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DNA linking number change induced by sequence-specific DNA-binding proteins

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ABSTRACT

Sequence-specific DNA-binding proteins play a key role in many fundamental biological processes, such as transcription, DNA replication and recombination. Very often, these DNA-binding proteins introduce structural changes to the target DNA-binding sites including DNA bending, twisting or untwisting and wrapping, which in many cases induce a linking number change (Δ*Lk*) to the DNA-binding site. Due to the lack of a feasible approach, Δ*Lk* induced by sequence-specific DNA-binding proteins has not been fully explored. In this paper we successfully constructed a series of DNA plasmids that carry many tandem copies of a DNA-binding site for one sequence-specific DNA-binding protein, such as λ O, LacI, GalR, CRP and AraC. In this case, the protein-induced Δ*Lk* was greatly amplified and can be measured experimentally. Indeed, not only were we able to simultaneously determine the protein-induced Δ*Lk* and the DNA-binding constant for λ O and GalR, but also we demonstrated that the protein-induced Δ*Lk* is an intrinsic property for these sequence-specific DNA-binding proteins. Our results also showed that protein-mediated DNA looping by AraC and LacI can induce a Δ*Lk* to the plasmid DNA templates. Furthermore, we demonstrated that the protein-induced Δ*Lk* does not correlate with the protein-induced DNA bending by the DNA-binding proteins.

INTRODUCTION

Sequence-specific DNA-binding proteins play a key role in many essential biological processes. For instance, DNA replication initiators, such as *Escherichia coli* DnaA protein and bacteriophage λ O protein, bind and restructure DNA replication origin elements (1,2) and subsequently prepare them for chromosome duplication (3–6). Transcription is another fundamental process in biology. The control of transcription initiation by transcriptional factors is extremely important for coordinating a sophisticated response to changes in the environment (7). Transcriptional factors either stimulate or inhibit the transcription initiation, depending on different scenarios (8,9). Among the best-characterized transcriptional factors are *E. coli lac* repressor (LacI) (10), *gal* repressor (GalR) (11), cAMP receptor protein (CRP) (12) and AraC protein of the L-arabinose operon (8). These transcriptional factors tightly bind to their recognition sites on the chromosome, restructure the recognition sites and regulate transcription from the nearby or distant promoters (8,13).

One common property of the sequence-specific DNA-binding proteins is to induce DNA structural change to their recognition sites, which includes DNA bending, twisting or untwisting, wrapping and other distortions (14,15). Among the DNA structural changes, protein-induced DNA bending has been extensively studied (16). For example, *E. coli* LacI, GalR, CRP and AraC all sharply bend their recognition sites (17,18). In fact, the protein-induced DNA bending was considered to be necessary for their biological functions (18). Nevertheless, other protein-induced DNA structural changes by sequence-specific DNA-binding proteins are rarely addressed (14). The main reason is that a reliable strategy has not been developed to carry out a systematic study for these protein-induced DNA structural changes. For instance, there were a few attempts to determine the linking number change (ΔLk) induced by LacI; however, the results were inconsistent (19,20). This controversy may be caused by the fact that each LacI causes a small ΔLk for each *lac* operator [much <1 (19)], it is experimentally not feasible to use a circular plasmid DNA containing one or two *lac* operators for these studies. In this case, it is required to construct a plasmid DNA template containing multiple tandem copies of the *lac* operator to determine LacI-induced ΔLk. As demonstrated by Sadler *et al.* (21), it was difficult to construct a stable plasmid containing

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many tandem copies of the *lac* O_1 operator, which made the determination of ΔLk challenging. In this article, we overcame this difficulty and constructed a series of circular plasmid DNA templates that carry many tandem copies of one DNA-binding site of a sequence-specific DNA-binding protein, such as LacI, GalR, CRP, AraC and λ O protein. Utilizing these unique plasmid DNA templates, we were able to simultaneously determine the protein-induced ΔLk and the DNA-binding constant for λ O protein and GalR. We also found that the protein-induced ΔLk is a unique property for each DNA-binding protein and does not correlate with the protein-induced DNA bending. In addition, protein-mediated DNA looping by AraC and LacI can introduce ΔLk to the DNA-binding sites.

MATERIALS AND METHODS

Purified proteins

Bacteriophage λ O protein was a generous gift of R. McMacken (Johns Hopkins University). *Escherichia coli* GalR was kindly provided by S. Adhya (National Institutes of Health). *Escherichia coli* CRP was a gift of J. C. Lee (The University of Texas Medical Branch, Galveston, TX, USA). *Escherichia coli* AraC protein is a gift of R. Schleif (Johns Hopkins University). *Escherichia coli* LacI was purified by the method of Chen and Matthews [(22); *E. coli* strains containing plasmids overexpressing these LacI mutants were kindly provided by K. S. Matthews at Rice University]. All restriction enzymes, T4 DNA ligase and calf intestinal alkaline phosphatase were purchased from New England Biolabs (Beverly, MA, USA). Recombinant Human topoisomerase I was obtained from TopoGen, Inc. (Port Orange, FL, USA).

Plasmid DNA templates

All plasmids are derived from low copy number plasmids pACYC184 or pACYCDuet-1. Plasmid pYZX43 was constructed by the insertion of a 24-bp synthetic DNA fragment, containing a BglII site, into the unique BspHI site of pACYC184. A 323-bp BamHI–BglII DNA fragment carrying phage λ sequences from 38 974 to 39 207, which includes the λ replication origin with four iterons (O protein-recognition sites), was cloned into the BamHI–BglII sites of pYZX43 to create pCB2. Next, the same 323-bp BamHI–BglII DNA fragment was inserted into the unique BglII site of pCB2 to produce pCB3 that contains two tandem copies of the λ replication origin. Plasmid pCB5 was made by the insertion of a 646-bp BamHI–BglII fragment, which contains two tandem copies of the λ replication origin, into the unique BglII site of pCB3. In this case, pCB5 contains four tandem copies of the λ replication origin, which has 16 O-recognition sites. Plasmid pCB21 was constructed by inserting a 145-bp DNA fragment, containing five tandem copies of iteron III of the λ replication origin, into the BamHI–BglII sites of pYZX43. The 145-bp fragment is the head-to-tail ligation product of a 29-bp synthetic BamHI–BglII DNA fragment (top strand: 5′-GATCCCTCAAATTGGGGGATTGCGCTGAA-3′; the underlined sequence is iteron III of λ replication origin). Since the O-recognition sequence iteron III is a 19-bp DNA sequence, the space between each iteron III is 10 bp. Plasmid pCB22 was produced by the insertion of the same 145-bp DNA fragment into the unique BglII site of pCB21 and contains 10 tandem copies of iteron III. Plasmid pCB28 was constructed by inserting a 290-bp BamHI–BglII fragment into the unique BglII site of pCB22. In this case, pCB28 contains 20 copies of head-to-tail iteron III multimers (20 O-recognition sites). Using similar approaches, the following plasmids have been made: pCB18, pCB32, pCB34 and pCB37, which contain 15, 20, 24 and 16 copies of head-to-tail iteron III multimers, respectively. The space between each iteron III is 5, 15, 20 and 25 bp for these plasmids, respectively (Table 1). These plasmids were used to examine the phase and distance effect on the O-protein-induced ΔLk or β.

