THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 268, No. 23, Issue of August 15, pp. 17571-17577, 1993
Printed in U.S.A.

RecA Binding to Bulge- and Mismatch-containing DNAs

CERTAIN SINGLE BASE MISMATCHES PROVIDE STRONG SIGNALS FOR RecA BINDING EQUAL TO MULTIPLE BASE BULGES*

(Received for publication, December 23, 1992, and in revised form, March 10, 1993)

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Studies from several laboratories have demonstrated that RecA protein can recognize a variety of pertubations in the DNA helix. Here, using a nitrocellulose filter binding assay, it was observed that RecA bound to bulge-containing DNAs more effectively than non-bulged DNA. The degree of binding of RecA protein to bulged DNA was dependent on the conformation of the bulged bases and the kinking angles produced by the bulges as determined by the type and number of bases in the bulge. Although a single base mismatch does not kink DNA, RecA protein showed preferential binding to DNAs containing certain single base mismatches. An A·C mismatch flanked by A·T base pairs in a 28-base pair (bp) DNA facilitated the binding of RecA protein to the same high level as when the 28-bp DNA contained a 4-base cytosine bulge. Chemical probing techniques were used to examine the structure of DNA within the RecA filament. It was found that upon binding of RecA protein, the DNA helix becomes accessible over at least 14 bp, and the degree of sensitivity agrees with the binding efficiency of RecA protein.

RecA protein of *Escherichia coli* is central to homologous recombination and the pathways of DNA repair. RecA protein has been shown to play a regulatory role in the SOS pathway by facilitating the cleavage of the LexA repressor, which leads to the expression of numerous SOS-regulated genes. RecA protein was also shown to process the UmuD protein into UmuD′, an active form in SOS mutagenesis (1–3). In addition, as described below, RecA protein may recognize a variety of lesions in DNA and has been shown to inhibit the editing activity of the DNA polymerase III holoenzyme (4). Rosenberg and Echols (5) have proposed that the binding of RecA protein at the damaged site could allow replication to by-pass the lesions and could increase the rate of mutagenesis and have suggested that RecA protein might be directly involved in mutagenesis. Recently, studies with a reconstituted *in vitro* system have shown that DNA polymerase III is able to by-pass a single abasic DNA lesion with the help of RecA, UmuC, and UmuD′ proteins (6). Among the many lesions that can be found in DNA in nature, base mismatches and bulged bases are particularly common, yet the interaction of RecA protein with these lesions has not been investigated.

Extra base bulges and mismatches in double-stranded DNA can arise from recombination between sequences that are not fully homologous or from errors of replication. In *E. coli*, mismatches can be repaired by the methyl-directed repair system and several short-patch mismatch repair pathways, and the repair efficiency depends on the type of mismatch and the sequence context (reviewed in Ref. 7). Genetic studies have shown that single base bulges can also be repaired by the methyl-directed repair system in *E. coli* (8); and using cell extracts, this repair system has been shown to repair DNAs containing 1–3-base bulges as efficiently as a G·T mismatch (9). A considerable amount of information is available about the structure of bulges and mismatches.

Structural studies using NMR suggest that bulged bases can exist in either stacked-in or looped-out conformations depending on the base composition of the bulge, temperature, and other factors (10–15). Gel electrophoretic studies have shown that bulges of 1–5 bases produce kinks in DNA, which result in decreased mobilities of bulged DNAs on polyacrylamide gels (16–19). Visualization of a DNA containing a 3-base bulge by electron microscopy has directly demonstrated kinks created by the bulges and provided a measure of the distribution of the kinking angles (20). Whereas single base bulges produce kinks, studies using NMR (reviewed in Ref. 21), x-ray crystallography (reviewed in Ref. 22), and gel electrophoresis (17, 23) show that single base mismatches do not produce detectable kinks. Furthermore, DNA containing multiple base mismatches shows no retardation of gel mobility on 15% polyacrylamide gels (16). These results suggest that mismatched bases remain stacked into the helix and produce at most only subtle perturbations in the DNA helix (23).

Previous studies have shown that thymine dimers (4), (6-4) photoproducts (5), psoralen cross-links (24), and intercalating drugs (25, 26) can promote the nucleation of RecA protein binding on double-stranded DNAs in the presence of ATPγS,[1] a very poorly hydrolyzed analog of ATP. Other structural variations such as B-Z junctions (27, 28), changes in DNA superhelicity (29), and an A·T-rich sequence (30) have also been shown to enhance the binding of RecA protein to double-stranded DNAs. These observations suggest that RecA protein recognizes perturbations in the DNA helix. To test the hypothesis that RecA protein is intimately involved in repair (reviewed in Refs. 31 and 32), it would be of value to examine the interaction of RecA protein with two lesions that are frequent products of DNA metabolism: bulged bases and single base mismatches.

Here, using a nitrocellulose filter binding assay, we show that RecA binds to bulge-containing DNAs more effectively than to the control DNA (no bulge and mismatch). Interestingly, RecA protein also showed preferential binding to DNAs containing single base mismatches. For bulged DNAs, the bind-

* The work was supported by American Cancer Society Grant NP-583 and National Institutes of Health Grants GM-31819 and GM-42342. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We dedicate this article to the memory of Dr. Harrison Echols.

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[1] The abbreviations used are: ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); DEP, diethyl pyrocarbonate.

ing of RecA appeared to depend on the conformation of the bulged bases and the kinking angle produced by the bulges. The binding of RecA protein to DNAs containing single base mismatches generally followed the degree of thermal instability of mismatches and their flanking sequences. Chemical probing techniques were also used to examine the structure of DNA within the RecA filament, and the results are described below.

