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Interaction of a *Saccharomyces cerevisiae* Strand Exchange Stimulatory Factor with DNA†

David Norris and Richard Kolodner*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, and Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115

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ABSTRACT: In the preceding paper (Norris & Kolodner, 1990), we described the purification of a M_r 33 000 polypeptide which dramatically stimulated the activity of SEP1, the yeast mitotic strand exchange protein. In this paper, we characterized this new protein, which was designated SF1, in the absence of SEP1. SF1 had a sedimentation coefficient of 1.7 S and a Stokes radius of 30 Å, which was consistent with a calculated native molecular weight of 31 000, indicating that SF1 existed in solution as a monomer. Filter binding assays showed that SF1 bound preferentially to single-stranded rather than double-stranded DNA. Fluorescence spectroscopy analysis indicated that SF1 occluded approximately eight nucleotides when bound to single-stranded DNA and exhibited a dissociation constant, K_D, of 2.83×10^{-6} M. The binding of SF1 to single-stranded DNA was noncooperative and appeared to involve at least one tyrosine residue. SF1, in the absence of SEP1, stimulated the renaturation of homologous single-stranded DNA, suggesting that it might act directly in some phase of the strand exchange reaction.

In the preceding paper (Norris & Kolodner, 1990), we described the purification of a M_r 33 000 polypeptide which dramatically stimulated the enzymatic activity of the SEP1 protein, the mitotic yeast strand exchange protein (Kolodner et al., 1987; Heyer et al., 1988). In the presence of this new protein, which was designated SF1 for *s*timulatory *f*actor *1*, the optimal amount of SEP1 in the in vitro strand exchange reaction dropped from 1 SEP1 monomer per 10–12 nucleotides to 1 SEP1 monomer per 725 nucleotides of single-stranded DNA. Even at levels as low as 1 monomer of SEP1 per 5800 nucleotides of single-stranded DNA, the rate of joint molecule formation in the presence of SF1 was equivalent to the maximal rate that could be catalyzed by SEP1 alone. The SF1 protein, however, did not appear to change any of the other characteristics of the SEP1-catalyzed reaction: the reaction requirements of the simulated and unstimulated reactions were the same, and the distributions of joint molecule products in the two reactions were nearly identical. To confer the observed levels of stimulation, SF1 had to be present at the relatively high stoichiometry of 1 SF1 monomer per 20 nucleotides of single-stranded DNA.

When compared to other purified strand exchange stimulatory factors (Cox & Lehman, 1981; Cox et al., 1983; Formosa & Alberts, 1986; Hamatake et al., 1989; Harris & Griffith, 1989; Heyer & Kolodner, 1989; Kodadek & Alberts, 1987; Kodadek et al., 1989; Muniyappa et al., 1984; Roman & Kowalczykowski, 1989; Yonesaki & Minagawa, 1989), SF1 imparted a level of stimulation on its cognate strand exchange protein which was unprecedented. Indeed, the observation that joint molecule formation in the presence of SF1 occurred at an SEP1 concentration of 1 monomer per 5800 nucleotides of single-stranded DNA called into question some accepted ideas about how strand exchange proteins work in vivo, particularly with regard to the presynaptic filament, a proposed obligatory intermediate in the strand exchange reactions catalyzed by the RecA and UvsX proteins (Cox & Lehman, 1987; Griffith & Harris, 1988). It was therefore of interest to understand the mechanism by which SF1 stimulated SEP1. As a first step toward this goal, we decided to characterize SF1 in the absence of SEP1. SF1 was found to be a monomer in solution which bound preferentially to single-stranded DNA

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* To whom correspondence should be addressed at the Dana-Farber Cancer Institute.

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with a $K_D = 2.83 \times 10^{-6}$ M. In addition, SF1 catalyzed the renaturation of homologous single-stranded DNA, a result which suggested several possibilities about how it might function in the stimulated strand exchange reaction.

Materials and Methods

Strains. *Escherichia coli* strains RDK1237 (*HfrH, thi-1, rel-1*) and 011′ (*thyA, deo, sup*) and bacteriophages M13mp19 and T7 were from laboratory stocks.

Enzymes. The purification of SF1 was described in the preceding paper (Norris & Kolodner, 1990); fraction VIII was used for all experiments. S1 nuclease was from Bethesda Research Laboratories. *E. coli* SSB was purified as previously described (Heyer & Kolodner, 1989), and the final preparation was >98% pure as demonstrated by SDS–PAGE.

