Protein-induced bending and DNA cyclization

(catabolite activator protein/DNA bending/phasing/thermodynamics)

JASON D. KAHN AND DONALD M. CROTHERS

Department of Chemistry, P.O. Box 6666, Yale University, New Haven, CT 06511

Contributed by Donald M. Crothers, March 26, 1992

ABSTRACT We have applied T4 ligase-mediated DNA cyclization kinetics to protein-induced bending in DNA. The presence and direction of a static bend can be inferred from J factors for cyclization of 150- to 160-base-pair minicircles, which include a catabolite activator protein binding site phased against a sequence-directed bend. We demonstrate a quasi-thermodynamic linkage between cyclization and protein binding; we find that properly phased DNAs bind catabolite activator protein ≈ 200 -fold more tightly as circles than as linear molecules. The results unambiguously distinguish DNA bends from isotropically flexible sites and can explain cooperative binding by proteins that need not contact each other.

Protein-induced DNA bending may be important in transcriptional regulation, control of replication and recombination, and DNA packaging into nucleosomes (1). Bending is likely to act by facilitating protein–protein or protein–DNA contacts. For example, integration host factor affects loop formation in recombination and transcriptional regulation (2–5), and catabolite activator protein (CAP) may activate transcription partially by stabilizing contacts between RNA polymerase and upstream DNA (6–8). The replacement of protein-induced bends by properly positioned sequence-directed bends or bends from unrelated proteins (2, 9, 10) shows that bending alone can suffice for function in some cases. Bending effects are generally sensitive to the helical phasing between the bending locus and the other DNA sites.

DNA bending by proteins has been studied by x-ray crystallography (8, 11) and gel electrophoresis methods. Circular permutation of the binding site within a DNA fragment (12) and phasing of the binding site against a sequence-directed bend (13–15) can reliably indicate bend location and direction. However, estimates for bend magnitudes have relied on empirical comparisons of gel mobility among different bends (16, 17). Estimates for the CAP-induced bend angle range from 90° from the cocrystal structure (8) to 140° from some comparative electrophoresis measurements (16); our value is 100° (17).

DNA ring closure, or cyclization, allows the study of DNA bending in solution (18). Cyclization efficiency is measured by the J factor, the effective concentration of one properly aligned end of a DNA molecule about the other. J is defined as K_c/K_a , the ratio of the equilibrium constants for unimolecular cyclization (K_c) and bimolecular association (K_a) (19). It can also be obtained from the kinetics of T4 DNA ligase-catalyzed covalent ring closure: $J = k_1/k_2$, where k_1 and k_2 are the rate constants for cyclization and bimolecular ligation, respectively (20). Measurements of J have been used to calculate the persistence length and torsional modulus of DNA and also intrinsic bend angles in curved DNA (21–24).

Rates or product distributions of ligase-catalyzed cyclization are affected by the binding of CAP (25, 26), HU (27), and *cro* repressor (28). In this paper, we show quantitatively that

CAP can dramatically increase or decrease J for cyclization of DNA molecules including CAP sites phased against a sequence-directed bend. In addition, we have verified that cyclization and protein-binding equilibria form a quasi-thermodynamic cycle. This concept provides an independent means of checking cyclization kinetics results and also suggests a general method for enhancing the stability of protein-bound DNA complexes by embedding the binding site in circular or prebent DNA. We discuss the importance of setting the rotational phase of curved DNA and the biological relevance of such mechanisms.

MATERIALS AND METHODS

Materials. Restriction enzymes, bovine serum albumin, BAL-31 nuclease, and T4 DNA ligase were purchased from New England Biolabs. Ligase concentrations are given as New England Biolabs ligation units/ml. Labeled compounds were purchased from NEN, ATP and dNTPs were from Pharmacia, and cAMP was obtained from Sigma. Oligonucleotides were synthesized on an Applied Biosystems model 380B synthesizer and gel-purified. CAP protein, generously provided by Steve Schultz and Tom Steitz (Yale University), was stored at 1.2 μ M in 0.5 $\times$ TE (1 $\times$ TE = 10 mM Tris Cl/1 mM EDTA, pH 7.5), 50% glycerol, 2 mM dithiothreitol, and 25 mM NaCl. Gel retardation titrations showed it to be about 60% active in DNA binding. CAP concentrations are in terms of active protein.

