GATA-1 Bends DNA in a Site-Independent Fashion

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Running Title: GATA-1 Bends DNA by 24°

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The DNA binding domain of GATA-1 consists of two adjacent homologous zinc fingers, of which only the C-terminal finger binds DNA independently. Solution structure studies have shown that the DNA is bent by about 15° in the complex formed with the single C-terminal finger of GATA-1. The N-terminal finger stabilizes DNA binding at some sites. To determine whether it contributes to DNA bending, we have performed circular permutation DNA bending experiments with a variety of DNA binding sites recognized by GATA-1. Using a series of full-length GATA-1, double zinc finger and single C-terminal finger constructs we show that GATA-1 bends DNA by about 24°, irrespective of the DNA binding site. We propose that the N- and C-terminal fingers of GATA-1 adopt different orientations when bound to different cognate DNA sites. Furthermore, we characterize circular permutation bending artifacts arising from the reduced gel mobility of the protein-DNA complexes.

DNA binding zinc fingers of the form CXXC-X_{17}-CNAC characterize the GATA family of transcription factors. GATA-1, the original member of the family, possesses two such zinc fingers. The C-terminal finger, accompanied by a basic linker region, is both necessary and sufficient for binding to the GATA recognition sequence, WGATAR (1-6). Even though the N-terminal finger does not bind DNA independently, it does stabilize GATA-1 interactions with some sites crucial for gene expression (7, 8, 9,
10, 11). These high affinity DNA sites are usually characterized by double GATA motifs, albeit arranged in diverse orientations and spacing. Since GATA-1 binds as a monomer to such sites, it has been proposed that both the N- and C-terminal fingers are involved in DNA recognition. Among these sites is an overlapping palindromic GATA sequence (ATCTGATA, referred to as GATApal), that is necessary for the activity of at least three vertebrate hematopoietic GATA-1 promoters, and requires both zinc fingers for high affinity interaction (11). Similarly, both zinc fingers of GATA-1 are requisite for the interaction with the double AGATA sites of the γ-globin promoter (7). In the accompanying manuscript we show that both the N- and C-terminal fingers of GATA-1 are necessary for the high affinity recognition of the overlapping AGATA sites of the ε-globin silencer (12). We also present similar results for an alternate binding site that contains a GATC consensus sequence.

Solution NMR studies of the complex formed between the C-terminal finger of GATA-1 and its cognate DNA sequence indicate that the DNA is bent by an overall angle of about 15° (3). This kink probably results from the insertion of the C-terminal basic residues required for DNA binding (6) into the minor groove. Unlike the single C-terminal zinc finger, it has been
proposed that the full length chicken GATA-1 introduces a bend of the order of 64° when bound to the chicken GATA-1 promoter (13), suggesting a possible role for the N-terminal finger in the overall DNA bend induced by the full length protein. Indeed, the double finger peptide of GATA-1 interacts with DNA probes containing double sites to yield fast and slow migrating 1:1 complexes. Based on a mutational analysis it has been proposed that the slow migrating complex is indicative of binding through only the C-terminal finger (11). The migration anomalies observed may therefore be due to the introduction of bends into the DNA target by the binding of the N-terminal finger. In order to evaluate the contribution of the N-terminal finger to DNA bending, we have performed gel mobility-shift assays using circular permuted probes containing different double and single GATA binding motifs. Using various GATA-1 constructs we show that the C-terminal finger is the sole contributor to DNA bending and that the bend angle previously reported to be induced by full length GATA-1 was overestimated. Because the bend angle is identical among double GATA sites separated by 0 to 9 base pairs, we propose that the N- and C-terminal fingers of GATA-1 necessarily adopt different relative orientations when bound to distinct cognate double GATA sites.
Materials and Methods

Constructions.