Plasmids pCB42 and pCB46 were constructed by the insertion, respectively, of a 648 or 738 bp BamHI–BglII DNA fragment into the BamHI–BglII sites of pYZX43. Both plasmids contain 18 tandem copies of the *gal* O_E operator. The difference between these two plasmids is the space between each *gal* O_E operator, which is 20 and 25 bp, respectively (the head-to-tail lengths for each repeating sequence are 36 and 41 bp, respectively). Plasmids pCB51 and pCB55 were made by insertion of an 1152 or 1272 bp BamHI–BglII DNA fragment into the BamHI–BglII site of pYZX43, respectively. Both plasmids contain 24 tandem copies of the CRP binding site of the *lac P1* promoter. The difference between these two plasmids is also the space between each neighboring CRP sites, which is 24 and 29 bp, respectively. Plasmid pYZX12 was constructed by the insertion of a 22-bp DNA fragment, containing a nicking restriction endonuclease Nt.BbvC1 site, into the unique BstEII site of pACYCDuet-1. A 476-bp XhoI fragment carrying seven copies of the head-to-tail araI site (a head-to-tail ligation product of a 68 bp synthetic XhoI DNA fragment containing one araI site of the L-arabinose operon) was then cloned into the unique XhoI site of pYZX12 to yield plasmid pYZX36. Plasmid pYZX42 was constructed by the insertion of a 588 bp EcoRV–AvrII fragment, containing the seven tandem copies of the araI site of pYZX36, between the BsaAI and NheI sites of pYZX36. In this case, pYZX42 contains 14 araI sites.

Plasmid pYZX46 was constructed in a few steps. In the first step, a synthetic oligonucleotide (92 bp; annealing product of oligonucleotides FL313 and FL314) containing two *lac* O_1 operators was inserted into the unique BamHI site of pYZX12 to produce pYZX29, which contains four *lac* O_1 operators (pACYCDuet-1 has two *lac* O_1 operators). Using similar approaches, we have made the following plasmids: pYZX34 (six *lac* O_1 operators), pYZX37 (eight *lac* O_1 operators) and pYZX40 (10 *lac* O_1 operators). Next, pYZX40 was digested by BamHI and BglII to produce 567, 790 and 3041 bp fragments. The 567-bp

Table 1. Plasmids constructed in this study

Plasmid	DNA-binding site	Space between each DNA-binding site (bp)	Copy number of the DNA-binding site	The DNA-binding sequences (top strand)
pCB5	O binding site	4	16	λ DNA replication origin[a]
pCB18	Iteron III[b]	5	15	5′-ATCCCTCAAATTGGGGGAT-3′
pCB28	Iteron III[b]	10	20	5′-ATCCCTCAAATTGGGGGAT-3′
pCB32	Iteron III[b]	15	20	5′-ATCCCTCAAATTGGGGGAT-3′
pCB34	Iteron III[b]	20	24	5′-ATCCCTCAAATTGGGGGAT-3′
pCB37	Iteron III[b]	25	16	5′-ATCCCTCAAATTGGGGGAT-3′
pYZX36	*ara*I	30	7	5′-TAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGA-3′
pYZX42	*ara*I	30	14	5′-TAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGA-3′
pCB42	*gal* O_E	20	18	5′-GTGTAAACGATTCCAC-3′
pCB46	*gal* O_E	25	18	5′-GTGTAAACGATTCCAC-3′
pCB51	The CRP binding site[c]	20	24	5′-CAATTAATGTGAGTTAGCTCACTCATTA-3′
pCB55	The CRP binding site[c]	25	24	5′-CAATTAATGTGAGTTAGCTCACTCATTA-3′
pYZX46	*lac* O_1	25	19	5′-AATTGTGAGCGGATAACAATT-3′

[a]λ DNA origin contains four DNA-binding sites for λ O protein.
[b]Iteron III is a DNA-binding site of λ O protein from λ DNA replication origin (the third repeating sequence of λ DNA replication origin).
[c]The CRP binding site is derived from the CRP binding site of *E. coli lac* P_1 promoter.

fragment, which contains nine *lac* O_1 operators, was then inserted into the BamHI sites of pYZX40 to generate pYZX46 that contains 19 head-to-tail tandem copies of *lac* O_1 operators.

Plasmids pBend2-λO, pBend2-lacO1, pBend2-galO_E and pBend2-CRP were created by insertion, respectively, of a synthetic XbaI DNA fragment containing either iteron III of λ DNA replication origin or *lac* O_1 operator or *gal* O_E operator or the CRP binding site of the *lac* P_1 promoter into the unique XbaI site of pBend2 [plasmid pBend2 is a generous gift of S. Adhya (National Institutes of Health)].

All DNA elements were confirmed by DNA sequencing.

The determination of protein-induced DNA Δ*Lk* or unwinding angle (*β*)

In order to determine the protein-induced Δ*Lk*, we used plasmids containing multiple tandem copies of a DNA-binding site of λ O protein, GalR, CRP, AraC or LacI (Table 1). Two methods were used: the ligation method and the Topo I (topoisomerase I) method. Since these plasmids each contain one nicking enzyme recognition site (Nb.BsrDI or Nt.BbvCI), in the ligation method, we nicked these plasmids using one of the nicking restriction enzymes, Nb.BsrDI or Nt.BbvCI, and then titrated the nicked plasmid with one of the DNA-binding proteins, i.e. λ O protein, GalR, CRP, AraC or LacI. After 30 min incubation, the DNA templates were ligated by T4 DNA ligase. The linking number (Lk) of the ligated DNA products was determined with 1% agarose gel electrophoresis in the presence of chloroquine (23) and calculated from the gel images stained with ethidium bromide or SYBR Gold using KODAK 1D Image Analysis Software. In the Topo I method, the supercoiled plasmid DNA templates were titrated with one of the DNA-binding proteins and then relaxed by human topisomerase I. The relaxation reaction was stopped by phenol extraction. The linking number of the DNA templates was also determined with 1% agarose gel containing chloroquine as described above. The observed linking number change was calculated by $\Delta Lk_{obs} = Lk - Lk^{\circ}$, where Lk and Lk° are the Boltzmann centers of the topoisomers band in the presence of the DNA-binding protein and in its absence, respectively. If we define the apparent protein-induced DNA-unwinding angle (β_{obs}) equals to $360 \times \Delta Lk_{obs}$, i.e. $\beta_{obs} = 360 \times \Delta Lk_{obs}$, then the protein-induced DNA-unwinding angle (β) per binding site was determined by the following equation if we assume that no interaction exists among the bound DNA-binding protein and context-dependent differences in binding affinity are ignored (for proteins cooperatively binding to its DNA-binding sites, more complex equations have to be derived) and the DNA-binding density (r) is equal to β_{obs}/β (see below for its derivation):

$$\frac{\beta_{obs}}{C_f} = K(n\beta - \beta_{obs}) \quad (1)$$

where K is the DNA-binding constant, n is the binding site size (n is the copy number of the DNA-binding sites on the plasmids that can be determined by DNA sequencing) and C_f is the free concentration of the DNA-binding protein (for the low protein concentrations, the free protein concentration was estimated using the DNA-binding affinity of the protein. For the high-protein concentrations, C_f is approximated to the total ligand concentration C_T). This analysis should give us the protein-induced β and K simultaneously. The intrinsic Δ*Lk* per binding site can be calculated by

$$\Delta Lk = \frac{\beta}{360} \quad (2)$$

To derive Equation 1, we consider a binding reaction between a sequence-specific DNA-binding protein and n independent binding sites on a plasmid DNA template with an intrinsic binding constant of K. Each of the n sites has the same affinity for the DNA-binding protein as any other and the binding is non-cooperative.

The binding properties can be described by Scatchard Equation:

$$\frac{r}{C_f} = K(n - r) \quad (3)$$

where r is the binding density (the number of moles of the DNA-binding protein bound per mole of the DNA template) and C_f is the concentration of the DNA-binding protein in the free form. If the DNA-binding protein binds to each of its n binding sites to cause the unwinding of β degrees of the DNA template, the DNA-binding density r can be calculated by

$$r = \frac{\beta_{obs}}{\beta} \quad (4)$$

Substitution of Equation 4 into Equation 3 yields Equation 1.