CAP Site–A-Tract Minicircles. CAP site DNA with *Eco*RI ends was derived from a pseudo-wild-type *lac* promoter CAP site with *Sty* I ends (29) by ligation of *Nco* I–*Eco*RI and *Avr* II–*Eco*RI adaptors (5'-CATGGAATCGATG/5'-AATTCATCGATTC and 5'-CTAGGTCTAG/5'-AATTCTAGAC), digestion with *Eco*RI, and cloning into pBluescript II KS(+) (Stratagene). The other half of the minicircle was constructed by ligation of *Msp* I–*Eco*RI phasing adaptors onto A-tract multimers [six A tracts, 63 base pairs (bp) (24)], followed by restriction with *Eco*RI. Each set of A-tract adaptors was gel purified, 4 nM adaptor and 2 nM *Eco*RI CAP site were ligated (6000 units/ml), and the products were separated by gel electrophoresis. Circular DNA was identified by BAL-31 resistance. Circles were eluted, restriction digested with *Cla* I or *Nco* I, amplified by PCR, re-restricted, and cloned into a modified pBluescript II vector with an *Nco* I site inserted at the *Cla* I site of the polylinker; thus, we initially selected phasing adaptor combinations that were capable of cyclization. Double-stranded plasmids were sequenced with Sequenase version 2.0 (United States Biochemical). In principle, 50 different circles can be generated by using five phasing adaptors by this approach, but we concentrated on four constructs with different bend phasings and varied the total length of some molecules by PCR (see below and Fig. 1).

Preparation of Cyclization Substrates. *Bst*NI-restricted plasmids were used as PCR templates. Reaction mixtures

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: CAP, catabolite activator protein.

(150 μ l) contained 50 mM KCl, 10 mM Tris Cl (pH 8.4), 2 mM $MgCl_2$, 30 μ Ci (1 Ci = 37 GBq) of [α - 32 P]dATP, each dNTP at 180 μ M, 6 units of *Taq* or Amplitaq polymerase (Perkin-Elmer/Cetus), 10% glycerol, gelatin at 100 μ g/ml, and each primer at 0.5–1 μ M and were subjected to 30 cycles of 1 min at 94°C, 1 min at 55°C, 1 min to 72°C, and 2 min at 72°C. PCR products were phenol extracted, incubated overnight at 37°C with 2.5 units of *Cla* I, purified on 8% native gels [40:1, acrylamide/*N,N'*-methylenebisacrylamide, TBE (50 mM Tris/50 mM boric acid/1 mM EDTA, pH 8.3)], and electroeluted. Specific activity was measured by UV absorbance (in 100- μ l Hellma cells) and scintillation counting; $\approx$ 1% of the DNA was labeled. This method of preparing labeled DNA avoids problems with incomplete dephosphorylation and rephosphorylation of restriction fragments, and PCR simplifies the synthesis of DNAs of slightly different lengths.

Cyclization Kinetics Measurements. Time course experiments were performed at 0.5–10 nM DNA and 0–24 nM CAP, with 105-bp A-tract multimer DNA (24) at $\leq$ 1 nM as an internal standard, all at 21°C. The ligation reaction buffer was 50 mM Tris Cl (pH 7.5), 5 mM KCl, 3.5 mM $MgCl_2$, bovine serum albumin at 40 μ g/ml, 0.01% Nonidet P-40 (Shell), 1 mM dithiothreitol, 1 mM ATP, and 100 μ M cAMP. T4 DNA ligase was stored at 6 units/ μ l at –20°C in ligation storage buffer [50 mM KCl, 10 mM Tris Cl (pH 7.6), 0.1 mM EDTA, 1 mM dithiothreitol, bovine serum albumin at 200 μ g/ml, and 50% glycerol]. Ligase concentration was varied between 2 and 600 units/ml depending on k_1 . It was diluted immediately before use into ligation storage buffer, without glycerol but including 0.01% Nonidet P-40.

Reactions were initiated by the addition of 7.5 μ l of diluted ligase for a final volume of 75 μ l, and eight 8- μ l time points over 15–30 min were quenched by addition to 4 μ l of 75 mM EDTA, proteinase K at 2 mg/ml, 15% glycerol, and dyes. Stopped reactions were heated to 55°C for 15 min and analyzed on 5–8% native gels (40:1, acrylamide/*N,N'*-methylenebisacrylamide, TBE, 0.8 mm thick, 20 cm long, run at 2.5 V/cm). Dried gels were quantified with a Betagen blot analyzer and autoradiographed.

