Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 8445–8449, October 1991
Biochemistry

SOS-inducible DNA repair proteins, RuvA and RuvB, of *Escherichia coli*: Functional interactions between RuvA and RuvB for ATP hydrolysis and renaturation of the cruciform structure in supercoiled DNA

(mutagenesis/recombination/DNA-binding protein/protein–protein interaction)

TOSHIKAZU SHIBA, HIROSHI IWASAKI, ATSUO NAKATA, AND HIDEO SHINAGAWA*

Department of Experimental Chemotherapy, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan

Communicated by Evelyn M. Witkin, June 3, 1991 (received for review October 5, 1990)

ABSTRACT **The *ruv* operon is induced by treatments that damage DNA and is regulated by the LexA repressor. It encodes two proteins, RuvA and RuvB, that are involved in DNA repair, recombination in RecE and RecF pathways, and mutagenesis. RuvB protein was previously purified and has ATP-binding activity and weak ATPase activity. To study the biochemical properties of RuvA and its interaction with RuvB, we purified RuvA protein to near homogeneity from an overproducing strain. RuvA bound more efficiently to single-stranded DNA than to double-stranded DNA. RuvA bound to DNA greatly enhanced the ATPase activity of RuvB; the enhancing effect of various forms of DNA was in the order of supercoiled DNA > single-stranded DNA > linear double-stranded DNA. UV irradiation further enhanced the ATPase stimulatory effect of supercoiled DNA dose dependently. The RuvA–RuvB complex has an activity that renatures the cruciform structure in supercoiled DNA. From these experiments and previous work, we infer that the RuvA–RuvB complex may promote branch migration in recombination and may correct irregular structures in DNA, such as cruciforms and hairpins, to facilitate DNA repair using ATP as the energy source.**

Escherichia coli ruv mutants are sensitive to various DNA-damaging agents, such as UV light, ionizing radiation, and chemical mutagens; they form multinucleate filaments after treatment with low doses of DNA-damaging agents (1–3). Nucleotide sequencing of the *ruv* region revealed the two genes, *ruvA* and *ruvB*, that constitute a LexA-regulated operon (4–6). Complementation tests with the mutants showed that both of them are involved in DNA repair (7, 8). *ruv* derivatives of *recBC sbcBC* and *recBC sbcA* are defective in genetic recombination, although *ruv* single mutants are nearly as proficient as the wild-type strain in recombination (9, 10). From these observations, it was inferred that the *ruv* operon is involved in a recombinational process for repair of damaged DNA along with other SOS-regulated recombination genes such as *recA*, *recN*, and *recQ* (11).

Since *ruv* strains can convert low molecular weight DNA produced immediately after irradiation into high molecular weight DNA (2), the deficiency would have to be in a step in the repair process subsequent to strand rejoining (9). It was suggested that *ruv* gene products may be involved in a late step of recombination such as resolution of the Holliday structure, a recombination intermediate with crossover junctions (9, 10, 12).

The *ruvAB* mutants were recently shown to be less sensitive to mutagenesis by UV and γ irradiation, and by some chemicals, than the wild-type strain (ref. 8; H.I., A.N., and H.S., unpublished results). These multifunctional properties of the *ruvAB* genes are reminiscent of those of the *recA* gene (13).

We started biochemical studies on RuvA and RuvB proteins to define the functions of these proteins in DNA repair, recombination, and mutagenesis. We have purified RuvB protein and demonstrated that it binds to ATP and ADP and has weak ATPase activity (14), which was predicted from the amino acid sequence (5, 6).

In this work, we purified RuvA protein and characterized its biochemical properties. We first studied the interaction of RuvA with various forms of DNA and also the functional interaction between RuvA and RuvB for ATPase activity. We then examined whether RuvA and RuvB had any effects on the cruciform structure in supercoiled DNA, which is similar in structure to the junction of the Holliday structure (15, 16).

MATERIALS AND METHODS

DNAs. Supercoiled DNA of M13mp18 (17), pBR322 (18), and pUC4 (19) were prepared by two cycles of CsCl equilibrium centrifugation with ethidium bromide (20). ^{3}H-labeled pBR322 DNA was prepared as described (21). Linear double-stranded DNAs (dsDNAs) were obtained by cutting supercoiled M13mp18 DNA with *Hin*cII and pBR322 with *Pvu* II. Single-stranded DNA (ssDNA) was isolated from M13mp18 phage particles as described (20) and further purified by hydroxylapatite (Koken; Tokyo) column chromatography. ssDNA was also prepared by heating ^{3}H-labeled linear pBR322 DNA at 100°C for 3 min and cooling it quickly on ice. All DNA concentrations are expressed in terms of the nucleotide concentration. DNA concentrations were calculated by taking an A_{260} of 1 as equivalent to 50 μg of dsDNA per ml and an A_{260} of 1 as equivalent to 36 μg of ssDNA per ml (22).

