran $I_f = 5$ Å and $I_u = 5.65$ Å. In titin the modules are tertiary
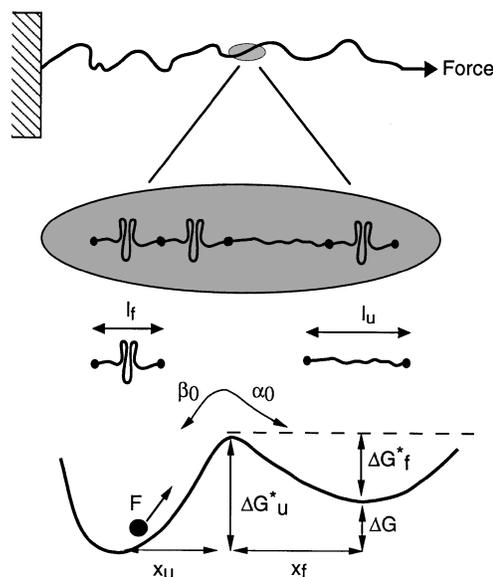

FIG. 3. Combination of WLC elasticity with a thermodynamic two-state model. Under the influence of an external force, the polymer extends according to the WLC model. Because each of the segments in the modular polymer can undergo a transition from a folded into an extended conformation, the total contour length of the polymer changes during a transition. The kinetics of the transition is modulated by the external force. The description of the parameters is given in the text. This model is used as a basis for a simple Monte Carlo simulation.

structural elements of the protein, the Ig domains. The low energy state is the folded state, and the high energy state is the unfolded state. Here $I_f = 4$ nm and $I_u = 32$ nm. In the following we call polymers that consist of such coupled two-state systems modular polymers.

In order to model the force vs extension relation of a modular polymer consisting of N modules, N_u of which are in the unfolded state and N_f of which are in the folded state, we first have to find an appropriate description for the elasticity of the polymer backbone of contour length $L = N_f I_f + N_u I_u$. Different models have been proposed. The wormlike chain model (WLC) [15–18] which includes enthalpic contributions via a bending elasticity has been shown to predict the elastic behavior of single polymer strands up to forces of several hundred piconewtons [2,5,17]. An analytical expression for the force F as a function of the polymer extension x was given in [17,18]

$$F(x) = \frac{k_B T}{p} \left(\frac{1}{4(1 - x/L)^2} - \frac{1}{4} + \frac{x}{L} \right). \quad (1)$$

The persistence length p describes the polymer stiffness, k_B is Boltzmann's constant, L the contour length, and T the temperature [19,20].

This force-extension relation needs to be extended by a kinetic description of the state of the individual modules which determines the actual polymer contour length at a given force. The parameters and kinetics of a two-state

system are depicted in Fig. 3. For the transition from the lower to the higher state, in the following referred to as unfolding, the rate α_0 is given by

$$\alpha_0 = \omega e^{-\Delta G_u^*/k_B T}. \qquad (2)$$

ΔG_u^* is the activation barrier for the unfolding process, ω is, as explained by Kramers theory, the reciprocal of a diffusive relaxation time (see [21] and [22]). The backreaction rate β_0 for the folding process is

$$\beta_0 = \omega e^{-\Delta G_f^*/k_B T}. \qquad (3)$$

ΔG_f^* is the activation barrier for folding.

Bell [23] and, in a more elaborate description, Evans [21] calculated the influence of an external force on the rate of unfolding. In Bell's linear approximation, the barrier ΔG_u^* is reduced by Fx_u, where x_u is the width of the activation barrier. This leads to a force dependent unfolding rate [22]:

$$\alpha(F) = \omega e^{-(\Delta G_u^* - Fx_u)/k_B T} = \alpha_0 e^{Fx_u/k_B T}. \qquad (4)$$

The folding rate is affected in the same way:

$$\beta(F) = \omega e^{-(\Delta G_f^* + Fx_f)/k_B T} = \beta_0 e^{-Fx_f/k_B T}. \qquad (5)$$

In contrast to unfolding, a stretching force will decrease the folding rate and the width of the activation barrier for folding x_f may be different from x_u (see Fig. 3).