Gel Retardation Competition. Circular DNAs were prepared from linear DNA of known specific activity under time course conditions (except at 3000 units of ligase per ml), phenol extracted, and gel purified; yields were >90%. Lightly labeled competitor DNA was either a linear construct DNA or the CAP site DNA with *Eco*RI ends used in minicircle construction, which gave identical results; the CAP site sequence in all circular and linear molecules was the same. Competitor concentrations were measured by UV absorbance and scintillation counting.

CAP and competitor DNA were incubated in ligation reaction buffer at 21°C for 30 min before the addition of circular DNA, and the mixtures were equilibrated overnight before analysis on 8% native gels (40:1, acrylamide/*N,N'*-methylenebisacrylamide, TBE, 0.8 mm thick, 100 μ M cAMP, with cAMP to 20 μ M added to the upper buffer every 2 hr). Gels were run for 12–15 hr at about 35°C, as elevated temperature improved resolution. CAP reduces the mobility of circular DNA only 1–5%. Gels were processed as above.

RESULTS

In applying cyclization kinetics to protein-induced DNA bends, we have measured J factors for members of a set of DNAs containing CAP-induced bends and A-tract multimer bends. Our constructs (Fig. 1) illustrate design considerations for DNA minicircles useful in studying protein-directed bends (18). Since several physical properties of DNA influence cyclization, it is necessary to use a family of DNAs to measure them individually. To maximize sensitivity to induced bending or twisting, the molecules are made as small

FIG. 1. CAP site-A-tract minicircles. Our nomenclature (e.g., 11A17) specifies the length of adaptor I (center of *Eco*RI site to center of *Msp* I site), the orientation of the A-tract sequence, and the length of adaptor II, in that order. Therefore, the second number describes bend phasing in linear DNA. "A" indicates that A-tract adenines are on the strand that reads 5' $\rightarrow$ 3' clockwise. The adaptor sequences are 5' . . . CCGGATCC[GTACAGCT]GAATTC . . . , with adaptors of increasing length including successive pairs of bracketed base pairs, starting from the left. PCR primers: 1, 5'-GCAGATATCGAT-TCCATGGCGCAACGCAATTAA; 2, 5'-CGCCATGGAATCGAT-GAATTC. Substrates 8T15 and 8A17 were amplified from plasmids 11T15 and 11A17 by using primers 2 and 1A (5'-GCAGATATCGAT-TCCATGGAACGCAATTAA), and 9T15 and 9A17 were similarly prepared by using primers 2 and 1B (5'-GCAGATATCGATTC-CATGGCAACGCAATTAA) (4 mM $MgCl_2$, with 4 cycles of 1 min at 94°C, 1 min at 37°C, 1 min to 55°C, and 2 min at 55°C preceding amplification as in the text).

as possible. Sequence-directed bends reduce the minimum length of DNA needed and allow the determination of the protein-induced bend direction; two sets of phasing adaptor DNA segments allow independent variation of the bend phasing and the total length. The two-base *Cla* I overhang minimizes K_a , the equilibrium constant for bimolecular association; this extends the range of accessible J factors (18, 24).

In-phase protein-induced and sequence-directed bends are expected to form the DNA into a C or paperclip shape, thereby reducing the average distance between the DNA ends, aligning them properly, and accelerating cyclization. Out-of-phase bends will form the DNA into an S shape, which represses cyclization. In this length range, cyclization also depends strongly on overall length, because the DNA ends must be torsionally aligned (21).

Cyclization Kinetics. The cyclization and bimolecular ligation of equal length constructs 11A17 and 17A11 (see the legend to Fig. 1 for nomenclature) illustrate the spectrum of ligation behavior with and without CAP (Fig. 2). For rapidly cyclizing molecules such as 11A17, linear DNA is converted mainly to circular product, with a small amount of linear dimers and dimeric circles. Binding of CAP to this in-phase construct accelerates cyclization and thereby suppresses the bimolecular reaction completely. In contrast, binding of CAP to construct 17A11, in which the bends are out of phase, suppresses the monomeric circle. Fig. 2 C and D shows representative determinations of k_1 and k_2 . The 105-bp A-tract multimer internal standard (24) allows correction for variations in ligase activity.