RuvB Protein. RuvB protein was purified from the overproducing strain as described and was >97% pure (14).

Other Materials. [α-^{32}P]ATP and [*methyl*-^{3}H]thymidine were purchased from Amersham; polyethylenimine (Polymin P) was obtained from BRL; nitrocellulose filters (HAWP25XX, pore size 0.45 μm) were purchased from Millipore; and polyethylenimine-cellulose plates were from Merck. All restriction and DNA modifying enzymes were obtained from Takara-Shuzo (Kyoto).

Construction of a Plasmid That Overproduces RuvA Protein. To construct a plasmid system that overproduces RuvA protein under the control of the *lac* promoter in pUC19 (17), a region upstream of the *ruvA* gene including the SOS box was removed, and the *ruvA* coding region was placed just

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; IPTG, isopropyl β-D-thiogalactoside.

*To whom reprint requests should be addressed.

downstream of the *lac* promoter as follows. Plasmid pHS202 (5), which contains nucleotides 1–1010 (the *Pvu* II–*Kpn* I chromosome fragment that encompasses *ruvA*) in the *Sma* I–*Kpn* I site of pUC19, was digested with *Bam*HI and *Pst* I. Various deletions from the upstream region of the *ruvA* gene on pHS202 were prepared by exonuclease III digestion followed by treatment with mung bean nuclease. After the protruding ends were filled in by Klenow polymerase, they were circularized with T4 DNA ligase and transformed into *E. coli*. Plasmids purified from the transformants were digested with *Eco*RI and *Hin*dIII, and the 700- to 800-base-pair (bp) DNA fragments, which are approximately the size of the fragments that delete the SOS box and still retain the intact *ruvA* coding region (5), were isolated by electrophoresis. The DNA fragments were ligated into the *Eco*RI–*Hin*dIII site of pUC19, and the resultant plasmids were transformed into UT481 (*lacI*^{q}, *lon*; ref. 23). Overproduction of RuvA protein in the lysates of the transformants grown in the presence of isopropyl β-D-thiogalactoside (IPTG) was analyzed by NaDodSO$_4$/PAGE. Plasmid DNAs were purified from the clones that overproduced a 22-kDa protein, which was likely to be RuvA (5, 6). One of the plasmids, pHS312, was found to carry the *ruvA* region from nucleotide 346 to nucleotide 1010 of pHS202 by DNA sequencing.

RuvA Purification. UT481 carrying pHS312 was grown at 37°C to an OD$_{600}$ of ≈0.4 in 3 liters of LB medium containing ampicillin. IPTG was added to the culture, and the culture was incubated for 10 hr. The bacteria were harvested by centrifugation. Subsequent steps were done at 4°C. The wet cell paste was suspended in 50 ml of R buffer (20 mM Tris·HCl, pH 7.5/0.1 mM EDTA/1 mM dithiothreitol/10% glycerol) containing 40 mM NaCl, and the cells were disrupted by sonication. The suspension was centrifuged at 31,000 × *g* for 50 min. Polymin P at pH 7.9 was added to the supernatant to 0.25%. After being stirred for 1 hr, the suspension was centrifuged at 25,000 × *g* for 20 min. The pellet was resuspended in R buffer containing 600 mM NaCl, stirred for 30 min, and centrifuged at 25,000 × *g* for 20 min. Ammonium sulfate was added to the supernatant to a final concentration of 60% saturation; the supernatant was stirred for 1 hr and centrifuged at 20,000 × *g* for 20 min. The pellet was resuspended in R buffer containing 50 mM NaCl and dialyzed against the same buffer. Precipitate formed during the dialysis was collected by centrifugation at 12,000 × *g* for 15 min. The pellet was dissolved in N buffer (20 mM Na_2HPO_4/NaH_2PO_4, pH 6.8/2 mM 2-mercaptoethanol/200 mM NaCl/10% glycerol) and dialyzed against the same buffer. The dialyzed solution was put on a hydroxylapatite column preequilibrated with N buffer. A linear gradient from 20 to 600 mM phosphate was used for elution. RuvA fractions eluting between 290 and 320 mM phosphate were pooled and dialyzed against storage buffer (20 mM Tris·HCl, pH 7.5/0.1 mM EDTA/2 mM 2-mercaptoethanol/200 mM NaCl/50% glycerol).