MATERIALS AND METHODS

DNA and Proteins—Oligonucleotides were synthesized on an Applied Biosystems Model 380B DNA synthesizer and purified by electrophoresis on 7.5 M urea, 20% polyacrylamide gels in 89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA (pH 8.0) buffer. The single-stranded oligonucleotides were labeled at the 5′-ends with T4 DNA kinase (Life Technologies, Inc.) and [γ-^{32}P]ATP (Amersham Corp.). DNA duplexes were prepared by heating equal amounts of the two complementary oligonucleotides at 65 °C for 10 min and then allowing the DNA to cool to room temperature over 6 h. The labeled DNA duplexes were further treated with T4 DNA polymerase (New England BioLabs, Inc.) to digest the excess single-stranded DNA and the 4 protruding bases on each end of the duplex. RecA protein was purified (33) using a RecA overproducer in an exo I$^-$ background. The purity of RecA protein was determined by analyzing each preparation on SDS-polyacrylamide gels, and the protein used in this study was >95% pure as measured by a densitometric scan of the gel. The protein was free of endo- and exonucleases as judged by lack of release of end labels or nicking of circular DNAs. The protein concentration was determined by the Bio-Rad protein assay, which is based on the dye binding procedure of Bradford (34).

Ligation and Gel Electrophoresis of Duplex DNAs—The DNA duplexes were ligated and electrophoresed as described (19). Briefly, the unlabeled DNA duplexes were prepared by annealing two complementary oligonucleotides, and the 5′-ends were phosphorylated with T4 DNA kinase. The phosphorylated DNAs were incubated with T4 DNA ligase (purified in this laboratory), and the ligated DNAs were electrophoresed on 15% polyacrylamide gels. DNA was visualized by staining with ethidium bromide and photographed under UV illumination.

RecA Binding Reactions—The labeled DNA (4 μg/ml or 6 μM in nucleotide pairs) was incubated with RecA protein at a concentration of 240 μg/ml (6.3 μM) at 37 °C for 5 min in reaction buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM EDTA, 30 mM NaCl, and 1 mM dithiothreitol. ATPγS (Boehringer Mannheim) was then added to 200 μM, and the incubation was continued at 37 °C. A control reaction containing no RecA protein was carried out in parallel with each RecA binding reaction. Aliquots (10 μl) were removed from the reaction mixture at various times and added to 100 μl of cold reaction buffer (4 °C) to stop the reaction. Each solution was filtered through a nitrocellulose filter (BA85, Schleicher & Schuell), which had been soaked in 1 mM ATP for 30 min, and then washed twice with 200 μl of cold reaction buffer (4 °C). Filters were dried, mixed with 5 ml of Scintiverse E solution (Fisher), and counted by scintillation counting. Background values for the binding of DNA to filters was determined from a DNA sample incubated without RecA protein. The efficiency of RecA binding was expressed by the percentage of DNA retained on the filter. Every data point shown was the average of at least three separate but identical reactions.

Chemical Modification Reactions—For each modification reaction, 5 μl of DEP (Sigma) or 34 μl of hydroxylamine (Aldrich; 4 M hydroxylamine hydrochloride freshly titrated with diethylamine to pH 7.0) was added to either 400 ng of labeled DNA in reaction buffer or 100 μl of a DNA-RecA binding reaction. The mixtures were incubated at 37 °C for 10 min and then terminated by adding 75 μl of 1 M sodium acetate (pH 5.2), 67 μg/ml tRNA, and 750 μl of cold ethanol. The precipitated DNA was dissolved in 250 μl of 0.3 M sodium acetate (pH 5.2), precipitated with cold ethanol again, washed with 70% ethanol, and dried *in vacuo*. Each dried DNA sample was dissolved in 100 μl of freshly diluted 1 M piperidine (Sigma) and incubated at 90 °C for 30 min to cleave the modified bases. The piperidine was removed by lyophilizing three times. Each time, the dried pellet was resuspended in 10 μl of distilled H_2O; the final pellet was dissolved in a solution of 80% (v/v) formamide, 10 mM NaOH, 1 mM EDTA, 0.1% (w/v) xylene cyanol, and 0.1% (w/v) bromphenol blue.

Sequence Analysis—The cleaved DNA was electrophoresed on 8.3 M urea, 20% polyacrylamide gels in 89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA (pH 8.0) buffer. The G or C + T base-specific cleavages to produce the marker DNAs followed the procedure of Maxam and Gilbert (35). The gels were exposed on Kodak XAR film. Densitometry was performed with a Sony XC-77 CCD video camera coupled to a Macintosh-based image processing system.

RESULTS

RecA Binding to Bulged DNA Duplexes—A filter binding assay was used to measure the binding of RecA to double-stranded DNA containing bulged bases. DNAs 27 base pairs in length containing 1–4-base cytosine bulges (Fig. 1) were prepared by annealing a ^{32}P-labeled single-stranded DNA to its complementary strand. These duplexes were further treated with T4 DNA polymerase to produce blunt-ended DNA molecules and also to digest any free single-stranded DNA. Upon incubation with RecA protein in the presence of ATPγS, DNA with a 1-base cytosine bulge showed similar kinetics of RecA binding as compared to DNA lacking a bulge (Fig. 1). RecA bound to DNA containing 2- or 3-base cytosine bulges with similar kinetics, and both showed higher levels of binding compared to the control DNA. DNA containing a 4-base cytosine bulge showed even higher binding (~30% more than the control). These results suggest that RecA protein recognizes the perturbation produced by the bulged bases.

Gel electrophoretic studies have demonstrated that single purine bulges in DNA cause greater retardation than single pyrimidine bulges (19). This suggests that purine bulges introduce kinks of a greater angle into the DNA helix as compared to pyrimidine bulges. To determine whether any correlation exists between the kinking angle and RecA binding, a set of oligonucleotides containing 1–4-base adenine bulges was prepared. The degree of kinking produced by these bulges was compared to DNA containing 1–4-base cytosine bulges by gel electrophoresis, and the nitrocellulose filter binding assay was used to measure the binding of the bulged DNA by RecA protein.

