Escherichia coli RecQ protein is a DNA helicase

(RecF recombination pathway/DNA unwinding/DNA-dependent ATPase)

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ABSTRACT   The *Escherichia coli recQ* gene, a member of the RecF recombination gene family, was set in an overexpression plasmid, and its product was purified to near-homogeneity. The purified RecQ protein exhibited a DNA-dependent ATPase and a helicase activity. Without DNA, no ATPase activity was detected. The capacity as ATPase cofactor varied with the type of DNA in the following order: circular single strand > linear single strand >> circular or linear duplex. As a helicase, RecQ protein displaced an annealed 71-base or 143-base single-stranded fragment from circular or linear phage M13 DNA, and the direction of unwinding seemed to be 3′ → 5′ with respect to the DNA single strand to which the enzyme supposedly bound. Furthermore, the protein could unwind 143-base-pair blunt-ended duplex DNA at a higher enzyme concentration. It is concluded that RecQ protein is a previously unreported helicase, which might possibly serve to generate single-stranded tails for a strand transfer reaction in the process of recombination.

The *recQ* gene of *Escherichia coli* (1) is a member of the so-called RecF gene family. The genes of this group, the other known members of which are *recA*, *recF*, *recJ*, *recN*, *recO*, *recR*, and *ruv* (for review, see refs. 2 and 3), share the common denominator that their mutations abolish conjugal recombination proficiency and UV resistance in a mutant lacking active exonuclease V (RecBCD enzyme) and exonuclease I (SbcB protein). In such a mutant, the conjugal recombination system operative in wild-type cells (the RecBCD pathway), which is also implicated in the postreplication recombinational repair of UV-damaged DNA (4), is no longer functional due to the loss of the key enzyme exonuclease V (see ref. 2). To explain the conjugal recombination proficiency and UV resistance of the mutant, it is generally assumed that the absence of exonuclease I somehow activates a substitute system, the RecF pathway (5), which is dependent on the RecF family genes (2). In addition to the above assignments, recent work has revealed the roles for the RecF family genes in plasmid recombination (6–8).

The genes of the RecF family have been cloned, and their products have been identified (2, 3, 9, 10). However, apart from the extensively characterized RecA protein, the activities of these products are still unknown except for RecJ and Ruv, whose nuclease and ATPase activities have recently been reported, respectively (11, 12). After cloning and sequencing the *recQ* gene (10, 13), we have been engaged in the construction of a RecQ overproducer and the purification of RecQ protein. Here, we report those experiments, which culminated in the demonstration that RecQ protein has a DNA-dependent ATPase and a DNA helicase activity.

MATERIALS AND METHODS

Plasmids, Phages, and Bacterial Strains. pKD148 is a pMC1403-based plasmid carrying a *recQ–lacZ* fusion (13).

pKD151 is a pUC8-based plasmid harboring an intact copy of the *recQ* gene in the multiple cloning site of the vector (13). pKD167, pKD175, and pKD176 are described below. pUC8 (14), pUC118 (15), and M13mp18 (16) have been described. *E. coli* K-12 strains CSH26 (17) and JM103 (16) were used as the hosts for pKD148 and pKD176, respectively. KD2257, a *recQ1802*::Tn*3* derivative of JM103, was constructed by P1 transduction with KD1996 (10) as a donor.

DNA Manipulations and Sequencing. Infection with phage M13mp18 and isolation of its single-stranded DNA were carried out as described (15). Isolation of plasmid DNA or M13mp18 replicative form (RF) was done by a modified alkaline lysis method (18), followed by CsCl/ethidium bromide equilibrium centrifugation when closed circular DNA was needed. M13mp18 RF II was recovered from the gels after electrophoresing an alkaline lysis preparation. Electrophoresis of DNA was carried out as described (18) with 0.8% agarose gels (type I; Sigma) or 4% NuSieve GTG agarose gels (FMC) and TAE buffer. Recovery of DNA from the gels was achieved by electroelution (18) or with a silica matrix (Geneclean; Bio 101, La Jolla, CA). Restriction endonucleases and T4 polynucleotide kinase were from Toyobo (Osaka). T4 DNA ligase, *E. coli* DNA polymerase I (Klenow fragment), and BAL-31 nuclease were from Takara Shuzo (Kyoto). The reaction conditions for these enzymes were as recommended by the suppliers. An *Eco*RI linker d(pGGAATTCC) was from P-L Biochemicals. A 25-mer oligonucleotide for mutagenesis, 5′-TCCGCCTGCGCCATAGCTGTTTCCT-3′, was made in an oligonucleotide synthesizer (model 380A; Applied Biosystems). DNA sequencing was carried out by a modified chain-termination method (19).