DNA Binding Assay. Binding of RuvA to DNA was measured by the protein-mediated alteration in the mobility of DNA during agarose gel electrophoresis (22). The standard assay (10 μl) contained in buffer A (20 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/0.1 mM EDTA/50 mM NaCl/2 mM 2-mercaptoethanol) 50 ng of DNA and proteins as indicated. The mixture was incubated at 37°C for 15 min, mixed with 2 μl of loading buffer (15% glycerol/0.1% bromphenol blue), and analyzed by electrophoresis in 0.7% agarose. DNA was stained with ethidium bromide and photographed under UV light. Binding efficiencies of RuvA for different types of DNA were measured by the filter-binding assay (22). Alkali-treated Millipore filters (24) were soaked in buffer A for 30 min prior to use. Reaction mixtures (100 μl) contained ^{3}H-labeled pBR322 DNA and RuvA in buffer A as indicated. The reaction mixtures were incubated at 37°C for 15 min, put on the filter under suction, and washed with 2.5 ml of buffer A. Filters were dried and assayed for radioactivity.

ATPase Assay. The ATPase activity of RuvB protein was assayed by measuring the radioactivity of ADP and ATP in the reaction products, which were separated by thin-layer chromatography, as described (14). The reactions were done in buffer A at 30°C for 30 min.

Assay for Renaturation of Cruciform DNA. Standard reaction mixtures (15 μl) contained preheated pUC4 DNA (15.2 μM), RuvA (2 μM), RuvB (1 μM), and ATP (0.67 mM) in an appropriate buffer as indicated. The mixtures were incubated at 37°C for 5 min, then mixed with *Pst* I (1 unit), and further incubated for 40 min. Reactions were stopped by adding 4 μl of the gel-loading buffer containing 0.1% NaDodSO$_4$. Samples were analyzed by electrophoresis in 0.7% agarose gels. DNA was stained with ethidium bromide.

RESULTS

Overproduction and Purification of RuvA. In the RuvA-overproducing plasmid pHS312, the *lac* promoter and the *ruvA* gene were connected at a site 5 bp downstream of the *Sph* I site in pUC19 and 14 bp upstream of the AUG initiator codon of *ruvA*. The amount of RuvA in *E. coli* UT481 carrying pHS312 reached about 7% of the total cell protein after incubation with IPTG for 10 hr (Fig. 1).

The purification of RuvA was followed by NaDodSO$_4$/PAGE (Fig. 1). The induced lysate (Fig. 1, lane 2) was centrifuged, and the supernatant (Fig. 1, lane 3) was fractionated by precipitation with Polymin P. The proteins were eluted from the precipitate with R buffer containing 0.6 M NaCl (Fig. 1, lane 4) and precipitated with 60% ammonium sulfate (Fig. 1, lane 5). The pellet was dialyzed against R buffer, and the major component of the precipitate was RuvA (Fig. 1, lane 6). This property of RuvA helped in its purification. The precipitate was dissolved in N buffer and purified by hydroxylapatite column chromatography (Fig. 1, lane 7). We estimate that the final fraction was about 97% RuvA protein by densitometric analysis of the stained gel.

The amino acid sequence from the amino terminus of the purified protein was analyzed by a gas-phase amino acid sequencer (model ABI477A; Applied Biosystems). The 10 residues from the amino terminus were Met-Ile-Gly-Arg-Leu-Arg-Gly-Ile-Ile-Ile. This sequence is exactly the same as that predicted from the DNA sequence of *ruvA* (5, 6).

RuvA Binds to Both dsDNA and ssDNA. Since *ruvA* is involved in DNA repair and recombination, we examined

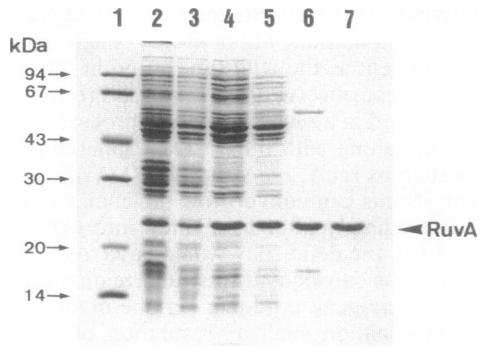

FIG. 1. Purification of RuvA protein. Samples were analyzed by electrophoresis in a 14% polyacrylamide gel and stained with Coomassie brilliant blue. The position of RuvA is indicated by an arrowhead. Successive fractions in the purification of RuvA: lane 1, molecular size markers; lane 2, induced lysate; lane 3, supernatant of the lysate; lane 4, fraction eluted with R buffer containing 0.6 M NaCl from the Polymin P precipitate; lane 5, precipitate with 60% ammonium sulfate; lane 6, precipitate formed during dialysis; lane 7, pooled peak fractions of hydroxylapatite column.

