Elastic energy of protein-DNA chimeras

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We present experimental measurements of the equilibrium elastic energy of protein-DNA chimeras, for two different sets of attachment points of the DNA "molecular spring" on the surface of the protein. Combining these with measurements of the enzyme's activity under stress and a mechanical model of the system, we determine how the elastic energy is partitioned between the DNA and the protein. The analysis shows that the protein is mechanically stiffer than the DNA spring.

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The normal function of proteins relies on the subtle interplay of nanoscale mechanics and chemistry. These macromolecular catalysts use molecular deformations to control chemical reactions [1,2], or, conversely, transduce chemical reactions into directed motion and work [3]. The specific mechanisms and general rules of the intramolecular transduction of chemical energy and mechanical work remain objects of ongoing investigation [4], as does the design of artificial allosteric mechanisms [5–11]. Protein-DNA chimeras, in which DNA is used as a "molecular spring" to deform proteins [5,6], offer a tool to examine protein mechanics as it relates to physiologically relevant conformational transitions. In these constructs, the DNA deforms the protein by exerting equal and opposite forces on two selected attachment points on the protein's surface (Fig. 1). The complete description of a protein's deformation state requires the entire set of displacement vectors at atomic scale. We propose instead a minimalistic characterization of the mechanical perturbation by a single quantity: the elastic energy of the chimera, which can be directly measured as follows. We construct a chimera with two separate DNA "arms," thus by construction the ds DNA spring contains a nick [Fig. 1(b)]. The elastic energy F_{el} of this construction can be relaxed by forming dimers, in which neither the DNA nor the protein is stressed. The monomer-dimer equilibrium provides a precise measurement of F_{el} [12]. In this paper, we report measurements of the elastic energy of a protein-DNA chimera for two different sets of attachment sites of the DNA spring. Because the mechanics of neither the protein nor sharply bent DNA is quantitatively known, it is not *a priori* clear which is stiffer: the protein or the DNA spring. Combining the experimental data of the elastic energy and the activity of the enzyme under stress with a mechanical model of the DNA spring, we show that the protein is stiffer than the DNA spring, i.e., most of the elastic energy of the chimera resides in the DNA.

Guanylate kinase (GK) from Mycobacterium tuberculosis (gene Rv1389c) was modified by site-directed mutagenesis (Stratagene) to remove the native cysteins from the wild type and add two cysteins at sequence sites 75 and 171 or 40 and 171 for later DNA conjugation. The mutant gene was cloned and expressed according to the method in Ref. [11]. Two 30mer DNA arms with different sequences: strand *A* 5′-GAGTGTGGAGCCTAGACCGTGAGTTGCTGG-3′ and strand *B* 5′-CAGTGGTGCGACCGACGTGGAGCC TCCCTC-3′ were purchased amino modified at the 5′ and 3′ end, respectively (Operon Biotech). The coupling scheme uses the heterobifunctional cross-linker NHS-PEO2-Maleimide (Pierce) and is described in detail in Ref. [14]. The two DNA arms are attached sequentially using high-pressure liquid chromatography (HPLC) purification of the intermediate products. The final synthesized two-armed chimera was purified by Ni-nitrilotriacetic acid (NTA) chromatography (Qiagen) through the His tag on the protein and verified by the corresponding molecular weight on SDS-polyacrylamide gel electrophoresis (PAGE). The construction of the two-armed chimera (Fig. 1) is more extensively described in Refs. [12,14].

We obtain samples that typically consist of 70% of the desired species and the rest various "impurities," such as

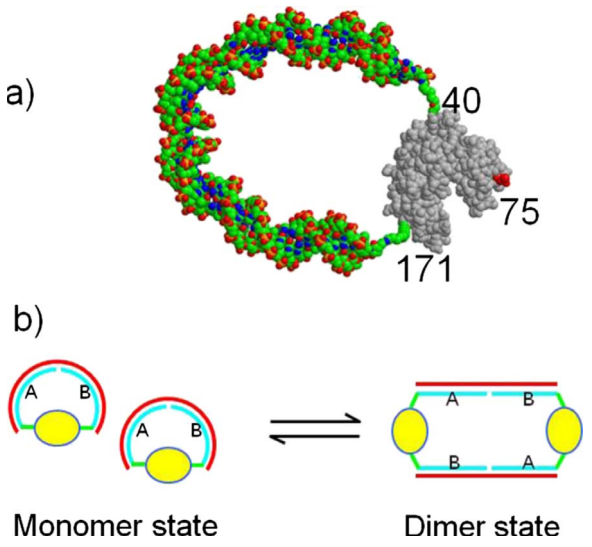

FIG. 1. (Color online) (a) Cartoon of the protein-DNA chimera with the 60 bp DNA spring attached at the sites 171⊗40. The protein (Guanylate Kinase) is the protein data bank (PDB) structure 1S4Q, the DNA is from the nucleosome structure 1KX5. (b) Cartoon of the elastic energy-driven dimerization process. We use the monomer-dimer equilibrium to measure the elastic energy F_{el} of the monomer.

