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A STABLE recA PROTEIN-SINGLE-STRANDED DNA COMPLEX: AN INTERMEDIATE IN DNA STRAND EXCHANGE[1]

Daniel A. Soltis, Michael M. Cox,[2]
Zvi Livneh and I. R. Lehman

Department of Biochemistry, Stanford University
School of Medicine, Stanford, California 94305

ABSTRACT The recA protein of Escherichia coli promotes the exchange of strands between linear duplex and homologous, circular, single-stranded DNAs to generate a nicked circular DNA duplex (RFII) and a displaced linear single strand. At an early stage in the reaction, recA protein exists in a stable, kinetically significant complex with the circular single-stranded DNA, whose formation requires single-stranded DNA binding protein (SSB) and ATP. After completion of strand exchange, the recA protein is bound to the RFII and the SSB is associated with the displaced single strand. The recA protein-RFII complex can be dissociated upon addition of ADP, permitting the recA protein to participate in another cycle of strand exchange.

INTRODUCTION

Mutations at a number of loci in the E. coli chromosome are known to affect general recombination; however, strains bearing mutations in the recA gene are particularly defective in this process (1,2). Studies of recA

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[2]Present address: Department of Biochemistry, University of of Wisconsin, Madison, WI 53706

mutants indicate that the recA gene product is essential during the early stages of homologous recombination, possibly in the transfer of strands between duplex DNA molecules (3-6). Models for general recombination propose that the formation of heteroduplex DNA is preceded by the pairing of strands from different DNA molecules to produce a heteroduplex joint (7,8), which is then extended by branch migration.

The product of the recA gene has been isolated (9-12) and shown to promote reactions in vitro that are relevant to its proposed role in homologous recombination in vivo. These reactions include the renaturation of complementary single-stranded (SS) DNAs (9) and the pairing of SS DNA molecules with homologous regions of duplex DNA (12,13). This latter reaction, termed strand assimilation or D-loop formation, is analogous to the pairing reaction believed to be an early step in homologous recombination.

Our studies have been aimed at defining the role of recA protein in homologous recombination. In particular, we have focused on a DNA strand exchange reaction in which a single strand is transferred from a linear duplex to a circular SS DNA, yielding a nicked circular duplex (RFII) and a linear SS DNA (14-16). This strand exchange reaction has been particularly useful in examining the role of recA protein in homologous recombination for several reasons. First, it is analogous to several of the steps in which the recA protein very likely participates during homologous recombination in vivo. Second, the substrates and products of the strand exchange reaction are well characterized and easily separated. Finally, there are a number of assays to follow this reaction including one which measures heteroduplex formation directly (14).

The exchange of strands is coupled to the hydrolysis of ATP and is stimulated by the single-stranded DNA binding protein of E. coli (SSB) (14,17,18). In fact, there is evidence which suggests that SSB stimulates homologous recombination in vivo (19,20). In this report, we describe a stable complex between recA protein and circular SS DNA that is formed in the presence of SSB and ATP and which is required for efficient DNA strand exchange.

RESULTS

Complex formation. The general strategy was to examine the strand exchange reaction at limiting recA protein concentrations. At various times, the reaction was challenged with a second set of DNA substrates to determine whether the recA protein was available for reaction with the challenging DNAs.

Typically, recA protein was preincubated with both the circular SS DNA and linear duplex DNA. After starting strand exchange with ATP and SSB, the reaction was challenged with another equivalent of SS and duplex DNAs. Heteroduplex formation occurring with the substrates in the preincubation is referred to as reaction 1; heteroduplex formation involving the challenging DNAs is termed reaction 2. By using [^{3}H]-labeled SS DNA in one or the other set of substrates, reactions 1 and 2 could be followed independently. The amount of recA protein present was sufficient to promote only half of the heteroduplex formation possible in either reaction 1 or reaction 2 alone. Sufficient SSB was added to provide optimal conditions for heteroduplex formation in both reactions. An ATP regenerating system was also included in each experiment to ensure that ATP was not limiting.

