**Complete Inhibition of *Streptococcus pneumoniae* RecA Protein-catalyzed ATP Hydrolysis by Single-stranded DNA-binding Protein (SSB Protein)**

**IMPLICATIONS FOR THE MECHANISM OF SSB PROTEIN-STIMULATED DNA STRAND EXCHANGE**

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The ATP-dependent three-strand exchange activity of the *Streptococcus pneumoniae* RecA protein (RecA(Sp)), like that of the *Escherichia coli* RecA protein (RecA(Ec)), is strongly stimulated by the single-stranded DNA-binding protein (SSB) from either *E. coli* (SSB(Ec)) or *S. pneumoniae* (SSB(Sp)). The RecA(Sp) protein differs from the RecA(Ec) protein, however, in that its ssDNA-dependent ATP hydrolysis activity is completely inhibited by SSB(Ec) or SSB(Sp) protein, apparently because these proteins displace RecA(Sp) protein from ssDNA. These results indicate that in contrast to the mechanism that has been established for the RecA(Ec) protein, SSB protein does not stimulate the RecA(Sp) protein-promoted strand exchange reaction by facilitating the formation of a presynaptic complex between the RecA(Sp) protein and the ssDNA substrate. In addition to acting presynaptically, however, it has been proposed that SSB(Ec) protein also stimulates the RecA(Ec) protein strand exchange reaction postsynaptically, by binding to the displaced single strand that is generated when the ssDNA substrate invades the homologous linear dsDNA. In the RecA(Sp) protein-promoted reaction, the stimulatory effect of SSB protein may be due entirely to this postsynaptic mechanism. The competing displacement of RecA(Sp) protein from the ssDNA substrate by SSB protein, however, appears to limit the efficiency of the strand exchange reaction (especially at high SSB protein concentrations or when SSB protein is added to the ssDNA before RecA(Sp) protein) relative to that observed under the same conditions with the RecA(Ec) protein.

*Streptococcus pneumoniae* is a naturally transformable bacterium that is able to take up DNA from its environment (in the form of ssDNA) and incorporate this DNA into its chromosome. It has been proposed that this process, known as transformational recombination, has evolved as a general mechanism that allows *S. pneumoniae* to change its genetic composition in response to environmental changes and stresses.

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This search identified an open reading frame encoding a protein similar in size (157 amino acids) and in sequence (31% identical, 50% similar at the amino acid level) to the SSB(Ec) protein (178 amino acids). We cloned the open reading frame, developed an efficient overexpression system, and purified the corresponding protein to greater than 99% homogeneity. We found that the purified protein binds to ssDNA in a manner similar to that of the SSB(Ec) protein and also stimulates the RecA(Sp) and RecA(Ec) protein-promoted strand exchange reactions. These results established that the protein was an S. pneumoniae analog of the SSB(Ec) protein (10).

In the course of evaluating the S. pneumoniae SSB protein (SSB(Sp)), it became apparent that the effect of this protein (as well as that of the SSB(Ec) protein) on the strand exchange activity of the RecA(Sp) protein was quite different from that seen with the RecA(Ec) protein. Our investigations into these differences provide insight into the mechanisms by which SSB proteins stimulate the RecA protein-promoted three-strand exchange reaction and are described in this report.

EXPERIMENTAL PROCEDURES

Materials—S. pneumoniae RecA protein (5), E. coli RecA protein (11), and S. pneumoniae SSB protein (10) were prepared as described. E. coli SSB protein was from Promega. ATP, dATP, [α-32P]ATP, [γ-32P]dATP, and dTTP were from Amersham Biosciences. Circular φX ssDNA (+strand) and circular φX dsDNA were from New England Biolabs. Linear φX dsDNA was prepared from circular φX dsDNA by PstI digestion as described (12). Single- and double-stranded φX DNA concentrations were determined by absorbance at 260 nm using the conversion factors 36 and 50 μg ml⁻¹ A260⁻¹, respectively. All DNA concentrations are expressed as total nucleotides.

NTP Hydrolysis Assay—ATP and dATP hydrolysis reactions were measured using a thin layer chromatography method as previously described (13). The specific conditions that were used for each set of reactions are given in the relevant figure legends.