Nucleic Acids. (*A*) *RF and Viral M13mp19 DNA. E. coli* strain RDK1237 was grown at 37 °C in LB medium (6 × 500 mL) to an $OD_{590} = 0.5$, at which point the culture was infected at an moi = 10 with M13mp19 bacteriophage. After 7 h of additional incubation at 37 °C, the culture was centrifuged at 4000 rpm for 20 min in a Beckman J-6M centrifuge. RF DNA was purified from the cell pellet by the cleared lysate method (Clewell & Helinski, 1969) followed by two cycles of equilibrium centrifugation in CsCl gradients containing ethidium bromide. Viral DNA was purified from the supernatant by a modification of previously published protocols (Messing, 1983). The supernatant was brought to 0.5 M NaCl, 20 mM Tris-HCl, pH 7.5, and 6% (w/v) PEG 6000 (Fluka AG) and was stirred gently at 4 °C for 12 h. The resulting PEG precipitate was collected by centrifugation at 4000 rpm for 20 min in a J-6M centrifuge. The pellet was resuspended in a wash solution [1 M NaCl, 0.1 M Tris-HCl, pH 7.5, and 1% (v/v) Sarkosyl] at 15 mL of wash solution per 500 mL of original culture. The resuspended phage solution was then cleared of insoluble material by centrifugation at 4 °C in a Sorvall SS34 rotor for 10 min at 10000 rpm. The supernatant was collected and brought to 6% (w/v) PEG 6000. The PEG precipitate was collected by centrifugation at 4 °C in a SS34 rotor at 10000 rpm for 10 min and was then resuspended in approximately 4 mL of 50 mM Tris-HCl, pH 7.5, per 500 mL of original cell culture. CsCl was added to 2.4 M, and the resulting solution was centrifuged in a Beckman 70.1 Ti rotor at 40000 rpm for 24 h. The translucent blue band containing the bacteriophage was collected and dialyzed against TE buffer (10 mM Tris, pH 8.0, and 1 mM EDTA). DNA was purified from the bacteriophage by phenol extraction followed by dialysis against TE.

To purify radioactive viral and RF M13mp19 DNA, *E. coli* strain RK1237 was grown in Fraser's medium to an $OD_{590} = 0.15$. At this point, [^{3}H]thymidine (Du Pont–New England Nuclear), adenosine, and deoxyadenosine were added to final concentrations of 0.125 mCi/500 mL, 1 mM, and 1 mM, respectively. The cells were then incubated at 37 °C with aeration until they reached an $OD_{590} = 0.7$, at which point they were infected with M13mp19 bacteriophage at an moi = 10. The cells were processed, and the DNA was purified as described above for nonradioactive DNA. The specific activities for the DNAs used in these experiments were 5280 cpm/nmol for the RF DNA and 3795 cpm/nmol for the viral DNA.

(*B*) *Radioactive T7 Phage DNA.* Radioactive T7 phage DNA was purified as described previously (Richardson, 1966) using *E. coli* 011′ as the host. The specific activity for the DNA used in these experiments was 25410 cpm/nmol.

DNA Binding Assays. DNA binding assays were performed as described previously (Heyer et al., 1988).

Renaturation and S1 Protection Assays. The renaturation and S1 protection assays were performed as described previously (Heyer et al., 1988).

Fluorescence Spectrophotometry. The fluorescence measurements for SF1 and SF1–nucleic acid complexes were made on a Kontron SFM 25 fluorescence spectrophotometer. The fluorescence was monitored at 23 °C in 1-cm path-length cells, with an excitation wavelength of 276 nm and an emission wavelength of 301 nm. To calculate the association constant, K, the fluorescence quenching data were plotted according to the equation described by Kelley et al. (1976):

$$1/\Delta F = 1/K[\mathrm{N}]_{\mathrm{FREE}}\Delta F_\infty + 1/\Delta F_\infty$$

where ΔF = the decrease in fluorescence intensity, $[\mathrm{N}]_{\mathrm{FREE}}$ = the concentration of free nucleotide binding sites, and ΔF_∞ = the decrease in fluorescence intensity at infinite DNA concentration.

To determine $[\mathrm{N}]_{\mathrm{FREE}}$, the concentration of free DNA binding sites, we used the equation described by McGhee and von Hippel (1974):

$$[\mathrm{N}]_{\mathrm{FREE}} = N(1 - n\nu)\{(1 - n\nu)/[1 - (n-1)\nu]\}^{n-1}$$

where N = the molar concentration of nucleotide, n = the binding site size, or the number of nucleotides occluded upon binding to SF1, and ν = the binding density, expressed in units of moles of bound ligand per mole of lattice residue.