Bending vectors. Oligonucleotides were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer and purified by denaturing PAGE. The EcoRV (GATATC) and BglII (AGATCT) restriction sites on pBEND5 (14) were modified into SfcI (CTCGAG) and BsrI (ACCAGT) sites, respectively, to remove potential GATA binding sites. Furthermore, the central Sall (GTCGAC) cloning site was modified into an AatII (GACGTC) site. The modified pBEND5, pB5RG, was prepared sequentially via pB5R. pB5R was prepared by insertion of the modified dsDNA containing the circularly permuted restriction sites into pBEND5 digested with EcoRI and XbaI; pB5RG was prepared by insertion of a similar dsDNA into pB5R digested with XbaI and HindIII (Figure 1). Bending vectors containing various double and single GATA binding sites (Table 2) were prepared in a similar fashion. dsDNA oligomers spanning the XbaI and HindIII sites of pB5RG containing the binding sites were inserted into pB5R digested with XbaI and HindIII. Each of the bending vector constructs was verified by DNA sequencing (dRhodamine terminator cycle sequencing followed by analysis on an ABI Prism 310 Genetic Analyzer).
GATA-1 expression vectors. The PCR primers used for amplification of chicken (2) and human (15) GATA-1 cDNAs are shown in Table 1. The PCR products were cleaved with the appropriate restriction enzymes, gel purified and cloned into the expression vector. Maltose binding fusion proteins were constructed by insertion of the digested PCR product into pMAL-c2x (New England Biolabs) restricted with EcoRI and SalI. Untagged DNA binding domain clones were constructed by insertion into pET-11a (Novagen) digested with NdeI and BamHI. Each vector (Table 1) was verified by DNA sequencing.

Expression and purification of GATA-1 proteins.

GST chicken GATA-1. Full-length chicken GATA-1 with an N-terminal glutathione-S-transferase (GST) fusion was prepared as described (16).

MBP GATA-1 fusion proteins. The maltose binding protein (MBP) N-terminal fusions to human GATA-1 DF, chicken GATA-1 DF and chicken GATA-1 CF were expressed in TB1 E. coli. The cells were grown at 37°C in rich medium with glucose containing 100 µg/mL of ampicillin and 50 µM Zn(OAc)$_2$ and induced with 0.5 mM IPTG for 4 hours. Approximately 30 g of cells (from a 4 L culture) were lysed in 80 mL of 20 mM Tris (pH = 7.4), 1
mM EDTA, 0.5 mM PMSF and 1 µM Pepstatin A by sonication. The lysates were clarified by centrifugation and loaded on a Q-Sepharose® Fast Flow column (Pharmacia) which had been equilibrated with the same buffer. The flow through and rinse were combined and loaded on an S-Sepharose® Fast Flow column (Pharmacia). A 0.0 to 1.0 M NaCl gradient in 20 mM Tris (pH = 7.4), 1 mM EDTA and 0.5 mM PMSF was used to elute the MBP GATA-1 protein. GATA-1 protein activity was detected by EMSA³; the fractions were pooled, adjusted to 10% glycerol (v/v) and stored at -80°C.

GATA-1 and GATA-1 zinc finger peptides. The double zinc finger domain of chicken GATA-1 was expressed in BL21(DE3) E. coli in LB broth containing 100 µg/mL of ampicillin and 50 µM Zn(OAc)₂. Expression and purification was carried out as described above. Peptides corresponding to the human GATA-1 double finger and chicken GATA-1 C-terminal single finger were prepared as previously described (6, 11). Full-length GATA-1 nuclear extracts were made by standard procedures from adult chicken erythrocytes (17).

DNA Bending experiments.

Preparation of the bending probes. Radioactively labeled, circularly permuted probes were prepared by PCR amplification of the EcoRI to
HindIII portion of the pB5RG vector and restriction enzyme treatment as described (18). PCR was carried out using CCCGGGCTGCAGGAATTCACG and GACGGTATGCATAAGCTTGGA as forward and reverse primers, respectively. Restriction digests to yield the circularly permuted products were performed with BsrI, Nhel, Clal, SpeI, Drai, MspI, NruI, KpnI, HinfI and BamHI. The DNA probes were gel purified on 5% native acrylamide gels and adjusted to the same concentration based on their specific activity.

GATA-1 DNA bending experiments. Each reaction (10 µl of 50 mM Tris (pH = 7.4), 3 mM MgCl₂, 1 mM CaCl₂, 1 mM DTT, 100 ng BSA, 0.125% Triton X-100 and 4% Ficoll,) contained 20 – 40 ng of labeled DNA, 500 ng poly(dI·dC), 500 ng of a cold pB5RG competitor containing no GATA sites prepared by PCR in addition to 2 – 4 µL of the GATA-1 protein preparation. The reactions were electrophoresed on polyacrylamide gels at 13 V/cm in 10 mM Tris base, 10 mM Hepes and 1 mM EDTA at room temperature. 10.2%, 7.8%, 6.0% and 4.2% gels were electrophoresed for 14, 6, 3 and 1.5 hours, respectively. The gels were fixed in 10% acetic acid for 30 minutes, dried and autoradiographed.