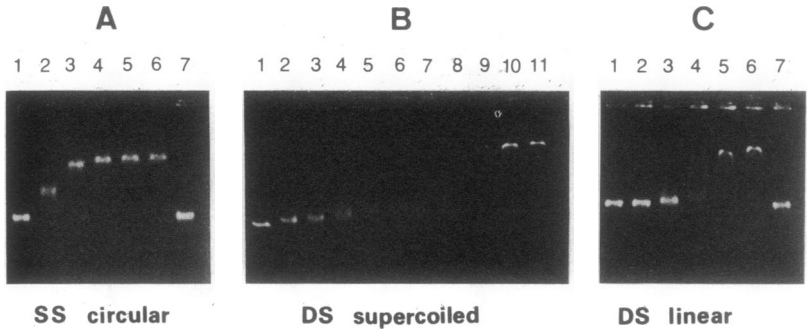

FIG. 2. Analysis of RuvA–DNA binding by gel electrophoresis. Reaction mixtures (10 μl) contained ssDNA (*A*), supercoiled DNA (*B*), or linear dsDNA (*C*) and the indicated amounts of RuvA. The concentration of DNA (M13mp18) in the mixtures was 15.2 μM. (*A* and *C*) Lane 1, no RuvA; lane 2, 1.11 μM RuvA; lane 3, 2.22 μM RuvA; lane 4, 3.33 μM RuvA; lane 5, 4.44 μM RuvA; lane 6, 5.56 μM RuvA; lane 7, bovine serum albumin (4.5 μM) as a negative control. (*B*) Lanes 1–6, same as in *A* and *C*; lane 7, 6.67 μM RuvA; lane 8, 7.78 μM RuvA; lane 9, 8.89 μM RuvA; lane 10, 10.0 μM RuvA; lane 11, 11.1 μM RuvA.

RuvA interaction with DNA by a gel retardation assay. Mixtures of various ratios of RuvA to dsDNA or ssDNA of M13mp18 were analyzed by electrophoresis in an agarose gel (Fig. 2). When the molar ratio of RuvA to ssDNA was 1:15, DNA migrated more slowly in the agarose gel. Increasing the molar ratio of RuvA to ssDNA to 3:15 resulted in conversion of most of the ssDNA into the most slowly migrating complex (Fig. 2*A*). Similarly, binding of RuvA to supercoiled DNA (Fig. 2*B*) and to linear dsDNA (Fig. 2*C*) was observed. Supercoiled DNA appears to require more RuvA for the binding saturation than linear dsDNA. The mobility patterns suggest that many molecules of RuvA can bind to each molecule of ssDNA and dsDNA. We also measured the relative binding efficiency of RuvA to different forms of DNA by the nitrocellulose filter-binding method (Fig. 3). RuvA bound more efficiently to ssDNA than to either form of dsDNA and appeared to bind more efficiently to supercoiled DNA than to linear dsDNA. These results appear to be consistent with the data shown in Fig. 2; the migration of ssDNA in an agarose gel was retarded by the smallest amount of RuvA. The concentration dependency of the binding of RuvA to ssDNA, as studied by the filter assay, suggests the cooperative interaction of RuvA molecules for binding to ssDNA (Fig. 3 *Inset*).

RuvA Bound to DNA Enhances the ATPase Activity of RuvB. Previous work has shown that RuvB has weak ATPase activity (14), and several lines of evidence indicate that RuvA and RuvB may interact functionally with each other in DNA repair *in vivo* (5–8). We examined whether RuvA had any effect on the ATPase activity of RuvB in the presence or absence of DNA (Fig. 4). RuvA itself did not show any ATPase activity in either the presence or absence of DNA. RuvB protein alone had weak ATPase activity as described in a previous paper (14), and this activity was not affected by the addition of DNA. However, addition of both RuvA and supercoiled DNA greatly enhanced the RuvB ATPase activity.

Different forms of DNA were tested for their efficiency as a cofactor for the enhancement of the RuvB ATPase activity by RuvA. At a fixed concentration of RuvB (0.49 μM), the RuvB ATPase was enhanced about 4- to 5-fold by linear dsDNA, 5- to 6-fold by ssDNA, and 8- to 12-fold by supercoiled DNA in the presence of saturating amounts of RuvA (data not shown).