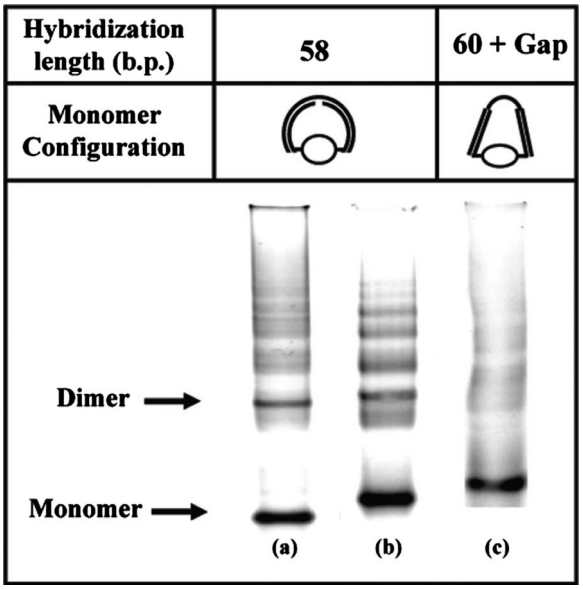

FIG. 2. Gel electrophoresis of a sample of hybridized chimeras, showing monomer and dimer bands, as indicated. For lane (a), the measured concentrations were 0.13 μM and 0.030 μM for monomers and dimers, respectively. Lane (a) is the $171 \otimes 75$ chimera, lane (b) is the $171 \otimes 40$ chimera, both constructed with the same 60 mer DNA and hybridized to the 58mer complementary. Lane (c) is a control: the same chimera of lane (b) is hybridized with a 68mer such that the 8 bases in the middle are unpaired. This relaxes the elastic energy and correspondingly dimers and higher-order polymers disappear.

chimeras, where both DNA arms are the same, one-armed chimeras, etc.; however, the different species are distinguishable on a gel. After hybridization with a complementary DNA strand, the elastic energy of the chimera can, at sufficiently high concentrations ($\sim \mu$M), drive the formation of dimers and higher-order polymers (Fig. 1), in which the elastic energy is relaxed. We measure the elastic energy of the monomer F_{el} from the monomer-dimer equilibrium, which can be visualized by gel electrophoresis (Fig. 2) (for control experiments to establish that bands on the gel correspond indeed to equilibrium populations in this system, see Ref. [12]),

$$F_{el} = \frac{1}{2} k_B T \ln\left(\frac{X_D}{X_M^2}\right), \tag{1}$$

where X_M (X_D) is the mole fraction of monomer (dimers), measured from the intensities of the corresponding bands in the gels, visualized using the DNA-binding dye SYBR Gold (Fig. 2). We examine F_{el} for two different sets of attachment points of the DNA spring on the protein (referred to as the "$171 \otimes 75$ mutant" and the "$171 \otimes 40$ mutant" shown in Fig. 1). Figure 2 shows a gel picture of the monomer-dimer equilibrium for the two mutants, for $\ell = 58$ bp, the length of the complementary DNA hybridized to the chimera. The amount of dimers is similar for the two mutants, so the elastic energy is almost the same. Quantitatively, we find

$$\Delta F_{el} = F_{el}(171 \otimes 75) - F_{el}(171 \otimes 40) \approx 0.4 \pm 0.2 k_B T \tag{2}$$

and $F_{el}(171 \otimes 75) \approx 9.2 \pm 0.2 k_B T$

These values are averaged over several experiments and hybridization lengths ℓ, for $42 < \ell < 60$ (the elastic energy vs ℓ is flat in this region); the quoted errors are the corresponding standard deviation. The question is now: how is the elastic energy partitioned between the protein and the DNA? This is equivalent to asking whether the DNA or the protein is stiffer. To see this, consider two springs with spring constants K_1, K_2 and relaxed end-to-end distances (EEDs) X_1, X_2. If the springs are now constrained to have the same EED, the system's elastic energy is