Results

Hydrodynamic Properties of SF1. In the preceding paper (Norris & Kolodner, 1990), the monomer molecular weight of SF1 was determined by SDS–PAGE to be approximately 33000. This result, however, did not indicate whether SF1 was a monomer or some higher order multimer in its native state. To answer this question, the native molecular weight of SF1 was calculated from its hydrodynamic properties. The Stokes radius of SF1, determined by size fractionation through HW55 gel filtration media (Toyosoda) according to the method of Laurent and Killander (1964), was 30 Å (standards used were chymotrypsin, 20.9 Å; ovalbumin, 30.5 Å; and bovine serum albumin, 35.5 Å). The sedimentation coefficient of SF1, determined by centrifugation through 5–20% (w/v) sucrose gradients according to the method of Martin and Ames (1961), was 1.7 S (standards used were lysozyme, 2.1 S; bovine serum albumin, 4.3 S; and catalase, 11.3 S). Assuming a partial specific volume of 0.725 cm^3/g, SF1 had a native molecular weight of approximately 31000 (Siegel & Monty, 1966). Since this value agreed quite closely with the value obtained by SDS–PAGE analysis, SF1 appeared to exist in solution as a monomer. Moreover, SF1 had a Perrin shape factor, f/f_{sph}, of 1.45, indicating an asymmetric shape in which one of its axes was roughly 8 times longer than its other two axes (Cantor & Schimmel, 1980b).

DNA Binding Properties of SF1. To determine the nucleic acid binding properties of SF1, the protein was incubated with radioactive single-stranded or double-stranded DNA under the same conditions used for the strand exchange reaction. The SF1–DNA complexes were then collected on KOH-treated nitrocellulose filters (McEntee et al., 1980), and the bound DNA was quantified by liquid scintillation counting. In the presence of 10 and 20 mM NaCl, 1–2 μg of SF1 bound >90% of the single-stranded M13mp19 DNA into complexes that could be collected on nitrocellulose filters (Figure 1A). In the presence of increasing NaCl concentrations, however, the ability of SF1 to bind to single-stranded DNA decreased, until no binding was evident at 100 mM NaCl. It is interesting to note that SF1 also began to lose its stimulatory effect above 20 mM NaCl (D. Norris and R. Kolodner, unpublished ob-

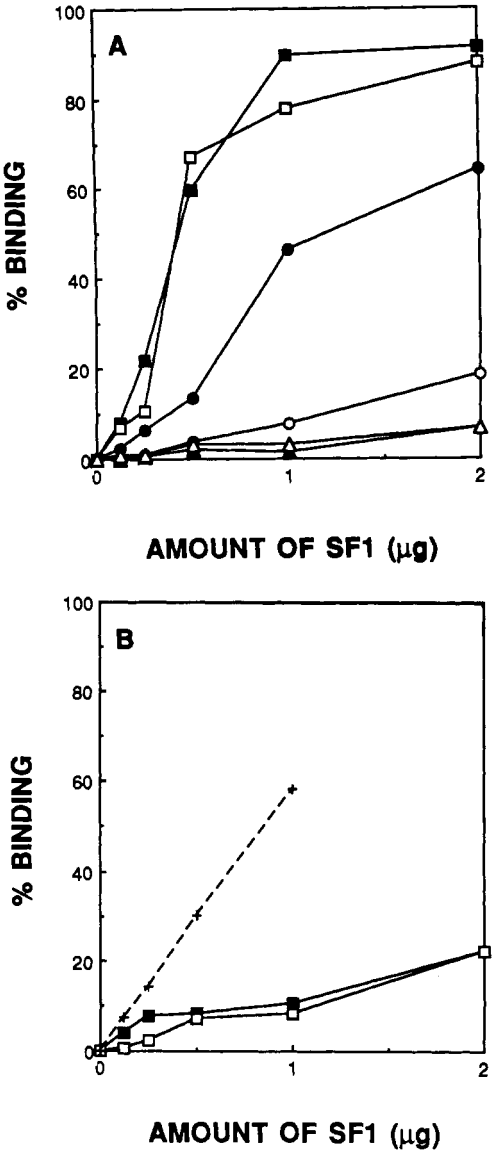

FIGURE 1: SF1 binding to single-stranded and double-stranded DNA as determined by nitrocellulose filter binding. (A) Reactions (15 μL) contained 33 mM Tris-HCl (pH 7.5), 13 mM $MgCL_2$, 1.8 mM dithiothreitol, 88 μg/mL BSA, 200 ng of ^{3}H-labeled M13mp19 viral DNA, the indicated amounts of SF1, and (■) 10, (□) 20, (●) 50, (○) 75, (▲) 100, or (△) 200 mM NaCl. The reactions were incubated for 5 min at 30 °C and were diluted with 500 μL of wash solution [33 mM Tris-HCl (pH 7.5) and 13 mM $MgCl_2$] containing the appropriate concentrations of NaCl. The diluted reactions were filtered through KOH-treated nitrocellulose filters (McEntee et al., 1980). The filters were washed 1 time with 1 mL of wash solution, containing the appropriate concentrations of NaCl, and the trapped radioactivity was determined by scintillation counting. (B) As in (A) except that reactions contained 400 ng of ^{3}H-labeled T7 bacteriophage DNA and (■) 10 or (□) 20 mM NaCl. Included is the binding of SEP1 to the same DNA preparation (dashed line).