Competition experiments demonstrated that the complexes formed were specific GATA-1 and DNA complexes. Bending experiments with probes based on the bent pB5RG-A5 and pB5RG-A55 were carried out in a
similar fashion using probes derived from pB5RG-G3 as a linear reference. The reactions contained approximately equal quantities of either the bent or linear probes.

Calculation of the DNA bending parameters. Gels were analyzed on a PhosphorImager (Molecular Dynamics) using ImageQuant 4.1. The mobilities of the protein-DNA complexes (or bent DNA) were normalized to the mobility of the free (or linear) DNA ($R_{\text{bound}}/R_{\text{free}}$) and fitted to a second order polynomial of $D/L$ to determine $a$, $b$ and $c$ (SigmaPlot 5.0, SPSS Inc.):

$$R_{\text{bound}}/R_{\text{free}} = a(D/L)^2 - b(D/L) + c$$

where $L$ represents the probe’s length and $D$ the distance of the GATA binding site from the 5’ end of the DNA molecule (19). The angle $\theta$, obtained from:

$$\cos(\theta) = (a/2c) - 1$$

$$\cos(\theta) = (-b/2c) - 1$$

yielded the bending angle $\alpha = 180^\circ - \theta$ as described (19). Excellent quadratic fits were obtained in all cases studied and the two values of $\theta$
for each fit were identical, within the precision of the method. Control bending experiments were carried out with a GATA-palindrome site fused to the A5 A-tract (Table 1) which has an intrinsic bend of 20° (Figure 3B):

AGAAGTCCA\textsc{AtCTGATA}AGACTTGGCCACG\textsc{AAAAACGGC\textsc{AAAAACGGCA\textsc{AAAAACGGTCGAGAC}}}

A visual inspection of the $R_{\text{bound}}/R_{\text{free}}$ data shows that GATA-1 double finger binding does not bend DNA significantly. A fit to the quadratic equation leads to an angle of $10 \pm 3°$, representing a lower limit angle that can be determined in this fashion. The bend introduced by the GATA-1 double finger binding is presumably out of phase with that of the A5 A-tract, resulting in an overall decrease of the bending angle.
RESULTS

**GATA-1 bends DNA in a site independent manner.** Gel mobility-shift assays using circularly permuted probes containing varying GATA binding motifs (Table 2) were carried out to determine the bend angle introduced by both chicken and human double zinc finger peptides. The GATA binding sites evaluated consisted of a series of double sites separated by 0 (GATApal (G2)), 1 (ε-globin silencer (εS) and GATC consensus site (GC)), 4 (GATA-1 promoter (G3)) or 9 (γ-globin promoter (γP)) base pairs, allowing us to evaluate how the combination of the two zinc fingers may contribute to the overall bend. It has already been shown that both the zinc fingers of GATA-1 interact with DNA when bound to the G2 and γP sites (7, 11). In the accompanying manuscript, we show that the same holds true for the εS and GC binding sites (12). In addition to these double GATA sites, two single GATA binding sites were analyzed in order to evaluate the contribution of the C-terminal finger to the overall DNA bend. One site (G1) represents the doubly mutated mouse GATA-1 promoter site, whereas the other (G0) is the consensus site used in obtaining the solution structure of the DNA complex formed with the C-terminal finger (6). Circular permutation bending experiments carried out with the human and chicken double finger peptides show, within the experimental precision of
the method, that the DNA bend induced by 1:1 complex formation is
independent of the GATA binding site. The average bend angle of $24 \pm 3^\circ$
obtained for these complexes is identical to the average bending angle
obtained with the chicken C-terminal finger peptide (Table 3). These data
demonstrate that GATA-1 bends DNA in a site independent manner and that
this bend arises solely from the binding of the C-terminal finger of GATA-
1 to the DNA (Table 3, sites G1 and G0).