RESULTS

Dependence of RecA Protein-promoted Strand Exchange on SSB Protein—The RecA(Sp) and RecA(Ec) proteins were analyzed for ATP-dependent three-strand exchange activity in the presence and absence of either SSB(Sp) or SSB(Ec) protein. In the three-strand exchange assay, a circular φX ssDNA molecule (5386 nucleotides) and a homologous linear φX dsDNA molecule (5386 base pairs) are recombined by RecA protein to form a nicked circular φX dsDNA molecule and a linear φX ssDNA molecule. The substrates and products of this reaction are readily monitored by agarose gel electrophoresis (12). The reaction solutions contained 5 μM circular φX ssDNA, 15 μM linear φX dsDNA, 5 mM ATP, and either 6 μM RecA(Sp) protein with or without 0.3 μM SSB(Sp) protein, or 6 μM RecA(Ec) protein with or without 0.3 μM SSB(Ec) protein, as indicated. The reactions were initiated by the addition of linear φX dsDNA with no SSB protein (minus SSB), or linear φX dsDNA followed by SSB protein (plus SSB). The final reaction solutions were incubated at 37 °C. At the indicated times, aliquots (20 μl) were removed from each reaction solution and quenched with SDS (1% final concentration)/EDTA (15 mM final concentration). The quenched aliquots were analyzed by electrophoresis on a 0.8% agarose gel using a Tris acetate-EDTA buffer system. The substrates and products of the reactions were visualized by ethidium bromide staining.

Essentially all of the circular ssDNA substrate was converted into the circular dsDNA product by the end of the 120-min reaction period. These results confirm that the strand exchange activity of the RecA(Ec) protein is stimulated by either SSB(Ec) or SSB(Sp) protein.
of intermediates and fully exchanged product was significantly lower in the RecA(Sp) reaction. These results indicate that although the strand exchange activity of the RecA(Sp) protein is strongly stimulated by either SSB(Sp) or SSB(Ec) protein, only a fraction of the circular ssDNA molecules are able to undergo strand exchange in the RecA(Sp) protein-promoted reaction.

Effect of SSB Protein on RecA Protein-catalyzed ssDNA-dependent ATP Hydrolysis—As noted in the Introduction, the SSB(Ec) protein is believed to stimulate the RecA(Ec) protein-promoted three-strand exchange reaction by facilitating the binding of RecA(Ec) protein to the circular ssDNA substrate. An experimental consequence of this SSB(Ec) protein-mediated increase in RecA(Ec) protein binding is that the observed rate of ssDNA-dependent ATP hydrolysis increases when SSB(Ec) protein is added to the reaction solution (9). In order to determine whether SSB protein stimulates the strand exchange reaction of the RecA(Sp) protein in a similar manner, the ssDNA-dependent ATP hydrolysis activities of the RecA(Sp) and RecA(Ec) proteins were measured in the presence and absence of SSB protein. The reaction solutions contained 5 μM φX ssDNA, 6 μM RecA protein, and either 0 or 0.3 μM SSB protein. Under these conditions (which simulate those used for the strand exchange reactions shown in Fig. 1), there is a sufficient amount of both RecA protein and SSB protein to cover all of the ssDNA present; maximal rates of ATP hydrolysis will be observed when the ssDNA is completely covered by RecA protein (linear φX dsDNA was not included in these reactions so that the effect of the SSB proteins on the RecA-φX ssDNA complexes could be monitored in the absence of an ongoing strand exchange reaction).