servations), suggesting that the binding of SF1 to single-stranded DNA was a prerequisite for stimulation.

In contrast to its affinity for single-stranded DNA, SF1 bound weakly to double-stranded DNA under the strand exchange conditions (Figure 1B). At 10 and 20 mM NaCl, concentrations at which maximal binding to single-stranded DNA occurred, less than 25% of double-stranded DNA was converted into complexes that could be collected on nitrocellulose filters by 2 μg of SF1. This low amount of binding was not attributable to some problem with the DNA preparation, since SEP1 bound with the expected stoichiometry to the same DNA preparation (Figure 1B) (Heyer et al., 1988). It is important to note that at SF1–DNA stoichiometries sufficient for maximal stimulation of the strand exchange reaction (Norris & Kolodner, 1990), less than 10% of double-stranded DNA but more than 90% of single-stranded DNA was converted into complexes that could be collected on nitrocellulose filters. This suggested that SF1 was stimulatory by virtue of its ability to bind to single-stranded DNA.

The observed differential binding of SF1 to single-stranded and double-stranded DNA explained why, in 50 mM NaCl, SF1 bound to single-stranded DNA–cellulose but flowed through double-stranded DNA–cellulose (Norris & Kolodner, 1990). The different affinities also accounted for the DNA aggregation characteristics of SF1 (Norris & Kolodner, 1990). SF1 aggregated both single-stranded and double-stranded DNA, but the aggregation of single-stranded DNA required less SF1 than the aggregation of double-stranded DNA. This probably resulted from the fact that its affinity for double-stranded DNA was less than that for single-stranded DNA. Moreover, more SF1 was required to aggregate double-stranded DNA in the presence than in the absence of single-stranded DNA. This could be explained by assuming that the single-stranded DNA titrated SF1 out of solution first, making it necessary to add more SF1 to see subsequent double-stranded DNA aggregation.

Determination of Binding Parameters by Fluorescence Spectroscopy. Filter binding assays are useful for qualitative determination of binding affinities, but fluorescence spectroscopy allows a more rigorous quantitative analysis of protein–DNA interactions. On the basis of the theoretical framework developed by McGhee and von Hippel (1974), this type of analysis allows one to determine three parameters of binding: the dissociation constant (K_D), the cooperativity constant (ω), and the binding site size (n). In order for fluorescence spectroscopy to be applicable, the protein of interest must fluoresce, and this fluorescence must be quenched when the protein binds to DNA. SF1 fulfilled both of these requirements for single-stranded DNA, but not for double-stranded DNA (see below).

To determine the binding site size, or the number of nucleotides occluded in an SF1–DNA complex, the fluorescence–spectrophotometric method developed by Alma et al. (1983) was used. According to this method, increasing quantities of SF1 were added to a reaction mixture that contained a fixed quantity of DNA. At low concentrations, the DNA in the reaction mixture titrated out the protein and, thus, quenched its intrinsic fluorescence. As more protein was added, however, the protein eventually saturated all of the DNA binding sites. From then on, the fluorescence of additional protein was no longer quenched, and therefore the fluorescence of the mixture as a whole increased more rapidly. The point where this change in slope occurred was used as an estimate of the binding site size (n). As seen in Figure 2, for single-stranded DNA, this change in slope occurred at 8.56 nucleotides per SF1 monomer. A second experiment conducted under essentially identical conditions resulted in a value of 7.56 nucleotides per SF1 monomer (data not shown). We therefore concluded that SF1 covered approximately 8 nucleotides upon binding to single-stranded DNA. It should also be noted that the first part of the biphasic fluorescence curve appeared to be linear, indicating that the binding of SF1 to single-stranded DNA was a noncooperative process (Alma et al., 1983). In other words, for SF1 binding to single-stranded DNA, $\omega = 1$.