**GATA-1 bends DNA by 24°.** The bend angle of 24° is significantly
different from the value of $64^\circ$ published for the full-length chicken
GATA-1 (13), and we have just shown that this difference cannot be
ascribed to the contribution of the N-terminal finger. To explain this
difference we performed a series of circular permutation mobility shift
assays with full-length chicken GATA-1 obtained from chicken
erythrocytes. Data obtained for the 1:1 complex formed with sites from
the mouse GATA-1 promoter (Table 3, site G3, essentially identical to the
chicken GATA-1 promoter used in (13)) lead to an angle of $59 \pm 4^\circ$ (Figure
2), a value identical to that previously published. As in the case of the
double and single C-terminal finger peptides, the angle was site
independent. Circular permutation experiments were also carried out with
an MBP\textsuperscript{2} fusion to the human double finger. As in the case of the double finger peptide, the bending angle obtained did not depend on the binding site. Unlike the peptide, however, an average bending angle of 83 ± 3° is noted (Table 3). Similar experiments carried out with a bacterially expressed GST\textsuperscript{1} fusion to the full-length chicken GATA-1 lead to a larger bending angle vis-à-vis the chicken GATA-1 (i.e. 104° versus 63°; Table 3). As neither the GST\textsuperscript{1} nor the MBP\textsuperscript{2} domains interact with DNA, these data indicate that the 63° bend observed with GATA-1 includes a contribution resulting from the decreased migration of the 1:1 complex. Indeed, a plot of the bending angle (α) as a function of the relative mobility of the complex (R\textsubscript{bound}/R\textsubscript{free}) suggests a direct relation between these parameters (Figure 3A).

In the reptation model describing DNA migration through a gel it is assumed that the presence of a single intrinsic bend imposes a large barrier to the motion of the DNA chain. Furthermore, it is assumed that the elastic force constant, B\textsubscript{eff}, describing the ‘interaction’ of the DNA chain with the acrylamide pores does not change within a particular set of probes (20). Measuring the bending angle as a function of the acrylamide concentration readily tests this assumption, which is critical to the
derivation of the quadratic equation relating mobility to bending. Bending of the mouse GATA-1 promoter (G3) by the double finger peptide of chicken GATA-1 leads to bending angles that are slightly dependent on the acrylamide concentration in a manner similar to the A-tracts used as controls (Figure 3B). This slight variation in the angle is therefore an intrinsic DNA property. However, both the full-length GATA-1 and the MBP-chicken double finger lead to bending angles that show a marked dependence on the acrylamide concentration (Figure 3B). In these cases, the elastic force constant of the complex varies as a function of the circularly permuted probe, leading to an overestimation of the true bend angle. Therefore, together with the observation that the double and single C-terminal zinc finger peptides lead to identical bending angles, we conclude that GATA-1 bends DNA by 24° in a site independent manner.
DISCUSSION

**GATA-1 bends DNA in a site independent manner.** We have shown that the GATA-1 proteins bend DNA by 24° in a site independent manner and that the C-terminal finger is sufficient to account for this bend. The NMR solution structure of the C-terminal finger peptide bound to G0 DNA shows a kink of about 15° in the direction of the major groove. It was proposed that the C-terminal basic arm required for binding (5, 6), is solely responsible for this bend (3). The site-independence of the induced angle shown here by circular permutation analysis confirms this hypothesis. The N-terminal finger of GATA-1 does not possess a basic arm and the bending angle induced by GATA-1 is not affected by the presence of N-finger binding sites. As both N- and C-terminal fingers simultaneously bind to a variety of double GATA sites (Table 2), any finger-finger interactions are necessarily constrained by their relative orientations and distance as little DNA bending is induced by either zinc finger.

DNA bending angles determined by circular permutation analysis represent a sum of the contributions of the true bend and the migration
anomaly produced by the structure of the protein-DNA complex. Indeed, it has been documented that circular permutation analysis usually overestimates the bend angle induced by DNA binding proteins (21, 22). For example, bending angles of 81° were obtained with this method for the thyroid hormone and retinoid X receptor dimers (TR/ TR and TR/ RXR) (23). A large contribution to this bend must be due to anomalous migration as phasing analysis leads to induced bends of about 10° (23). Similar conclusions have been reached in a study of the GCN4-DNA complex, where it was shown that the full-length GCN4 leads to an anomalous circular permutation analysis due to its size (24).