As shown in Fig. 2, the initial rate of ATP hydrolysis by the RecA(Sp) protein in the absence of SSB protein was ~20 μM min⁻¹. Since the turnover number for ssDNA-dependent ATP hydrolysis by the RecA(Sp) protein (determined with ssDNA in excess relative to RecA(Sp) protein) is ~35 min⁻¹ (5) (data not shown), the maximal rate of ATP hydrolysis that would be expected under the conditions of the reaction shown in Fig. 2 (with 5 μM φX ssDNA) would be ~58 μM min⁻¹ (1.7 μM RecA(Sp) protein bound, assuming a maximum binding stoichiometry of 1 RecA monomer/3 nucleotides of ssDNA (6, 7)). Therefore, the observed rate of 20 μM min⁻¹ indicates that only one-third of the ssDNA was covered with RecA(Sp) protein under the conditions in Fig. 2. Since the concentration of RecA(Sp) protein (6 μM) was ~4-fold greater than that required to completely cover the ssDNA, the observed rate of ATP hydrolysis suggests that approximately two-thirds of the ssDNA was inaccessible to the RecA(Sp) protein, presumably due to the existence of secondary structure in the ssDNA that impedes RecA(Sp) protein binding (see Ref. 9). This conclusion is consistent with the RecA(Sp) protein titration curves shown in Fig. 3. When ATP hydrolysis was measured at a fixed concentration of φX ssDNA (5 μM), the observed rate of hydrolysis increased with increasing RecA(Sp) protein concentration until reaching a maximal value of 20–24 μM min⁻¹ at RecA(Sp) protein concentrations above 2 μM. By comparison, when a similar titration was performed with a fixed concentration of dT₂₀₀ (5 μM) as the ssDNA effector (dT₂₀₀ does not form secondary structure), the observed rate of ATP hydrolysis was much higher and approached values close to the expected maximum of 58 μM min⁻¹ at RecA(Sp) concentrations above 2 μM (Fig. 3). These results demonstrate that the concentration of RecA(Sp) protein (6 μM) that was employed in the experiments shown in Fig. 2 was sufficient to cover all of the accessible binding sites in the φX ssDNA and indicate that the submaximal rate of ATP hydrolysis was due to only a limited amount of the φX ssDNA being available for RecA(Sp) protein binding.

The initial rate of ATP hydrolysis by the RecA(Ec) protein in the absence of SSB protein was 18 μM min⁻¹ (Fig. 2). Since the turnover number for ATP hydrolysis by the RecA(Ec) protein is ~22 min⁻¹ (14) (data not shown), the maximal rate of ATP hydrolysis that would be expected under the conditions of the reaction shown in Fig. 2 (with 5 μM φX ssDNA) would be ~37 μM min⁻¹ (1.7 μM RecA(Ec) protein bound). Therefore, the observed rate of 18 μM min⁻¹ indicates that approximately one-half of the φX ssDNA was covered with RecA(Ec) protein under the conditions in Fig. 2. Since the concentration of RecA(Ec) protein (6 μM) was again 4-fold greater than that required to completely cover the ssDNA, the observed rate of hydrolysis suggests that approximately one-half of the ssDNA was inaccessible to the RecA(Ec) protein. This conclusion is consistent with the RecA(Ec) protein titration curves in Fig. 3, which show that when ATP hydrolysis was measured at a fixed
concentration of δX ssDNA (5 μM) and increasing concentrations of RecA(Ec) protein, the observed rate of hydrolysis only reached a value of 20–24 μM min⁻¹, whereas when a similar titration was performed with δT₂₀₀ (5 μM), the rate reached the expected maximum of 37 μM min⁻¹.

The time courses of the ATP hydrolysis reactions that were catalyzed by the RecA(Ec) and RecA(Sp) proteins in the presence of SSB protein are also shown in Fig. 2 (SSB protein was added to the otherwise complete reaction solution at 10 min). The initial rate of ATP hydrolysis by the RecA(Ec) protein increased from 18 to 37 μM min⁻¹ when either SSB(Ec) protein (Fig. 2) or SSB(Sp) protein (not shown) was added to the reaction solution. This rate is equivalent to the expected maximal value of 37 μM min⁻¹ and indicates that both SSB proteins are able to facilitate the binding of RecA(Ec) protein to δX ssDNA such that essentially all of the δX ssDNA is covered with RecA(Ec) protein when either SSB protein is added to the reaction solution. In contrast, the ATP hydrolysis activity of the RecA(Sp) protein was completely inhibited as soon as either SSB(Sp) protein (Fig. 2) or SSB(Ec) protein (not shown) was added to the reaction solution. The total elimination of ATP hydrolysis activity suggests that the SSB proteins act not to facilitate the binding of RecA(Sp) protein but rather to displace the RecA(Sp) protein from the δX ssDNA.