When the reaction 1 and 2 substrates were preincubated with recA protein and the reaction initiated with ATP and SSB, approximately 25% of both SS DNA substrates were incorporated into heteroduplex DNA indicating that they could compete successfully for the recA protein (Table 1). When recA protein was preincubated with reaction 1 substrates and the ATP and SSB added with the reaction 2 substrates, a competition for recA protein again occurred. However, in this case, reaction 2 proceeded more efficiently than reaction 1 (Table 1, experiments 1A and 1B). The lower extent of reaction 1 relative to reaction 2 in this experiment was probably due to the formation of unproductive intermediates between the reaction components during the preincubation in the absence of ATP. In contrast, when recA protein was preincubated with the substrates of reaction 1, and ATP and SSB were added to initiate the reaction 20 min prior to the addition of the reaction 2 substrates, reaction 1 was approximately 12-fold more efficient than reaction 2 (experiments 2A and 2B). Taken together, these results indicate that early in strand

TABLE 1
REQUIREMENTS FOR COMPLEX FORMATION

Experiment	Time of addition (min) -40	-30	-20	0	Extent of heteroduplex formation (%)
1A	[^{3}H]SS DNA DS DNA	recA		SS DNA DS DNA ATP/2xSSB	23.5
B	SS DNA DS DNA	recA		[^{3}H]SS DNA DS DNA ATP/2xSSB	45.7
2A	[^{3}H]SS DNA DS DNA	recA	ATP SSB	SS DNA DS DNA SSB	58.8
B	SS DNA DS DNA	recA	ATP SSB	[^{3}H]SS DNA DS DNA SSB	4.7
3A	[^{3}H]SS DNA DS DNA	recA	ATP	SS DNA DS DNA 2x SSB	26.2
B	SS DNA DS DNA	recA	ATP	[^{3}H]SS DNA DS DNA 2x SSB	38.4

TABLE 1. Reactions were performed as described (18). The extent of heteroduplex formation is the average of three measurements taken at 40, 50 and 60 minutes after t=0. The concentrations resulting from the indicated additions are: unlabeled circular SS DNA (SS DNA), 3.3 μM; [^{3}H]-labeled circular SS DNA ([^{3}H]SS DNA), 3.3 μM; linear duplex DNA (DS DNA), 5.6 μM; recA protein, 0.9 μM; SSB, 0.15 μM; ATP, 1.2 mM. An ATP regenerating system was also included in each experiment.

exchange, the recA protein becomes sequestered in a complex with the substrate DNAs which is largely irreversible when both ATP and SSB are present.

Requirements for complex formation. When reaction 1 was initiated without SSB, both reactions 1 and 2 proceeded to similar extents (experiments 3A and 3B); thus SSB is necessary for the formation of the stable recA protein-SS DNA complex. Omission of duplex DNA from the preincubation gave results similar to those in experiments 2A and 2B, demonstrating that the duplex DNA is not required for complex formation. However, when reaction 1 was initiated without ATP, and ATP was added with the reaction 2 substrates, the extent of reaction 1 decreased and the extent of reaction 2 increased relative to experiments 2A and 2B. These results indicate that recA protein had not been sequestered within the complex and was able to interact with both sets of substrates. Similarly, when SS DNA was omitted from the preincubation and added with the reaction 2 substrates, recA protein was not sequestered as in experiments 3A and 3B. Thus, SS DNA, SSB and ATP are required for the formation of the stable recA protein-SS DNA complex.

Interaction of recA protein with SS DNA. A complex formed between recA protein and circular SS DNA in the presence of SSB and ATP can be observed by sedimentation in sucrose density gradients if the complex is stabilized with the nonhydrolyzable ATP analog, ATPγS, prior to sedimentation (Fig. 1). This complex corresponds to the one inferred from the kinetic analyses described above and shown to be an early intermediate in the strand exchange reaction (18). The sedimentation coefficient of the recA protein-SS DNA complex was approximately 78S. Addition of 150 mM NaCl to the sucrose gradient did not affect this value, indicating that it is an accurate measure of the density of the recA protein-SS DNA complex.

The kinetic analyses summarized in Table 1 showed that SSB is required for the formation of a stable complex between recA protein and SS DNA and suggested that SSB might function in complex formation by interacting directly with the recA protein. Thus, it might be expected that SSB would be found associated with the recA protein-SS DNA complex. As shown in Fig. 1, however, SSB sedimented near the top of the gradient and not with the recA protein in the 78S peak. Attempts to crosslink SSB chemically to the recA protein-SS DNA complex using formaldehyde and/or glutaraldehyde were unsuccessful. These results suggest that SSB does not interact stably with the recA protein-SS DNA complex, however, an alternative possibility is that