The determination of the protein-induced DNA bending angle

The protein-induced DNA bending angles were determined using the circular permutation assay (24). Plasmids pBend2 derivatives containing a recognition sequence of a sequence-specific DNA-binding protein, such as λ O, GalR, CRP and LacI were digested by restriction enzymes MluI, BglII, NheI, SpeI, XhoI, DraI, EcoRV, NruI and BamHI to produce a set of fragments with identical length and base composition in which the position of the protein binding site is variable. The DNA fragments were labeled with ^{32}P at 5′-termini by T4 polynucleotide kinase in the presence of γ-^{32}P-ATP. The DNA–protein complexes were formed by addition of 1.0 to 3.0×10^{-9} M of the protein to a solution containing 1×10^{-10} M of ^{32}P-labeled DNA. After equilibration for 60 min at 22°C, the samples were loaded on a polyacrylamide gel to determine the mobility of DNA–protein complexes, which is dependent upon the position of the bound protein, with the lowest mobility present when the protein is bound to the center of the fragment. The bending angle α by which the DNA is bent from linearity was estimated by

$$\mathrm{Cos}\left(\frac{\alpha}{2}\right) = \frac{\mu_M}{\mu_E} \quad (5)$$

where μ_M and μ_E are the mobility of the complex with protein bound at the center and the end of DNA, respectively (25).

Electrophoretic mobility shift assay

Electrophoretic mobility shift assays (EMSA) were used to determine the apparent DNA-binding constant of λ O protein, CRP, GalR and LacI. DNA oligomers containing a DNA-binding site of one of the DNA-binding proteins (the EcoRV fragment of the pBend2 derivatives) were labeled with ^{32}P at 5′-termini by T4 polynucleotide kinase in the presence of γ-^{32}P-ATP. The DNA–protein complexes were formed by addition of appropriate amounts of the protein to a solution containing 0.1 nM of ^{32}P-labeled DNA in the 1 × DNA-binding buffer containing 20 mM Tris–HCl (pH 8.0), 200 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$ and 5% glycerol. After equilibration for 60 min at 22°C, the samples were loaded on an 8% native polyacrylamide gel in 0.5 × TBE buffer [0.045 M Tris–borate (pH 8.3) and 1 mM EDTA] to separate free and bound DNA. The gels were subsequently dried and visualized by autoradiography or quantified using a Fuji FLA 3000 image analyzer. The radioactivity of the free and bound DNA was determined and used to calculate the binding ratio (R), which is equal to the ratio of the radioactivity of the bound DNA divided by the sum of the radioactivity of the bound and free DNA. The apparent DNA-binding constant (K_{app}) was obtained by nonlinear-least-squares fitting the following equation using the program Scientist.

$$R = \frac{(a + x + 1/K_{app}) - \sqrt{(a + x + 1/K_{app})^2 - 4ax}}{2a} \quad (6)$$

where a and x represent the total DNA and the total protein concentration, respectively.

RESULTS

A strategy to study protein-induced DNA Δ*Lk* of circular plasmid DNA by sequence-specific DNA-binding proteins

Here, we successfully made a series of DNA plasmids containing many tandem copies of one DNA-binding site, such as *lac* O_1 operator, *gal* O_E operator, the CRP binding site of *lac P1* promoter, *araI* site of the L-arabinose operon and iteron III of phage λ DNA replication origin (a DNA-binding site of λ O protein). Our cloning strategy was to use a low-copy number plasmid pACYC184 or pACYCDuet-1 to minimize the recombination among the tandem copies of the DNA-binding sequences. In this case, plasmids carrying multiple copies of one DNA-binding site are stable in *E. coli* strains, such as DH5α and Top10. We also cloned one nicking endonuclease recognition site (either Nt.BbvCI or Nb.BsrDI) into these plasmids. The distance between the nicking endonuclease recognition site and the DNA-binding sites is ~0.8 or 1.8 kb (Figure 1) to minimize the interference between the specific binding of the DNA-binding proteins to the multiple DNA-binding sites and the ligation reaction for determining the protein-induced Δ*Lk* (see below for details). Figure 1 and Table 1 summarize the main properties of the plasmids.

As described in 'Materials and Methods' section, we employed two methods, a ligation method and a Topo I method to determine the protein-induced Δ*Lk* by sequence-specific DNA-binding proteins. Since Δ*Lk* of −1 is equivalent to an unwinding angle of 360° for a circular plasmid DNA template, we chose to use the unwinding angle (β) for our calculation, i.e. $\beta = 360 \times \Delta Lk$. If the concentration of the DNA template is sufficiently low ($<<K_d$) and the interaction between individual DNA-binding proteins is negligible, we should be able to simultaneously determine β or Δ*Lk* and the DNA-binding constant (K). Since the initial

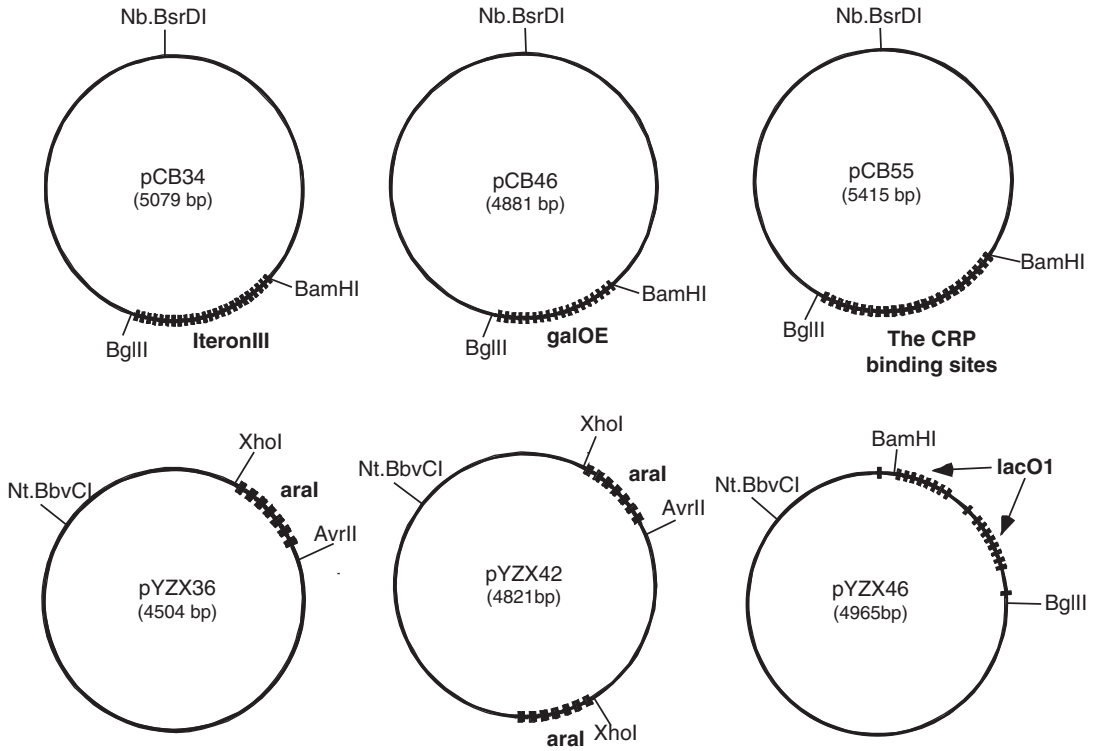

Figure 1. Plasmids used to determine protein-induced ΔLk by λ O protein, GalR, CRP, AraC and LacI. Plasmids pCB34, pCB46 and pCB55 were derived from pACYC184; plasmids pYZX36, pYZX42 and pYZX46 were derived from pACYCDuet-1. These plasmids were constructed as detailed under 'Materials and Methods' section. The restriction enzyme sites for BamHI, BglII, XhoI, AvrII, Nb.BsrDI and Nt.BbvCI are shown. The small closed rectangles represent the site specific recognition sequence for λ O protein (Iteron III), GalR (*gal* O_E), CRP, AraC (araI) and LacI (*lacO1*).

topological status of the DNA templates is quite different for these two methods, β or ΔLk determined by these two methods may also be different (see below for details). Our strategy is summarized in Supplementary Figure S1.