To test the idea that the RecA(Sp) protein is unable to compete with SSB protein for binding to δX ssDNA, a ssDNA-dependent ATP hydrolysis reaction was carried out in which the δX ssDNA was incubated with SSB(Sp) protein before the RecA(Sp) protein was added to the reaction solution. As shown in Fig. 2, there was no ATP hydrolysis when this order of addition was followed, indicating that the RecA(Sp) protein was unable to displace SSB(Sp) protein from the δX ssDNA. Equivalent results were obtained with the SSB(Ec) protein (not shown). In contrast, the RecA(Ec) protein exhibited ATP hydrolysis activity even when it was added to the reaction solution after the δX ssDNA had been incubated with SSB(Ec) protein (Fig. 2) or SSB(Sp) protein (not shown). There was a delay in ATP hydrolysis with this order of addition, however, presumably reflecting the time-dependent displacement of SSB(Ec) protein from the ssDNA by the RecA(Ec) protein (Fig. 2).

**Time Course of ATP Hydrolysis during RecA Protein-promoted Strand Exchange**—The results in Fig. 1 demonstrate that the strand exchange activity of the RecA(Sp) protein is strongly stimulated by the SSB(Sp) protein. The results in Fig. 2, however, show that the ssDNA-dependent ATP hydrolysis activity of the RecA(Sp) protein is completely inhibited by SSB(Sp) protein. In order to clarify the relationship between the ATP hydrolysis and strand exchange activities of the RecA(Sp) protein, the time course of ATP hydrolysis during an ongoing RecA(Sp) protein-promoted strand exchange reaction was determined (Fig. 4). The reaction conditions were identical to those described for the strand exchange reactions shown in Fig. 1 (either linear dsDNA alone (no SSB protein) or linear dsDNA followed by SSB protein were added to the otherwise complete reaction solution at 10 min).

As shown in Fig. 4, the RecA(Sp) protein-catalyzed ATP hydrolysis reaction proceeded at an observed rate of ~20 μM min⁻¹ during the initial 10-min incubation period. When linear dsDNA alone was added to the reaction solution, there was little change in the rate of ATP hydrolysis (Fig. 4), and no strand exchange occurred (Fig. 1). In contrast, when the addition of linear dsDNA was followed by SSB(Sp) protein, the rate of ATP hydrolysis began to decrease immediately, resulting in the complete cessation of ATP hydrolysis within 20 min (Fig. 4). A comparison of this ATP hydrolysis time course with the time course of the strand exchange reaction (Fig. 1) shows that the strand exchange products were formed in the 20-min time period following the addition of SSB(Sp) protein.

The ATP hydrolysis activity of the RecA(Ec) protein under strand exchange conditions is also shown in Fig. 4. In contrast to the results that were obtained with the RecA(Sp) protein, the initial rate of the RecA(Ec) protein-catalyzed ATP hydrolysis reaction was higher when both linear dsDNA and SSB(Ec) protein were added to the reaction solution (36 μM min⁻¹) than when linear dsDNA alone was added (27 μM min⁻¹). Furthermore, the RecA(Ec) protein-promoted ATP hydrolysis reaction continued for at least 120 min in the presence of SSB(Ec) protein (Fig. 4), although most of the strand exchange products were formed within the first 30 min of the reaction (Fig. 1).

A comparison of the results in Figs. 2 and 4 indicates that the SSB(Sp) protein-mediated inhibition of the RecA(Sp) protein-catalyzed ATP hydrolysis reaction that was observed under strand exchange conditions (Fig. 4) was not as immediate as that observed with ssDNA alone (Fig. 2). This indicates that the RecA(Sp) protein may not be as readily displaced from a complex containing both ssDNA and dsDNA as it is from a complex containing only ssDNA. It is apparent that SSB(Sp)
protein does effect some change in the RecA(Sp)-ssDNA-dsDNA complex, however, since strand exchange does not occur until SSB(Sp) protein is added to the reaction solution. The eventual cessation of ATP hydrolysis indicates that the RecA(Sp) protein does not bind to the displaced linear ssDNA that is generated during the strand exchange reaction (or remain associated with the circular dsDNA reaction product). Instead, since RecA(Sp) protein is unable to compete with SSB protein for ssDNA binding under these conditions (Fig. 2), it is likely that the displaced linear ssDNA (as well as any unreacted circular ssDNA substrate) will be covered by SSB protein.

