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.

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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 $\beta$-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.](image)
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 \( I_f \) and \( I_u \) 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]

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

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
The unfolding process is depicted in Fig. 3. For the transition from the lower to the higher state, in the following referred to as unfolding, the rate $\alpha_0$ is given by

$$\alpha_0 = \omega e^{-\Delta G_u/k_BT}. \tag{2}$$

$\Delta G_u$ is the activation barrier for the unfolding process, $\omega$ is, as explained by Kramers theory, the reciprocal of a diffusive relaxation time (see [21] and [22]). The backreaction rate $\beta_0$ for the folding process is

$$\beta_0 = \omega e^{-\Delta G_f/k_BT}. \tag{3}$$

$\Delta G_f$ 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 $\Delta G_u^*$ is reduced by $Fx_u$, where $x_u$ is the width of the activation barrier. This leads to a force dependent unfolding rate [22]:

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

The folding rate is affected in the same way:

$$\beta(F) = \omega e^{-(\Delta G_f^* + Fx_u)/k_BT} = \beta_0 e^{-Fx_u/k_BT}. \tag{5}$$

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 $\Delta x$ at each time interval $\Delta t$ of

$$\Delta x = v_c \Delta t. \tag{6}$$

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 $\Delta t$ is

$$dP_u = N_f \alpha(F) \Delta t. \tag{7}$$

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

$$dP_f = N_u \beta(F) \Delta t. \tag{8}$$

In each interval $\Delta t$ 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 $\phi$ 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.

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 $\alpha_0$ and $\beta_0$ 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
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 \(v_c = 1 \mu m/s\) 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 \(\mu m/s\) indeed shows that the unfolding force is reduced. Values of \(a_0 = 3 \times 10^{-5} \text{s}^{-1}\) for the thermal unfolding rate and \(\Delta x_\nu = 3 \text{Å}\) 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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