SDS/PAGE and Protein Assay. SDS/PAGE was done by the method of Laemmli (20) as adapted to 10-cm slab gels and followed by silver staining (21). Recovery of protein from the gel and its renaturation were done as reported (22). For protein determination, a dye-binding assay reagent (Bio-Rad) was used with bovine gamma globulin as a standard.

Purification of RecQ–LacZ Fusion Protein. Cells of CSH26/pKD148 were grown in 10 liters of L broth (1) supplemented with ampicillin (50 μg/ml) at 37°C with shaking to 1.2 × 10⁸ cells per ml. Mitomycin C (1 μg/ml) was added to induce the *recQ* expression, and incubation was continued for 14 hr. The RecQ–LacZ fusion protein was purified essentially by the method for β-galactosidase (17), except that crude extracts were prepared as described below for RecQ and that sucrose gradient centrifugation was replaced by gel filtration with Sepharose 6B (Pharmacia). The final preparation (0.92 mg) gave a single band in SDS/PAGE.

Purification of RecQ Protein. Cells of JM103/pKD176 were grown in 6 liters of L broth (1) supplemented with ampicillin (50 μg/ml) at 37°C with aeration to 6 × 10⁸ cells per ml. Isopropyl β-D-thiogalactopyranoside (1 mM) was added, and incubation was continued for 3 hr at 42°C to increase the copy number of the plasmid (23). After being harvested by cen-

trifugation, the cells were suspended in 12 ml of buffer A (0.01 M Tris·HCl, pH 7.5/0.1 M NaCl/1 mM EDTA/10 mM 2-mercaptoethanol), lysozyme (0.2 mg/ml) was added, and the suspension was kept on ice for 30 min. It was frozen at −80°C, then quickly thawed at 30°C, and sonicated at 20 kHz for 40 sec in an ice-water bath. After addition of phenylmethylsulfonyl fluoride (1 mM), the lysate was clarified by centrifugation at 77,000 × *g* for 30 min, and nucleic acids were removed by treatment with 3% (wt/vol) streptomycin sulfate followed by centrifugation at 9000 × *g* for 10 min (crude extract). The subsequent purification was carried out at 4°C by following a M_r 74,000 band in SDS/PAGE (see *Results*). The crude extract was applied to a column (1.5 × 5.0 cm) of single-strand DNA cellulose (Pharmacia) equilibrated against buffer A. After being washed with 5 bed volumes of buffer A supplemented with 0.1 M NaCl, the column was eluted with 3 bed volumes of buffer A supplemented with 0.5 M NaCl (DNA cellulose fraction). Solid ammonium sulfate was added to this fraction to 60% saturation, and the precipitate was dissolved in 2 ml of buffer A (ammonium sulfate fraction). This was fractionated on a column (2.0 × 100 cm) of Sephacryl S-200 (Pharmacia) with buffer A, and peak fractions (around M_r 69,000) were pooled (gel filtration fraction). The pooled fractions were mixed with solid ammonium sulfate to give 30% saturation and subjected to hydrophobic chromatography on a column (1.0 × 12 cm) of Butyl-Toyopearl 650 (Tosoh, Tokyo) equilibrated with buffer A 30% saturated with ammonium sulfate. After washing the column with 3 bed volumes of buffer A 30% saturated with ammonium sulfate, elution was effected with 200 ml of a linear downward gradient from 30% to 0% ammonium sulfate saturation in buffer A. Peak fractions (around 12% saturation) were pooled, dialyzed against 0.1 M Tris·HCl, pH 7.5/0.1 M NaCl/0.2 mM EDTA/2 mM dithiothreitol (hydrophobic chromatography fraction), and stored at −20°C after being mixed with an equal volume of glycerol until used.