The combination of (1) with (4) and (5), which describes the extension of the modular polymer by an AFM cantilever, can be realized in a simple Monte Carlo simulation. The AFM cantilever extends the polymer with a speed v_c starting from $x = 0$. This leads to an additional extension Δx at each time interval Δt of

$$\Delta x = v_c \Delta t. \qquad (6)$$

After each time step the actual force is calculated according to (1), and the transition rates are determined using (4) and (5). The probability dP_u of observing the unfolding of any of the N_f folded modules in the chain during Δt is

$$dP_u = N_f \alpha(F) \Delta t. \qquad (7)$$

The probability for observing the folding of any of the $N_u = N - N_f$ unfolded modules dP_f is

$$dP_f = N_u \beta(F) \Delta t. \qquad (8)$$

In each interval Δt the probabilities for a transition are calculated using (7) and (8). Based on a random number decision, the respective transition is executed by changing the polymer contour length accordingly. The structure of (7) and (8) implies that there is no cooperativity between the unfolding of the various modules. The time steps need to be kept small enough so that both dP_u and dP_f are always well below 1.

This simulation was applied to the stretching experiments of a dextran strand consisting of $N = 275$ modules (monomers). Using a persistence length p of 1.5 Å [20], $\Delta x_f = \Delta x_u = 0.32$ Å and an equilibrium constant K: $\beta_0/\alpha_0 = 5.7 \times 10^5$ [cf. Fig. 4(a)] the curve shown in Fig. 4(b) was obtained. The congruence between data and simulation is striking.
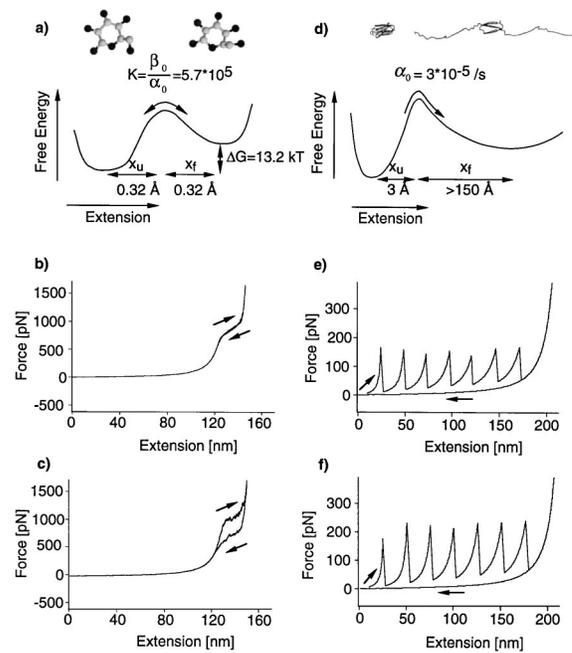

FIG. 4. (a) Parameters for the two-state model that reproduce in the Monte Carlo simulation the measured elasticity of dextran. Because the dextran strand is always in equilibrium at the given pulling speeds, only the equilibrium constant and the equilibrium free energy can be determined but not both rates independently. (b) Simulation of the dextran extension at a speed of 1 μm/s which is comparable to the experimental speeds used in Fig. 2(a). Because the gain in length upon each flip is very small (0.65 Å) compared to the contour length, the individual flips are not resolved but give rise to the plateau at around 700 pN. The relaxation trace superimposes well with the extension trace which supports the experimental observation that pulling at this speed is an equilibrium process. The simulation reproduces the data in Fig. 2(a) very well. (c) Simulation was performed at high speed (1 cm/s). At these extension speeds the flips occur at a time scale comparable to that of the experiment. Thus the experiment is performed in nonequilibrium and extension and relaxation traces show a hysteresis. (d) Parameters for the two-state model that reproduces the measured unfolding forces of titin Ig domains. Here the potential is highly asymmetric ($x_u \ll x_f$) and the experiment occurs in nonequilibrium. A simulation at low speed (0.01 μm/s) is shown in (e); a simulation at higher speed (1 μm/s) which is identical to the experimental speed in Fig. 2(b) is shown in (f).

