Bulge Defects Do Not Destabilize Negatively Supercoiled DNA

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ABSTRACT The conformational properties of DNA lesions such as extrahelical bulges are presumed to be essential for recognition of defects in DNA structure by a cell's genomic repair machinery. Efficient recognition and repair of lesions by DNA-repair systems occurs despite the wide range of normal heterogeneities in DNA structure, including features such as sequence-dependent bends. The effects of global negative supercoiling on the structure of DNA lesions have been largely unexplored. We have investigated the behavior of several plasmid DNAs containing bulge defects with up to five extrahelical adenine residues. Using two-dimensional agarose-gel electrophoresis, we show that there is no spontaneous cooperative unwinding of these bulge loci up to native levels of negative supercoiling ($\sigma = -0.055$) under our conditions.

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DNA-repair systems recognize a multitude of lesions in DNA, including bulge defects and nonhomologous regions, base substitutions, and DNA-damaging covalent adducts. The efficient recognition and repair of such lesions occurs despite the wide range of normal heterogeneities in DNA structure. There is strong evidence that DNA lesions cause significant alterations in DNA structure and dynamics (1); however, almost all information about the structural properties of DNA lesions comes from studies on linear DNA molecules. Hence, the effects of negative, (−), supercoiling on these structures have not generally been assessed. Because almost all DNA in living cells is (−) supercoiled, it is important to ask whether global DNA unwinding can significantly affect the local helical parameters of DNA lesions, which are presumed to be the molecular determinants of recognition by DNA-repair systems. In this study we investigated whether (−) supercoiling promotes local unwinding transitions for a series of plasmid DNAs containing extrahelical adenine bulges.

We constructed plasmids containing bulge defects by a modification of the method used by Kodadek and Gamper (2), in which a DNA primer containing the defect, hybridized to a single-stranded DNA template, is extended by DNA synthesis (Fig. 1). The ssDNA template is one strand of a duplex plasmid that carries a bacteriophage-f1 replication origin and is isolated as phage-encapsulated ssDNA by an M13 helper-phage-dependent single-strand rescue procedure. After the primer was annealed to the ssDNA circle, phage T4 gene-32 protein was added at a 10-fold molar excess (gp32/template) to prevent the inhibition of primer extension reactions by template secondary structure. T4 DNA polymerase was added to the reaction and primer extension was carried out at 25°C overnight. Incubation of the extension reactions at temperatures below 37°C was essential to prevent displacement of the primer by the polymerase. The overall yield of this procedure is high; judging from the fraction of template converted to dsDNA circles under these conditions, yield is in the range of 80–90% (data not shown). Fig. 2 *A* shows the sequences of primer and template strands as well as the positions of the A_n bulges incorporated into the respective plasmids.

We were greatly concerned about the fidelity of the primer-extension procedure, particularly whether bypass of the lesion by the polymerase might have taken place during the extension reaction. We therefore isolated restriction fragments that contained the lesion sites and analyzed their behavior in polyacrylamide-gel electrophoresis. Bulge defects can cause large reductions in polyacrylamide-gel mobility because of the significant intrinsic bend induced by the extrahelical bulge. The mobility reduction has previously been demonstrated to increase with the size of the bulge (1,3). As shown in Fig. 2 *B*, DNA fragments derived from our plasmids that are identical with respect to size and the location of the bulges all have gel mobilities that are significantly, and in some cases substantially, reduced relative to the fully duplex fragment of the same size and sequence. The effects of the bulges on fragment mobility are in the range of those reported in previous studies (4,5); moreover, the absence of aberrant primer-extension products or other contaminants in Fig. 2 *B* attests to the fidelity of the extension reaction.

