Artificial Allosteric Control of Maltose Binding Protein

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(Received 2 April 2004; published 26 January 2005)

We demonstrate the allosteric control of a protein based on mechanical tension. When substrate binding is accompanied by a significant change of conformation of the protein, a mechanical tension favoring one or the other conformation will alter the binding affinity for the substrate. We have constructed a chimera where the two lobes of the maltose-binding protein are covalently coupled to the ends of a DNA oligomer. The mechanical tension on the protein is controlled externally by exploiting the difference in stiffness between single stranded and double stranded DNA. We report that the binding affinity of the protein for its substrates is significantly altered by the tension.

DOI: 10.1103/PhysRevLett.94.038103 PACS numbers: 87.15.He

Introduction.—Allosteric control [1] is the mechanism whereby a control molecule binds to a site on a protein, inducing a conformational change at a distant site, which affects the function of the protein. It is a fundamental molecular control mechanism in the cell [2]: enzymes are typically allosterically controlled (e.g., hexokinase [3,4]); gene expression is regulated locally by allosteric control of repressors (e.g., the Tryptophan repressor (reviewed in [5]), and nonlocally through looping induced by DNA-binding proteins (reviewed in [6]). The latter is a simple example of how binding at one site can effectively modify the chemistry at a distant site: the essence of allosteric control.

An allosterically controlled enzyme is a chemical amplifier: it takes one molecule to switch the enzyme on, but many molecules are synthesized as products of the catalytic reaction. Building artificial molecular devices with similar ''chemical transistor'' properties has evident scientific and technological interest.

If the function of a protein is coupled to a change in conformation, there is the possibility of affecting the function through mechanical forces which favor one or the other conformation. We have built an allosteric control module into a protein, by creating a chimera where a DNA oligomer coupled to the protein exerts a mechanical tension on it. The tension on the protein can be varied externally by changing the stiffness of the DNA component of the chimera, through hybridization with different sequences; this changes the binding affinity of the protein for its substrates.

Our system utilizes the Maltose-binding protein (MBP) of *E. coli*, the periplasmic component of the maltose transport system [7]. Maltose binds in the cleft between the two lobes of the structure (Fig. 1), inducing a large ($\sim$1 nm amplitude) conformational change which brings the two lobes closer together, clamping down on the maltose. This is known from the crystal structure of the bound and unbound forms [8–10], and also from EPR spectroscopy [11]. The general idea of the present experiment is to control this conformational change through the mechanical tension derived from the bending elasticity of a second polymer coupled to the protein. For this purpose, MBP was modified by site directed mutagenesis (see *Materials and methods*) to introduce two ''chemical handles'' on opposite sides of the two lobes (Fig. 1): Lys 202 (red in the figure) was mutated to Cys, and a hexahistidine tag was appended at the N terminus (purple in the figure). These specific binding sites were used to attach a 60 bases long single stranded (ss) DNA oligomer [Fig. 1(a)] by one end to the Cys residue [through a covalent (disulfide) bond] and by the opposite end to the His tag (through a metal ion complex). For a second chimera (a Cys double mutant), both

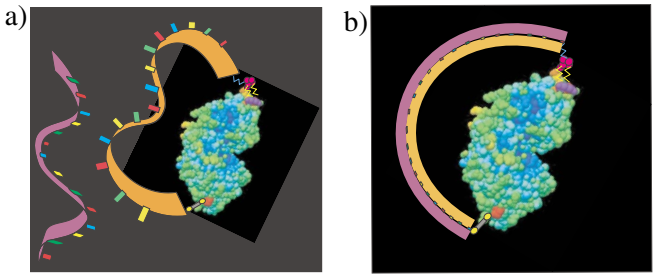

FIG. 1 (color). (a) The MBP-DNA ss construct. The MBP structure is from the Protein Data Bank. The location of the Lys 202 → Cys mutation is shown in red, the location of the His tag in purple. The distance between these two groups is $\sim$7 nm. The ss DNA 60mer is flexible and exerts only a small compression on the molecule. (b) After hybridization with a complementary strand, the DNA part of the chimera is more rigid and exerts a mechanical stress on the protein.

sides were covalently attached through disulfide bonds. The functionality of the chimera is intact: the measured binding affinity K for maltose and malto-triose ($K_1 = 0.9\ \mu M^{-1}$; $K_2 = 5.3\ \mu M^{-1}$) is within the the range of literature values for the wild-type MBP (0.7–1.1 μM^{-1} for maltose, 5–6 μM^{-1} for malto-triose [7,12]).