HPLC. The hydrophobic chromatography fraction was dialyzed against buffer B (10 mM potassium phosphate, pH 6.8/1 mM dithiothreitol/3 mM $CaCl_2$) and loaded on a hydroxyapatite column (4.0 × 50 mm; Pentax, Tokyo) equilibrated with buffer B, which was then washed with 4 column volumes of buffer B and developed with 20 column volumes of a linear gradient from buffer B to 0.4 M potassium phosphate, pH 6.8/1 mM dithiothreitol/7.5 μM $CaCl_2$ at a flow rate of 0.5 ml/min.

Determination of Amino-Terminal Sequence and Amino Acid Composition. These were carried out, respectively, by automated Edman degradation in a gas-phase peptide sequencer (model 470A; Applied Biosystems) and with an amino acid analyzer (model 835; Hitachi, Tokyo) after hydrolysis in 6 M HCl at 110°C for 24 hr *in vacuo*.

NTPase Assay. The reaction mixture (20 μl) contained 40 mM Tris·HCl (pH 7.5), 2 mM $MgCl_2$, 6 mM dithiothreitol, 50 μg of bovine serum albumin per ml, 2.5% glycerol, 2.5 mM NaCl, 3 μM (as nucleotides) DNA, 0.55 mM [³H]NTP (50 μCi/ml; 1 Ci = 37 GBq), and an appropriate amount of the enzyme, unless otherwise indicated. After incubation at 37°C for 10 min or otherwise as specified, the mixture was chilled in an ice-water bath, and 20 μl of a solution containing 35 mM EDTA, 6 mM NDP, and 6 mM NTP was added. Samples (10 μl) were spotted on polyethyleneimine cellulose thin-layer plates (Polygram Cel 300 PEI; Macherey & Nagel) in duplicate, separated, and quantified (24). One unit of the enzyme was defined as the amount that hydrolyzes 1 nmol of ATP under the conditions described above with M13mp18 single-stranded DNA as a cofactor.

Helicase Assay. A circular and a linear partially duplex substrate were prepared as described by Matson (25). Blunt-ended duplex substrates were prepared by filling the ends of the 141-base-pair (bp) and 200-bp *Cla* I–*Hae* III fragments of

M13mp18 RF with [α-³²P]dCTP and dGTP by using DNA polymerase I. The reaction mixture (20 μl) was the same as for the ATPase assay except that the final ATP concentration was 1.8 mM, and the reaction mixture was incubated at 37°C for 10 min. Termination of the reaction and electrophoretic analysis of the products were carried out as described (25).

RESULTS

Translational Initiation Site for *recQ*. Nucleotide sequence analysis of the *recQ* region has revealed four potential initiation codons for the *recQ* gene (13). To determine the actual site for initiation, we took advantage of a *recQ–lacZ* protein fusion gene (13). The product of the fusion gene was purified from the cells of CSH26/pKD148 to near-homogeneity, and its amino-terminal sequence was determined. The revealed sequence, Ala-Gln-Ala-Glu-Val, is consistent with the nucleotide sequence, on the assumption that the translation starts with the second (GTG) of the four potential initiation codons (13) followed by removal of the terminal methionine by the methionine-specific amino peptidase (26). The initiation codon is preceded by an atypical Shine–Dalgarno sequence, AGG, which may possibly cooperate with the GTG initiation in restraining the expression of the *recQ* gene.
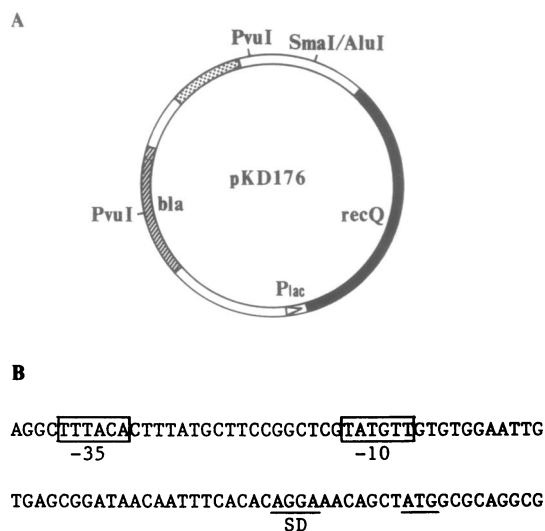