Simultaneous determination of protein-induced ΔLk or β and the DNA-binding constant of λ O protein and GalR

Due to availability, we first decided to determine λ O protein-induced ΔLk or β. λ O protein is the DNA replication initiator of bacteriophage λ and specifically binds to the four repeating sequences (iterons) of λ DNA replication origin (26). Upon λ O binding, the λ DNA replication origin is wrapped around O protein to form a nucleoprotein complex, the 'O-some' (6) and subsequently unwind the DNA sequence of λ DNA replication origin (27). In this case, λ O protein should induce a ΔLk to the DNA templates containing multiple copies of its binding sites. Figure 2A shows results of λ O protein titrating into a solution containing 0.0625 nM of the nicked plasmid pCB5 carrying four λ replication origins (16 λ O protein binding sites; the concentration of the λ O protein binding site is 1 nM). After the ligation reaction, the plasmid DNA samples were subjected to electrophoresis in a chloroquine–agarose gel to determine the linking number (Lk). In the absence of λ O protein, the DNA template is relaxed with a superhelical density, σ, of approximately zero. In the presence of the DNA intercalator chloroquine (0.5 μg/ml), this DNA migrated during agarose gel electrophoresis as if it contained a few (+) supercoils

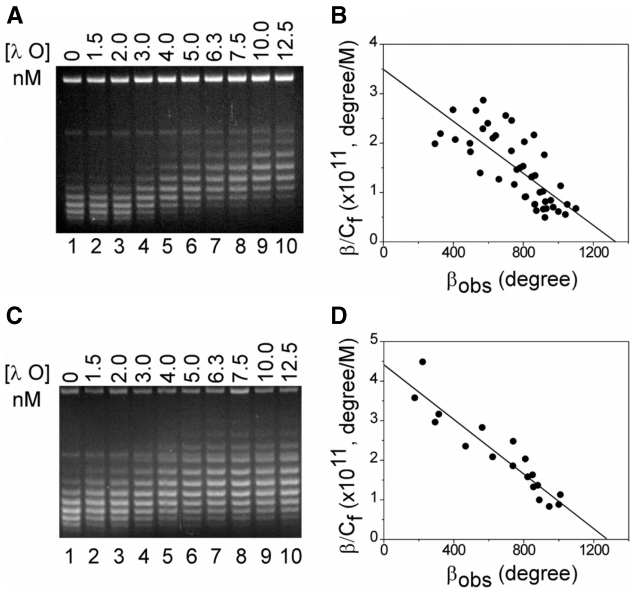

Figure 2. Simultaneous determination of λ O protein-induced DNA unwinding angle (β) or ΔLk and the DNA-binding constant (K). (**A**) and (**C**) λ O protein titration assays were performed as described under 'Materials and Methods' section using the ligation method (A) or the Topo I method (C). In addition to 0.0625 nM of plasmid pCB5, the reaction mixtures for the DNA samples applied to lanes 1–10 also contain 0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.3, 7.5, 10.0, 12.5 nM of λ O protein (as dimer), respectively. DNA topoisomers were resolved with electrophoresis in 1% agarose gel containing 0.5 μg/ml chloroquine. (**B**) and (**D**) Quantification analysis of the binding data from the λ O titration experiments. β and K were calculated according to Equation 1. ΔLk was calculated by Equation 2.

(Figure 2A, lane 1). Binding of λ O protein to the λ O binding sites on plasmid pCB5 introduced about 4 (−) supercoils into the DNA template (Figure 2A, lanes 1–10). The λ O-induced ΔLk or β required the presence of O binding sites in the DNA templates (Figure S2). When plasmid pYZX43, which is identical to pCB5 except that it does not contain λ O binding sites, was used in the ligation method, λ O protein was not able to induce a ΔLk or β to the plasmid (Figure S2A). The results in Figure 2 were used to fit Equation 1 to yield the λ O protein–induced β ($80.0° \pm 5.6°$) or ΔLk (-0.222 ± 0.016) and the DNA-binding constant [$3.0(\pm 1.5) \times 10^8\,M^{-1}$] (Figure 2B). The DNA-binding constant of λ O protein is almost identical to the one determined with EMSA assay (Figure 3). Similar results were also obtained using the Topo I assay (Figure 2C and D; Table 2).

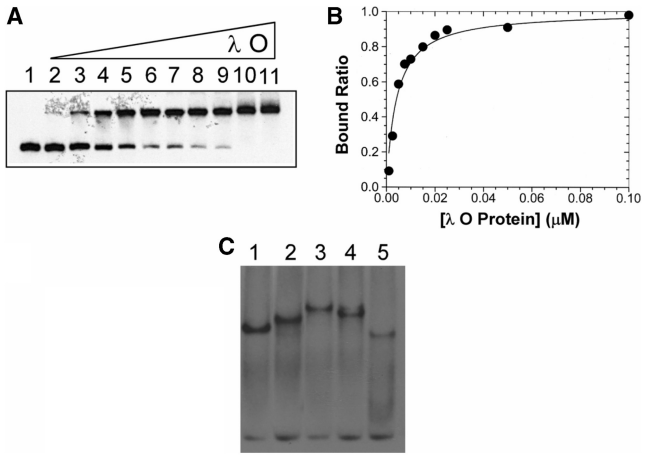

Figure 3. The Determination of the DNA-binding constant (K) and the protein-induced DNA bending angle for λ O protein. The EMSA assays and the DNA bending assays were performed as described under 'Materials and Methods' section. (**A**) Binding of λ O protein to the DNA oligomer containing the iteron III of coliphage λ DNA replication origin. A 145 bp ^{32}P-labeled EcoRV fragment of pBend2-Iteron III was incubated with increasing concentrations of λ O protein in 50 μl of 1× EMSA binding buffer containing 10 mM Tris–HCl (pH 7.4), 20 mM KCl, 0.5 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$ and 5% glycerol. The autoradiogram was shown. The radioactivities were quantified with a PhosphorImager. Lane 1 is free DNA fragment. In addition to the ^{32}P-labeled DNA fragment, the reaction mixtures for the DNA samples applied to lanes 2–11 also contains 0, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 50 and 100 nM of λ O protein (as dimer), respectively. (**B**) Quantification analysis of the binding data from the EMSA experiments. The bound ratio of DNA was plotted against the protein concentration. The curve was generated by fitting the data to Equation 6 as described under 'Materials and Methods' section to yield the DNA-binding constant of $2.5 \times 10^8\,M^{-1}$. (**C**) The DNA bending assay. After the binding of λ O protein to the permutated DNA fragments, an 8% polyacrylamide gel was used to separate the bound and free DNA fragments. The autoradiogram of ^{32}P-labeled DNA fragments was shown. Lanes 1–5 are the λ iteron III fragments produced by digestion of plasmid pBend2-Iteron III with restriction enzymes of MluI, NheI, EcoRV, SspI and BamHI, respectively. The DNA fragments in the bottom of the gels are free DNA, and those in the upper part are protein–DNA complexes.

We next varied the distance between the individual O binding sites (iteron III of λ replication origin) to examine whether the phase match or the distance between these DNA-binding sites affects the induced ΔLk or β. To our surprise, O protein-induced ΔLk or β is independent of the phase match and the distance between these DNA-binding sites (Table 2). Again, the DNA-binding constant was determined to be $\sim 2.0–3.0 \times 10^8\,M^{-1}$, which is consistent with the EMSA results (Figure 3) and the published results (2). However, some variations in the DNA-binding constants are observed in these assays.