In the RecA(Ec) protein-promoted reaction, in contrast, the continuing ATP hydrolysis reaction that is observed after the completion of the strand exchange reaction may arise from RecA(Ec) protein bound to the displaced linear ssDNA (since RecA(Ec) protein can compete with SSB protein for ssDNA binding under these conditions) or from RecA(Ec) protein that remains associated with the circular dsDNA reaction product (15).

To more clearly define the conditions under which the SSB(Sp) protein is able to displace RecA(Sp) protein from the dsX ssDNA substrate, a strand exchange reaction was carried out in which SSB(Sp) protein was added to the reaction solution for 10 min after the dsX ssDNA had been incubated with RecA(Sp) protein but before the homologous linear dsDNA had been added to initiate the strand exchange reaction. There was no detectable strand exchange reaction when this order of addition was followed, indicating that the SSB(Sp) protein was able to displace the RecA(Sp) protein from the dsX ssDNA during the time period before the linear dsDNA was added to the reaction solution (gel not shown). There also was no detectable strand exchange when the dsX ssDNA was incubated with SSB(Sp) protein before the RecA(Sp) protein was added to the reaction solution (Fig. 5), indicating that the RecA(Sp) protein was unable to displace the SSB(Sp) protein from the dsX ssDNA. The RecA(Ec) protein, in contrast, exhibited strand exchange activity even when it was added to the reaction solution after the dsX ssDNA had been incubated with SSB(Ec) protein before the RecA(Ec) protein was added to the reaction solution (Fig. 5), presumably reflecting the time-dependent displacement of SSB(Ec) protein from the dsX ssDNA by the RecA(Ec) protein prior to the initiation of strand exchange (Fig. 5).

Dependence of RecA Protein-promoted Strand Exchange on SSB Protein Concentration—The results described above indicate that the strand exchange activity of the RecA(Sp) protein is both stimulated and inhibited by SSB(Sp) protein. To char-
characterize these competing effects further, a series of strand exchange reactions was carried out in which the concentrations of circular \( \phi X \) ssDNA (5 \( \mu M \)), linear \( \phi X \) dsDNA (15 \( \mu M \)), and RecA protein (6 \( \mu M \)) were kept constant, and the concentration of SSB(Sp) protein was varied from 0 to 3 \( \mu M \) (the concentration of SSB(Sp) or SSB(Ec) protein required to saturate the \( \phi X \) ssDNA (5 \( \mu M \)) under our reaction conditions was \( -0.3 \mu M \)).

As shown in Fig. 6, the efficiency of the RecA(Sp) protein-promoted strand exchange reaction increased as the concentration of SSB(Sp) protein was increased from 0 to 0.3 \( \mu M \) (the concentration used in the reaction shown in Fig. 1) and then decreased markedly as the SSB(Sp) protein concentration was increased further from 0.3 to 3.0 \( \mu M \). These results indicate that the strand exchange activity of the RecA(Sp) protein is stimulated optimally by moderate concentrations of SSB(Sp) protein (roughly equivalent to that required to saturate the ssDNA substrate) and that higher concentrations of SSB(Sp) protein act to counter this stimulatory effect. By comparison, the maximal level of strand exchange by the RecA(Ec) protein was achieved with 0.05 \( \mu M \) SSB(Ec) protein, and the efficiency of the reaction remained undiminished even at the highest SSB(Ec) protein concentration examined (3 \( \mu M \)) (Fig. 6).

**RecA(Sp) Protein-promoted dATP Hydrolysis and dATP-dependent Strand Exchange**—The results described above indicate that the RecA(Sp) protein-promoted strand exchange reaction is stimulated by SSB(Sp) protein only if (i) the SSB(Sp) protein is present at moderate concentrations and (ii) it is added to the reaction solution after the RecA(Sp) protein has been allowed to associate with both the circular \( \phi X \) ssDNA and linear \( \phi X \) dsDNA substrates. Even under optimal conditions, however, the RecA(Sp) protein-promoted strand exchange reaction does not proceed to the same extent as the RecA(Ec) protein-promoted reaction (Fig. 6), presumably because the SSB(Sp) protein displaces the RecA(Sp) protein from a significant fraction of the circular \( \phi X \) ssDNA molecules, rendering them inactive in strand exchange. We have shown, however, that the RecA(Ec) protein binds more tightly to ssDNA in the presence of dATP than with ATP (16) (also see Refs. 17 and 18), and this suggested to us that the RecA(Sp) protein might be able to compete more favorably with SSB protein if dATP were used in place of ATP as the nucleotide cofactor. To test this idea, we examined the ssDNA-dependent NTP hydrolysis and strand exchange activities of the RecA(Sp) protein in the presence of dATP.