FIG. 1. RecQ-overproducing plasmid pKD176. Starting with pKD151 (13), we first constructed pKD167 consisting of the following three DNA fragments: (*i*) the larger *Sma* I–*Eco*RI fragment of pUC8, (*ii*) a *recQ*-containing 2.2-kbp *E. coli* chromosomal fragment (prepared by BAL-31 nuclease digestion) spanning from the first base of the *recQ* initiation codon (GTG) to the *Alu* I site situated about 400 bp downstream of the *recQ* termination codon (13), and (*iii*) the *Eco*RI linker connecting the *recQ* initiation codon and the *Eco*RI site of pUC8. In this plasmid, the *recQ* gene was translationally fused with *lacZ*, its product carrying, by inference, seven extra non-RecQ amino acid residues at the amino terminus. For the production of the unmodified RecQ protein, we first replaced the 896-bp *Pvu* I fragment of pKD167 with the 1372-bp *Pvu* I fragment of pUC118 containing the intergenic region of phage M13 to make pKD175 and then deleted a 21-bp segment from the second codon of *lacZ* through the *recQ* initiation codon using oligonucleotide-directed mutagenesis (27). pKD176 thus obtained was shown by sequencing to have a modified *recQ* gene with an ATG initiation codon instead of GTG and the promoter/ribosome-binding region replaced by that of *lac*. (*A*) Overall structure. Solid segment, modified *recQ* coding sequence; dotted segment, intergenic region of phage M13; hatched segment, ampicillin-resistance gene; triangle, *lac* promoter. (*B*) Nucleotide sequence of the 5′ region of the modified *recQ*. Boxed segments, the −35 and −10 regions of the *lac*-derived promoter; SD, the *lac*-derived Shine–Dalgarno sequence; ATG with underline, the *lac*-derived initiation codon.

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RecQ-Overproducing Plasmid. Fig. 1 shows the structure of plasmid pKD176 carrying a *recQ* gene engineered for RecQ overproduction. It was constructed as described in the legend to Fig. 1.

Purification of RecQ Protein. RecQ protein has previously been shown by the maxicell technique to give a band with an apparent M_r of 74,000 in SDS/PAGE (10). Not knowing its activity, we purified provisional RecQ as described in *Materials and Methods* by monitoring the SDS/PAGE profiles of fractions for such a band. Although this band was hardly visible with the crude extract, this method worked well in the subsequent steps of purification. A typical purification is shown in Fig. 2. The final preparation consisted almost exclusively of the suspect protein as judged by SDS/PAGE.

The identity of the purified protein with RecQ was established as follows. Analysis of amino-terminal residues for the final preparation revealed a sequence, Ala-Gln-Ala-Glu-Val, which coincided with the one for the RecQ-LacZ fusion protein. Further, the amino acid composition of the preparation agreed, within the limit of experimental error, with the one predicted from the nucleotide sequence of *recQ* (13) (data not shown).

DNA-Dependent NTPase Activity. As shown in Fig. 3, the purified preparation of RecQ protein hydrolyzed ATP in the presence of DNA; the ATPase activity was hardly detectable without DNA. Single-stranded DNA was apparently much more effective as cofactor than double-stranded DNA. Notably, even the least active cofactor, the linear double-stranded DNA, effected reproducible, albeit very weak, stimulation of the reaction. Table 1 shows that the ATP hydrolysis was dependent on divalent cations such as Mg^{2+} and that dATP, but not GTP, was also hydrolyzed. This ATPase preparation had specific activity of 600 units/μg of protein when assayed with M13mp18 single-stranded DNA as a cofactor.