The rate constants k_1 and k_2 are linearly dependent on ligase concentration up to at least 1200 units/ml (in the presence of 0.01% Nonidet P-40) and are independent of DNA concentration (data not shown). Rate constants per units/ml of ligase were derived from graphs of rate constants vs. three or four ligase concentrations used in each experiment. J is reproducible to $\leq$ 15%. The effect of CAP reaches its maximum by 6 nM (for low concentrations of DNA) and

$$[C]_t = \frac{k_1}{4k_2} \ln(1 + 4[M]_0(1 - e^{-k_1 t})k_2/k_1)$$

$$[B]_t = \frac{1}{2} \left\{ [M]_0 - [C]_t - \frac{[M]_0 e^{-k_1 t}}{1 + 4[M]_0(1 - e^{-k_1 t})k_2/k_1} \right\},$$

where $[C]_t$ and $[B]_t$ are the concentrations of monomolecular circular product and total bimolecular products at time t , $4k_2$ is the rate constant for bimolecular ligation of DNA with two ligatable ends (30), and $[M]_0$ is the concentration of ligatable DNA, determined at

is roughly constant up to 30 nM; at higher concentrations ligation is inhibited. We routinely performed experiments at 12 and 24 nM CAP to ensure complete CAP binding and to check for nonspecific binding, and all results reported here were essentially the same at the two concentrations. Gel retardation showed little nonspecific binding to the linear DNAs at these CAP concentrations (data not shown).

To calculate $J = k_1/k_2$ when cyclization is rapid, k_2 must be estimated from control reactions. The average ratio $[k_2/(k_1 \text{ for } 105\text{-mer})]$ for several slowly cyclizing molecules is $7.0 \times 10^6 \text{ M}^{-1}$ in the absence of CAP (data not shown), allowing us to calculate an unknown k_2 (in the absence of CAP) from the observed 105-mer k_1 . CAP at 12–24 nM consistently gives k_2 (in the presence of CAP) $\approx 0.65 \times k_2$ (in the absence of CAP), and k_1 (in the absence or presence of CAP) is standardized to the 105-mer k_1 . This bimolecular calibration is our major source of experimental error. Others have addressed the problem by adjusting the DNA concentration so that the bimolecular reaction is always observable (30), but this is impossible for our rapidly cyclizing molecules: it would require DNA, CAP, and ligase concentrations up to 1000 times those used here, leading to nonspecific protein binding or aggregation and changes in solution conditions.

Effect of CAP on J factors. The results in Table 1 show that J has the expected dependence on phasing between sequence-directed and protein-induced bends. When the two bends are in phase, CAP binding can increase J up to 120-fold, whereas when the bends are out of phase, CAP can decrease J at least 10-fold. A parallel but much weaker effect in the absence of CAP confirms that CAP site DNA is intrinsically slightly bent (32). We expected that slight unwinding induced by CAP (33) would prefer cyclization of molecules overwound relative to the optimum length. This trend is observed for the 8A17/9A17/11A17 set, where the cyclization of 11A17 is more strongly enhanced by CAP, but the effect is not seen for the 8T15/9T15/11T15 series, nor is it observed in the binding experiments below. The discrepancy may be due to alterations in twist imposed by the requirement that the two bends be nearly coplanar for cyclization.

These results confirm that cyclization kinetics can indicate the presence of DNA bending (25–27). In addition, phasing the CAP binding site against a known bend allows determination of the bend direction and demonstrates the existence of a static bend as opposed to a region of exceptional flexibility (14). Cyclization reactions are done entirely in solution, they do not require gel-stable complexes, and the measured J factors span 5 orders of magnitude.

Thermodynamic Linkage Between Cyclization and Binding. The marked effect of CAP binding on DNA cyclization, which reflects an effect on the energetics of DNA conformation, must be accompanied by a corresponding effect of conformation (i.e., cyclization) on CAP binding. In other words, if a protein enhances cyclization of a DNA molecule, it should also bind more tightly to the circular product than to the linear reactant. This concept is illustrated in the quasi-thermodynamic cycle of Fig. 3, in which it is assumed that the rate constant for ligation is proportional to the equilibrium constant K_c for transient cyclization (20). We see that the ratio of binding constants of CAP to circular and linear DNA should be the same as the ratio of J factors for cyclization of DNA with and without CAP. This effect is analogous to the improved binding of unwinding ligands to supercoiled DNA (34).

times at which the reaction has gone substantially to completion. The curves shown in C and D are theoretical curves using best-fit values from the two equations above. C, circular; L, linear.