We also determined GalR-induced ΔLk or β. GalR is a dimer and specifically binds to the O_E and O_I operators of *E. coli* galactose operon to inhibit transcription from two *gal* promoters *P1* and *P2* (28). As described in 'Materials and Methods' section, we made two plasmids pCB42 and pCB46 that carry 18 *gal* O_E operators (Figure 1 and Table 1). The difference of these two plasmids is the space between the neighboring O_E operators: pCB42, 20 bp and pCB46, 25 bp (the head-to-tail distance of the O_E operators are 36 and 41 bp, respectively). Since the O_E operator is a 16 bp DNA sequence, it is reasonable to assume that GalR binds to the neighboring O_E operators of pCB42 on the opposite side; in contrast, GalR binds to the neighboring O_E operators of pCB46 on the same side. In this case, we were able to examine whether the phase match affects the induced ΔLk or β. Our results are summarized in Figure 4 and Table 3. For the ligation method, the binding of GalR to both plasmids pCB42 and pCB46 induced about 3 (−) supercoils into the

Table 2. λ O protein-induced DNA-unwinding angle (β), ΔLk and DNA-binding constant (K)

Plasmid	The space between each DNA-binding site (bp)	Copy number	β (°)	ΔLk	K ($\times 10^8\,M^{-1}$)
pCB5[a]	4	16	80.0 ± 5.6	−0.222 ± 0.016	3.0 ± 1.5
pCB5[b]	4	16	79.1 ± 1.5	−0.220 ± 0.004	3.6 ± 0.5
pCB18[a]	5	15	79.4 ± 1.4	−0.221 ± 0.004	1.9 ± 0.4
pCB28[a]	10	20	80.0 ± 1.8	−0.222 ± 0.005	3.9 ± 0.7
pCB32[a]	15	20	80.8 ± 1.8	−0.224 ± 0.005	1.5 ± 0.3
pCB34[a]	20	24	80.0 ± 1.3	−0.222 ± 0.004	2.3 ± 0.3
pCB37[a]	25	16	79.5 ± 1.3	−0.221 ± 0.004	1.9 ± 0.4

[a]The protein-induced ΔLk, β and the DNA-binding constant was determined by the ligation method as described under 'Materials and Methods' section.
[b]The protein-induced ΔLk, β and the DNA-binding constant was determined by the Topo I method as described under 'Materials and Methods' section.

DNA templates (Figure 4A, lanes 1–10). Control experiments indicated that GalR-induced ΔLk or β was specific for the GalR–O_E protein–DNA interaction. GalR failed to induce DNA supercoiling when its inducer, D-galactose, was present (Figure 4B) or the plasmid template does not contain the gal operators (Figure S2B). These results were used to fit Equation 1 to yield the GalR-induced β ($60.9° \pm 1.5°$) or ΔLk (-0.169 ± 0.004) and the DNA-binding constant of $3.2(\pm 1.5) \times 10^8\,M^{-1}$ (Figure 4C and Table 3). Interestingly, there is no difference for the β or ΔLk values between these two plasmids, indicating that phase match does not influence GalR-induced ΔLk or β. Similar results were obtained by using the Topo I method for GalR-induced ΔLk or β (Table 3).

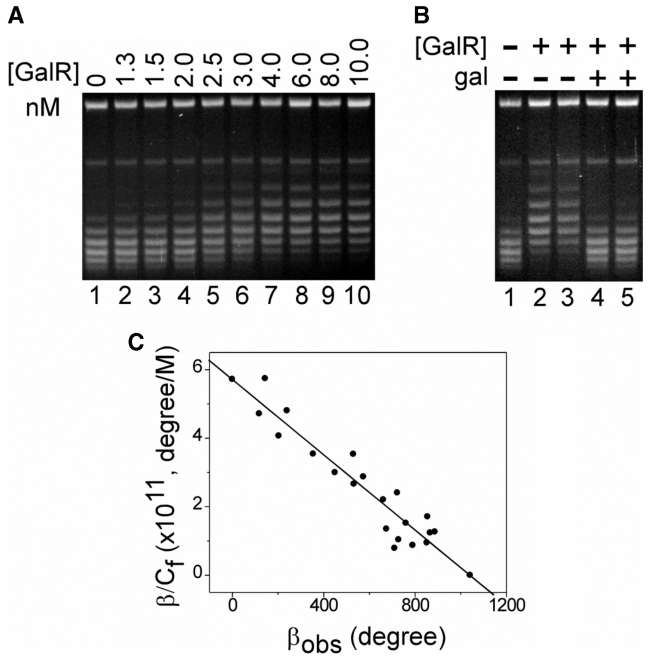

Figure 4. Simultaneous determination of GalR-induced DNA-unwinding angle (β) or ΔLk and the DNA-binding constant (K). GalR titration assays were performed as described in 'Materials and Methods' section using the ligation method. DNA topoisomers were resolved with electrophoresis in 1% agarose gel containing 0.5 μg/ml chloroquine. (**A**) The GalR titration experiment. In addition to 0.055 nM of plasmid pCB42, the reaction mixtures for the DNA samples applied to lanes 1–10 also contain 0, 1.3, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0 nM of GalR (as dimer), respectively. (**B**) GalR failed to unwind the DNA when D-galactose was present. The reaction mixture for the DNA sample applied to lane 1 contains neither GalR nor D-galactose. As indicated at the top of the image, the reaction mixtures for the DNA samples applied to lanes 2–4 contain GalR (lanes 2 and 4, 5 nM; lanes 3 and 5, 10 nM) and D-galactose (lanes 4 and 5, 1 mM). (**C**) Quantification analysis of the binding data from GalR titration experiments. β and K were determined according to Equation 1. ΔLk was calculated by Equation 2.

ΔLk or β induced by DNA looping proteins AraC and LacI

Since DNA loops are topological structures, we decided to examine whether protein-mediated DNA looping affects protein-induced ΔLk or β. For this purpose, we chose AraC and LacI, two well-characterized DNA-binding proteins that cause DNA looping *in vivo* and *in vitro* (29). In the absence of L-arabinose, AraC, a homodimer, binds *in trans* to I_1 and O_2 half-sites of the L-arabinose operon to form a DNA loop and therefore inhibits transcription from the P_{BAD} promoter (8). In the presence of L-arabinose, AraC also tightly binds in cis to the neighboring I_1 and I_2 half-sites; however, it does not form a DNA loop. In this case, AraC stimulates transcription from the P_{BAD} promoter (8). LacI, a homotetramer, forms DNA loops through specifically binding to the *lac* operators, such as O_1, O_2 and O_3, to negatively control gene transcription of *E. coli lac* operon (30). To study AraC-induced ΔLk or β, we constructed two plasmids pYZX36 and pYZX42 (Figure 1). pYZX36 and pYZX42 contain 7 and 14 head-to-tail *araI* sites, respectively. The 14 araI sites of plasmid pYZX42 are located in two different locations with seven araI sites for each location. The distance between these two groups of araI sites is 1.2 kb on one side and 2.7 kb on the other side. In this situation, AraC should be able to induce DNA looping between these two groups of araI sites (31). As expected, AraC did not induce significant DNA supercoiling for plasmid pYZX36. Nevertheless, AraC greatly supercoiled plasmid pYZX42, and the binding of AraC to araI sites on plasmid pYZX42 introduced about 5 (−) supercoils into the DNA template (compare lane 1 to lane 2 of Figure 5A). Interestingly, as the AraC concentration was increased, the plasmid DNA template became less supercoiled (Figure 5A). Our interpretation is that AraC-mediated DNA looping is the cause for AraC-induced ΔLk or β. Since we only used 1 nM of the araI site for the assay, a low concentration of AraC,

Table 3. GalR protein-induced DNA-unwinding angle (β), ΔLk and DNA-binding constant (K)

