Helical phasing between DNA bends and the determination of bend direction

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ABSTRACT

The presence and location of bends in DNA can be inferred from the anomalous mobility of DNA fragments or protein-DNA complexes during electrophoresis in polyacrylamide gels. Direction of bending is not so easily determined. We show here that a protein-induced bend, when linked to a protein-independent DNA bend by a segment of variable length, exhibits an electrophoretic mobility that varies in a sinusoidal manner with the length of the linker. Mobility minima occur once for each addition to the linker of one helical turn of DNA. Since minima should occur when two bends reinforce one another, the direction of one bend relative to the other can be determined from the distances between the two centers of bending at which minima occur. Our results strongly support the idea that the A5-6 tracts in kinetoplast DNA bend towards the minor groove while the bend at the recombination site of the $\gamma\delta$ resolvase (binding site I of the $\gamma\delta$ res site) bends towards the major groove.

INTRODUCTION

Proteins that induce or stabilize bends in specific DNA sequences are involved in a variety of cellular processes including site-specific recombination (1), DNA replication (2-6), chromatin organization (7), and gene regulation (8). A gel mobility assay has been developed which can identify bends in DNA molecules and protein-DNA complexes (8). Since the electrophoretic mobility of DNA fragments in polyacrylamide gels is dependent on their average shape (in particular, the mean square average end-to-end distance; ref. 9-11), a bent DNA molecule migrates at a different rate (more slowly) than a linear fragment with the same number of base pairs; bent protein-DNA complexes behave in an analogous manner.

Hypothetically, the mobility of DNA fragments that contain more than one bend should depend on the phase relationship between

the bend loci. If two bends lie in the same plane they can either reinforce each other's curvature (in-phase configuration) and reduce the average end-to-end distance of the fragment, or oppose each other (out-of-phase configuration) and favor a more extended conformation. For protein-independent bends this reasoning appears to be correct, since the mobility of DNA fragments formed through ligation of oligonucleotides depends on the phase relationship between individual bend elements (*ie.* A tracts) (12-15).

In this report we investigate whether the concept of phasing can be used to determine the direction of a protein-induced bend relative to an intrinsic (sequence-determined) bend. $\gamma\delta$, a procaryotic transposable element from *E. coli*, encodes a site-specific recombinase (resolvase) that binds to three recognition sites within a DNA region called res (16, for a review see 17). Previous studies have implied that resolvase protein bends res DNA at each binding site (18, Salvo and Grindley, in preparation). Appropriate phasing of these bends might serve to organize a specific three dimensional protein-DNA structure. Independent evidence has suggested that at site I (which contains the recombinational crossover point) the minor groove is on the outside of the bend (19).

MATERIAL AND METHODS

Plasmids with resolvase binding site I joined to a K-DNA segment.

pJS50 contains site I as a 95 bp SalI-EcoRI fragment (from res $\Delta 24R$; ref. 20) joined to a 126 bp EcoRI-HindIII fragment containing the K-DNA segment (from pHW132; ref. 8), cloned between the SalI and HindIII sites of pNG16 (ref. 21). To alter the spacing between site I and the K-DNA segment insertions were made at the intervening EcoRI site using a variety of linkers, combined with filling and recircularization by standard procedures. All derivatives were subjected to DNA sequence analysis and details of their structure are shown in Fig. 1. pJS55 has the EcoRI site of pJS50 filled. pJS60, 61, 62(1) and 63 contain an inserted BamHI linker of 6 bp, 8 bp, 10 bp and 12 bp respectively. pJS62(9) was an aberrant clone derived in the construction of pJS62(1) but with an extra base pair inserted. pJS81-84 contain an 8 bp BglII linker inserted into the filled BamHI site of pJS61, 62(1), 62(9) and 63 respectively. pJS102-105 are chimeras made by joining a site I fragment terminated at a BamHI site (from a pJS60-series plasmid) to a K-DNA fragment terminated at a BglII site (from a pJS80-series plasmid) in the following combinations: pJS102 = pJS61/83; pJS103 = pJS61/84; pJS104 = pJS62(1)/84; pJS105 = pJS63/84.

Polyacrylamide gel electrophoresis of resolvase-DNA complexes.