Table 1. Effects of CAP on DNA cyclization and of DNA cyclization on CAP binding

DNA	Total length		Phasing		<i>J</i> , nM		$\frac{J(+CAP)}{J(-CAP)}$	$\frac{K_B(C)}{K_B(L)}$	<i>K</i> ratio
	bp	Turns	bp	Turns	- CAP	+ CAP			
8A17	157	15.1	53	5.1	300	12,000	39	120	3.1
9A17	158	15.2	53	5.1	150	10,500	69	225	3.3
11A17	160	15.4	53	5.1	17	2,000	120	160	1.4
17A11	160	15.4	47	4.5	5.2	1.0	0.19	≈0.4*	≈2*
8T15	155	14.9	51	4.9	365	17,000	47	83	1.8
9T15	156	15.0	51	4.9	425	19,500	46	95	2.1
11T15	158	15.2	51	4.9	95	4,200	44	55	1.3
15A9	156	15.0	45	4.3	33	3.3	0.094	ND	ND

See Fig. 1 for DNA nomenclature. The helical repeat of B-DNA is estimated to be 10.45 bp per turn, from the dependence of *J* on total DNA length for cyclizations of in-phase constructs in the absence of CAP, assuming 10.33 bp per turn for A tracts (31). Phasing specifies the distance (in bp) from the center of the CAP bend to the center of the first A tract. An integral number represents in-phase bends. No correction is made for possible unwinding by CAP. When CAP was present, the active CAP concentration was 12 nM. ND, not done; C, circular; L, linear.

*Relative binding constants in this case were estimated from the half-saturation points in a protein titration (data not shown).

The proposed relationship between cyclization and binding was tested by using gel retardation competition, in which limiting CAP partitions between circular DNA and linear competitor DNA. In the experiment of Fig. 4 and Table 1, six DNA circles were analyzed using the same CAP and competitor solutions. The data were fit to the equation below, which is derived from the equilibrium expressions for protein binding to the two DNAs assuming that a small fraction of competitor DNA is bound:

$$\theta = \frac{1}{2[C]} \left\{ [C] + [P] + \frac{[L] + 1/K_B(L)}{K_B(C)/K_B(L)} - \left[\left([C] + [P] + \frac{[L] + 1/K_B(L)}{K_B(C)/K_B(L)} \right)^2 - 4[C][P] \right]^{1/2} \right\}, \quad [1]$$

where θ is the fraction of circular DNA bound to CAP, $[C]$ is the total concentration of circular DNA, $[P]$ is the total active CAP concentration, $[L]$ is the total concentration of linear (competitor) DNA, and $K_B(C)$ and $K_B(L)$ are the binding constants of CAP with C and L, respectively. We measured $[C]$ (≈ 1.3 nM) and $[L]$ (0–500 nM) by scintillation counting and $[P]$ (0.9 nM) by titration with excess DNA. The observed $[C]$ was allowed to vary by up to 15% to fit θ at 0 competitor (a mixture with 3.6 nM CAP showed no dissociation of the complex during electrophoresis; Fig. 4). Curve fitting each titration individually gave initial values for $K_B(C)$ and $K_B(L)$, and the average $1/K_B(L)$ (3.0 nM, confirmed by direct titration) was then assumed for the whole set to give final values for $K_B(C)/K_B(L)$, as shown in Fig. 4C. Repeated

FIG. 3. Quasi-thermodynamic cycle relating cyclization and binding equilibria. Cyclization reactions are shown for free DNA (top line) and bound DNA (second line). The observed $k_1 = K_c \times k_{lig}$, where k_{lig} is the rate constant (per ligase units/ml) for covalent joining of associated ends (20). The derivation of binding ratios from *J* ratios is shown below: the three equalities reflect the lack of a CAP effect on K_a , the path independence of the overall equilibrium constant between linear DNA and nicked circle-CAP, and equivalent CAP binding to the nicked and closed circles.

experiments gave values of $K_B(C)/K_B(L)$ up to 50% larger than those here, because the ratio is sensitive to errors in $[P]$. However, relative binding ratios for different circles were consistent to within 15%.

The results of direct binding and cyclization kinetics measurements are in good quantitative agreement (within a factor of ≈ 2), and CAP binds ≈ 200 -fold more tightly to a well-phased circle than to linear DNA. CAP binds to the 9A17 in-phase circle with an apparent dissociation constant of 15 pM.