Plasmid	The space between the individual DNA-binding site (bp)	Copy number	β (°)	ΔLk	K ($\times 10^8\,M^{-1}$)
pCB42[a]	20	18	60.9 ± 1.5	−0.169 ± 0.004	4.4 ± 1.7
pCB42[b]	20	18	102.6 ± 0.9	−0.285 ± 0.003	7.2 ± 0.5
pCB46[a]	25	18	60.0 ± 0.9	−0.167 ± 0.003	3.8 ± 1.2
pCB46[b]	25	18	66.5 ± 3.8	−0.185 ± 0.011	7.7 ± 0.9

[a]The protein-induced ΔLk, β and the DNA-binding constant was determined by the ligation method as described under 'Materials and Methods' section.
[b]The protein-induced ΔLk, β and the DNA-binding constant was determined by the Topo I method as described under 'Materials and Methods' section.

e.g. 2.5 or 5 nM, should favor the binding of the protein *in trans* to form a DNA loop. In this scenario, AraC greatly supercoiled the DNA template. In contrast, a high concentration of AraC was in favor of the binding of the dimeric AraC to a single half site without forming a DNA loop, i.e. the DNA loop was titrated out. In this case, AraC should not significantly supercoil the DNA template. Our explanation was confirmed by the results shown in Figure 5B in which AraC failed to induce ΔLk or β when L-arabinose was present in the reaction mixtures (compare lanes 2 and 3 with lanes 4 and 5 of Figure 5B). These results suggest that AraC-mediated DNA looping is the reason for AraC-induced ΔLk or β under our experimental conditions. Because AraC-mediated DNA looping is a cooperative process, we cannot use Equation 1 to derive AraC-induced ΔLk or β and the DNA-binding constant. However, with an assumption of AraC binding to all 14 araI sites of pYZX42, we estimated the maximum ΔLk and β induced by AraC to be -0.333 ± 0.039 and $120 \pm 14°$, respectively (Table 4). Similar results were also obtained using the Topo I assay. The ΔLk and β induced by AraC may be different if a different plasmid containing multiple AraC binding sites is used.

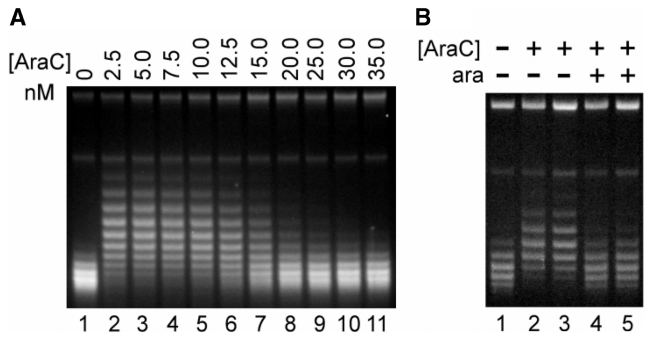

Figure 5. AraC-induced ΔLk. Experiments to determine AraC-induced ΔLk were performed as described under 'Materials and Methods' section. DNA topoisomers were resolved with electrophoresis in 1% agarose gel containing 0.5 μg/ml chloroquine. (**A**) The AraC titration experiment. In addition to 0.0714 nM of plasmid pYZX42, the reaction mixtures for the DNA samples applied to lanes 1–11 also contain 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0, 35.0 nM of AraC (as dimer), respectively. (**B**) AraC failed to induce ΔLk of pYZX42 when L-arabinose was present. The reaction mixture for the DNA sample applied to lane 1 contains neither AraC nor L-arabinose. As indicated at the top of the image, the reaction mixtures for the DNA samples applied to lanes 2–5 contain AraC (lanes 2 and 4, 3 nM; lanes 3 and 5, 6 nM) and L-arabinose (lanes 4 and 5, 1 mM).

We also made a few plasmids to study LacI-induced ΔLk or β. Among them is pYZX46 that carries 19 *lac* O_1 operators (Figure 1 and Table 1). The 19 *lac* O_1 operators are divided into two different locations with each location containing 9 or 10 *lac* O_1 operators. The distance between these two locations is 224 bp (Figure 1). According to previous results (32), LacI should induce a DNA loop between these two groups of *lac* O_1 operators. In this case, it should also induce DNA supercoiling. Figure 6A and B show results of the DNA supercoiling assay (the ligation method) by LacI in which the DNA concentration was kept sufficiently low to minimize the nonspecific DNA-binding. As expected, LacI induced 2–3 (−) supercoils into the DNA templates (compare lane 1 to lanes 2–4 of Figure 6A and B), which is consistent with the published results (20). Interestingly, although *lac* operators are required for LacI-induced ΔLk or β (Figure S2D), IPTG inhibited the LacI-induced DNA supercoiling only when the concentration of both LacI and *lac* O_1 operator was very low (compare lane 2 with lane 5 of Figure 6A). When LacI's concentration and/or *lac* O_1's concentration was increased, IPTG stimulated LacI-induced DNA supercoiling (compare lanes 6 and 7 with lane 2 of Figure 6A; also compare lanes 5–7 with lanes 2–4 of Figure 6B). We believe that the stimulation of LacI-induced DNA supercoiling by IPTG stems from the LacI-mediated DNA looping which has a different topology. As demonstrated before (20,32), LacI, even in the presence of IPTG, is still able to induce DNA looping. We demonstrated here that the topology of the DNA loops in the presence and absence of IPTG is different. We estimated the maximum ΔLk induced by LacI in the absence or presence of IPTG are, respectively, −0.08 (β, 29°) and −0.21 (β, 75.8°) (Table 4). We also

Table 4. Protein-induced DNA-unwinding angle (β), ΔLk, DNA bending angle (α) and DNA-binding constant (K)

Protein	β (°)	ΔLk (°)	α (°)	K (M^{-1})[a]
λ O	80.0 ± 5.6[b]	−0.222 ± 0.016	89 ± 2	$2.3 \pm 1.7 \times 10^8$
GalR	60.9 ± 1.5[b]	−0.169 ± 0.004	119 ± 4	$10.4 \pm 3.6 \times 10^8$
GalR + galactose	0[b]	0	N/A	N/A
CRP + cAMP	0[b]	0	89 ± 5	$2.8 \pm 1.1 \times 10^9$
AraC[d]	120.0 ± 14.0[b]	−0.333 ± 0.039	90	5×10^9
AraC + arabinose[d]	0[b]	0	90	2×10^{11}
LacI	29.0 ± 5.8[b]	−0.081 ± 0.016	73 ± 4	$1.2 \pm 0.4 \times 10^{10}$
LacI + IPTG	75.8 ± 6.3[b]	−0.211 ± 0.018	N/A	N/A
LacI	107.5 ± 5.5[c]	−0.299 ± 0.015	73 ± 4	$1.2 \pm 0.4 \times 10^{10}$
LacI + IPTG	90.7 ± 2.7[c]	−0.252 ± 0.008	N/A	N/A

[a]The DNA-binding constants (K) were determined by using EMSA as described under 'Materials and Methods' section. The values are the average of at least three independent determinations.
[b]The protein-induced β or ΔLk was determined by the ligation method as described under 'Materials and Methods' section.
[c]The protein-induced β or ΔLk was determined by the Topo I method as described under 'Materials and Methods' section.
[d]The DNA-bending angle of AraC in the absence or presence of L-arabinose was obtained from reference (36). The DNA-binding constant of AraC was obtained from reference (44).