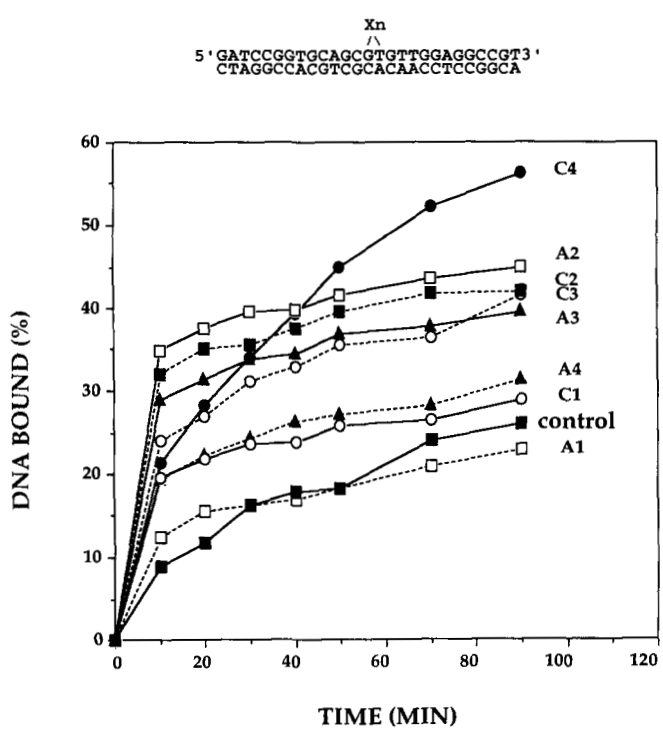

FIG. 1. **Binding of RecA to DNAs containing 1–4-base cytosine or adenine bulges.** Duplex DNAs containing central 1–4-base cytosine bulges (*C1–C4*) and 1–4-base adenine bulges (*A1–A4*) and the control DNA were prepared by annealing the ^{32}P-labeled upper strand (the strand containing extra bases) with the complementary lower strand (see "Materials and Methods"). The labeled DNA (4 μg/ml) was incubated with RecA protein at a concentration of 240 μg/ml in the presence of ATPγS at a final concentration of 200 μM. The efficiency of RecA binding was determined by the filter binding assay.

DNA duplexes containing 1–4-base adenine or cytosine bulges and the control DNA were ligated head to tail, and their mobilities on 15% polyacrylamide gels were examined (Fig. 2). All bulged DNAs showed slower gel mobilities than the non-bulged control, and the DNAs containing adenine bulges showed greater gel retardation than the cytosine bulged DNA. The differences in retardation between adenine and cytosine bulged DNAs increased as the number of bases in the bulge increased from 1 to 4. These results showed that, up to 4 bases, adenine bulges produce a greater degree of kinking compared to bulged cytosines, confirming our earlier results (19).

The kinetics of RecA protein binding to DNAs containing either 4-base adenine or cytosine bulges shown in Fig. 1 demonstrated that RecA bound to DNA containing a 4-base cytosine bulge much more efficiently than to DNA with a 4-base adenine bulge, although 4-base adenine bulges produce kinks of a greater angle than 4-base cytosine bulges. The results suggest that the degree of kinking is not the only determinant of RecA binding. Two possible explanations are 1) cytosine bulges might accommodate certain conformations that allow RecA to bind more efficiently, and 2) the larger kinking angle produced by 4-base adenine bulges might inhibit the binding of RecA.

To examine the conformation of DNAs containing 4-base adenine or cytosine bulges, structural probing with DEP and hydroxylamine was carried out. DEP reacts with adenines that are not base-paired (16, 23, 36), while hydroxylamine has been shown to react specifically with exposed cytosines (23, 37). DNA containing a central 4-base adenine bulge was ^{32}P-labeled at the 5′-end of the bulge-containing strand and then was incubated with DEP at 37 °C for 10 min in the RecA reaction buffer; DNA containing a 4-base cytosine bulge was probed with hydroxylamine under the same conditions. The modified DNAs were cleaved with piperidine and analyzed by 8.3 M urea, 20% polyacrylamide gel electrophoresis. The results (Fig. 3) suggested that the 4 cytosine bases in the bulge have similar reactivities to hydroxylamine, whereas the 4 adenines in the bulge showed different chemical reactivities, with the 5′-adenine being less sensitive. The difference in conformation between DNAs containing 4-base adenine or cytosine bulges could thus account for the differential binding of RecA, but these results do not rule out the possibility that 4-base adenine bulges produce kinking angles too large to allow the most efficient binding of RecA protein.