DISCUSSION

We have shown that the binding of CAP to 150- to 160-bp DNA minicircles containing a CAP site and a sequence-directed bend separated by variable phasing adaptors can greatly stimulate or depress DNA ring closure in a phasing-dependent manner. The effect of protein on cyclization is

FIG. 4. Gel retardation competition experiments. (A) Two autoradiograms showing displacement of CAP from circular DNA with increasing linear competitor. Circular DNA was at ≈ 1.3 nM, and the circles had migrated about 20 cm. The 3.6 nM CAP lanes and the outermost lanes were used to correct for smearing between bands ($<10\%$). At higher concentrations of CAP, we observe doubly shifted DNA. (B and C) The curves show fits to the fractional saturation of each circular DNA vs. the concentration of competitor DNA, modeled with Eq. 1 as in the text. For presentation, the 0 competitor points are plotted at 0.4 nM. The dashed vertical lines indicate half-saturation competitor concentrations for DNA circles 9T15 and 9A17. The DNA concentrations of the 8A17/9A17/11A17 set were slightly higher than those for 8T15/9T15/11T15, giving a lower θ at 0 competitor.

mirrored by the effect of cyclization on protein binding: CAP binds ≈ 200 -fold more tightly to a binding site in a properly phased minicircle than to the same site in linear DNA. The cyclization assay can be used qualitatively to determine the direction of an unknown bend, even if a gel-stable complex is not formed.

Verification of T4 Ligase-Mediated Cyclization Measurements. Agreement among the relative binding constants for CAP binding to linear vs. circular DNA predicted from ligation kinetics and those measured by direct binding is excellent, though our data suggest that molecules that are closer to optimum torsional alignment bind CAP slightly better (as circles) than expected from cyclization. The discrepancy could be due to ligation of slightly misaligned DNAs, but it is clear that the ligation kinetics method is not prey to any large errors due to joining of dramatically distorted DNA backbones. (Incorrect ligation may affect bimolecular ligations at the much higher ligase concentrations often used in cloning.) This verification of ligase fidelity by an independent technique also inspires confidence in Monte Carlo simulations of cyclization, which assume that DNA can be ligated only if the ends are perfectly aligned.

Applications of Constrained Binding Sites. Enhanced binding of CAP to a binding site constrained in a circle has practical implications: by providing a DNA binding site already in an optimal conformation, it may be possible to isolate unstable protein-DNA complexes or to select a particular complex from a mixture. Exonuclease resistance allows cyclization to be used to select DNA circles with optimum binding conformations.

Sequence-directed bends in these minicircles enhance binding and selectivity. The rotational preorganization of the binding site may contribute substantially to binding free energy: since the A tracts in our constructs presumably set the rotational phasing of the DNA, defining an inside and an outside of the circle (35), CAP binding does not incur the entropy cost of restricting the rotation of DNA about its helix axis. The A-tract bend also reduces the minicircle size; it introduces more curvature into the binding site upon cyclization.

Relevance to Looping and Cooperativity. Transcriptional activation at a distance is probably mediated by looping of the intervening DNA (36). Since the thermodynamic linkage discussed above applies to any means of constraining DNA ends, our results provide further support for bending as a plausible mechanism for phasing-dependent control of looping or for the influence of looping on the binding of distant bending proteins. The resulting cooperativity or anticooperativity between proteins does not depend on direct protein-protein interaction or on their binding to neighboring DNA sites. Experimental examples include the phasing-dependent disruption of the araC repression loop by CAP binding (37), the enhancement or repression of looping-mediated transcriptional activation upon integration host factor binding between σ^{54} promoter sites and enhancer sites occupied by NIFA or *ntnC* (4, 5), and the regulation of λ phage excision by a set of proteins that can switch the DNA from one looped conformation to another (38). DNA cyclization has been used to detect looping mediated by the plasmid R6K replication initiator protein (39). Finally, we note that upstream RNA polymerase DNA contacts (6–8) would require bending the CAP binding site, leading to cooperative binding of CAP and polymerase. The ≈ 200 -fold effect we observe for prebending the DNA is sufficient to explain the magnitude of CAP-induced gene activation.

We thank J. Drak and members of the Crothers lab for discussions and S. Schultz and T. Steitz for CAP protein. This work was

supported by National Institutes of Health Grant GM 21966. J.D.K. was supported by National Institutes of Health postdoctoral fellowship GM 14122.