DNA Helicase Activity. DNA helicases, among other enzymes, are known to exhibit DNA-dependent ATPase activity. We therefore examined our RecQ preparation for such activity by measuring the release of labeled, single-stranded DNA fragments from duplex DNA with three different kinds of substrates. The results with the partially duplex circular substrate (25) are shown in Fig. 4 *A* and *B*. Clearly, the purified preparation effected the release of the 71-base fragment from the single-stranded M13mp18 circle in a time-dependent manner. This reaction required ATP, which could not be replaced by adenosine 5'-[γ-thio]triphosphate, indicating that the helicase activity depends on ATP hydrolysis. The unwinding activity also shared a Mg^{2+} requirement with the DNA-dependent
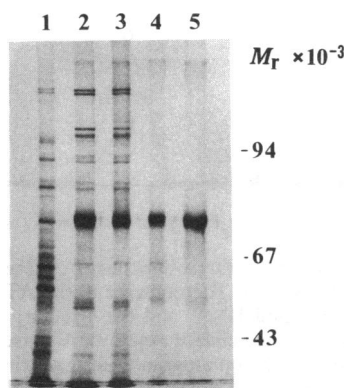

FIG. 2. Purification of provisional RecQ protein as monitored by SDS/PAGE. A crude extract from JM103/pKD176 containing 1360 mg of protein was fractionated. Lane 1, 9.6 μg of the crude extract; lane 2, 4 μg of the DNA cellulose fraction (total protein, 4.6 mg); lane 3, 3.5 μg of the ammonium sulfate fraction (4.1 mg); lane 4, 2 μg of the gel filtration fraction (2.8 mg); lane 5, 1 μg of the hydrophobic chromatography fraction (1.3 mg).
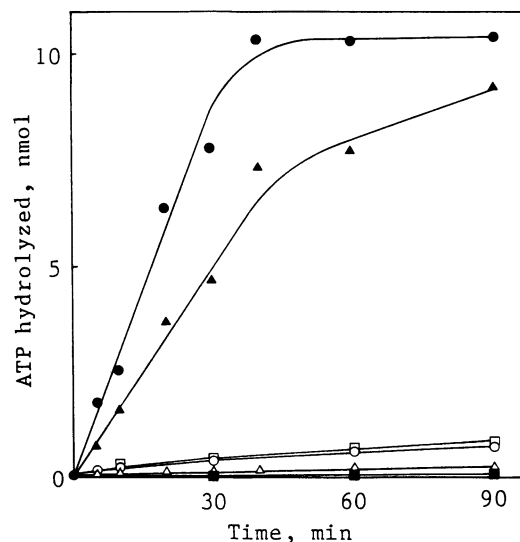

FIG. 3. ATPase activity of RecQ preparation in the presence of different DNAs. ATPase activity was measured with 3 μM DNA effectors and 2 ATPase units (3 ng or 44 fmol) of RecQ protein (hydrophobic chromatography fraction). ○, M13mp18 RF I; ●, M13mp18 single-stranded DNA; △, M13mp18 RF I linearized at the *Sma* I site; ▲, linearized M13mp18 RF denatured at 95°C for 5 min; □, M13mp18 RF II; ■, no DNA.

ATPase activity. These results suggest that the two activities are somehow coupled.

Fig. 4*C* presents the results with the linear, partially duplex substrate (25), which was used to uncover a possible polarity of the reaction. Our RecQ preparation was able to displace only the 143-base annealed fragment from the 5' end of the linear M13mp18 DNA when 2 ATPase units of the enzyme was used. This suggests that the presumptive RecQ helicase unwinds a duplex portion in the 3' → 5' direction with respect to the single strand to which it supposedly binds. However, when 20 ATPase units of the enzyme was used, the 202-base fragment at the other end was also released. This raised the possibility of an unwinding reaction initiated at the blunt end, and the results shown in Fig. 4*D* indicate that this was actually the case. Thus, our preparation was able to unwind the 143-bp blunt-ended duplex DNA at 20 units but not at 2 units. The 200-bp blunt-ended duplex DNA gave a similar result, but the amount of unwound DNA was considerably less than that for the 143-bp substrate (data not shown). The unwinding of the blunt-ended duplex DNA required ATP and Mg^{2+} like that of the partially duplex substrate. We therefore conclude that this helicase can unwind also blunt-ended