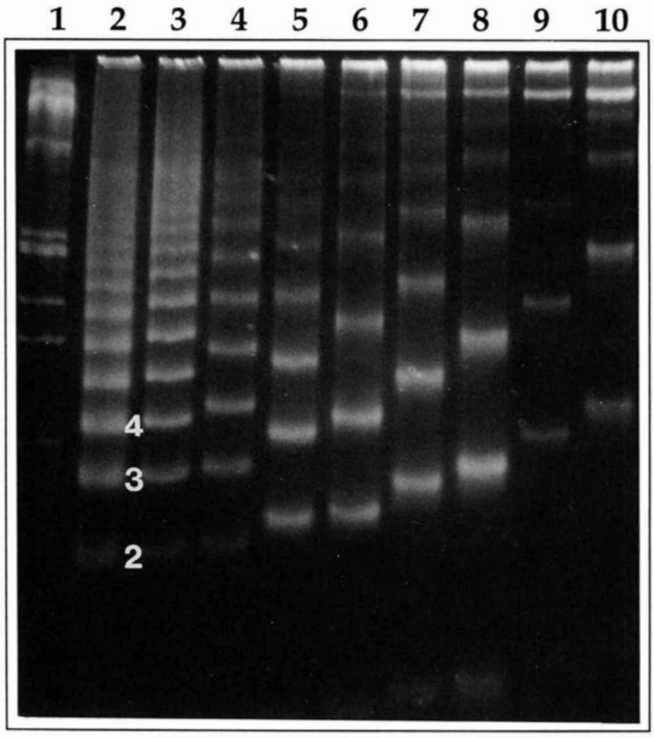

FIG. 2. **Electrophoretic analysis of bending of DNAs containing 1–4-base adenine or cytosine bulges.** DNAs 27 base pairs in length containing central base bulges were prepared, ligated head to tail, electrophoresed on a 15% polyacrylamide gel, and then visualized by staining with ethidium bromide. *Lane 1*, *Hae*III digest of ϕX174 DNA; *lane 2*, a control DNA containing no bulged bases (the numbers indicate dimer, trimer, and tetramer bands); *lanes 3*, *5*, *7*, and *9*, DNAs containing 1–4-base cytosine bulges, respectively; *lanes 4*, *6*, *8*, and *10*, DNAs containing 1–4-base adenine bulges, respectively.

To examine whether the greater kinking angle produced by 4-base adenine bulges might inhibit RecA binding, the binding of RecA to DNAs containing 1–4-base adenine bulges was compared to the binding to DNA with 1–4 cytosines in a bulge (Fig. 1). Interestingly, RecA bound to 2- and 3-base adenine bulged DNA with higher affinity than to the 4-base adenine bulged DNA. These results suggest that the greater kinking angle produced by 3- and 4-base adenine bulges may inhibit the binding of RecA.

Structural Probing of Bulged DNAs within the RecA Filament—To examine the state of the bulged DNA within the RecA protein filament, DEP was used to probe the accessibility of the adenines in the strand opposite the strand containing the 4 extra adenines or cytosines. DNAs containing 4-base adenine or cytosine bulges were prepared (the lower strand was labeled with ^{32}P at the 5′-end) and incubated with RecA protein under the conditions used for the filter binding assays. For each sample, three conditions were examined: no RecA protein and incubation with RecA for 10 min and for 80 min. DEP was added at 37 °C for an additional 10 min, and the modified DNAs were cleaved with piperidine and separated by urea-20% polyacrylamide gel electrophoresis. The autoradiogram of the gel is shown in Fig. 4*A*, and the densitometry scan is in Fig. 4*B*. In the absence of RecA protein (Fig. 4, *A*, *lanes 2* and *3*; and *B*, *lower panels*), only the adenine immediately adjacent to the bulge was accessible to DEP, indicating local distortion around the bulge. When RecA protein bound to DNAs containing 4 bulged adenines or cytosines, the regions on both sides of the bulge became sensitive to chemical cleavage. Moreover, especially in samples incubated with RecA for 80 min, the degree of reactivity on the 3′-side of the bulge was different between 4-base adenine and cytosine bulged DNAs, but was similar on the 5′-side of the bulge (Fig. 4, *A*, *lanes 6* and *7*; and *B*, *upper panels*). Similar results were also observed when the upper strand of the DNA duplex was labeled (data not shown). Thus, the binding of RecA protein to the bulge-containing DNA opens the helix on both sides of the bulge for at least 14 base pairs, and it appears that the growth of the RecA filament on the double-stranded DNA may have a 5′ to 3′ polarity relative to the strand containing the bulge.

RecA Binding to DNAs Containing Mismatches—Although NMR, x-ray crystallographic, and gel electrophoretic studies show that a single base mismatch does not produce kinks in the DNA helix (reviewed above), it was of interest to examine whether RecA protein would recognize the subtle instability of a single mismatched base pair. DNAs containing the eight possible mismatches flanked by either G·C or A·T neighbors were prepared (Fig. 5*A*), and filter binding assays of the 16 DNAs plus the control were performed. A 40-min time course for DNAs containing single base mismatches flanked by A·T or G·C base pairs is shown in Fig. 5 (*B* and *C*). These results clearly demonstrate that RecA protein can recognize mismatched bases and further show preferential binding among mismatched DNAs. For example, RecA bound to the DNA containing a single A·C mismatch with A·T flanking base pairs