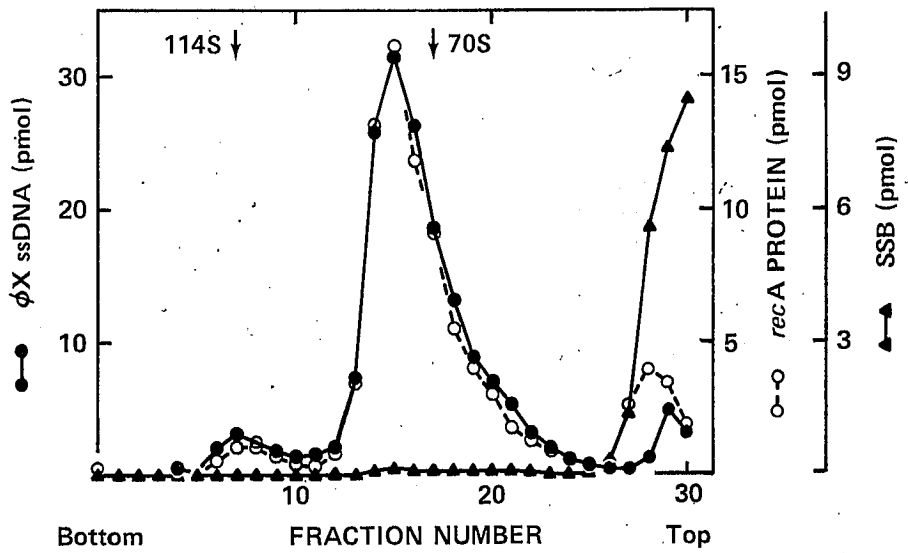

FIGURE 1. RecA protein-SS DNA complex formation in the presence of SSB. The reaction and sedimentation were performed as described (21). The migration of normal (114S) and eclipsed (70S) forms of bacteriophage φX174 are indicated in this and succeeding figures.

SSB cannot be crosslinked to the recA protein-SS DNA complex by the methods used.

An analysis by sucrose gradient sedimentation of the interaction of recA protein with SS DNA in the absence of SSB is shown in Fig. 2. Under these conditions, approximately 80% of the recA protein sedimented near the top of the gradient and was not bound to the SS DNA, whereas in the presence of SSB, essentially all of the recA protein was found associated with the SS DNA (Fig. 1). Since ATPγS was added to stabilize any interaction between the recA protein and SS DNA, the recA protein found in the 78S peak would appear to represent a steady state level of recA protein bound to SS DNA in the absence of SSB. Thus, in the absence of SSB, a stable complex does not form between recA protein and SS DNA, a result which is in agreement with our kinetic analysis.

RecA protein-RFII complexes. DNA challenge experiments similar to those described above indicated that recA

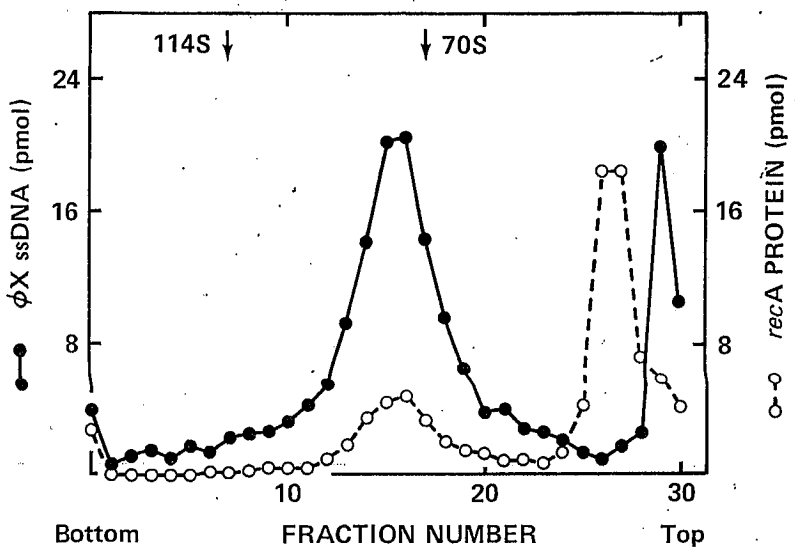

FIGURE 2. Interaction of recA protein with SS DNA in the absence of SSB. The reaction and sedimentation were performed as described (21).

protein was unavailable for further reaction after the completion of strand exchange when the ATP/ADP ratio was maintained at sufficiently high levels (18). The sucrose density gradient analysis of the products of a strand exchange reaction performed with labeled circular SS DNA, in the presence of SSB is shown in Fig. 3. Once again, ATPγS was added to stabilize any recA protein-DNA interactions. At the end of the reaction, most of the recA protein cosedimented with the labeled, circular SS DNA which had been incorporated into the RFII product. The sequestering of recA protein after completion of strand exchange was therefore the result of the formation of a complex between recA protein and the product RFII.