In contrast, the fluorescence curve for binding to double-stranded DNA was completely linear, having no apparent point

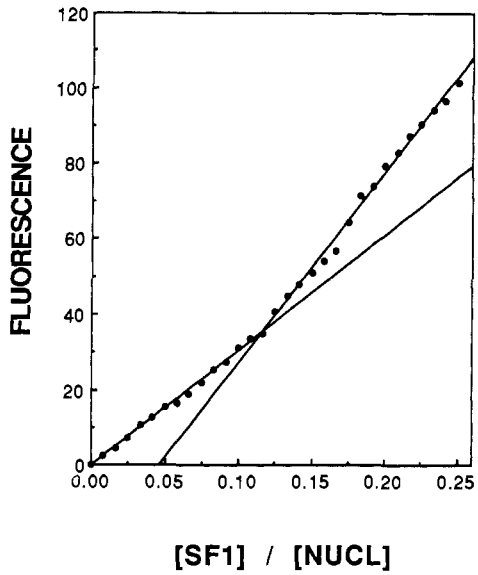

FIGURE 2: Binding of SF1 to single-stranded DNA as determined by fluorescence spectrophotometry. Reaction mixtures (3 mL) contained 33 mM Tris-HCl (pH 7.5), 13 mM $MgCl_2$, 20 mM NaCl, and 1.2 μg of M13mp19 viral DNA. For each point, 1 μg of SF1 was added, and the reaction mixture was gently mixed and allowed to equilibrate for 3 min at 23 °C. Fluorescence was measured at an excitation wavelength of 276 nm and an emission wavelength of 301 nm.

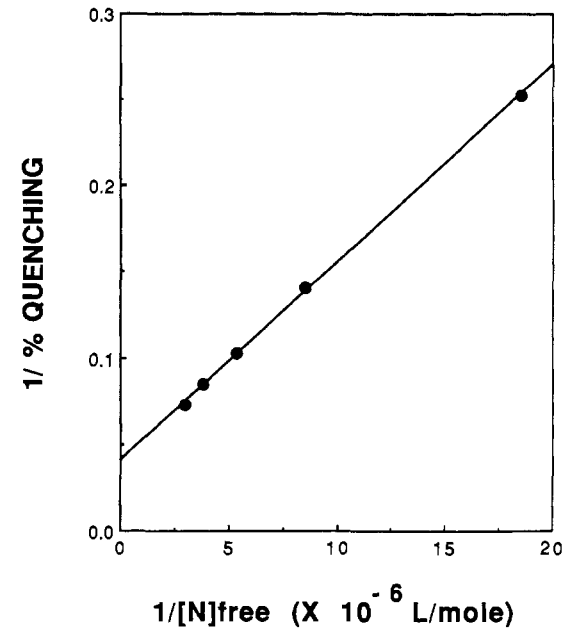

FIGURE 3: Determination of the affinity of SF1 for single-stranded DNA by fluorescence spectroscopy (Materials and Methods). The reaction mixture (3 mL) contained 33 mM Tris-HCl (pH 7.5), 13 mM $MgCl_2$, 20 mM NaCl, and 30 μg of SF1. For each point, 1 μg of viral M13mp19 DNA was added, and the reaction mixture was gently mixed and allowed to equilibrate for 3 min at 23 °C. Fluorescence was measured at an excitation wavelength of 276 nm and an emission wavelength of 301 nm.

where the slope changed (data not shown). One interpretation of this result was that SF1 was unable to bind to double-stranded DNA. This interpretation, however, seemed unlikely on the basis of the aggregation (Norris & Kolodner, 1990) and filter binding experiments (Figure 1B). A more likely interpretation was that the mechanism of association with double-stranded DNA differed from that with single-stranded DNA. According to the latter interpretation, this different mode of binding resulted in a lower affinity for double-stranded DNA (Figure 1) and altered quenching properties (Figure 2). Furthermore, the fluorescent properties of SF1 in these experiments suggested how the binding of SF1 to double-stranded DNA might differ from its binding to single-stranded DNA. The wavelengths used for excitation and emission in these experiments, which were the points of maximum excitation and emission of SF1 (data not shown), corresponded to the excitation and emission wavelengths of tyrosine (Cantor & Schimmel, 1980a). The results therefore suggest that a tyrosine residue(s) was (were) intimately involved in the binding of SF1 to single-stranded DNA but that this (these) same tyrosine(s) was (were) not involved in the binding of SF1 to double-stranded DNA.

The binding of SF1 to single-stranded DNA was further characterized by using the method of Kelley et al. (1976). According to this method, fluorescence quenching was monitored as increasing quantities of DNA were added to a fixed concentration of protein. A double-reciprocal plot of quenching vs free DNA binding sites was linear, as expected for noncooperative binding, the y intercept was equal to the reciprocal of the fluorescence quenching at infinite DNA concentrations, and the slope was inversely proportional to the molecular dissociation constant (Figure 3). From the y intercept, we calculated that the fluorescence quenching at infinite DNA concentrations was 25%. From the slope of the curve, the K_D for the binding of SF1 to single-stranded DNA was calculated to be 2.83×10^{-6} M.