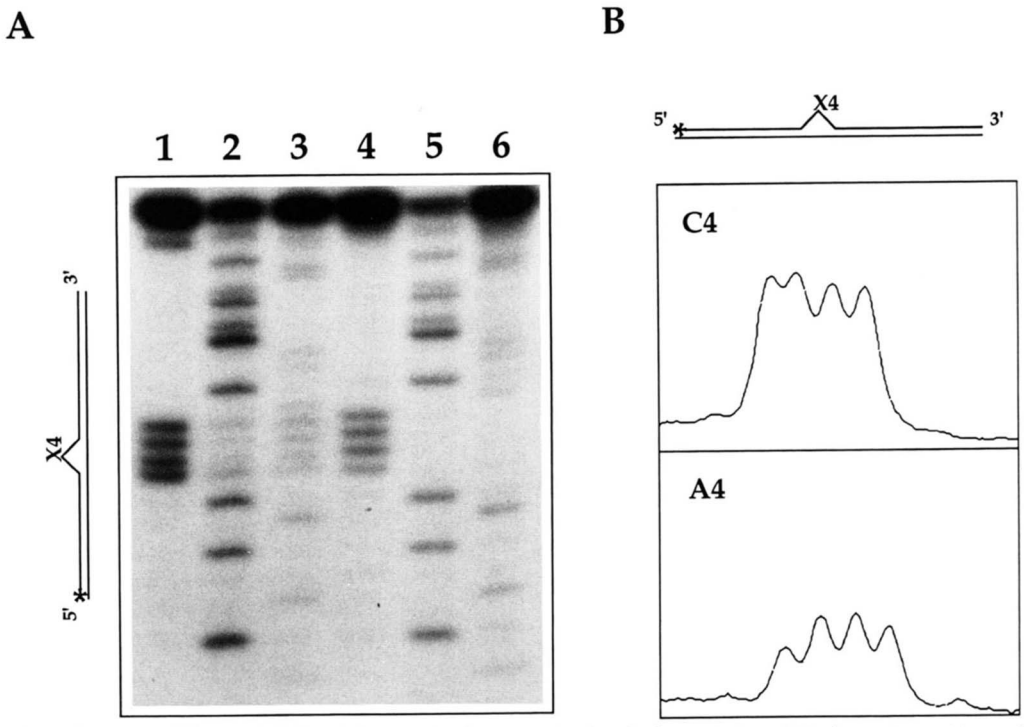

FIG. 3. **Chemical probing of DNAs containing 4-base adenine or cytosine bulges.** *A*, in a 100-μl reaction, 400 ng of ^{32}P-labeled DNA containing 4-base cytosine (*lane 1*) or adenine (*lane 4*) bulges was treated with 5 μl of DEP and 34 μl of hydroxylamine, respectively, at 37 °C for 10 min in the RecA reaction buffer. The position of the ^{32}P label is indicated (*). The reaction mixtures were terminated by ethanol precipitation, further treated with 1 M piperidine to cleave the modified bases, and finally separated by 8.3 M urea, 20% polyacrylamide gel electrophoresis. *Lanes 2* and *3*, G and C + T base-specific sequencing reactions of 4-base cytosine bulged DNA, respectively; *lanes 5* and *6*, G and C + T base-specific reactions for DNA containing 4-base adenine bulges, respectively (35). *B*, densitometry of *lane 1* (*C4*) and *lane 4* (*A4*) of the gel.

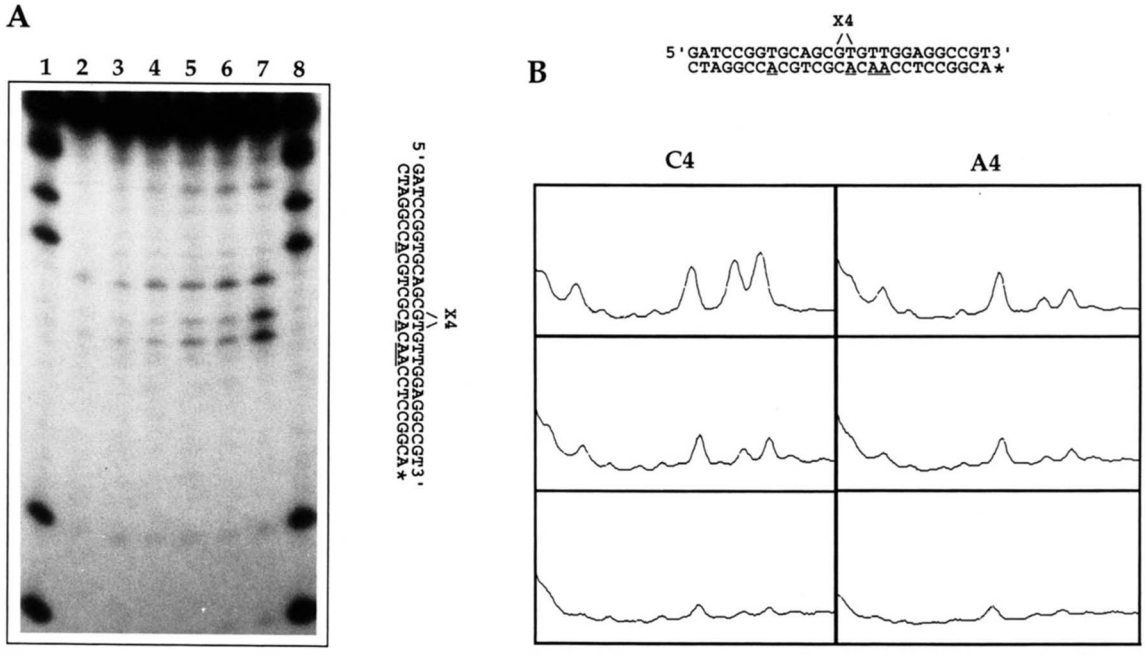

FIG. 4. **DEP probing of bulged DNA-RecA complexes.** *A*, DNA containing 4-base adenine (*lanes 2*, *4*, and *6*) or cytosine (*lanes 3*, *5*, and *7*) bulges was prepared (the lower strand was labeled with ^{32}P at the 5′-end, as indicated (*)), and RecA binding reactions were performed under the same conditions as for the filter binding assay. Three conditions were examined for each DNA sample: no RecA protein (*lanes 2* and *3*) and incubation with RecA for 10 min (*lanes 4* and *5*) and for 80 min (*lanes 6* and *7*). DEP was added at 37 °C for an additional 10 min, and the modified DNAs were cleaved with 1 M piperidine and separated by 8.3 M urea, 20% polyacrylamide gel electrophoresis. *Lanes 1* and *8*, G-specific cleavage reactions to serve as markers (35). *B*, densitometry of the autoradiogram. *Lower panels*, no RecA protein; *middle panels*, RecA binding for 10 min; *upper panels*, RecA binding for 80 min.