The RecA(Sp) protein-catalyzed dATP hydrolysis reactions were carried out under the same conditions as the ATP hydrolysis reactions shown in Fig. 2. As shown in Fig. 7, the initial rate of dATP hydrolysis by the RecA(Sp) protein in the absence of SSB(Sp) protein was 38 \( \mu M \) min\(^{-1}\). Since the turnover number for dATP hydrolysis by the RecA(Sp) protein is \( -44 \) min\(^{-1}\) (5), the maximal rate of dATP hydrolysis that would be expected under the conditions of the reactions shown in Fig. 7 (with 5 \( \mu M \) \( \phi X \) ssDNA) would be \( -75 \mu M \) min\(^{-1}\) (1.7 \( \mu M \) RecA(Sp) protein bound). Therefore, the observed rate of 38 \( \mu M \) min\(^{-1}\) indicates that approximately one-half of the \( \phi X \) ssDNA was covered with RecA(Sp) protein under the conditions in Fig. 7. In contrast to the results that were obtained with ATP, however, the rate of the RecA(Sp) protein-catalyzed dATP hydrolysis reaction increased to 68 \( \mu M \) min\(^{-1}\) when SSB(Sp) protein was added to the reaction solution (Fig. 7). This rate is close to the expected maximal value of 75 \( \mu M \) min\(^{-1}\) and indicates that essentially all of the \( \phi X \) ssDNA was covered with RecA(Sp) protein under these conditions (the decrease in dATP

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2 S. E. Steffen and F. R. Bryant, unpublished results.
Hydrolysis after 60 min is probably due to the accumulation of dADP in the reaction solution. Furthermore, the RecA(Sp) protein exhibited dATP hydrolysis activity even when the dsDNA ssDNA was incubated with SSB(Sp) protein before the RecA(Sp) protein was added to the reaction solution (Fig. 7). These results indicate that the RecA(Sp) protein can displace SSB(Sp) protein from the dsDNA ssDNA when dATP is present in the reaction solution. This conclusion is consistent with the strand exchange reactions shown in Fig. 8. In contrast to the results that were obtained with ATP, the RecA(Sp) protein exhibited dATP-dependent strand exchange activity when it was incubated with the dsDNA ssDNA before SSB(Sp) protein was added to the reaction solution and when it was added to the reaction solution after the dsDNA ssDNA had been incubated with the SSB(Sp) protein (there was no detectable dATP-dependent strand exchange activity in the absence of SSB protein (gel not shown)).

**DISCUSSION**

SSB protein has been shown to stimulate the RecA(Ec) protein-promoted three-strand exchange reaction by removing secondary structure from the circular ssDNA substrate, thereby allowing the ssDNA to be more completely covered by RecA(Ec) protein (9). This increase in RecA(Ec) protein binding is reflected by an increase in the observed rate of ssDNA-dependent ATP hydrolysis when SSB protein is added to the reaction solution (9). The RecA(Sp) protein differs dramatically from the RecA(Ec) protein, however, in that its ssDNA-dependent ATP hydrolysis activity is completely inhibited by SSB protein, apparently because SSB protein displaces the RecA(Sp) protein from the ssDNA. Nevertheless, the ATP-dependent three-strand exchange activity of the RecA(Sp) protein is strongly stimulated by SSB protein. These results indicate that, in contrast to the mechanism that has been established for the RecA(Ec) protein, SSB protein does not stimulate the RecA(Sp) protein-promoted strand exchange reaction by increasing the binding of RecA(Sp) protein to the circular ssDNA substrate.