UV Irradiation of Supercoiled DNA Further Enhances the ATPase Activity. Since the products of *ruvAB* are required for the repair of DNA lesions inflicted by UV irradiation, we examined the specific interaction of RuvA with the DNA lesions by measuring the ATPase-enhancing activity of RuvA bound to the DNA. We studied the effects of UV irradiation to various forms of DNA on the ATPase activity. The ATPase assay was done with constant amounts of RuvA and RuvB proteins and DNAs that had been exposed to a range of UV doses (Fig. 5). The RuvB ATPase activity was enhanced by UV irradiation of supercoiled DNA in a dose-dependent fashion. However, no enhancement by UV irra-

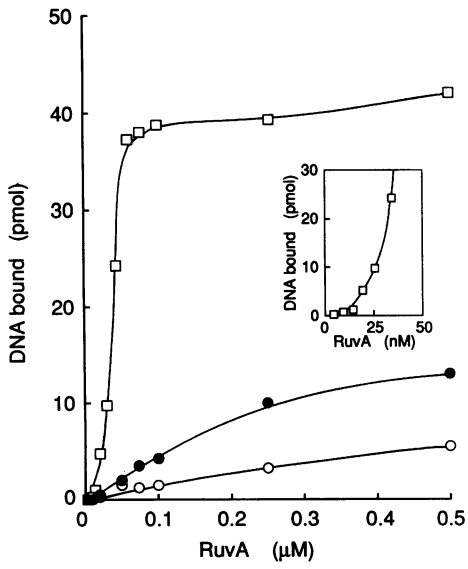

FIG. 3. Binding of RuvA to different forms of pBR322 DNA. ^{3}H-labeled pBR322 DNA (1 μM) was incubated with various concentrations of RuvA, and formation of RuvA–DNA complexes was assayed by the filter-binding method. (*Inset*) Binding of RuvA to ssDNA at lower concentrations of RuvA. ●, Supercoiled DNA; □, ssDNA; ○, linear dsDNA.

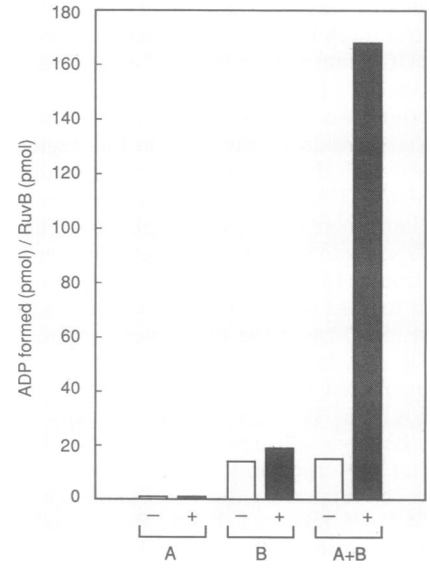

FIG. 4. Effects of RuvA and DNA on RuvB ATPase activity. Reaction mixtures (10 μl) contained only RuvA at 0.68 μM (A), only RuvB at 0.49 μM (B), or RuvA at 0.68 μM and RuvB at 0.49 μM (A+B). +, Addition of 121 μM supercoiled DNA (M13mp18) to the reaction mixture; −, no DNA added. Each reaction mixture contained 150 μM ATP.

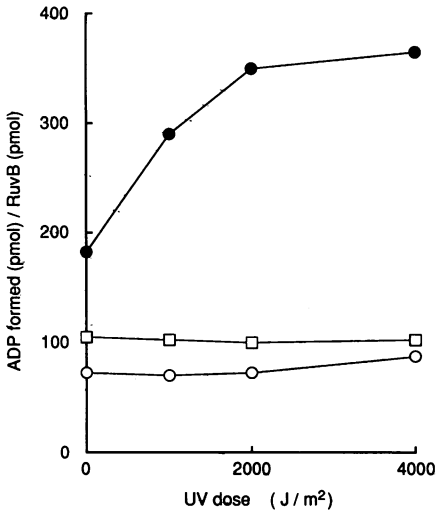

FIG. 5. Enhancement of the ATPase activity of RuvB by UV irradiation to supercoiled DNA. The ATPase activity of RuvB was measured in a reaction mixture containing RuvA (0.68 μM), RuvB (0.49 μM), and M13mp18 dsDNA (121 μM) or M13mp18 ssDNA (61 μM) irradiated with various doses of UV light. ●, Supercoiled DNA; □, ssDNA; ○, linear dsDNA.

diation to either ssDNA or linear dsDNA was observed. Therefore, DNA lesions such as pyrimidine dimers and 6–4 photoproducts (i.e., 6–4 pyrimidine–pyrimidone diadducts) themselves were not responsible for the enhancement, but the conformational changes in the supercoiled DNA brought about by the formation of these adducts might be.