The principle of the experiment is as follows. A DNA oligomer with a length intermediate between the persistence length of ss DNA ($\ell_{ss} \sim 1$ nm or three bases) and that of double stranded (ds) DNA ($\ell_{ds} \sim 50$ nm or 150 bases) is flexible in the ss form, but stiff in the ds form. Thus the 60mer ss DNA of the chimera [Fig. 1(a)] does not exert a tension on the protein (actually, it exerts a small entropic compression). However, if this DNA strand is hybridized with a complementary strand, it will exert a mechanical tension on the protein, because the stiff ds DNA molecule has to bend [Fig. 1(b)]. The mechanical equivalent is a strung bow, where the protein is the string and the DNA is the bow. This tension favors the "open" conformation of MBP described above, and therefore lowers the binding affinity for the substrates. We obtained similar results using both malto-triose or maltose as the substrate; here we report the former.

Results. —The preparation of the chimera is described in [13]. Through native gel electrophoresis we confirmed that the samples consist primarily of protein-DNA complexes. For these samples, we measured the binding affinity for malto-triose and maltose, for the chimera, and for the chimera hybridized to DNA of different lengths L. The binding constant K was measured by titration, from the fraction of proteins with a bound substrate f determined by monitoring Trp fluorescence, which is quenched by about 20% upon substrate binding [7]. Within a two-state description, the change in fluorescence normalized between 0 and 1 gives the fraction of proteins in the bound (or closed) state, f.

Figure 2 shows titration curves f vs malto-triose concentration $[M]$ for the ss chimera, and the chimera hybridized to DNA with 12 and 60 base complementarity. For the 60 bp hybrid, the binding affinity for malto-triose is lowered by 35%. The 12 bp hybrid is a control, where no mechanical tension is expected to develop. This complementary DNA is actually 60 bases long, but only 12 are complementary to the DNA of the chimera. It shows that the presence of a second DNA strand attached to the chimera does not by itself change the binding affinity.

Varying the length of DNA hybridized to the chimera changes the mechanical tension on the protein. We performed experiments with hybrids of lengths $L = 0$, 12, 20, 30, 40, 50, and 60 bp. The corresponding malto-triose binding affinities are plotted in Fig. 3. To estimate the mechanical tension F for a given hybridization length L we constructed a mechanical model based on known pa-

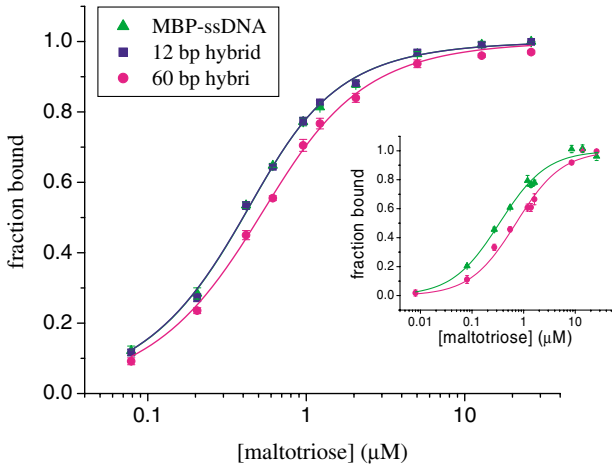

FIG. 2 (color). Titration curves (obtained from the change in Trp fluorescence normalized between 0 and 1) displaying the fraction of proteins with a bound substrate (malto-triose) vs substrate concentration. Each point is the average of four to six different experiments; the error bars are ±1 SD. The lines are fits using Eq. (1), from which the binding constants are extracted. The 60 bp hybrid shows a ~35% reduction in binding affinity K with respect to the controls (MBP: ss DNA and 12 bp hybrid). Inset: results for a double Cys chimera where the DNA is coupled covalently on both sides; the 60 bp hybrid now shows a ~60% reduction in K.