In addition to facilitating the formation of the presynaptic RecA(Ec)-ssDNA complex, it has been reported that SSB protein also stimulates the RecA(Ec) protein-promoted strand exchange reaction postsynaptically, by binding to the partially displaced linear single strand that is generated when the circular ssDNA invades the homologous linear dsDNA. The binding of SSB protein is believed to enhance the formation of the initial DNA pairing intermediates, prevent secondary DNA pairing reactions (which may arise from the invasion of the partially displaced strand into a second dnaDNA molecule), and drive the reaction forward to the formation of the completely exchanged circular dsDNA product (19, 20). Since our results clearly indicate that SSB protein will bind to ssDNA in the presence of the RecA(Sp) protein, it is reasonable to propose that the stimulatory effect of SSB protein on the RecA(Sp) protein-promoted strand exchange reaction may be due to the postsynaptic binding of the displaced single strand of the dsDNA substrate.

The SSB protein-stimulated strand exchange reaction of the RecA(Sp) protein is curious inasmuch as the rate of ATP hydrolysis that was measured prior to the addition of SSB protein indicated that only about one-third of the circular dsDNA ssDNA was covered by RecA(Sp) protein (presumably as a result of secondary structure in the ssDNA that impedes RecA(Sp) protein binding; see Ref. 9). If the dsDNA ssDNA substrate was only partially covered by RecA(Sp) protein (and if SSB protein does not facilitate the binding of additional RecA(Sp) protein to the ssDNA), it is not clear how the circular dsDNA and linear ssDNA strand exchange products would be able to form, since it is generally believed that the ssDNA substrate has to be completely covered with a continuous filament of RecA protein before strand exchange can occur (6, 7). One possibility is that a distribution of RecA(Sp)-ssDNA complexes exists under our reaction conditions, some of which are much more covered by RecA(Sp) protein and others that are much less covered (with the average coverage being approximately one-third). Those dsDNA ssDNA molecules that are more covered by RecA(Sp) protein would be more likely to interact with the homologous linear dsDNA substrate; SSB protein may then activate these complexes for strand exchange by binding to the displaced strand of the linear dsDNA substrate. Those dsDNA ssDNA molecules that are less covered by RecA(Sp) protein, on the other hand, would not interact effectively with the linear dsDNA substrate; SSB protein may act to displace the RecA(Sp) protein from these dsDNA ssDNA molecules, thereby rendering them inactive for strand exchange. This scenario would account for our results, which showed that although the time required for the initial appearance of partially exchanged intermediates and fully exchanged products was similar to that observed with the RecA(Ec) protein, only a fraction of the dsDNA ssDNA substrate molecules were converted to intermediates and products in the RecA(Sp) protein-promoted strand exchange reaction. With the RecA(Ec) protein, in contrast, SSB protein would not only enhance the strand exchange reactivity of those dsDNA ssDNA molecules that were initially covered by RecA(Ec) protein (by binding to the displaced strand of the linear dsDNA substrate) but would also facilitate the binding of additional RecA(Ec) protein to those dsDNA ssDNA molecules that were initially less covered by RecA(Ec) protein, thereby activating these ssDNAs for strand exchange as well. As a result, essentially all of the circular dsDNA ssDNA is converted to the fully exchanged dsDNA product in the RecA(Ec) protein-promoted reaction.

The competing stimulatory and inhibitory effects of SSB

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3 Since the effects of the SSB(Ec) and SSB(Sp) proteins on the NTP hydrolysis and strand exchange activities of the RecA(Ec) and RecA(Sp) proteins were experimentally indistinguishable (see "Results"), we will refer to these two proteins collectively as "SSB protein" under "Discussion."
protein that are proposed above are consistent with the pronounced dependence of the RecA(Sp) protein-promoted strand exchange reaction on SSB protein concentration. If the concentration of SSB protein is too low, there will not be enough SSB protein to bind all of the displaced single strands, and only a limited amount of strand exchange will occur. If the SSB protein concentration is too high, however, SSB protein may displace the RecA(Sp) protein from the ssDNA substrate before strand exchange can be initiated. The optimum SSB protein concentration probably represents a concentration high enough to activate most of the RecA(Sp)-ssDNA-dsDNA complexes, while low enough to minimize the rate of the competitive displacement of RecA(Sp) protein from the ssDNA substrate. Even so, no ATP-dependent strand exchange is observed (even at the optimal SSB protein concentration) if (i) the ssDNA substrate is incubated with SSB protein before the RecA(Sp) protein is added to the reaction solution or (ii) the SSB protein is added to the RecA(Sp)-ssDNA complex before linear dsDNA is added to the reaction solution. In these cases, the SSB protein would either prevent the RecA(Sp) protein from binding to the ssDNA (case i), or displace the RecA(Sp) protein from the ssDNA before strand exchange can be initiated (case ii). The RecA(Ec) protein, in contrast, is able to promote strand exchange efficiently in the presence of high concentrations of SSB protein and regardless of the order in which the RecA(Ec) and SSB(Ec) proteins are added to the reaction solution.