The RuvA–RuvB Complex Renatures Cruciform Structures in Supercoiled DNA. We reasoned that the DNA lesions that disrupt hydrogen bonds between complementary bases in supercoiled DNA might accumulate stress and the stress might be released by forming cruciform or hairpin structures in the DNA. Furthermore, the configuration of the DNA strands at the base of the junction of the cruciform is analogous to that of the Holliday junction, which was suggested to be resolved by the products of *ruvAB* (9). These speculations prompted us to examine whether the RuvA–RuvB complex in the presence of ATP had any effect on the cruciform structures formed in supercoiled DNA.

Plasmid pUC4 contains an inverted-repeat sequence of 48 bp with a unique *Pst* I site at the center of the repeat (Fig. 6*A*). This restriction site allows estimation of the fraction of molecules that contain a cruciform in this region. The cruciform formed with the inverted repeat by heating and slow cooling of pUC4 DNA makes the *Pst* I site single stranded and thus makes it resistant to *Pst* I digestion. The purpose of heating the DNA is to overcome a kinetic barrier to extrusion of the palindrome (15, 16). About 10% of the untreated DNA was resistant to *Pst* I digestion, but after heating the DNA at 60°C for 2 hr, 50–70% of the molecules became resistant to digestion (Fig. 6*B*, lanes 3 and 4). When the heated pUC4 DNA was incubated with the RuvA–RuvB complex in the presence of ATP at 37°C for 45 min, virtually all the DNA appeared to become sensitive to *Pst* I digestion (lane 10), indicating that the cruciform was converted to a standard duplex. The conversion to the *Pst* I-sensitive form of the heated pUC4 DNA required RuvA, RuvB, and ATP, since the conversion did not take place in the absence of any one of them (Fig. 6*B*, lanes 5–10). The RuvA–RuvB complex did not mediate the reaction with adenosine 5′-[γ-thio]triphosphate, which is poorly hydrolyzed (data not shown). These results suggest that the RuvA–RuvB complex renatures the cruciform structure in the supercoiled DNA by using ATP as the energy source to overcome the kinetic barrier as schematically shown in Fig. 6*A*. The disappearance of the band that corresponds to the closed circular form of DNA in Fig. 6*B*, lane 10, was not due to the binding of RuvA–RuvB complex to DNA or to the endonuclease activity of RuvAB, since the presence of the RuvA–RuvB complex did not affect the electrophoretic mobility of the DNA (Fig. 6*B*, lane 13).