The use of labeled linear duplex DNA in a strand exchange reaction with unlabeled circular SS DNA performed in the presence of SSB provided additional evidence for the association of recA protein with the RFII product. As shown in Fig. 4, there were two major peaks of labeled DNA. The more rapidly sedimenting peak comigrated with the recA protein and sedimented to the same position in the gradient

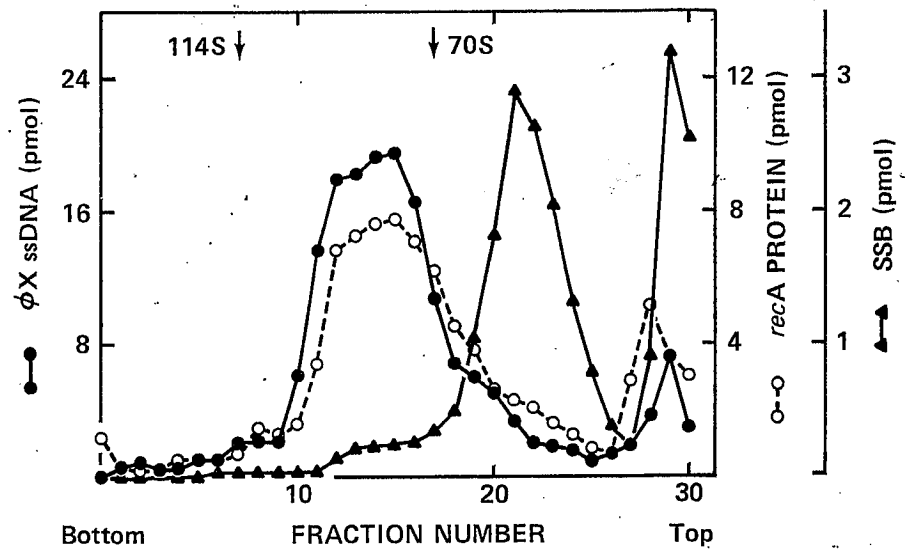

FIGURE 3. Association of recA protein with the RFII product of DNA strand exchange. Strand exchange was performed in the presence of SSB under standard conditions and the products sedimented as described (21).

as the recA protein-RFII complex shown in Fig. 3. The slower sedimenting peak comigrated with SSB (see Fig. 3). When analyzed by agarose gel electrophoresis, the DNA in the more rapidly sedimenting peak containing the recA protein was in the form of RFII, and the slower sedimenting SSB-DNA peak contained linear SS DNA. Thus, one strand of the linear duplex DNA is incorporated into the RFII product which is complexed with the recA protein and the other strand is associated with SSB.

The sedimentation coefficient of the recA protein-RFII complex shown in Figures 3 and 4 is approximately 85S. This value was not affected by the addition of 150 mM NaCl to the sucrose gradient indicating that the S value is again a reasonable measure of the size and density of the complex. However, when the products of the strand exchange reaction were incubated with 4 mM ADP prior to sedimentation, the recA protein and labeled DNA sedimented near the top of the gradient indicating that the recA protein-RFII complex had dissociated (Fig. 5). The dissociation of the