In contrast to the inhibitory effects that were observed on the ssDNA-dependent ATP hydrolysis reaction, SSB protein had a stimulatory effect on the RecA(Sp) protein-catalyzed hydrolysis of dATP. When RecA(Sp) protein was incubated with δX ssDNA in the presence of δATP and SSB protein, the rate of the ensuing dATP hydrolysis reaction was close to that which would be expected if the δX ssDNA was completely covered by RecA(Sp) protein. We recently found that RecA(Ec) protein binds to ssDNA much more tightly in the presence of δATP than with ATP (16), and preliminary experiments indicate that the RecA(Sp) protein behaves in a similar manner. It is likely that the tighter binding that is induced by δATP allows the RecA(Sp) protein to compete favorably with SSB protein for binding to ssDNA, and as a result, the RecA(Sp) protein is able to completely cover δX ssDNA when SSB protein is added to the reaction solution. Consistent with this conclusion, the RecA(Sp) protein is able to catalyze δATP hydrolysis and to promote δATP-dependent strand exchange, even when the circular ssDNA substrate is incubated with SSB protein before the RecA(Sp) protein is added to the reaction solution. Thus, the RecA(Sp) protein behaves in a manner similar to that of the RecA(Ec) protein, when δATP is provided as the nucleotide cofactor.

The biochemical properties of the RecA(Sp) protein are intriguing when considered in the context of the mechanism of transformational recombination. It has been reported that when ssDNA is transported into the S. pneumoniae cell during transformation, it is coated by an SSB-like protein that protects it from degradation by cellular nucleases (21). Presumably, this SSB-like protein (which has not yet been characterized) must be displaced from the ssDNA by RecA(Sp) protein before the ssDNA can be integrated into the S. pneumoniae chromosome. If this SSB-like protein (Mₚ = 19,500, as estimated by SDS-PAGE mobility (21)) and the SSB(Sp) protein (Mₚ = 17,400 (10)) are the same protein, however, it is not clear how a RecA(Sp)-ssDNA complex would be able to form, since our results indicate that the RecA(Sp) protein is unable to displace SSB(Sp) protein from ssDNA, at least in the presence of the presumed natural cofactor, ATP. It is possible that either our experimental conditions do not adequately mimic the conditions inside the S. pneumoniae cell, or δATP is somehow able to serve as a cofactor for the RecA(Sp) protein in vivo. It is also conceivable that the inhibition of ssDNA binding by the SSB(Sp) protein may serve as a regulatory mechanism and that the assembly of a RecA(Sp)-ssDNA filament may require other recombinational accessory proteins. Alternatively, an inspection of the S. pneumoniae genome sequence reveals that in addition to the gene encoding the SSB(Sp) protein described here (designated ssb in the genome sequence), there is a second gene (designated ssbB in the genome sequence) that also appears to encode an SSB-like protein (22, 23). A recent genetic study indicates that the ssbB gene (referred to as cilA in the study) may be part of a competence-induced operon, suggesting that it may play a role in transformational recombination (24).

Although the predicted molecular weight of the ssbB protein is only 14,800 (as calculated from the gene sequence), it is conceivable that it is the same protein as the SSB-like protein that was previously identified as being associated with the exogeneous ssDNA during transformational recombination (24). It will therefore be of interest to examine the effect of the ssbB protein on the strand exchange activity of the RecA(Sp) protein.

The isolation and characterization of the ssbB protein are in progress.

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