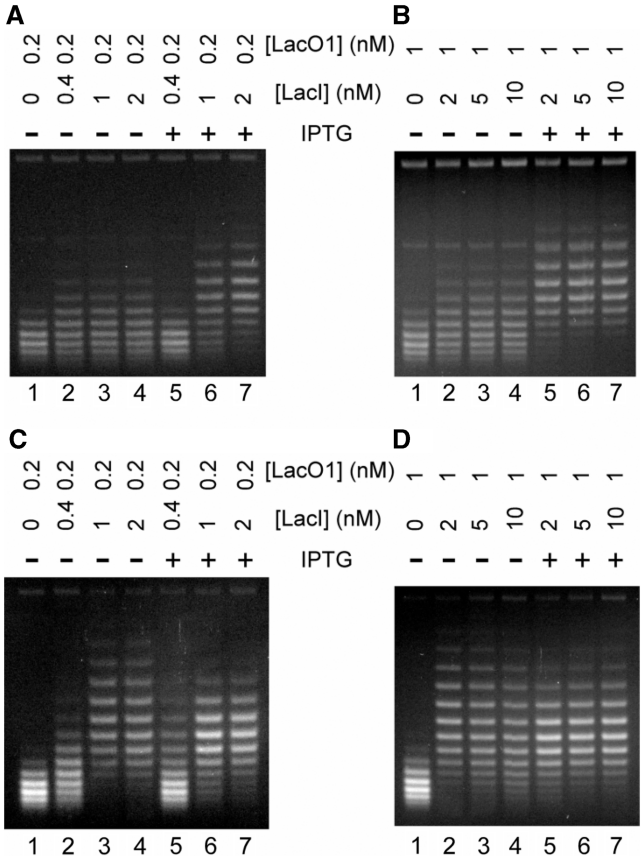

Figure 6. The LacI-induced ΔLk in the presence or absence of IPTG. The experiments to determine LacI-induced ΔLk were performed according to the ligation method (**A** and **B**) or the Topo I method (**C** and **D**) as described under 'Materials and Methods' section. Plasmid pYZX46 that contains 19 *lac* O_1 operators was used as the DNA template. The concentration of *lac* O_1 operator and LacI (as tetramer) are shown at the top of the images. The reaction mixtures for the DNA samples applied to lanes 5–7 also contain 1 mM of IPTG.

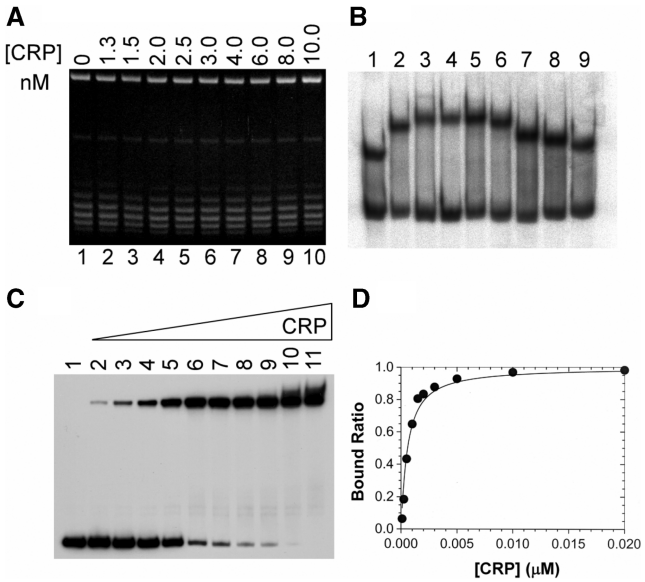

Figure 7. CRP-induced ΔLk, the DNA-bending angle (α), and the DNA-binding constant. (**A**) CRP titration experiments were performed according to the ligation method as described under 'Materials and Methods' section. In addition to 0.0417 nM of plasmid pCB51 and 20 μM of cAMP, the reaction mixtures for the DNA samples applied to lanes 1–10 also contain 0, 1.3, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0 nM of CRP (as dimer), respectively. DNA topoisomers were resolved with electrophoresis in 1% agarose gel containing 0.5 μg/ml chloroquine. (**B**) DNA bending assays were carried out as described under 'Materials and Methods' section. After the binding of CRP to the permutated DNA fragments in the presence of 20 μM of cAMP, an 8% polyacrylamide gel was used to separate the bound and free DNA fragments. The autoradiogram of ^{32}P-labeled DNA fragments was shown. Lanes 1–9 are the *lac* P_1 promoter's CRP fragments produced by digestion of plasmid pBend2-CRP with restriction enzymes of MluI, BglII, NheI, SpeI, XhoI, DraI, EcoRV, NruI and BamHI, respectively. The DNA fragments in the bottom of the gels are free DNA, and those in the upper part are protein-DNA complexes. (**C**) The EMSA assays were performed as described under 'Materials and Methods' section. A 159-bp ^{32}P-labeled EcoRV fragment of pBEND2-CRP was incubated with increasing concentrations of CRP in 50 μl of 1 × EMSA binding buffer containing 20 mM Tris–HCl (pH 8.0), 20 μM cAMP, 200 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 5 mM $MgCl_2$ and 5% glycerol. The autoradiogram was shown. The radioactivities were quantified with a PhosphorImager. Lane 1 is free DNA fragment. In addition to the ^{32}P-labeled DNA fragment, the reaction mixtures for the DNA samples applied to lanes 2–11 also contains 0.1, 0.25, 0.5, 1, 1.5, 2.0, 3.0, 5.0, 10.0 and 20.0 nM of CRP (as dimer), respectively. (**D**) Quantification analysis of the binding data from the EMSA experiments. The bound ratio of DNA was plotted against the protein concentration. The curve was generated by fitting the data to Equation 6 as described under 'Materials and Methods' section to yield the DNA-binding constant of $2.1 \times 10^9\,M^{-1}$.

performed DNA supercoiling assay using the Topo I method. Intriguingly, LacI induced 6 to 7 (−) supercoils into the DNA templates (compare lane 1 with lanes 3 and 4 of Figure 6C), which is 4 more (−) supercoils than those in the ligation method. The induced ΔLk or β was determined to be −0.30 or 107.5°, which is significantly greater than those calculated from the ligation method (Table 4). These results suggest that LacI may 'keep' some superhelical energy in the LacI–DNA loop complex.

ΔLk or β induced by CRP

Previously, it was shown that *E. coli* cAMP receptor protein (CRP) may cause a small ΔLk upon binding to CRP binding sites (33,34). We therefore decided to study CRP-induced ΔLk or β. Two plasmids pCB51 and pCB55, each containing 24 CRP binding sites, were used for this study (Table 1). As described in 'Materials and Methods' section, the difference between these two plasmids is the head-to-tail distance of the neighboring CRP binding sites. Figure 7A shows results of the ligation assay of CRP titrating into a solution containing 0.0417 nM of plasmid pCB51 (the total concentration of the CRP binding site is 1 nM). To our surprise, CRP in the presence of cAMP did not induce DNA supercoiling under our experimental conditions. Control experiments demonstrated that CRP bound to and bent the CRP binding site (Figure 7B and C). The DNA-binding constant was determined to be $2.8 \pm 1.1 \times 10^9\,M^{-1}$ (Figure 7D). These results indicated that CRP-induced ΔLk or β is close to 0 (Table 4). Similar results were also obtained for plasmid pCB55 and for both plasmids using the Topo I method.

Protein-induced ΔLk or β does not correlate with protein-induced DNA bending by sequence-specific DNA-binding proteins

So far, all sequence-specific DNA-binding proteins used here sharply bend the DNA-binding sequences (Table 4). Since DNA bending is a geometric property which may alter DNA supercoiling (35), we decided to examine whether there is a correlation between the protein-induced DNA bending and ΔLk or β. Our results in Table 4 showed that the protein-induced ΔLk or β does not correlate with the protein-induced DNA bending. For instance, λ O protein bends each of the iterons ~90°, and the ΔLk and β were determined to be −0.22 and 80°, respectively. In contrast, CRP bends its binding site ~90° as well; it does not have an induced ΔLk or β. Although AraC bends its binding site ~90° in the presence or absence of L-arabinose (36), AraC-induced ΔLk or β is exclusively dependent on AraC-mediated DNA looping (Figure 5); in the presence of L-arabinose, it did not have an induced ΔLk or β (Table 4 and Figure 5). These results suggest that there is no relationship between the protein-induced ΔLk or β and the DNA bending. Our results also showed that there is no correlation between the DNA-binding constant and the protein-induced ΔLk or β (Table 4).