DNA Renaturation Promoted by SF1. The preceding analysis showed that SF1 existed in solution as a monomer and bound with greater affinity to single-stranded than to double-stranded DNA. These results, however, shed little light on the mechanism by which SF1 stimulated strand exchange. The observation that strand exchange occurred when SEP1 and SF1 were present at 1 monomer every 5800 nucleotides and 1 monomer every 20 nucleotides, respectively, suggested that SF1 might catalyze a subreaction required for joint molecule formation. According to this hypothesis, SEP1 provided a function that SF1 lacked, a function that was required at relatively low levels. On the basis of the analysis of RecA-mediated strand exchange, particularly with regard to the high protein stoichiometry required for activity, one hypothetical mechanism of stimulation would be that SF1 was directly involved in the strand renaturation process (Cox & Lehman, 1987; Griffith & Harris, 1988).

To test this hypothesis, SF1 was analyzed to determine whether it renatured homologous single-stranded DNA. Double-stranded DNA was denatured by heat treatment, the resulting single-stranded DNA was incubated for 2 min with SF1, and the reaction mixture was electrophoresed on a 1% agarose gel. Previous work showed that SEP1, like RecA protein, renatured homologous single-stranded DNA into large DNA networks which were too large to enter a gel and, hence, remained at the top of the gel after electrophoresis (McEntee et al., 1980; Heyer et al., 1988). Similar results were seen with SF1 (Figure 4). When SF1 was incubated with heat-denatured T7 DNA, renaturation products were formed within 2 min (Figure 4, lane 4). The SF1-mediated renaturation required homologous DNA as no renatured product was seen when M13 viral DNA was substituted for the denatured T7 DNA (Figure 4, lane 6). Therefore, SF1 catalyzed the renaturation of homologous single-stranded DNA.

An S1 nuclease assay was used to more quantitatively analyze the SF1-mediated renaturation of DNA (Figure 5). Radioactive T7 DNA was denatured and renatured in the same fashion as for the electrophoretic assay; the end products of the reaction, however, were treated with S1 nuclease, the nuclease-resistant DNA was precipitated with TCA, and the

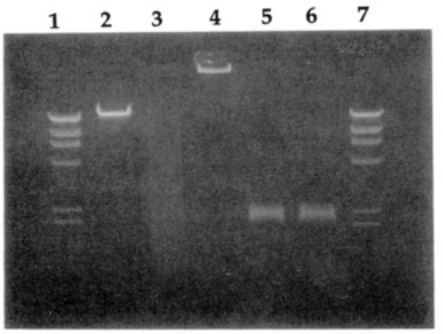

FIGURE 4: Analysis of SF1-mediated renaturation by agarose gel electrophoresis. Reaction mixtures (15 μL) contained 33 mM Tris-HCl (pH 7.5), 13 mM $MgCl_2$, 1.8 mM dithiothreitol, 88 μg/mL BSA, and (lane 2) T7 DNA, (lane 3) heat-denatured T7 DNA, (lane 4) heat-denatured T7 DNA and 2 μg of SF1, (lane 5) viral M13mp19, and (lane 6) viral M13mp19 and 2 μg of SF1. Reactions were incubated for 2 min at 30 °C, diluted to 30 μL with H_2O, and processed for gel electrophoresis as described (Kolodner et al., 1987). (Lanes 1 and 7) λ *Hin*dIII size markers.

acid-precipitable radioactivity was determined by liquid scintillation counting (Materials and Methods) (Heyer et al., 1988). Homologous single-stranded DNA renatured quickly in the presence of SF1; maximal levels of renaturation occurred within 1–2 min after initiation (Figure 5A). The renaturation appeared to occur in a stoichiometric fashion since the final amount of product was dependent upon the amount of SF1 present in the reaction mixture. The stoichiometric nature of the reaction, however, was not perfect. In a classic stoichiometric reaction, the amount of product is directly proportional to the amount of protein present. In the case of SF1, this was not strictly true (Figure 5A). Whether this reflected a technical deficiency in the assay or an actual characteristic of SF1-mediated renaturation is at present unclear.