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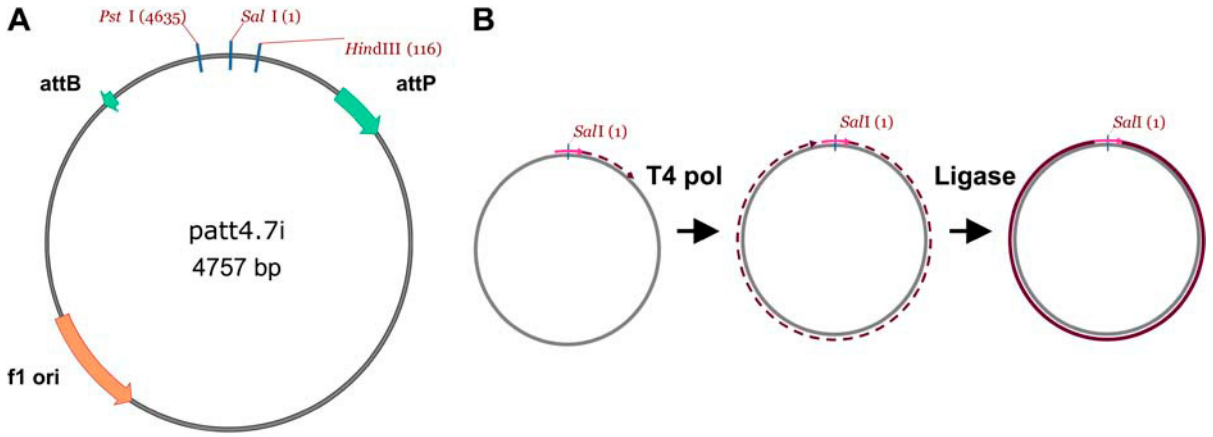

FIGURE 1 (*A*) Plasmid from which bulge-containing DNAs were derived. patt4.7i is a phagemid that also carries the attachment sites for λ-integrative recombination (attP, attB). A 37-bp sequence corresponding to the bottom strand of the sequences shown in Fig. 2 was cloned into the *Sal*I site of patt4.7i, propagated in *Escherichia coli*, and purified according to standard procedures. (*B*) Strategy for preparing covalently closed, bulge-containing plasmid DNAs. Primer is depicted in pink; synthesized DNA in magenta.

Next, the open-circular plasmids that were generated by primer extension were covalently closed by T4 DNA ligase in the presence of ATP. Ligation reactions included ethidium bromide at concentrations ranging from 0 to 2.5 μg mL^{-1}. Populations of (−) −supercoiled plasmid topoisomers were recovered after extraction with buffer-saturated butanol and ethanol precipitation. The highest ethidium concentration used results in an average superhelix density of −0.055 for a fully duplex control plasmid under identical temperature and buffer conditions.

Distinct topoisomer populations of covalently closed, bulge-containing plasmids were prepared by ligation in the presence of different concentrations of ethidium. These reaction products were pooled and subjected to two-dimensional (2D) agarose-gel electrophoresis (6) (Fig. 3). All gels were run in the first dimension in the absence of an intercalating agent whereas the second dimension in all gels contained 1.5 μg mL^{-1} chloroquine phosphate. By including sufficiently high concentrations of intercalator in the second dimension the helical repeat of the DNA duplex is increased to a level such that all of the topoisomers are (+) supercoiled. Thus, secondary-structure transitions that are promoted by (−) supercoiling and affect the mobility of some topoisomers in the first dimension are reversed in the second