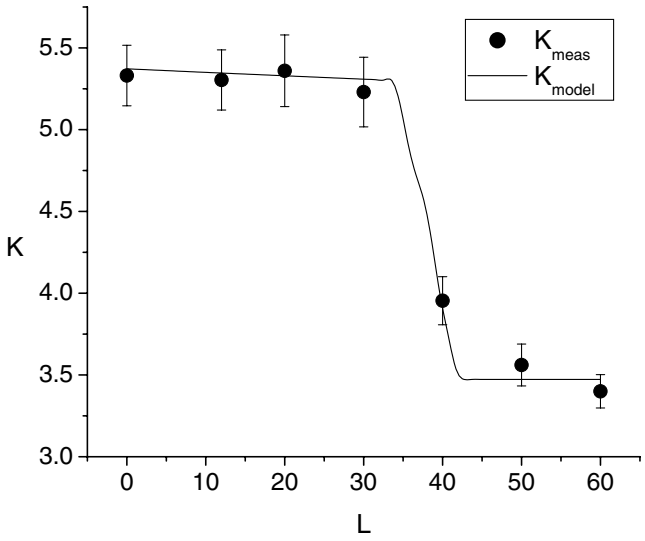

FIG. 3. Binding affinities K (in μM^{-1}) for malto-triose vs length of the complementary (L) hybridized to the 60mer DNA of the chimera. Each point represents the average of four to six titration curves; error bars are ±1 SD. The mechanical tension on the protein is expected to set in for $L > 34$ (Fig. 4). The data therefore confirm that the change in K is caused by the mechanical tension. The $L = 12$ data point is an especially significant control, because the length of the complementary strand for this case is actually 60 bases, but only 12 are complementary to the DNA of the chimera, so no tension is expected to develop. The continuous line is the prediction of a simple thermodynamic model [Eq. (2)] described in the text.

rameters of DNA elasticity. In the model (discussed in more detail in [13]) the ds part of the DNA bends like a bow, while the ss ends act as (nonlinear) springs (see the Fig. 4 inset). The force exerted by the bow is calculated from the work per unit length required to bend it: $W/s = B/(2R^2)$, where s is the contour length of the bow, B is the bending modulus, and R is the radius of curvature. For ds DNA, $B \approx 200$ pN $\times$ nm^2 [14]. The extension of the ss DNA "springs" is calculated from the mechanical equilibrium condition that the tension in the springs is the same as the force needed to bend the bow (Fig. 4 inset). For the force-extension characteristic of the springs we use the published force-extension curves for ss DNA [16,17]. The prediction of this model (which contains no adjustable parameters) is that substantial tension sets in for $L \geq 36$ (Fig. 4). Comparing Fig. 4 to the data of Fig. 3 supports the interpretation that the change in binding affinity is caused by the mechanical tension exerted by the DNA on the protein. Note that while the elastic energy stored in the DNA competes with the binding energy of hybridization, the total free energy of the system still favors hybridization (Fig. 4 inset). This conclusion is supported by experiments in which an intercalating fluorescent dye was used to monitor the degree of DNA hybridization on the chimera (see Fig. S3 in [13]).

Materials and methods.—The mutagenesis and purification, and the MBP-DNA Complex conjugation, are detailed in [13].

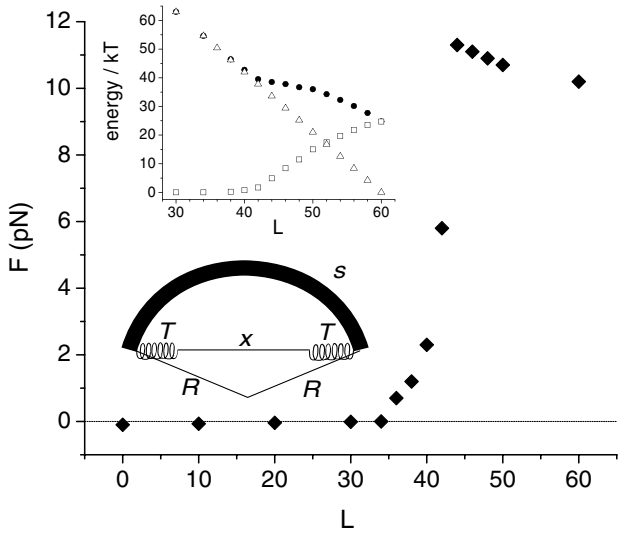

FIG. 4. Mechanical tension F on the protein vs length L of the complementary hybridized to the chimera, as predicted by the mechanical model shown in the inset. Inset: the elastic and bond free energies in the DNA (in units of kT), from the model and [15]. Open squares: the total elastic energy E_{elastic} (for details, see Fig. S3 in [13]). Open triangles: the free energy of hybridization $F_{\text{bond}} \approx 2.1$ kT per open bp. Filled circles: the total free energy of the system $F_{\text{tot}} = E_{\text{elastic}} + F_{\text{bond}}$.