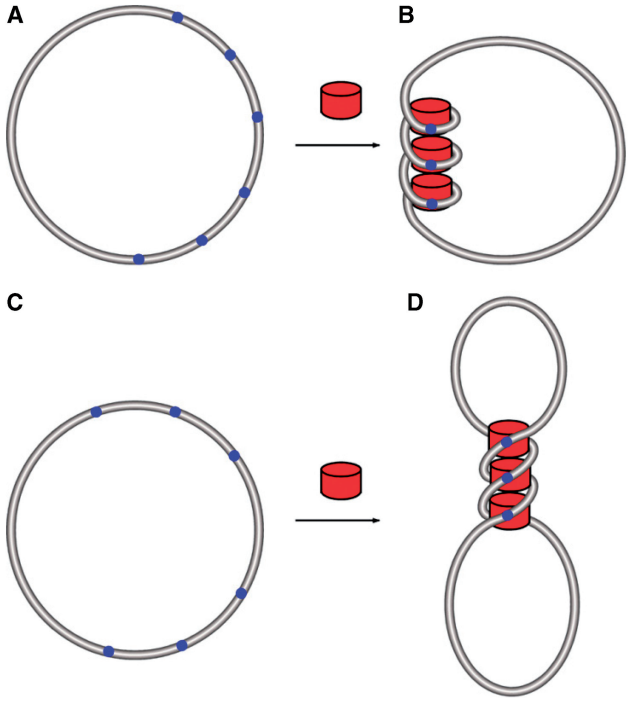

Figure 8. Proposed models to explain the protein-induced ΔLk by sequence-specific DNA-binding proteins. Models (**A** to **B**) show that certain site-specific DNA-wrapping proteins, such as λ O protein, bind and wrap the DNA recognition sites to induce the ΔLk of the DNA template. Models (**C** to **D**) demonstrate that DNA-looping proteins, such as AraC and LacI, form a topological nucleoprotein complex and therefore induce the ΔLk of the DNA template. Blue circle and red cylinder, respectively, represent the DNA recognition sequence for a site-specific DNA-binding protein and the site-specific DNA protein.

DISCUSSION

White *et al.* (14,37) previously pointed out that closed circular DNA can be used as a probe for protein-induced structural changes. The primary quantity that can be experimentally determined is ΔLk. We showed here that it is feasible to simultaneously derive ΔLk and the DNA-binding constant from a simple titration experiment (Figures 2 and 4). Two proteins, λ O protein and GalR were chosen for these determinations. Both DNA-binding proteins bind to their recognition sites with moderate binding affinity (2,38) and, more importantly, without cooperativity (39). In this case, Equation 1 can be used to calculate ΔLk or β and the DNA-binding constant from experimental results shown in Figures 2 and 4. The DNA-binding constants of λ O protein and GalR were determined to be $\sim 2.6 \times 10^8\,M^{-1}$ (Table 2) and $\sim 5.8 \times 10^8\,M^{-1}$ (Table 3), respectively. These results are consistent with our EMSA results (Table 4) and the published results (2,38). These results further suggest that these two proteins bind to their recognition site non-cooperatively. Interestingly, the protein-induced ΔLk by both λ O protein (~−0.22) and GalR (~−0.18) is independent of the distance and phase match between each neighboring binding site (Tables 2 and 3), suggesting that the protein-induced ΔLk is a unique property for these sequence-specific DNA-binding proteins. White *et al.* also showed that ΔLk can be described by two geometrical terms: the surface linking number (SLk) and the winding number (ϕ(14,37)). In this case, $\Delta Lk = \Delta SLk + \Delta\phi$. For λ O protein binding to its recognition site, i.e. λ DNA replication origin, we believe that ΔLk mainly results from the wrapping of the DNA around λ O protein (Figure 8A and B), which forms a unique nucleoprotein structure, the O-some (6). Our unpublished atomic force microscope results strongly support this interpretation. For GalR binding to its recognition site, i.e. the O_E operator, we also believe that DNA wrapping is the main cause for the induced ΔLk, although further studies are needed to support this interpretation.

As shown in Figures 5 and 6, the protein-mediated DNA looping by AraC or LacI can supercoil the DNA-binding sites. For AraC, the DNA looping is exclusively responsible for the induced DNA supercoiling since AraC in the presence of L-arabinose cannot supercoil the DNA-binding sites (Figure 5B). Intriguingly, LacI-induced DNA supercoiling is dependent on the initial topological status of the DNA template in the protein-induced DNA supercoiling assays (Figure 6 and Table 4). The LacI-induced ΔLk in the Topo I assay (the initial σ of the DNA template is ~−0.06) is much greater than the ΔLk in the ligation assay (the initial σ of the DNA template is near 0). These results suggest that LacI is able to keep superhelical energy within the LacI–O_1 nucleoprotein complexes in a thermodynamically stable status (Supplementary Figure S3). Models in Figure 8C and D present a possible case to explain how the DNA looping proteins bring the two groups of the DNA-

binding sites together to fold into a topological nucleoprotein complex. It is feasible to form a well-defined nucleoprotein complex, including the topological folding of DNA and the DNA regulatory proteins, which allow multiple protein–protein interactions *in vivo*. These nucleoprotein complexes may also be able to serve as DNA supercoiling barriers or dividers (23) to block the localized supercoiling diffusion to assist DNA transactions, such as replication, transcription and recombination.

In this article, we also demonstrated that CRP did not induce a ΔLk upon binding to its recognition site although CRP bent the site sharply [Figure 7B; (17)]. According to the crystal structure of the CRP–DNA complexes (40), CRP wraps around the DNA-binding site, which should result in a surface linking number change (ΔSLk). However, it is possible that this ΔSLk is compensated by changes in the local DNA winding in the CRP binding sites (the ΔSLk-$\Delta\phi$ or twist-writhe compensation). In this situation, the DNA helical axis lies in a plane and the overall ΔLk is equal to zero. This explanation resembles the interpretation of the so-called 'linking number paradox' for the topological changes in a minchromosome (15). It has been known for a long time that 146 bp of DNA wrap around the histone octamer core 1.8 turns in a left-handed superhelix. In this case, $\Delta SLk = -1.8$. Initially, it was mis-expected that $\Delta Lk = \Delta SLk$, which resulted in the 'linking number paradox' in literature (14,15). As mentioned above, $\Delta Lk = \Delta SLk + \Delta\phi$, where $\Delta\phi = -0.8$ and significantly compensated the ΔSLk to yield $\Delta Lk = -1$ that is corresponding to ~ -1 per nucleosome in the topoisomerase relaxation assays (41). Another example of the ΔSLk-$\Delta\phi$ compensation is the binding of the TATA box binding protein (TBP) to its recognition site which resulted in very little negative supercoiling in the topoisomerase relaxation assay (42). The crystal structures of the TBP–DNA complexes showed that the negative $\Delta\phi$ from DNA unwinding is canceled by the positive ΔSLk gained from 'wrapping' TBP around DNA (42,43).

In summary, we have constructed a series of DNA plasmids that carry many tandem copies of a DNA-binding site for sequence-specific DNA-binding proteins, such as λ O protein, GalR, CRP, AraC and LacI. Using these plasmid DNA templates, we determined the protein-induced ΔLk and demonstrated that the protein-induced ΔLk is an intrinsic property of these DNA-binding proteins. We also demonstrated that the protein-mediated DNA looping by AraC and LacI can introduce a ΔLk to the DNA-binding sites. Moreover, our results showed that the protein-induced ΔLk does not correlate with the protein-induced DNA bending and the DNA-binding constant.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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