$$E = E_1 + E_2 = \frac{1}{2}(X_1 - X_2)^2 \frac{K_1 K_2}{K_1 + K_2}, \tag{3}$$

and the ratio of the energies in the individual springs is $E_1/E_2 = K_2/K_1$, which demonstrates that most of the energy is stored in the softer spring. To make progress, we need a more quantitative treatment of the elastic energy of the protein-DNA chimera, such as can be obtained from the analysis of a bent, inextensible, and elastic rod (DNA) [13] coupled to a linear spring (the protein) by freely rotating linkers. We assume initially that the local bending modulus of the DNA is constant and thus sequence independent; additionally, given that the total arc length of the DNA in the experiments is less than half its thermal persistence length, the free-energy change associated with the formation of the chimera is dominated by the elastic energy stored in the deformed structure rather than the changes in configurational entropy of the DNA. This justifies our reliance on a zero-temperature mechanical analysis. The application of linear elasticity to the protein mechanics cannot be similarly justified *a priori*. Clearly, one expects to observe significant deviations from the linear elastic response of the protein for large elastic energies; but for small enough protein deformations, linear response is expected. The linearity assumption introduces the minimum number of protein elastic parameters (one spring constant that can be related to the protein's Young's modulus) and allows for the determination of a unique solution for the protein deformation and spring constant from the experimental data.

We use the conditions for mechanical equilibrium of a bent rod [16]. The problem is identical to the mechanical equilibrium of a strung bow as used in archery; here the DNA plays the role of bow and the protein, the tensed bowstring. We parametrize the shape of the DNA in terms of the angle $\theta(s)$ made by its local tangent vector with respect to the $\hat{z}$ axis as a function of arc length s. By symmetry, we restrict the domain to the upper half of the problem and measure the arc length from the midpoint of the DNA strand. The shape of the DNA strand of length L in the presence of a force F (applied to it by the protein) is given by the solution of the differential equation $\kappa\theta''(s) - F \sin(\theta) = 0$, where the prime denotes differentiation with respect to arc length and κ is the bending modulus of DNA. The boundary condition at the midpoint of the DNA is $\theta(s=0) = 0$, and, since

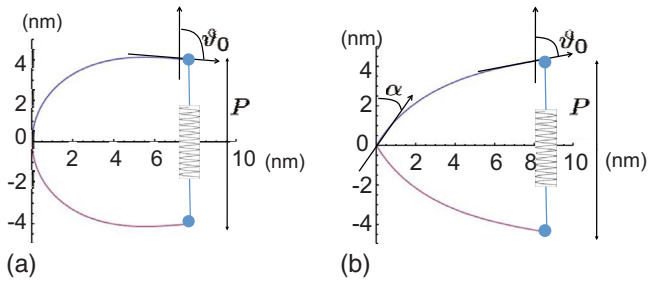

FIG. 3. (Color online) Calculated shapes of a 20-nm-ds DNA strand as it is deformed by the protein represented as a spring of rest length 8 nm. (a) The upper (blue) and lower (red) curves represent the unkinked solution (no discontinuity in the local tangent vector) of the DNA. Due to the reflection symmetry of the problem, these represent a single solution reflected about the x axis. The same holds for the kinked solution shown in (b). Here the hypothetical highly incompliant protein is extended by 0.02 nm under an applied force of 7.4 pN. The total elastic energy is $16.9k_BT$. (b) Allowing for a kink, with a total elastic energy of $9.1k_BT$ and the protein extended by 0.8 nm, the solution is $q=0.5$ and half-angle $\alpha=0.53$ of the kink, which reduces the applied force to 3.8 pN. Here the elastic energy is primarily stored in the DNA (52%) and kink (44%), while the protein contains only 4% of the total.

the attachment point of the DNA to the protein cannot exert torques, the boundary condition at the point of attachment is $\theta'(s=L/2)=0$. The existence of a first integral of the motion permits us to write the arc length of the DNA in terms of an incomplete elliptic integral of the first kind

$$L/2 = \sqrt{\frac{\kappa}{2F}} \int_0^{\theta_0} \frac{dz}{\sqrt{\cos(z) - \cos(\theta_0)}}, \tag{4}$$

where the final angle θ_0 is the angle that the local tangent of the DNA makes with $\hat{z}$ axis at the point of attachment (see Fig. 3). We require that the total arc length of the DNA be fixed at $L=20$ nm so that Eq. (4) is an implicit relation between the final angle and the force applied to the DNA by the protein. Similarly, as shown in Fig. 3, we demand that the DNA extends the necessary vertical distance P to attach to the end of the protein by requiring

$$P/2 = \sqrt{\frac{\kappa}{2F}} \int_0^{\theta_0} \frac{\cos(z)dz}{\sqrt{\cos(z) - \cos(\theta_0)}}. \tag{5}$$