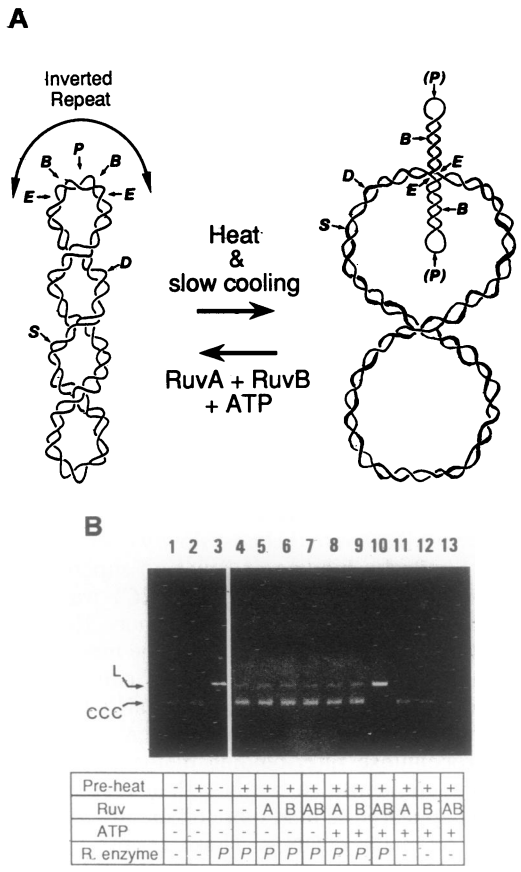

FIG. 6. Renaturation of cruciform structures in pUC4 DNA by the RuvA–RuvB complex. (*A*) Schemes of cruciform formation and readsorption in plasmid pUC4. (*B*) Sensitivity of pUC4 DNA, after various treatments, to *Pst* I digestion as analyzed by agarose gel electrophoresis. pUC4 DNA was heated, then slowly cooled to form the cruciform structure (Pre-heat, +), and treated with RuvA and RuvB (Ruv, AB) in the presence of ATP (ATP, +). The effects of the treatments on the cruciform were examined by the sensitivity of the DNA to *Pst* I digestion (R. enzyme, *P*), which were analyzed by gel electrophoresis. The kinds and conditions of these treatments are indicated at the bottom of each lane. The reactions were done in P buffer (20 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/100 mM NaCl/2 mM 2-mercaptoethanol). The faint bands observed near the position of linear DNA in lanes 1, 2, 11, 12, and 13 are open circular DNA. CCC, covalently closed circular DNA; L, linear DNA; R. enzyme, restriction enzyme; P, *Pst* I; S, *Ssp* I; D, *Dra* II; B, *Bam*HI; E, *Eco*RI.

We also confirmed the RuvA–RuvB-mediated cruciform renaturation by gel electrophoresis of the DNA in the presence of chloroquine as described by Gellert *et al.* (ref. 16; data not shown). These results show that the RuvA–RuvB complex facilitates the renaturation of the cruciform structure in pUC4 DNA and that this activity is dependent on ATP hydrolysis.

DISCUSSION

This work showed that RuvA and RuvB, the products of the two genes in the same operon, functionally interact with each other. Therefore, RuvB, which alone cannot bind to DNA, can bind to DNA through the interaction with RuvA. Since RuvA enhances the ATPase activity of RuvB only when it

binds to DNA, RuvA should undergo a conformational change by binding to DNA, and the DNA-bound RuvA may modify the conformation of RuvB into a form that is more active as an ATPase. Physical interaction among RuvA, RuvB, and DNA was demonstrated by sedimentation analyses of a RuvA and RuvB mixture and also of a RuvA, RuvB, and DNA mixture in glycerol gradients (unpublished results).

Supercoiled DNA was more effective in enhancing the RuvB ATPase activity than ssDNA or linear dsDNA. RuvA appeared to bind to ssDNA more efficiently than to dsDNA. UV irradiation enhanced the ATPase cofactor activity only in the supercoiled DNA. Therefore RuvA may interact more efficiently with some particular conformations formed in supercoiled DNA that are different from the orderly B form and that may be increased by UV irradiation to the DNA. Differences in the degrees of the RuvB ATPase enhancement among the three forms of DNA may depend on the amount of such conformations that become the substrates for a dynamic reaction mediated by the RuvA–RuvB complex. ATP may be consumed as the energy for the reaction that changes the DNA conformation. Photoproducts such as pyrimidine dimers and the 6–4 adducts in UV-irradiated DNA should disrupt hydrogen bonds in the vicinity of the adducts and consequently should produce physical stresses in supercoiled DNA. Formation of cruciform structures or conversion from the B form to the Z form may relieve such tension in the DNA. These structures may be similar to the intermediates in DNA recombination (25) and may be the substrates for the dynamic reactions mediated by the RuvA–RuvB complex.

These concepts are compatible with the finding that the RuvA–RuvB complex renatures the cruciform structure in pUC4 DNA in the presence of ATP. The requirement of ATP in this reaction suggests that cruciform readsorption does not result from nonspecific protein binding. The RuvA–RuvB complex did not possess the activity to underwind supercoiled DNA and the activity to generate negative supercoils under the conditions that were employed in the cruciform DNA renaturation assay (unpublished data). Although the RuvA–RuvB complex shares some biochemical properties with RecA, it did not mediate heteroduplex formation between circular ssDNA and linear dsDNA as examined by the method of Cox and Lehman (26). RecA did not facilitate the transition of the cruciform into a standard duplex in either the presence or absence of ATP (data not shown).

We pose the following possibilities for the biological significance of the cruciform renaturation activity of the RuvA–RuvB complex. First, the RuvA–RuvB complex may facilitate branch migration by itself, in combination with RecA, or with another unknown protein involved in recombination. Renaturation of the cruciform, which is facilitated by the RuvA–RuvB complex, involves disrupting and reforming hydrogen bonds between the complementary bases of DNA as in branch migration. When the branch migration is inhibited by some special DNA conformations that are induced by DNA lesions, RuvA–RuvB may resolve such conformations, restore the native conformation, and promote the branch migration. Second, the RuvA–RuvB complex may be involved in the replication that is required for recombination. The tertiary structure of DNA containing the cruciform structure or other unusual structures that are formed during recombination or by DNA lesions may inhibit DNA replication. The RuvA–RuvB complex may resolve such structures in DNA to allow replication to proceed.