As can be seen from Fig. 2(a), the force versus extension curve on a dextran strand is fully reversible in an AFM experiment. This means that the bond angle flips occur much faster than the time the experiment takes (<1 s) and pulling occurs in equilibrium. This is the reason why only the equilibrium constant K has an influence on the shape of the simulated curve. As long as K is kept constant, the rates α_0 and β_0 can be changed over a wide range without any effect. That the system is in equilibrium is also shown by the fact that the transition force both in the simulation and in the experiments does not depend on the pulling speed over a wide range. Only above a critical pulling speed ($\sim$1 cm/s), not yet accessible in the

experiment, we would be able to see a hysteresis between a pulling and a relaxing cycle. In this case both rate constants could be obtained separately. This is shown by the simulation at high speed [Fig. 4(c)]. Here a marked hysteresis becomes apparent and at the same time the transition forces become speed dependent, indicating that the polymer is in nonequilibrium.

In Fig. 4(f) a curve that simulates the unfolding of seven titin domains in series at a pulling speed of $v_c = 1$ μm/s is shown. The parameters of the simulation were chosen as shown in Fig. 4(d). In the case of titin the folding potential is highly asymmetric. The width of the activation barrier for forced unfolding is only 3 Å (see [2]). At a small force of 10 pN, on the other hand, an unfolded polypeptide chain will be stretched already to half its contour length. So at this force the width of the activation barrier for folding x_f is 15 nm. This means that according to (5) the rate of refolding, which was assumed to be $\beta_0 = 2$ s^{-1} [24] at zero force, is reduced by a factor of e^{-35} and thus refolding is totally prevented. Therefore, at the typical time scales of an AFM stretching experiment titin unfolding is a nonequilibrium process. As a consequence the unfolding force of the titin domains should depend on the pulling speed. The curve in Fig. 4(e) which was simulated with a pulling speed of 0.01 μm/s indeed shows that the unfolding force is reduced. Values of $\alpha_0 = 3 \times 10^{-5}$ s^{-1} for the thermal unfolding rate and $\Delta x_u = 3$ Å for the width of the folding potential were obtained by comparing the experimentally measured speed dependence of the unfolding forces and the simulations [2] [Fig. 4(d)].

To summarize, we could show that a combination of classical polymer elasticity with the kinetics of a thermodynamic two-level system is well suited to describe the measured force versus extension characteristics of a variety of modular polymers.

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[19] One could argue that the different states of the modules could lead to different persistence lengths for the one or the other state. The approximation of using one persistence length for both states is justified in the case of dextran by the fact that the two states differ by only 10% in length and thus the modules are very similar in both states. For the titin domains the unfolded conformation is 8-fold longer than the folded module. Although the folded domains are much stiffer than the unfolded polypeptide, they are so short that most of the contour length will be determined by the unfolded peptide. Attributing too short a persistence length to the small rest consisting of folded domains will thus not lead to a considerable error. If necessary, the model can be easily extended using two wormlike chains in series with two different persistence lengths as suggested in [5].

[20] It has to be emphasized that the WLC model contains just one parameter, the persistence length, to describe the elasticity of a polymer over the complete range of extension forces. However, as described in the text, the sources of elasticity in the low force regime (entropic) and in the high force regime (enthalpic) are different. Obviously one parameter will not perfectly describe the polymer elasticity for the whole force range. In describing the AFM extension traces, we are mainly concerned with high forces (50 pN $< F <$ 1 nN). In this case the WLC model leads to persistence lengths p that seem too short. Persistence lengths of 1.5 Å for dextran and 4 Å for titin used in this study describe the data well for forces above 50 pN. These p values, however, must not be compared to measurements of p on polymer coils in the absence of mechanical tension. Indeed a fit to the force range below 50 pN leads to $p = 4$ Å for dextran and $p = 8$ Å for titin.

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