8-fold more efficiently than to a DNA containing a C·C mismatch with G·C flanking base pairs at 40 min (61 *versus* 7%). The effect of mismatches on the binding of RecA to the mismatch-containing DNAs can be divided into three classes: 1) promoting RecA binding (the A·C mismatched DNA with A·T flanking base pairs is one example of this class), 2) inhibiting RecA binding (the T·T mismatched DNA with A·T flanking base pairs represents this group of mismatches), and 3) no effect (for example, in a G·T mismatch with A·T flanking base pairs, RecA bound to the mismatched DNAs and the control DNAs

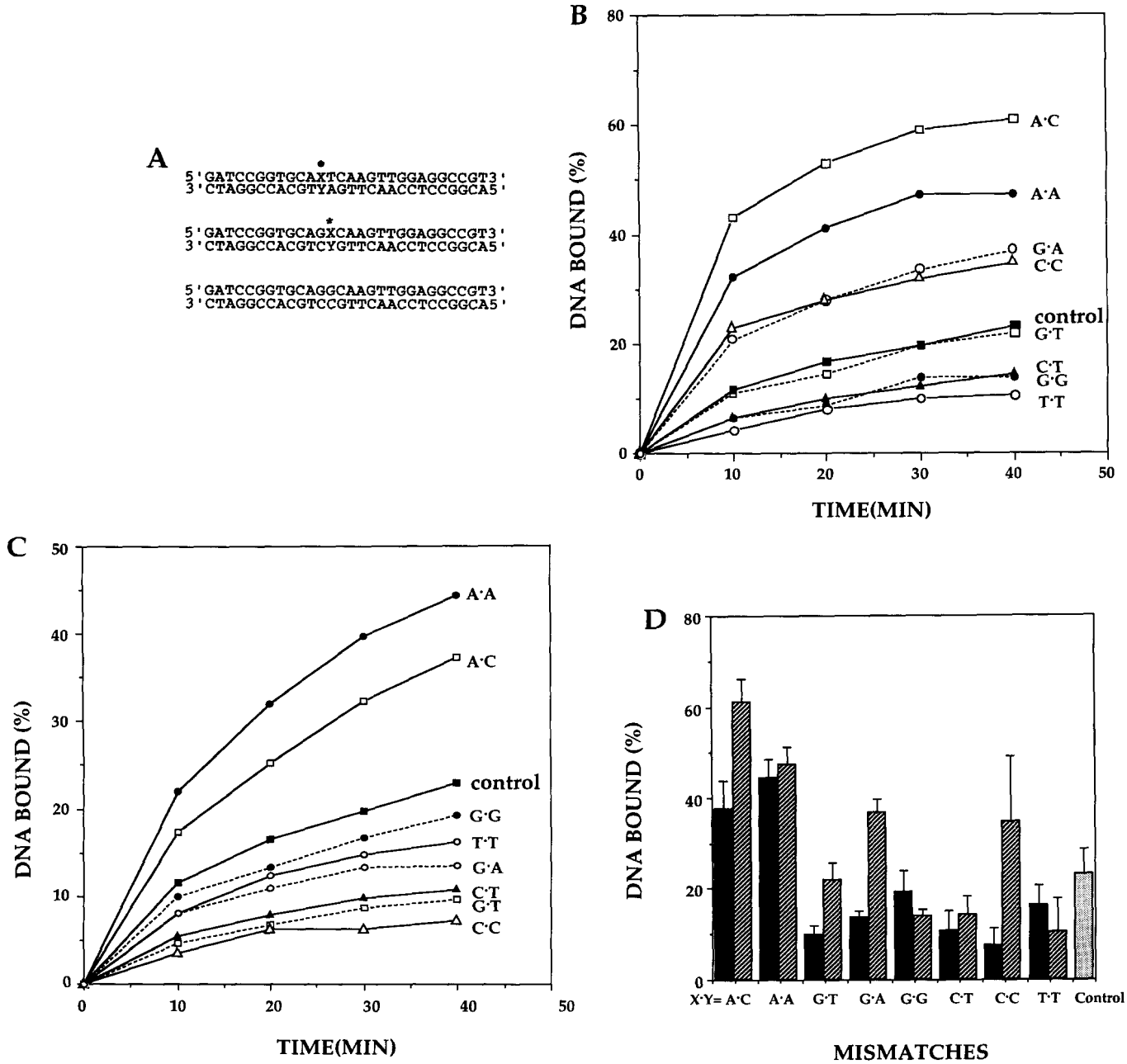

FIG. 5. **Binding of RecA to DNAs containing single base mismatches.** *A*, sequences of DNAs containing eight possible mismatches (positions indicated by *X·Y*) with 2 different flanking base pairs (G·C or A·T) and the control DNA (the lower sequence) are shown; *B*, time course analysis of RecA binding to eight possible mismatched DNAs flanked by A·T base pairs and the control DNA; *C*, time course analysis of RecA binding to eight possible mismatched DNAs flanked by G·C base pairs and the control DNA; *D*, the 40-min time points of RecA binding to all 16 mismatched DNAs and the control DNA. *Solid bars*, mismatches flanked by G·C base pairs; *hatched bars*, mismatches flanked by A·T base pairs.

with similar efficiency).

To compare the effect of the flanking base pairs on the binding of RecA protein to the mismatch-containing DNAs, the 40-min time points of all 17 DNAs were compared in a single histogram (Fig. 5*D*). Each data point shown was the average of at least three separate reactions. In all cases, RecA bound to DNAs with A·T flanking base pairs with equal or better efficiency than to DNAs containing the same mismatch, but with G·C flanking base pairs. In general, the efficiency of RecA binding to mismatch-containing DNAs correlated with the known thermal stability of the mismatches and flanking base pairs (38).