Recently, *ruvC* mutations, whose phenotypes are indistinguishable from *ruvAB* mutations, have been found to be located upstream of the *ruvAB* operon (27, 28). The product of *ruvC* has been highly purified, and it has an endonuclease activity that specifically resolves the Holliday structure *in vitro* (H.I., M. Takahagi, T.S., A.N., and H.S., unpublished results). RecA initiates recombination by pairing and exchanging homologous DNA strands, leading to the formation of the Holliday structure. The RuvA–RuvB complex may bind to the Holliday structure and promote branch migration. RuvC may specifically interact with the RuvA–RuvB complex and may replace the complex to resolve the Holliday structure. The specific interaction of RuvA–RuvB with RuvC may explain the indistinguishable phenotypes among the *ruvA*, *ruvB*, and *ruvC* mutants.

We thank Drs. T. Horii, K. Makino, and I. Kobayashi for helpful suggestions. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Science, Education, and Culture of Japan.

1. Otsuji, N., Horiuchi, T., Nakata, A. & Kawamata, A. (1978) *J. Antibiot.* **31**, 794–796.
2. Otsuji, N., Iyehara, H. & Hideshima, Y. (1974) *J. Bacteriol.* **117**, 337–344.
3. Otsuji, N. & Iyehara-Ogawa, H. (1979) *J. Bacteriol.* **138**, 1–6.
4. Shurvinton, C. E. & Lloyd, R. G. (1982) *Mol. Gen. Genet.* **185**, 352–355.
5. Shinagawa, H., Makino, K., Amemura, M., Kimura, S., Iwasaki, H. & Nakata, A. (1988) *J. Bacteriol.* **170**, 4322–4329.
6. Benson, F. E., Illing, G. T., Sharples, G. J. & Lloyd, R. G. (1988) *Nucleic Acids Res.* **16**, 1541–1549.
7. Iwasaki, H., Shiba, T., Nakata, A. & Shinagawa, H. (1989) *Mol. Gen. Genet.* **219**, 328–331.
8. Sargentini, N. J. & Smith, K. C. (1989) *Mutat. Res.* **215**, 115–129.
9. Lloyd, R. G., Benson, F. E. & Shurvinton, C. E. (1984) *Mol. Gen. Genet.* **194**, 303–309.
10. Lloyd, R. G., Buckman, C. & Benson, F. E. (1987) *J. Gen. Microbiol.* **133**, 2531–2538.
11. Mahajan, S. K. (1988) in *Genetic Recombination*, eds. Kucherlapati, R. & Smith, G. R. (Am. Soc. Microbiol., Washington), pp. 87–140.
12. Holliday, R. (1964) *Genet. Res.* **5**, 282–304.
13. Walker, G. C. (1984) *Microbiol. Rev.* **48**, 60–93.
14. Iwasaki, H., Shiba, T., Nakata, A. & Shinagawa, H. (1989) *J. Bacteriol.* **171**, 5276–5280.
15. West, S. C., Parsons, C. A. & Picksley, M. (1987) *J. Biol. Chem.* **262**, 12752–12758.
16. Gellert, M., O'Dea, M. H. & Mizuuchi, K. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5545–5549.
17. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33**, 103–119.
18. Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heynecker, H. L., Boyer, H. W., Crosa, J. H. & Falkow, S. (1977) *Gene* **2**, 95–113.
19. Vieira, J. & Messing, J. (1982) *Gene* **19**, 259–268.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
21. Yeung, A. T., Mattes, W. B. & Grossman, L. (1986) *Nucleic Acids Res.* **14**, 2567–2582.
22. McEntee, K., Weinstock, G. M. & Lehman, R. (1981) *J. Biol. Chem.* **256**, 8835–8844.
23. Wertman, K. F. & Mount, D. W. (1985) *J. Bacteriol.* **163**, 376–384.
24. Smolarsky, M. & Tal, M. (1970) *Biochim. Biophys. Acta* **199**, 447–452.
25. Holliday, R. (1989) *Trends Genet.* **5**, 355–356.
26. Cox, M. M. & Lehman, I. R. (1982) *J. Biol. Chem.* **257**, 8523–8532.
27. Sharples, G. J., Benson, F. E., Illing, G. T. & Lloyd, R. G. (1990) *Mol. Gen. Genet.* **221**, 219–226.
28. Takahagi, M., Iwasaki, H., Nakata, A. & Shinagawa, H. (1991) *J. Bacteriol.*, in press.