The plasmids diagrammed in Fig. 1 were digested with HindIII, BanI and NruI, and labeled at their 3'-OH ends by filling with DNA polymerase I (Klenow fragment) in the presence of [α - 32 P]-dATP and the other three deoxynucleoside triphosphates. In the case of pJS50, site I and the K segment are contained on a 335 bp fragment. The ends of this fragment are 184 bp to the left of the center of site I and 105 bp to the right of the center of K-DNA segment. Fragments were incubated with resolvase (approx. 50nM) in 20mM Tris.HCl (pH 7.5), 10mM MgCl₂, 100mM NaCl, and 100 μ g/ml calf thymus DNA. After 10 min. samples were loaded on an 8% polyacrylamide gel (30:1 acrylamide: bis-acrylamide) in 50mM Tris.borate (pH 8.3), 1 mM EDTA. Electrophoresis was at 7 volts/cm for 12 hours. The gel was dried and autoradiographed.

RESULTS AND DISCUSSION

We have constructed a set of DNA fragments that contain site I of the $\gamma\delta$ res site separated from an intrinsic (protein-independent) bend by a spacer of variable length (see Fig.1). For the intrinsic bend we chose a segment of kinetoplast DNA (K-DNA) from *Leishmania tarentolae*, which has been studied in detail and is bent in solution and during polyacrylamide gel electrophoresis (8, 22-24). The K-DNA acquires its bend from a series of A tracts

Figure 1. Plasmids with a variable spacer separating resolvase binding site I and a K-DNA segment. Asterisks mark the centers of site I (shown by the two inverted arrows) and the K-DNA segment, and distances between these are as indicated. Wu and Crothers (8) have shown that the center of the K-DNA bend corresponds to the indicated center of the segment. The A tracts that constitute the K-segment are marked with "+" while the A tract found adjacent to site I in the $\gamma\delta$ res site is marked "#". See Materials and Methods for further details.

Figure 2. (A) Polyacrylamide gel electrophoresis of DNA and resolvase-DNA complexes. Labeled DNA fragments derived from the plasmids shown in Fig. 1 were incubated with resolvase and analysed by electrophoresis on an 8% polyacrylamide gel (see Methods). The free DNA fragment containing site I and the K segment is marked (.), each protein-bound fragment is marked (p). The lanes are labeled with the distance (bp) between the centers of site I and the K segment. (B) Graph of the relative mobility M_b/M_f of each resolvase-DNA complex plotted against the distance between the centers of site I and the K segment.

(A5 to A6) whose centers are spaced 1 helical turn apart. In the constructs the distance between the centers of site I and of the K-DNA segment was increased in small increments from 50 bp to 79 bp (Fig. 1).

The electrophoretic behavior of the DNA fragments, both free and complexed with resolvase, in a polyacrylamide gel is shown in Figure 2A. The naked DNA fragments themselves have mobilities that vary in a cyclical manner with the separation of the two DNA segments. The minima are separated by 10-12 bp. The variation of mobility is not seen in an agarose gel (data not shown) confirming that it results from deformation of the DNA and not from fragment size differences. We infer that the site I segment contains an intrinsic bend that exhibits a helical phasing with the K-DNA segment. Examination of the DNA sequence of the site I fragment shows there is an A5 sequence immediately to the right of site I (see Fig. 1). Since the mobility minima occur when the center of this A tract is 2, 3 or 4 helical turns (20 bp, 30 bp or 42 bp) from the center of the first A5 tract of the K-segment, it seems likely that the A tract adjacent to site I is responsible, at least in part, for the intrinsic bend in the site I segment.

The electrophoretic mobilities of the resolvase-complexed fragments also display a cyclical behavior dependent upon the spacer length. Clearly the mobility minima for the complexes do not coincide with those of the uncomplexed DNA but rather occur at interbend distances that are 2-3 bp shorter. If we calculate the mobility of each complex relative to the mobility of its uncomplexed fragment (M_b/M_f), and plot these relative mobilities as a function of the distance between the center of site I and the center of the K segment (Fig. 2B) we obtain a smooth sinusoidal curve with minima (and maxima) separated by 10-12 bp. If the intrinsic bend in the site I segment and the resolvase-induced bend are independent of one another, the effect of the intrinsic bend on electrophoretic mobility of the complexes should be factored out in this curve. Minima occur at center-to-center distances of 63 bp and 73 bp, that is, 6.0 and 7.0 helical turns assuming 10.5 bp per turn). We conclude that the K-DNA bend and the resolvase-induced bend are in phase and show maximum reinforcement of one another when their centers are separated by

an integral number of helical turns. These two bends, therefore, are in the same direction relative to the DNA structure (for example, both into the major groove). We can draw a further conclusion from our results. Since the mobility curve has a periodicity of about 10 bp, the resolvase-site I bend (like the K-DNA bend) must have specific directionality and cannot be ascribed to increased isotropic or symmetrical planar flexibility.