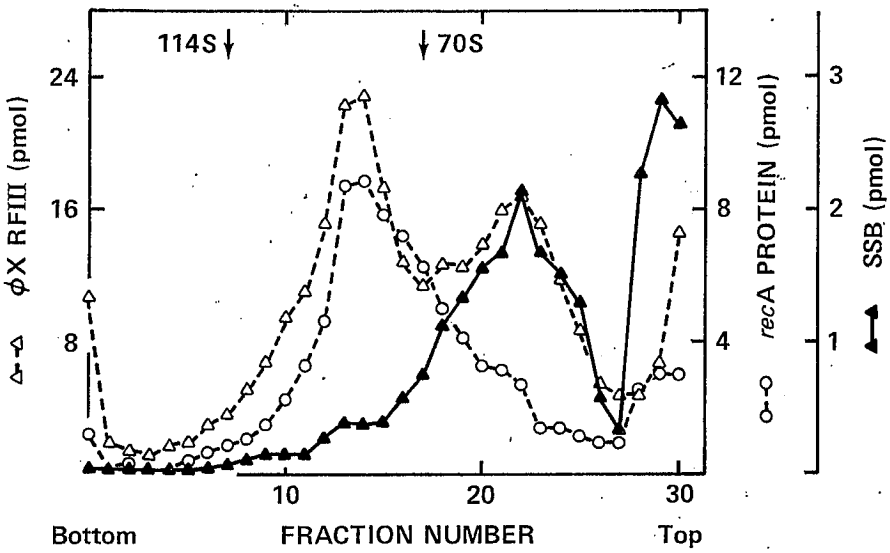

FIGURE 4. RecA protein-RFII complex formation during DNA strand exchange. The reaction was performed in the presence of SSB and the products sedimented as described (21).

recA protein-RFII complex by ADP had been inferred previously from kinetic analyses of the strand exchange reaction (17).

DISCUSSION

We have presented here a summary of our recent studies on the mechanism of recA protein-promoted DNA strand exchange. A plausible interpretation of the results of our DNA challenge experiments is that a specific recA protein-DNA complex is formed during strand exchange. The existence of this complex was confirmed by sedimenting the reaction intermediates and products in sucrose density gradients. Although SSB is necessary for the formation of the stable complex, we have been unable to demonstrate that SSB interacts directly with either the recA protein or SS DNA present in the complex as predicted by the model (18). It is possible, however, that the techniques used to exam-

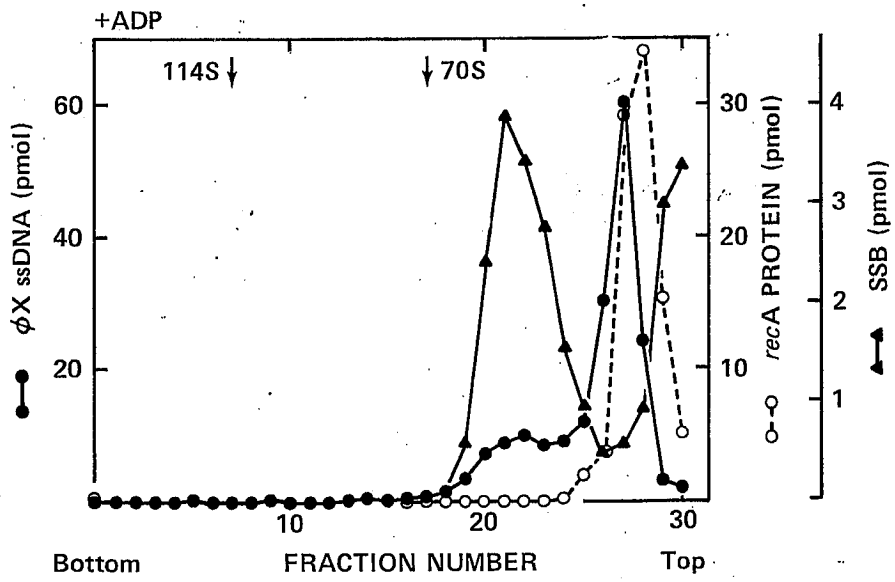

FIGURE 5. ADP mediated dissociation of recA protein from the RFII product of DNA strand exchange. Strand exchange in the presence of SSB was performed under standard conditions followed by incubation of the reaction products with ADP. Sedimentation was performed as described (21).

ine these complexes disrupted the interactions between the SSB and the recA protein-SS DNA complex. Flory and Radding (22) have reported differences in the structure of recA protein-SS DNA complexes formed in the presence and absence of SSB but they did not show directly that SSB was present in the complexes.

In the absence of SSB, the stable recA protein-SS DNA complex is not formed. Instead, recA protein equilibrates rapidly between free and bound forms. The inability of recA protein to form a stable complex with SS DNA in the absence of SSB causes the strand exchange reaction to proceed at a lower rate and to a lesser extent than when SSB is present (17,18). Thus, the formation of a stable complex between recA protein and SS DNA is necessary for efficient DNA strand exchange.

After completion of strand exchange in the presence of SSB, most of the recA protein is found in association with

the RFII product and the SSB is bound to the displaced linear SS DNA. The recA protein remains associated with the RFII as long as the level of ATP is maintained at a sufficiently high level. The accumulation of ADP causes the disruption of the recA protein-RFII complex and enables the released recA protein to interact with additional DNA substrates once the excess ADP has been converted to ATP. These results account for the previously observed inaccessibility of recA protein after the completion of strand exchange and are in good agreement with the model for the reaction proposed earlier (18).

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