To obtain the MBP-Maltose binding affinity, fluorescence measurements were performed with a Photon Technology Instruments fluorimeter, in 3 mL cuvettes, at $20 \pm 2\,^{\circ}$C. The excitation and emission wavelengths were $\lambda_{\text{ex}} = 281$ nm, $\lambda_{\text{em}} = 341$ nm. The concentration of MBP-DNA chimera was approximately 50 nM in phosphate buffered saline.

The fraction of proteins with a bound maltose is $f = [M]/(K^{-1} + [M])$, where K is the binding constant, M is the maltose, P is the protein, and [ ] means equilibrium concentration. In terms of the initial maltose concentration $[M]_0$ the relation is [18]

$$[P]_0 f^2 - (K^{-1} + [M]_0 + [P]_0) f + [M]_0 = 0. \quad (1)$$

The binding constant K was determined by fitting the data to Eq. (1), after correcting for dilution effects.

Discussion.—We have built an artificial allosteric control module into a protein. The principle is to use mechanical tension to influence the conformation of the protein. The tension is derived from the elasticity of another polymer coupled to the protein. Using a protein-DNA chimera we show that the tension can be controlled externally, in this case by the DNA sequence which is allowed to hybridize to the chimera. Based on the elastic properties of DNA, we estimate that we can obtain a significant tension on the protein, up to $\sim$10 pN. Nonetheless, the effect on the binding affinity K is relatively small ($\sim 35\%$ reduction). We believe that at present the observed effect is limited by the yield of complete chimeras in the samples. In addition, MBP binds the substrates also in the open conformation, only with a smaller K. A study where the conformation was forced permanently open by mutagenesis reports a $\sim$50% reduction in binding constant [19]. Finally, the labile protein-DNA connection on the Ni^{2+} side probably limits the average tension. Indeed, our results with a Cys double mutant chimera, where the DNA is covalently attached at both ends (and the yield probably increased), show a larger ($\sim$60%) effect on K (inset of Fig. 2). To support our interpretation, we also show in Fig. 3 (continuous line) the result from the simplest thermodynamic model for K, which takes into account the above. Given a conformational motion of size s, an applied force F alters the free energy difference between the two states by $F \times s$. We assume for the binding affinity

$$K(F) = K_{\text{closed}} e^{-F \times s/\text{kT}} \quad (2)$$

for $F \leq F_0$ such that $K(F_0) = K_{\text{open}}$, and $K(F) = K_{\text{open}}$ for $F \geq F_0$. Here K_{closed}, K_{open} are the binding affinities in the "closed" and open states: $K_{\text{closed}} \approx 5.3\ (\mu\text{M})^{-1}$ and $K_{\text{open}} \approx \frac{1}{2} K_{\text{closed}}$ [19]. With a yield p of correct chimeras (see [13]), the values for F of Fig. 4, and the parameters above, we plot this model (for $s = 0.9$ nm, $p = 0.6$) as the continuous line in Fig. 3. The fit has one adjustable parameter (the yield p, or equivalently K_{open}).

To summarize the controls, we have shown, by hybridizing complementaries of different lengths L, that the threshold length to obtain an effect on K coincides with the estimated threshold where mechanical tension sets in ($L \approx 34$). The decrease in K for $L > 40$ cannot be attributed simply to the presence of an extra DNA strand in close proximity to the protein, because a 60mer with only 12 base complementarity ($L = 12$), which therefore produces similar steric effects as the 60mer true complementary, but no tension, does not affect K. Finally, if in the hybridized chimera we cut off one side of the protein-DNA connection (adding imidazole, which competes with the His tag for binding to the Ni^{2+}), K returns to its original value (before hybridization).

This study opens a new approach to the control of protein function. It provides a new tool to study the relationship between protein function and conformation, because it allows external control of the conformation. In addition, such control of the catalytic rate of enzymes would open tremendous possibilities for applications. We envision amplified molecular probes, applied for instance to the detection of specific DNA sequences. One can even dream of ''smart drugs,'' which are turned on or off in the presence of certain transcription products. As a first step, we will pursue the artificial allosteric control of an enzyme.

We thank Martin Phillips for help with the fluorescence spectroscopy, Elena Zocchi and David Bensimon for valuable suggestions, and Albert Libchaber for commenting on the manuscript. This work was partially supported by NSF Grant No. DMR-0405632.

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