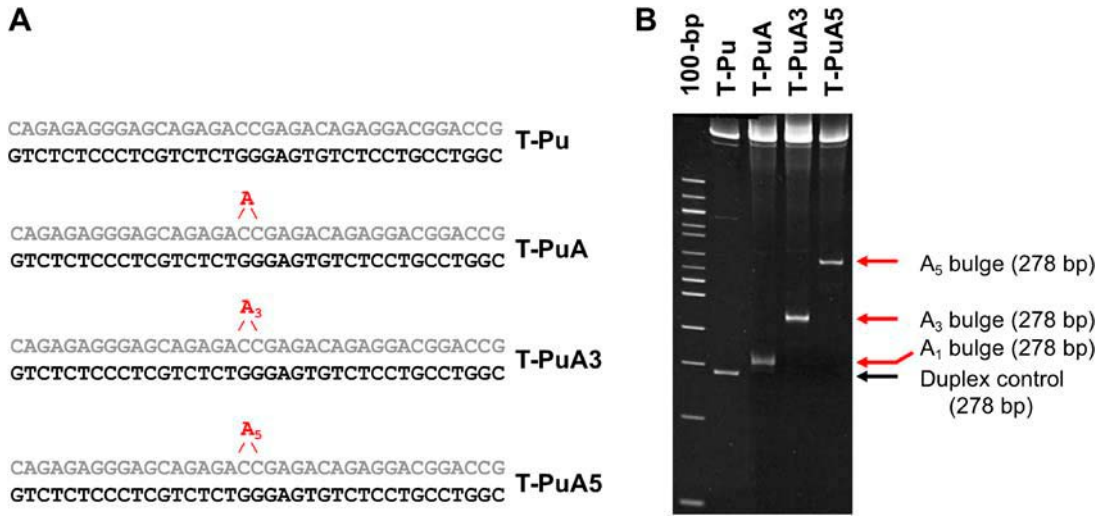

FIGURE 2 (*A*) Sequences of full duplex and extrahelical bulge-containing inserts generated according to the procedure outlined in Fig. 1 *B*. Annealed primer sequences, either fully complementary or containing a bulge of 1, 3, or 5 A residues are in gray. (*B*) 278-bp DNA fragments containing centrally positioned A1, A3, or A5 bulges analyzed on a 5% native polyacrylamide gel. Fragments were generated by *Pst*I/*Hin*dIII digestion of constructs derived from patt4.7i. The mobility of the 278-bp fragments decreases monotonically with increasing bulge size, a consequence of the increasing magnitude of intrinsic bends conferred by bulges of increasing size. No mobility differences were observed between linearized fragments generated from nicked or covalently closed bulge-containing plasmids.

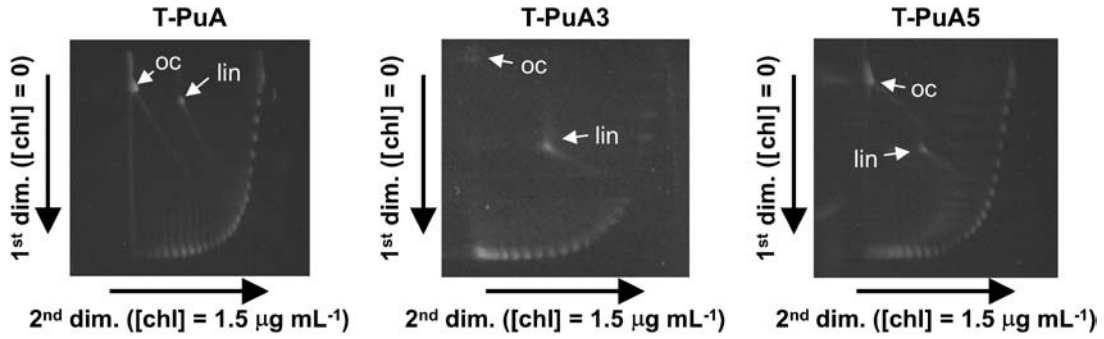

FIGURE 3 Analysis of T-PuA, T-PuA3 and T-PuA5 topoisomers by 2D agarose-gel electrophoresis. First- and second-dimension runs took place in TBE buffer (50 mM Tris-borate, 1 mM Na_2EDTA; pH 8.5) including the indicated concentrations of chloroquine. There are well-defined, arc-shaped patterns of topoisomers in all cases, indicating the absence of sharp, supercoiling-dependent secondary-structure transitions. Positions indicated by oc and lin are those of open-circular and linear forms, respectively. Note also the high proportion of covalently closed molecules to nicked DNAs in these preparations.

dimension. The signature for such structural transitions is a discontinuity in the arc of topoisomer bands, commonly seen in plasmids containing sequences that undergo transitions to the Z-form or cruciform structures (6).

As shown in Fig. 3, none of the 2D agarose gels show evidence of structural transitions in the bulge-containing topoisomers. The absence of structural transitions in bulge-containing plasmids is rather surprising given the fact that our electrophoresis conditions correspond to a relatively low ionic-strength environment. Low-salt conditions promote DNA unwinding, which can act synergistically with (−) supercoiling to promote secondary-structure transitions (7). We conclude on the basis of our data that DNA bulges containing up to five extrahelical adenine residues do not introduce strongly destabilizing effects on duplex regions adjacent to bulge loci. This conclusion suggests that dynamic changes in DNA supercoiling such as those occurring in transcription-coupled DNA repair (8) may not have dramatic effects on the intrinsic structure of bulge defects.

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