The right-hand side of Eq. (5) can be written in terms of a combination of incomplete elliptic integrals of the first and second kinds. The protein has a known rest length $X=8$ nm (this includes the length of the cross linkers used, in the experiments, to attach the DNA to the protein) and an unknown extension δ in mechanical equilibrium so that $P=X+\delta$. Using Eqs. (4) and (5) and the values of the protein length (rest length plus extension) and DNA contour length, we compute both end angle of the DNA and the force F applied to the DNA by the protein. From the latter and Hooke's law, we may write the total elastic energy of the chimera $F_{el}=E_{\mathrm{DNA}}(\delta)+\frac{1}{2}F\delta$ as a function of solely the extension of the protein. This quantity is not known *a priori*, but the total elastic energy of the chimera is measured experimentally, allowing for a solution of the protein's extension and its effective spring constant via $k=F/\delta$.

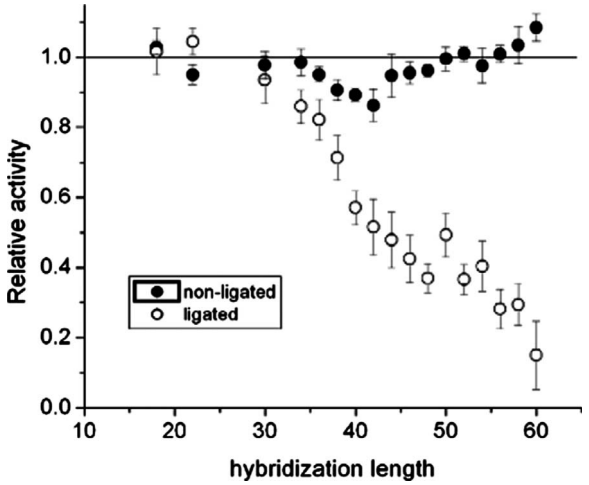

FIG. 4. Relative activity vs hybridization length (in bp) for the $171\otimes 40$ chimera, with (filled circles) and without (open circles) the nick in the DNA. The ligated curve is corrected for the yield of ligated chimeras measured from a gel.

The total elastic energy of the chimera is a monotonically decreasing function of protein extension δ with a value of about $17k_BT$ in the limit of an elastically noncompliant protein [see Fig. 3(a)]. To obtain the experimental value of $F_{el}=9.2\pm0.2k_BT$, we uniquely determine the protein's extension to be $\delta=9.6$ nm. However, this is incompatible with measurements of the enzymatic activity of the chimera under stress. Figure 4 shows measurements of the enzymatic activity relative to the activity of the unstressed (ss) chimera, for the two cases: the two-arm chimera, for which there is a nick in the DNA spring, and the chimera with the two arms ligated, i.e., without nick in the DNA spring, as a function of hybridization length. It is apparent that the DNA spring with the nick has no effect on the enzymatic activity, while without the nick the activity is suppressed. An elongation of the protein by ~ 10 nm, as calculated above in order to obtain the elastic energy measured for the chimera with the nick, is incompatible with the absence of change in its enzymatic activity (Fig. 4, filled circles). To resolve this paradox, we allow for a kink in the DNA spring at the position of the nick, modeled by introducing a locally softer bending modulus at that point. In fact, even without the nick, the DNA might lower its elastic energy by nucleating a localized bubble [15]. We analyze the mechanics of the kinked configuration by relaxing the constraint on the DNA tangent at the midpoint; now $\theta(s=0)=\alpha$, where α is the kink half-angle [see Fig. 3(b)]. We assume that the elastic energy associated with the kink is also harmonic $E_{\mathrm{Kink}}=1/2\gamma(2\alpha)^2$ and that the new bending elastic constant $\gamma=q\kappa/a$ can be written in terms of the DNA bending modulus, a microscopic distance ($a=3.4$-nm-one turn of the DNA helix) and an unknown dimensionless parameter $q<1$, expressing the fact that the kinked DNA is locally softer with respect to bending than the DNA in its native state. We now adjust α to minimize the total elastic energy of the system. Due to the introduction of this softening parameter q, there is a one-parameter family of

mechanical equilibria having protein deformation $\delta(q)$. The combination of the experimentally determined total elastic energy and the assumption that the protein's deformation not be more than one nanometer (given that enzymatic activity is retained) determines the range of admissible values of q: $0.5 < q < 0.75$. The solution shown in Fig. 3(b), using $q = 0.5$, requires a protein extension of 0.8 nm under an applied force of 3.8 pN leading to a protein spring constant of 5 pN/nm. Assuming that the protein deforms as an elastic continuum and using simple estimates for the length and cross-sectional area of the protein, we estimate the Young's modulus of the protein to be in the range of 1–80 MPa. Both the effective spring constant and the estimated Young's modulus are consistent with values determined via atomic force microscopy measurements of other proteins [17].