Table 1. Requirements for NTP hydrolysis

Reaction mixture	NDP formed, nmol
Complete	3.19
− enzyme	0.04
− Mg^{2+}	0.04
− Mg^{2+}, + Mn^{2+} (2 mM)	3.07
− Mg^{2+}, + Ca^{2+} (2 mM)	2.85
− Mg^{2+}, + Zn^{2+} (2 mM)	0.75
+ EDTA (20 mM)	0.06
− dithiothreitol	1.01
− bovine serum albumin	0.14
− ATP, + dATP	3.15
− ATP, + GTP	0.02

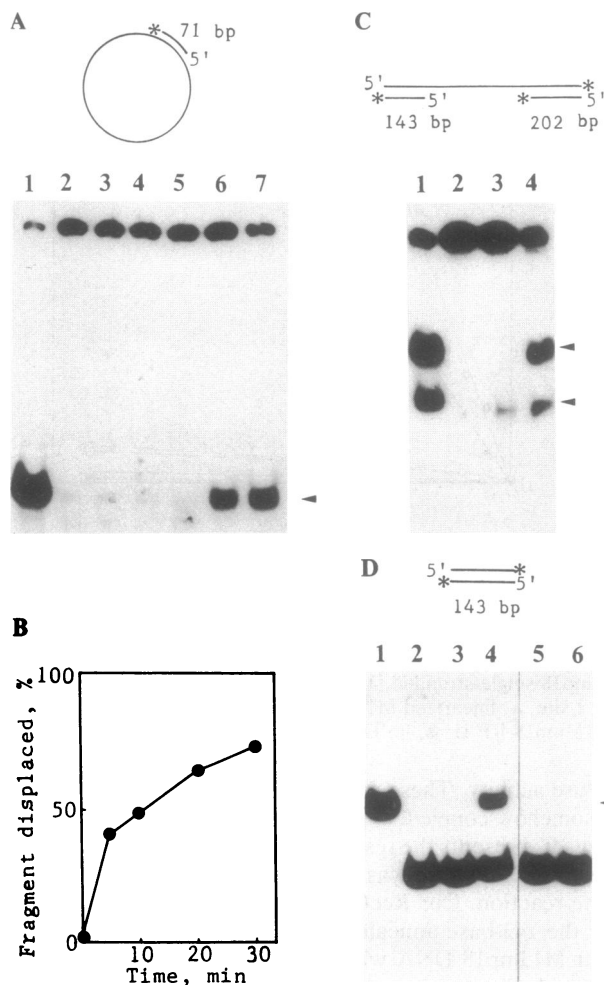The complete system was as described in the text with 2 ATPase units of RecQ, ATP, and M13mp18 single-stranded DNA.

nor helicase activity was detected at the corresponding position in the resulting hydrophobic chromatogram for either strain, where the protein peak was also absent. From these results, we conclude that the DNA-dependent ATPase and helicase activities are attributable to the amplified *recQ* gene on the overproducing plasmid. Also, the failure of JM103/pUC8 cells to show either activity in the parallel purification clearly indicates that the RecQ-overproducing strain actually produces the protein in an increased amount compared with the normal strain, although the magnitude of amplification could not be assessed.