In a comparative study we have shown that the non-bending migration anomaly, due in part to ‘trailing’ portions of the protein, contributes significantly to the apparent bending angle in the case of the full-length GATA-1, the MBP and GST fusion proteins. (Figure 3A). Because their apparent bending angles vary significantly as a function of the acrylamide concentration, the angles consequently are not a reflection of the true bend induced.
Implied properties of GATA-1. In the accompanying manuscript we demonstrate that the N- and C-terminal zinc fingers of GATA-1 influence one another in DNA binding (12). This change is most likely the result of an intramolecular interaction between the GATA fingers and (or) linkers. Indeed, it has been shown that GATA-1 dimerizes with low affinity on DNA. This occurs through an association between the N- and C-terminal zinc fingers of separate molecules, indicating that the fingers associate specifically with one another (25). For similar interactions to occur intramolecularly, the fingers must be capable of movement relative to one another through the amino acids linking them. This linker region of GATA-1 has recently been shown by NMR to have little secondary structure and is therefore likely to allow sufficient movement of the fingers relative to each other (26). As the DNA is apparently unconstrained by the orientations of the two zinc fingers, the bending experiments support a model in which the N- and C-terminal zinc fingers adopt different geometries when bound to different double GATA sites.
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**Abbreviations.**

1. GST, glutathione-S-transferase
2. MBP, maltose binding protein
3. EMSA, electrophoretic mobility shift assay
Figure 1. Representation of the modified pBEND5: pB5RG. Sequence of the 247 base pair EcoRI-HindIII fragment of pB5RG. Unique sites are shown in bold; the duplicated sites used to generate DNA fragments of identical length are indicated. The GATA binding sites and A-tracts shown in Table 2 are inserted into the XbaI and AatII sites.

Figure 2. Cyclic-permutation analysis of the binding of full-length GATA-1 to the GATA-palindrome site. (A) 7.8% acrylamide electrophoretic analysis of circularly permuted GATA-1 DNA complexes. The locations of the restriction enzyme generated free probes (F) and complexes (C) are shown. (B) The bending angle was determined based on the mobility of the GATA-1 DNA complex ($R_{\text{bound}}$) normalized to the mobility of the corresponding free DNA ($R_{\text{free}}$). The value of $R_{\text{bound}}/R_{\text{free}}$ was plotted as a function of the distance of the center of the G2 site from the 5' end of the probe divided by the total length of the probe. The best-fit quadratic polynomial shown was used to estimate the bending angle.

Figure 3. Understanding the anomalous bending angles observed for the full-length chicken GATA-1. (A) The average relative
mobility of the complex is proportional to the apparent bending angle. The relative mobility, which depends on where the protein is bound, is averaged for each experiment and plotted as a function of $\alpha$. A bending angle of 15° is obtained on extrapolation to a relative mobility of 1.0. (B) Bending angles for complexes formed between full-length chicken GATA-1 (●), MBP-chicken double finger (▼) and chicken double finger peptide (○) as a function of the acrylamide concentration. Bending angles obtained from control experiments using A5 (▼) and A55 (■) A-tracts are shown.
Figure 1

EcoRI  BsrI  NheI  ClaI  SpeI  DraI
GAATTCACGC GTACCATCGAT TAGCATCGAT CCATGGACTA GTCTCGAGTT TAAACTCGAG

MspI  NruI  KpnI  HinfI  BamHI  XbaI
CAGCTGCCCG GGAAGGCTTC GCAGAAATATT GGTACCCCAT GGAATCGAGG GATTCCTCTAG

AatII  BsrI  NheI  ClaI  SpeI  DraI
AGTTAAACGAC GTCACCGCGTA CCAGTCTAG CATCGATCCA TGGACTAGTC TCGAGTTTAA

MspI  NruI  KpnI  HinfI  BamHI
ACTCGAGCAG CTGCCGCCGGA GGCCTTCGCG AAATATTGGT ACCCATGGGA ATCGAGGGATC

HindIII
CAAGCTT
Table 1

GATA protein expression vectors

| Expression vector | GATA protein expressed | Forward (EcoRI) and Reverse (SalI) PCR primers |
|-------------------|------------------------|-----------------------------------------------|
| pMALhDF           | MBP-hDF (200 – 317)    | CCGGAATTTCGGTGAGCCAGGGAGTGTTGTAAC
                      |                        | ACGCGTCGACTCATCACCCTTCTTTTTTTTTTTTTTCTTTT |
| pMALcDF           | MBP-cDF (106 – 223)    | CCGGAATTTCGGCGAGCCGCGTGGTCAAC
                      |                        | ACGCGTCGACTCATCAGCGCTTTTTTACCCCTT |
| pMALcCF           | MBP-cCF (156 – 223)    | CCGGAATTCAAGCGCCAGCGACAGTGTCGAGC
                      |                        | ACGCGTCGACTCATCAGCGCTTTTTTACCCCTT |
| pET11cDF          | cDF (106 – 223)        | GGGAATTCATATGGGGCGAGGGGCGTGATGCCTCAAC
                      |                        | CGCGGATCCTCATCAGCGCTTTTTTACCCCTT |