The renaturation reaction also showed a salt sensitivity which was reminiscent of its DNA binding and strand exchange stimulatory activities (Figure 5B). When NaCl was present at concentrations above 20 mM, the renaturation activity was inhibited, with complete inhibition occurring above 75 mM NaCl. Also included in Figure 5B is an experiment illustrating single-stranded DNA renaturation by the *E. coli* SSB protein. The lack of renaturation catalyzed by the SSB suggested that SF1 was not renaturing DNA solely as a result of its ability to bind to single-stranded DNA under these conditions (Kowalczykowski et al., 1981).

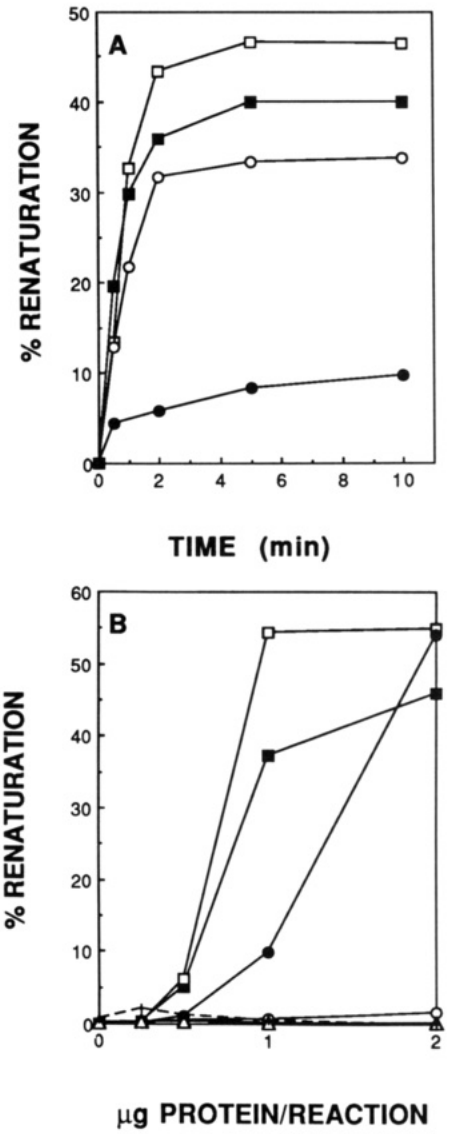

FIGURE 5: Renaturation of single-stranded DNA by SF1. Reaction mixtures (15 μL) contained 33 mM Tris-HCl (pH 7.5), 13 mM $MgCl_2$, 1.8 mM dithiothreitol, 88 μg/mL BSA, and 500 ng of ^3H-labeled T7 phage DNA that had been denatured by boiling for 2 min. (A) Reaction mixtures containing (□) 2, (■) 1, (○) 0.75, or (●) 0.5 μg of SF1 were incubated for the indicated amounts of time. The percent renaturation for each reaction was determined by S1 nuclease digestion as described previously (Heyer et al., 1988). (B) Reaction mixtures containing (■) 10, (□) 20, (●) 50, and (○) 75 mM NaCl were incubated for 2 min in the presence of the indicated amount of SF1. Percent renaturation was determined as in (A). Controls: reaction mixtures containing (△) ^3H-labeled, viral M13mp19 DNA rather than denatured T7 DNA, or (dashed line) *E. coli* SSB rather than SF1, were processed as described above.

Discussion

In the preceding paper (Norris & Kolodner, 1990), we described the purification and initial characterization of the SF1 strand exchange stimulatory factor. In this paper, we characterized SF1 in the absence of SEP1. SF1 was found to exist in solution as an asymmetric monomer and to bind preferentially to single-stranded DNA in a noncooperative fashion. Approximately eight nucleotides were occluded when SF1 bound to single-stranded DNA, and the binding reaction between SF1 and single-stranded DNA exhibited a dissociation constant (K_D) of 2.83×10^{-6} M. In addition, the protein promoted the renaturation of homologous single-stranded DNA.

The biochemical characteristics of the yeast SEP1–SF1 system differ from those in previously described strand exchange stimulatory systems. For example, in the *E. coli* RecA–SSB system, the amount of stimulation conferred on RecA by SSB is much lower than that seen in this study (Cox et al., 1983; Muniyappa et al., 1984). Moreover, the stimulation of RecA by *E. coli* SSB is nonspecific in the sense that SSBs from other organisms also have stimulatory effects (Muniyappa et al., 1984; Egner et al., 1987; Heyer & Kolodner, 1989). By contrast, SF1, although dramatically stimulating strand exchange catalyzed by SEP1, showed no stimulatory effect on strand exchange catalyzed by RecA (data not shown). This suggests that the mechanism of stimulation by SF1 differs from that of SSB. This conclusion is consistent with the observation that SF1 and *E. coli* SSB interact with DNA differently: SSB apparently interacts with nucleic acids through lysine rather than tyrosine residues (Kowalczykowski et al., 1981).