1. Travers, A. A. (1990) *Cell* **60**, 177–180.
2. Goodman, S. D. & Nash, H. A. (1989) *Nature (London)* **341**, 251–254.
3. Moitoso de Vargas, L., Kim, S. & Landy, A. (1989) *Science* **244**, 1457–1461.
4. Claviere-Martin, F. & Magasanik, B. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1631–1635.
5. Hoover, T. R., Santero, E., Porter, S. & Kustu, S. (1990) *Cell* **63**, 11–22.
6. Buc, H. (1986) *Biochem. Soc. Trans.* **14**, 196–199.
7. Zinkel, S. S. & Crothers, D. M. (1991) *J. Mol. Biol.* **219**, 201–215.
8. Schultz, S. C., Shields, G. C. & Steitz, T. A. (1991) *Science* **253**, 1001–1007.
9. Bracco, L., Kotlarz, D., Kolb, A., Diekmann, S. & Buc, H. (1989) *EMBO J.* **8**, 4289–4296.
10. Gartenberg, M. R. & Crothers, D. M. (1991) *J. Mol. Biol.* **219**, 217–230.
11. Brennan, R. G., Roderick, S. L., Takeda, Y. & Matthews, B. W. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8165–8169.
12. Wu, H.-M. & Crothers, D. M. (1984) *Nature (London)* **308**, 509–513.
13. Zinkel, S. S. & Crothers, D. M. (1987) *Nature (London)* **328**, 178–181.
14. Kerppola, T. K. & Curran, T. (1991) *Science* **254**, 1210–1214.
15. Salvo, J. J. & Grindley, N. D. F. (1987) *Nucleic Acids Res.* **15**, 9771–9779.
16. Thompson, J. F. & Landy, A. (1988) *Nucleic Acids Res.* **16**, 9687–9705.
17. Zinkel, S. S. & Crothers, D. M. (1990) *Biopolymers* **29**, 29–38.
18. Crothers, D. M., Drak, J., Kahn, J. D. & Levene, S. D. (1992) *Methods Enzymol.* **212**, 3–29.
19. Jacobsen, H. & Stockmayer, W. H. (1950) *J. Chem. Phys.* **18**, 1600–1606.
20. Shore, D., Langowski, J. & Baldwin, R. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4833–4837.
21. Shore, D. & Baldwin, R. L. (1983) *J. Mol. Biol.* **170**, 957–981.
22. Levene, S. D. & Crothers, D. M. (1986) *J. Mol. Biol.* **189**, 61–72.
23. Levene, S. D. & Crothers, D. M. (1986) *J. Mol. Biol.* **189**, 73–83.
24. Koo, H.-S., Drak, J., Rice, J. A. & Crothers, D. M. (1990) *Biochemistry* **29**, 4227–4234.
25. Kotlarz, D., Fritsch, A. & Buc, H. (1986) *EMBO J.* **5**, 799–803.
26. Dripps, D. & Wartell, R. M. (1987) *J. Biomol. Struct. Dyn.* **5**, 1–13.
27. Hodges-Garcia, Y., Hagerman, P. J. & Pettijohn, D. E. (1989) *J. Biol. Chem.* **264**, 14621–14623.
28. Lyubchenko, Y., Shlyakhtenko, L., Chernov, B. & Harrington, R. E. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5331–5334.
29. Gartenberg, M. R. & Crothers, D. M. (1988) *Nature (London)* **333**, 824–829.
30. Taylor, W. H. & Hagerman, P. J. (1990) *J. Mol. Biol.* **212**, 363–376.
31. Drak, J. & Crothers, D. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3074–3078.
32. Liu-Johnson, H.-N., Gartenberg, M. & Crothers, D. M. (1986) *Cell* **47**, 995–1005.
33. Douc-Rasy, S., Kolb, A. & Prunell, A. (1989) *Nucleic Acids Res.* **17**, 5173–5189.
34. Davidson, N. (1972) *J. Mol. Biol.* **66**, 307–309.
35. Drew, H. R. & Travers, A. A. (1985) *J. Mol. Biol.* **186**, 773–790.
36. Bellomy, G. R. & Record, M. T., Jr. (1990) *Prog. Nucleic Acid Res. Mol. Biol.* **39**, 81–128.
37. Lobell, R. B. & Schleif, R. F. (1991) *J. Mol. Biol.* **218**, 45–54.
38. Moitoso de Vargas, L. & Landy, A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 588–592.
39. Mukherjee, S., Erickson, H. & Bastia, D. (1988) *Cell* **52**, 375–383.