(a) The GATA-1 domains expressed correspond to (DF) the double zinc finger DNA binding domain and (CF) the C-terminal single zinc finger. The corresponding human (h) or chicken (c) GATA protein residue positions are indicated in parenthesis.
Table 2
GATA binding sites incorporated into pB5RG

| Plasmid      | Insert name      | Sequence (XbaI-insert-AatII) \(^a\)                                      |
|--------------|------------------|-------------------------------------------------------------------------|
| PB5RG        | No insert        | AGAGTTAACGAC                                                            |
| PB5RG-γP     | γ-globin promoter| AGACACACTATCTCAATGCAAAATCTGTCTGGAC                                       |
| pB5RG-εS     | ε-globin silencer| AGAGAATGGGAGAGATGGATATCATTTGGAAAGGAC                                    |
| pB5RG-G3     | GATA-1 promoter  | AGAGTCCATCTGATAAGACTATCTGTGCCCAGGAC                                     |
| pB5RG-G2     | GATA-palindrome  | AGAGTCCATCTGATAAGACTTCAGTGCTGCCCAGGAC                                   |
| pB5RG-G1     | Single GATA site | AGAGTCCAGTGATGACTTCAGTGCTGCCCAGGAC                                      |
| pB5RG-G0     | NMR single GATA  | AGAGTTGCAAGATACATTGAC                                                   |
| pB5RG-GC     | Consensus GATC   | AGAAPCTTGGGGATAGATCTAAATTCAGGATC                                       |
| pB5RG-A5     | A-tract          | AGACGAAAAACCGGAAAAACGGGAAAAACGGGTCAGGAC                                  |
| pB5RG-A55    | Phased A-tract   | AGACGAAAAACCGGAAAAACGGGAAAAACGGGTCAGGAC                                  |

\(^{a}\) Flanking partial sequences of the central XbaI and AatII restriction sites are shown in italics. GATA binding sites are shown in bold.
Table 3

GATA-1 bends DNA in a site independent manner

| PB5RG probes | GST c GATA-1 | MBP-hDF Chicken GATA-1 | Human DF | Chicken DF | Chicken CF |
|--------------|--------------|-----------------------|---------|----------|----------|
| γP           | 97 ± 4       | 61 ± 4                | 29 ± 3  | 26 ± 2   |
| εS           | 107 ± 2      | 64 ± 4                | 22 ± 4  |
| G3           | 102 ± 8      | 87 ± 3                | 59 ± 4  | 26 ± 2   | 19 ± 3   | 23 ± 4   |
| G2           | 109 ± 6      | 81 ± 6                | 67 ± 4  | 25 ± 3   | 20 ± 2   | 16 ± 2   |
| G1           | 86 ± 9       | 25 ± 2                | 24 ± 2  | 26 ± 2   |
| G0           | 81 ± 5       | 24 ± 2                | 23 ± 2  | 22 ± 3   |
| GC           |              |                       | 28 ± 3  |
| Average      | 104 ± 6      | 83 ± 3                | 63 ± 4  | 25 ± 2   | 24 ± 4   | 23 ± 4   |

(a) Values of the bending angles (°) obtained by circular permutation analysis as a function of GATA binding sites shown in Table 2. The angles represent the average of at least three experiments and the error represents the standard error of the mean. Experiments were also carried out for the MBP-cDF and MBP-cCF fusion proteins. Within the
error of the method, angles independent of the DNA binding sites were observed. Average angles of 76 ± 8° and 88 ± 10° were obtained for the MBP-cDF and MBP-cCF, respectively.
Figure 2

(A) Gel electrophoresis showing restriction enzyme digestion patterns of DNA samples. The enzymes used are BsrI, NheI, Clal, SpeI, DraI, MspI, NruI, KpnI, HinfI, and BamHI.

(B) Graph showing the relative mobility ($R_{bound}/R_{free}$) as a function of flexure displacement ($D/L$) with a curve fit to the data points.

The graph shows a trend where the relative mobility increases as the flexure displacement increases, indicating a change in mobility due to the presence of the enzyme-bound complexes.
Figure 3