To compare the binding of RecA protein to DNAs containing either bulges or mismatches, the filter binding assay was used with two bulged DNAs (1- and 4-base cytosine bulges), two mismatch-containing DNAs (A·C and G·T mismatches flanked by A·T base pairs), and the control DNA. As shown in Fig. 6, the DNA containing the A·C mismatch showed a RecA binding pattern that was very similar to the 4-base cytosine bulged DNA, whereas RecA protein bound to the G·T mismatched DNA, the 1-base cytosine bulged DNA, and the control DNA with equal affinity. It has been shown that a 4-base cytosine bulge produces marked distortion in the DNA helix (Fig. 2), whereas no significant pertubation could be detected in the A·C mismatched DNA (17). Here, however, we observe that RecA protein recognizes 4-base cytosine bulges equally well compared to the A·C mismatch flanked by A·T base pairs.

The results of DEP probing of DNA-RecA complexes containing A·C mismatches with either A·T or G·C flanking base pairs and the control DNA are shown in Fig. 7. Chemical probing

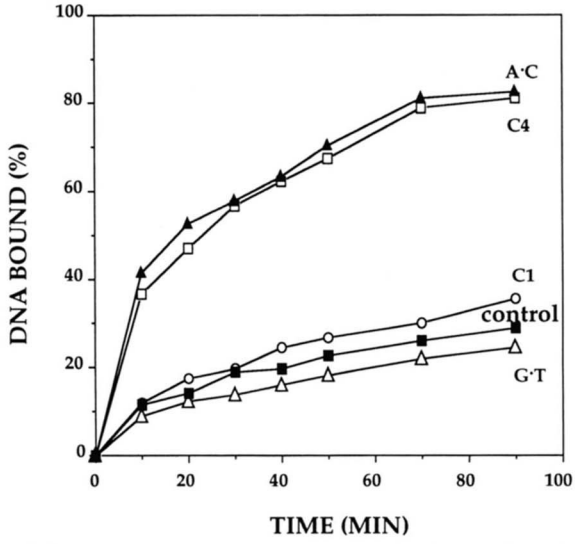

FIG. 6. **Comparison of RecA binding to bulged and mismatched DNAs.** DNAs containing 1-base cytosine bulges (*C1*), 4-base cytosine bulges (*C4*), an A·C mismatch flanked by A·T base pairs (*A·C*), and a G·T mismatch flanked by A·T base pairs (*G·T*) and the control DNA were compared using the filter binding assay as described for Fig. 1.

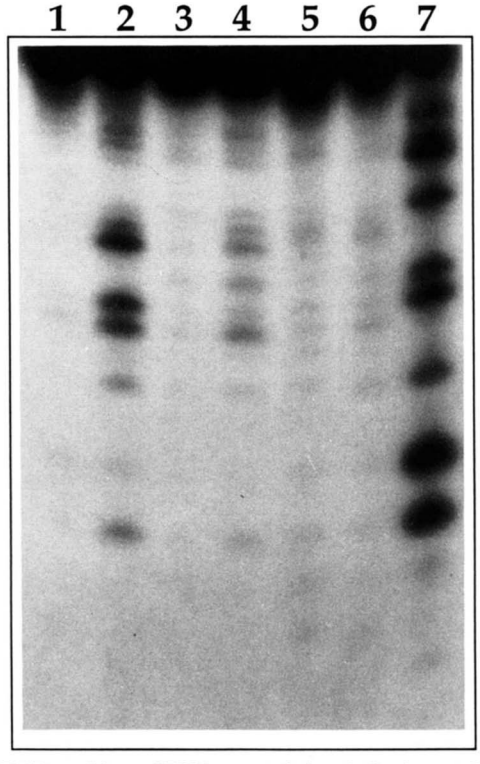

FIG. 7. **DEP probing of DNAs containing A·C mismatches.** DNAs containing an A·C mismatch flanked by A·T base pairs (*lanes 1* and *2*) or by G·C base pairs (*lanes 3* and *4*) and the control DNA (*lanes 5* and *6*) were incubated without (*lanes 1*, *3*, and *5*) or with (*lanes 2*, *4*, and *6*) RecA protein for 40 min at 37 °C. DEP was added at 37 °C for an additional 10 min, and the modified DNAs were cleaved with piperidine and analyzed by electrophoresis on an 8.3 M urea, 20% polyacrylamide gel. *Lane 7*, a G-specific cleavage reaction to serve as markers (35).

demonstrated that the adenines in the mismatch and in flanking base pairs became more accessible to chemicals when both types of mismatched DNA were bound by RecA protein, whereas the control DNA showed no difference in its cleavage pattern upon RecA binding. Densitometry revealed that in the presence of RecA protein, the mismatched adenines in the DNAs containing mismatches flanked by A·T or G·C base pairs showed 10- and 3-fold increases in sensitivity to DEP, respectively. This increased accessibility among these DNAs agreed with the increased binding of RecA shown by the filter binding assay.

DISCUSSION

Here we have used a combination of filter binding and chemical probing techniques to investigate the ability of RecA protein to bind to and alter the structure of DNA containing multiple base bulges and single base mismatches. We show that RecA protein binds to DNA containing some bulges or single base mismatches with much higher efficiency than to the perfectly paired duplex DNA controls. The binding of RecA protein to DNA containing bulges is affected by the composition and number of bases in the bulge. These factors determine the conformation of the bulged bases and the kinking angles produced by the bulges, which (as shown here) strongly affect the binding of RecA protein. For the mismatch-containing DNAs, DNAs containing single base mismatches flanked by A·T base pairs showed equal or better efficiency of RecA binding than when the mismatches were flanked by G·C base pairs. Among the eight possible mismatches flanked by A·T base pairs, the A·C, A·A, G·A, and C·C mismatches promoted RecA protein binding in the presence of ATPγS. In the absence of RecA protein, the bases in and immediately adjacent to the bulges showed sensitivity to DEP and hydroxylamine. However, following RecA binding to the lesion-containing DNA, the DNA acquired sensitivity to these chemicals over at least 14 base pairs. Comparison of the chemical sensitivity of two DNAs, one containing a 4-base adenine bulge and the other a 4-base cytosine bulge, while bound by RecA protein revealed a similar chemical accessibility at the 5′-side of the bulges, but higher accessibility at the 3′-side of the bulge for the cytosine bulged DNA. Chemical probing of DNAs containing a single A·C mismatch flanked by either A·T or G·C base pairs showed the mismatch-containing DNA to be more sensitive, in particular when bound by RecA protein.