Determination of the absolute direction of one bend requires that the direction of the other is known. There is suggestive evidence concerning the direction of both the site I bend and the K-DNA bend that turns out to be totally consistent with the results described above. Hatfull *et al.* (19) showed that binding of resolvase to site I resulted in increased intercalation of the footprinting reagent, methidiumpropyl-EDTA.(FeII) (MPE; ref. 25-26), at the exact center of the site. The enhanced intercalation was dependent upon the protein-induced bend; a mutant form of resolvase that bound to site I but failed to bend it, did not show the enhancement. Since MPE intercalates into the minor groove of the DNA (25-26), we concluded that the minor groove was on the outside of the bend and, as a result (or cause) of bend induction, was opened up, perhaps as a localized kink, with the central base pairs partially unstacked to provide a preferred substrate for intercalation (19). If, as we suggested, site I is bent into the major groove, then our phasing experiments suggest that the K segment is also bent into the major groove.

The K-DNA segment used consists of four A tracts helically phased together; thus, the center of the K segment lies precisely between two A tracts. Two general classes of models for binding of A tracts, the "wedge" model (27-29) and the "junction bend" model (8,14,30), have been proposed. Although these models differ in overall concept, both predict that the effective bend of an A5 tract will be towards the minor groove at the center of the tract. Experimental evidence for narrowing of the minor groove at A or T tracts has come both from studies of the preferred positions of such sequences in nucleosome core particles (31-32) and from the hydroxyl radical cleavage patterns of A tracts in larger DNA fragments (33). Finally, in an experiment identical in concept to the one described here (and using the same K-DNA fragment), Zinkel

and Crothers (34) have demonstrated a helical phasing between K-DNA and the bend induced by the cyclic AMP binding protein, CAP, of *E. coli*. These authors have assumed that the CAP-induced bend is effectively into the minor groove at the center of its binding site (based on protection and model building studies; ref. 35-38), and conclude from their phasing results that the K-DNA bend is effectively into the major groove at the center of the segment, that is, into the minor groove at the centers of each A tract.

CONCLUSIONS

The experiments described above show that a protein-dependent bend in DNA exhibits "helical phasing" with an intrinsic DNA bend located on the same DNA fragment. This is detected as a periodic variation of gel mobility dependent upon the inter-bend distance, and can be used to determine the relative direction of two independent bends. Our data show that the resolvase-dependent bend at site I of the $\gamma\delta$ res site, and the intrinsic bend of an A5 tract are in the opposite direction. Together with the data of Zinkel and Crothers (34) and the experiments of Hatfull et al. (19), our results are most consistent with the conclusion that the center of site I is bent (or kinked) into the major groove whereas the effective bend at the center of an A5 tract is into the minor groove. Regardless of whether this conclusion is correct, the two independent sets of phasing data (34, this paper) show clearly that the CAP-induced and resolvase-induced bends are effectively in opposite directions (if each bend is considered as a single discontinuity of the helix located at the center of the protein binding site). One explanation for this arises from the geometries of the two binding sites. CAP binds to two adjacent major grooves (35-36) whereas resolvase at site I interacts with non-adjacent major grooves separated by a single unoccupied major groove (39). If the DNA is bent towards the DNA-binding domains of both proteins, the central minor groove in the case of CAP, and the intervening major groove in the case of resolvase, will lie on the inside of the bend.

Our data show that phase relationships between bends are maintained with spacers exceeding 50 bp in length (or 70 bp in ref. 34). Thus complex curvatures can be fashioned from separate

non-abutting bend elements. We suspect that appropriately spaced bends, both resolvase-dependent and protein-independent, help organize res into a precise three dimensional structure that contributes to the specificity and near topological invariance of the recombination process (40). Clearly, the method described in this paper (and in ref. 34) can be used to investigate the direction of the other resolvase-induced bends in res, as well as any other bend in DNA.

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