DISCUSSION

We purified the product of the *E. coli recQ* gene to apparent homogeneity and showed it to possess a DNA-dependent ATPase and a DNA helicase activity. These activities seem significant, being comparable in capacity to those of some well-characterized ATPase/helicases. Thus, RecQ ATPase showed a similar specific activity to those of helicase II, helicase IV, and Rep of *E. coli* (28–30), and, judging from the time course of the reaction (Fig. 4B) as compared with analogous data in the literature (28, 29), the unwinding capacity of RecQ helicase seems to be on the same order as that of *E. coli* helicases II and IV. It should also be added that RecQ as an ATPase/helicase can be distinguished from any

FIG. 4. DNA helicase activity of RecQ preparation. The substrates used in the assays are shown above the respective autoradiograms. Asterisks denote the positions of radioactive label, and arrowheads indicate the positions for the displaced fragments. Unless otherwise mentioned, all incubations were done with 2 ATPase units (3 ng or 44 fmol) of RecQ (hydrophobic chromatography fraction). (A) Assay with the circular, partially duplex substrate (M13mp18 DNA with a 71-bp duplex portion). Lanes: 1, heat-denatured substrate; 2, no enzyme; 3, no ATP; 4, adenosine 5'-[γ-thio]triphosphate instead of ATP; 5, no Mg^{2+}; 6, 2 units of RecQ; 7, 20 units of RecQ. (B) Time course of helicase reaction with the same substrate as for A. (C) Assay with the linear, partially duplex substrate (linear M13mp18 DNA with a 143-bp and a 202-bp duplex portion at the 5' and 3' ends, respectively). Lanes: 1, heat-denatured substrate; 2, no enzyme; 3, 2 units of RecQ; 4, 20 units of RecQ. (D) Assay with the 143-bp blunt-ended duplex substrate. Lanes: 1, heat-denatured substrate; 2, no enzyme; 3, 2 units of RecQ; 4, 20 units of RecQ; 5, no ATP; 6, no Mg^{2+}.

duplex DNA perhaps from its ends, albeit in much lower efficiency than with the partially duplex DNA.

Association of the DNA-Dependent ATPase and Helicase Activities with RecQ Protein. To establish that the ATPase and helicase activities are actually associated with RecQ protein, we monitored the final step of the purification for both activities and protein concentration (Fig. 5A). The profiles of both enzyme activities and protein coincided perfectly with one another, each forming a single symmetrical peak. Moreover, this association survived further fractionation of a hydrophobic chromatography fraction by SDS/PAGE (Fig. 5B) or HPLC on hydroxyapatite (data not shown).

We also fractionated extracts from KD2257/pUC8 (no functional *recQ*) and JM103/pUC8 (chromosomal *recQ* only) up to the hydrophobic chromatography step. Neither ATPase
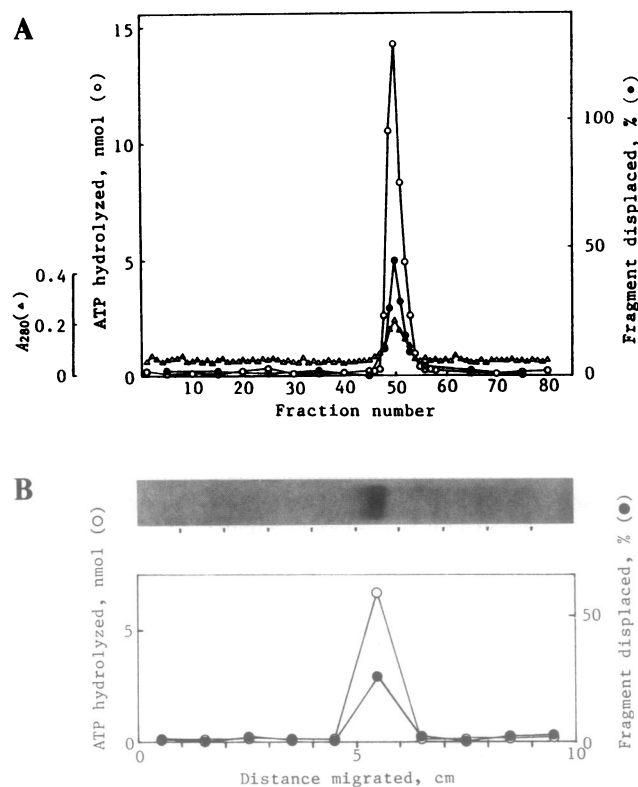
FIG. 5. Association of DNA-dependent ATPase and helicase activity with RecQ protein. The cofactor in the ATPase assay was M13mp18 single-stranded DNA, and the circular, partially duplex substrate was used for the helicase assay. ○, ATPase; ●, helicase; △, A_{280}. (A) Hydrophobic chromatography of a gel filtration fraction. Both enzyme activities were measured with a 0.5-μl portion of each fraction (2.5 ml). (B) SDS/PAGE of a hydrophobic chromatography fraction. A lane containing 14 μg of protein was cut into pieces at 1-cm intervals, from which the protein was recovered and allowed to renature (22). A 1-μl portion of each eluate (50 μl) was used for each enzyme assay. Shown above the graph is an adjacent silver-stained lane containing 1 μg of protein, with graduations indicating the positions of cutting the gel. The recovery of the ATPase activity was 3.8%; that of the helicase activity was not estimated.