In previous studies of others using NMR, gel electrophoresis, and x-ray crystallography, it was concluded that single base mismatches do not create structural distortions in the DNA helix (see above). Here, however, we have shown that RecA protein binds preferentially to certain mismatch-containing DNAs and thus may "see" very subtle perturbations in the DNA helix in addition to more global distortions such as B-Z junctions, intercalated drugs, and photodamage. Indeed, some severe distortions in the helix may inhibit RecA protein binding as evidenced by the greater binding of RecA to a DNA containing a 2-base adenine bulge as contrasted to a 4-base adenine bulge. Among the 16 mismatch-containing DNAs that were examined, the effect of mismatches on the binding of RecA protein could be classified into three groups: promoting RecA binding, inhibiting the binding, and no effect. Aboul-ela *et al.* (38) examined the thermostability of eight possible mismatches in DNA and showed that single A·C and C·C mismatches are the least stable ones. An effect of flanking sequences on the binding of RecA protein to mismatched DNAs was also observed in this study. RecA bound to DNAs containing single base mismatches flanked by A·T base pairs with equal or better efficiency than to the G·C base-paired counterpart. Therefore, our results (Fig. 5) using RecA binding as a probe generally parallel the decreased thermostability of the mismatches and their flanking sequences, although some differences were observed. Clearly in addition to the nature of the mismatch, the sequence context within which it lies will be highly important in determining the ability of RecA protein to bind.

We found that the DNA containing a single A·C mismatch flanked by A·T base pairs showed the highest RecA binding

among all the mismatch-containing DNAs examined (Fig. 5) and produced a degree of RecA binding equal to that of a DNA containing a 4-base cytosine bulge (Fig. 6). It has been shown that the pertubation produced in a DNA helix by a single A·C mismatch is far less than for a 4-base cytosine bulge (Fig. 2) (17). The binding of RecA protein to bulges and mismatches is not as specific as the binding of UvrAB protein to thymine dimers or MutS protein to single base mismatches. However, our results suggest that the nucleation step of RecA binding to damage-containing DNA depends not only on the pertubations produced in the DNA helix by these damages, but also on specific recognition of certain lesions by RecA protein. A difference in the values for the binding of RecA protein to the 4-base cytosine bulged DNA was observed in Fig. 1 compared to Fig. 6, which could be due to the use of different preparations of RecA protein and the age of the protein samples used in these two experiments. Therefore, we routinely perform the binding reactions with DNAs for which comparisons are being made within the same experiment. Su *et al.* (39) showed that in the methyl-directed mismatch repair system, the binding affinity of MutS protein to the site of the mismatch parallels the efficiency with which the mismatch is corrected, with the exception of an A·C mismatch. They observed that MutS protein binds at the site of an A·C mismatch 7-fold better than at a C·T mismatch, yet the repair efficiency was similar for both. In the future, it will be interesting to determine if RecA protein facilitates or inhibits the repair of certain mismatches by the methyl-directed mismatch repair system. Possibly the stronger binding of RecA protein to the A·C mismatch than to others may occlude the binding of MutS protein *in vivo* and result in less efficient repair of this mismatch.

RecA protein assembles onto single-stranded DNA in a 5′ to 3′ direction (40–42), the same direction as that of the strand-exchange reaction relative to the single-stranded DNA (43–45). Chemical probing of DNAs containing 4-base adenine and cytosine bulges revealed that in the presence of RecA protein, the flanking sequences on the 5′-side of the bulge for both DNAs had similar chemical accessibility. However, the flanking sequences on the 3′-side of the bulge showed different chemical accessibility, suggesting that the assembly of RecA protein onto double-stranded DNA has a 5′ to 3′ directionality relative to the strand containing the bulge.

The chemical probing experiments revealed that the N-7 group of the adenines in the DNA-RecA complexes was available for DEP modification, implying that RecA protein does not make close contact with this group. However, in the same complexes, the cytosines of the flanking sequences were not accessible to hydroxylamine (data not shown), and these data might indicate that in the DNA-RecA protein complexes, RecA protein interferes with the interaction between the 5–6 bond of cytosines and hydroxylamine and suggest that RecA protein is in close proximity to this group.

Echols and co-workers have demonstrated that RecA protein shows preferential binding to DNAs containing thymine dimers (4) and (6-4) photoproducts (5) and proposed a model in which the binding of RecA protein at the damaged sites could inhibit the editing activity of polymerase III and allow *trans*-lesion replication (5). Recently, *in vitro* studies have shown that polymerase III is able to by-pass a single abasic DNA lesion in a reconstituted system with RecA, UmuC, and UmuD′ proteins (6). However, in the case of bulges and mismatches, these lesions become "transparent" when the two strands of DNA are separated during replication. Therefore, bulges and mismatches would have to be detected by RecA and other repair systems prior to strand separation. In addition to the direct role of RecA protein in mutagenesis, the binding of RecA protein to damaged DNA may facilitate recombinational repair by increasing the local concentration of RecA protein at the site of the damage.

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