Of the $9k_BT$ of total elastic energy of the chimera, $8.7k_BT$ is stored in the DNA and only $0.3k_BT$ in the protein. Taking the above value of the protein's spring constant, the model predicts that the elastic energy of the chimera without kinks in the DNA, corresponding to the chimera with ligated nick (Fig. 4, open circles), is $14.6k_BT$, of which now $1k_BT$ is in the protein. Thus, by introducing one extra parameter corresponding to a kink at the position of the nick, the mechanical model accounts simultaneously for (i) the measurement of the elastic energy of the nicked chimera, (ii) the observation that for different attachment points of the DNA spring, the elastic energy of the chimera is almost the same [Eq. (2)], since the energy is stored primarily in the DNA and not in the protein, (iii) the absence of a change in the enzymatic activity of the protein with the nicked DNA (Fig. 4, filled circles), and conversely (iv) the substantial effect on enzymatic activity of the DNA spring without nick (Fig. 4, open circles). For the latter case, we find that the elastic energy in the protein is $1k_BT$. If the total work of protein deformation "allosterically" lowered the binding energy of the substrates, we would expect a reduction in enzymatic rate by $e^{-1} \simeq 0.4$, compatible with the observations (Fig. 4).

In conclusion, we have answered the question of which of the two elements of the chimera shown in Fig. 1 is mechanically softer: the protein or the DNA spring. Combining equilibrium measurements of the elastic energy of the chimera for different attachment points of the DNA spring, measurements of the enzymatic activity of the chimera under stress and modeling of the DNA spring mechanics, we show that both in the presence and absence of a nick in the DNA most of the elastic energy of the chimera resides in the DNA spring, which is thus the softer element. Therefore, a stiffer molecular spring, such as might be constructed, for example, from DNA and PNA, would increase the dynamic range of mechanical control of the enzyme. At the same time, these results show that the present DNA spring is well suited to study the equilibrium mechanical response of the protein in the functionally relevant regime, where the protein structure is not greatly deformed.

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[1] D. E. Koshland, Proc. Natl. Acad. Sci. U.S.A. **44**, 98 (1958).
[2] J. Monod, J. P. Changeux, and F. Jacob, J. Mol. Biol. **6**, 306 (1963).
[3] W. A. Engelhardt and M. N. Lyubimova, Nature (London) **144**, 668 (1939).
[4] Y. Savir and T. Tlusty, PLoS ONE **2**, e468 (2007).
[5] B. Choi, G. Zocchi, S. Canale, Y. Wu, S. Chan, and L. J. Perry, Phys. Rev. Lett. **94**, 038103 (2005).
[6] B. Choi, G. Zocchi, Y. Wu, S. Chan, and L. J. Perry, Phys. Rev. Lett. **95**, 078102 (2005).
[7] C. V. Miduturu and S. K. Silverman, J. Am. Chem. Soc. **127**, 10144 (2005).
[8] E. Zelin and S. K. Silverman, ChemBioChem **8**, 1907 (2007).
[9] A. Saghatelian *et al.*, J. Am. Chem. Soc. **125**, 344 (2003).
[10] L. Röglin, F. Altenbrunn, and O. Seitz, ChemBioChem **10**, 758 (2009).
[11] B. Choi and G. Zocchi, Biophys. J. **92**, 1651 (2007).
[12] A. Wang and G. Zocchi, Biophys. J. **96**, 2344 (2009).
[13] M. E. Hogan and R. H. Austin, Nature (London) **329**, 263 (1987).
[14] Y. Wang, A. Wang, H. Qu, and G. Zocchi, J. Phys.: Condens. Matter **21**, 335103 (2009).
[15] J. Yan and J. F. Marko, Phys. Rev. Lett. **93**, 108108 (2004).
[16] L. D. Landau and E. M. Lifshitz, *Theory of Elasticity*, 3rd ed. (Elsevier, Oxford, 1986).
[17] A. Ikai, Biophys. Chem. **116**, 187 (2005); R. Afrin, M. T. Alam, and A. Ikra, Protein Sci. **14**, 1447 (2005).