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of the six chromosome-encoded proteins with helicase activity thus far identified in *E. coli* (29–34) by biochemical properties (32–34) or genetic map positions (1, 35–38). Incidentally, our RecQ preparation did not exhibit, as far as examined, any DNase and DNA topoisomerase activities in the presence or absence of ATP (our unpublished results).

The helicase reaction required ATP, and the nonhydrolyzable analog adenosine 5′-[γ-thio]triphosphate could not substitute for it, indicating that the ATP hydrolysis fuels the RecQ-mediated unwinding reaction. However, the ATPase activity itself was strongest when the single-stranded DNAs were supplied as cofactors, which are not thought to have extensive secondary structure. The ATP hydrolysis stimulated by such cofactors, therefore, should be independent of the unwinding reaction. As suggested for other helicases (24, 28), this type of ATP breakdown may be associated with the translocation of the enzyme on the DNA single strand. Alternatively, it may not be related to such kinetic activity but represent a conformational change of RecQ caused by binding to single-stranded DNA, which results in the manifestation of the ATPase activity.

With the linear, partially duplex substrate (Fig. 4*C*), RecQ protein at a lower concentration selectively displaced the short fragment at the 5′ end of the long single strand, suggesting that the enzyme binds to the single strand portion of the substrate and then unwinds the duplex part in the direction 3′ → 5′ with respect to the single strand. This finding is similar to the results obtained with Rep protein (39), helicase II (25), and helicase IV (29). However, a larger amount of the RecQ helicase was able not only to displace the other short fragment at the 3′ end of the single strand (Fig. 4*C*) but also to unwind the blunt-ended duplex (Fig. 4*D*). Presumably, RecQ protein can initiate the unwinding reaction also at a blunt end of duplex DNA, a feature not noted with the other three helicases mentioned above (25, 29, 39). It should be pointed out, however, that these observations do not eliminate the possibility that the enzyme bound to the single strand portion of the linear partial duplex may go on to unwind the duplex part in the 5′ → 3′ direction at a much slower rate than in the other direction.

In accordance with the demonstrated activities, the predicted amino acid sequence of RecQ protein (13) reveals a putative ATP-binding site (residues 44–60) with a considerable homology to the consensus sequence for such sites (40). Surprisingly, however, the whole sequence shows no discernible homology, except for the ATP-binding site, with those of Rep protein (41), helicase II (42), and helicase IV (38), all of which bear resemblance to RecQ with respect to the polarity of unwinding.

The central question regarding RecQ protein is whether the observed helicase activity accounts for its role in recombination. Conceivably, RecQ helicase may participate in recombination by generating a 3′ single-stranded tail, which should serve as a substrate in a RecA-mediated strand exchange reaction (2). In this connection, it is interesting to note that RecJ protein, supposed to work in the same pathway, has recently been shown to possess a single-strand-specific DNase activity, which is greater on 5′ tailed than on 3′ tailed duplex DNA (11). Thus, a plausible idea would be that RecJ protein may possibly stabilize the 3′ tail of a product of the RecQ-mediated unwinding reaction by degrading the complementary 5′ tail. More precise understanding of the role for RecQ requires elucidation of the functions of the other RecF family gene products and more detailed characterization of RecQ itself. In this regard, we must reserve the possibility that some key features of the RecQ helicase or even its nonhelicase function is yet to be discovered.

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