SF1 also appears to be distinguishable from the previously described strand exchange stimulatory factors from phage T4. The dda helicase stimulates the strand exchange activity of

UvsX to a much lower extent and, unlike SF1, is not required in stoichiometric amounts (Kodadek & Alberts, 1987). The UvsY protein, which is similar to SF1 in being unable to stimulate the strand exchange activity of RecA, stimulates strand exchange by UvsX approximately 2 orders of magnitude below those reported in this study (Harris & Griffith, 1989; Kodadek et al., 1989; Yonesaki & Minagawa, 1989). Of all the previously isolated prokaryotic strand exchange stimulatory factors, the gene 32 protein of phage T4 shows the most similarity to SF1. Like SF1, gene 32 protein is required in stoichiometric amounts, shows specificity for its cognate strand exchange protein, stimulates strand exchange quite dramatically, and promotes renaturation of DNA (Kowalczykowski et al., 1981; Kodadek et al., 1988). However, even in the presence of gene 32 protein, UvsX must be present at a high concentration (approximately one monomer per five nucleotides of single stranded DNA) to catalyze strand exchange, a characteristic which distinguishes gene 32 protein and SF1 (Kodadek et al., 1988). Moreover, the gene 32 protein, a true helix-destabilizing protein, binds cooperatively and tightly (K_D of approximately 10^{-8} M) to single-stranded DNA and elutes from single-stranded DNA–cellulose at 0.6–2.0 M NaCl (Bittner et al., 1979; Kowalczykowski et al., 1981). By comparison, SF1 binds noncooperatively and less tightly (K_D of approximately 10^{-6} M) to single-stranded DNA and elutes from single-stranded DNA–cellulose at approximately 0.25 M NaCl. Therefore, by these criteria, SF1 appears to constitute a different type of strand exchange stimulatory factor.

A number of proteins have also been purified from *Saccharomyces cerevisiae* that stimulate SEP1 and STPα, the yeast strand exchange proteins. A single-stranded DNA binding protein, now known to be the yeast analogue of the RP-A protein, stimulates the strand exchange activity of SEP1, although not to the same extent as SF1 (Heyer & Kolodner, 1989; Heyer et al., 1990). Because of its ability to substitute for the *Escherichia coli* SSB protein in reactions with RecA (Heyer et al., 1989), and because of its role in DNA synthesis in vitro (Wold & Kelly, 1988; Fairman & Stillman, 1988), the yeast RP-A protein is thought to function in a manner similar to *E. coli* SSB protein, therefore making it different from SF1. A number of DNA binding proteins called ySSBs have been shown to stimulate STPα (Hamatake et al., 1989). As only limited information has been published to date on the purification and DNA binding properties of these proteins, it is difficult to directly compare them with SF1. Nonetheless, the ySSBs stimulate STPα only modestly and appear to do so by aggregating the substrate DNAs (Hamatake et al., 1989). Therefore, SF1 differs from these ySSBs [see also Norris and Kolodner (1990)]. By the same criteria, SF1 differs from three additional DNA binding proteins which were originally purified as stimulatory factors for topoisomerase II (Goto & Wang, 1982). Like the ySSBs, these latter proteins, known as DBPs, modestly stimulate STPα by the nonspecific mechanism of DNA aggregation (Hamatake et al., 1989). To our knowledge, stimulatory factors for other eukaryotic strand exchange proteins have not yet been described. In summary, primarily owing to the dramatic stimulation of its cognate strand exchange protein, SF1 appears to be unique among previously characterized strand exchange stimulatory factors.

How then can we account for the levels of stimulation imparted on SEP1 by SF1? There are two general ways in which SF1 *might* be stimulatory. One possibility is that SF1 plays an indirect role in the strand exchange reaction. It is unlikely that this indirect role would be to disrupt inhibitory secondary structure on the single-stranded DNA substrate in a fashion analogous to the prokaryotic SSBs (Cox et al., 1983; Muniyappa et al., 1984) since, for the reasons enumerated above, the biochemical properties of SF1 are different from those of typical SSBs. Moreover, *E. coli* SSB has been shown to have no stimulatory effect on the strand exchange activity of SEP1 (Heyer & Kolodner, 1989). SF1, however, might function to generate productive—or prevent nonproductive—binding of SEP1 to DNA. Although this type of model is reminiscent of one proposed to account for UvsY stimulation of UvsX (Kodadek et al., 1989), the molecular details of the interaction between SF1 and SEP1 would probably differ owing to the vastly different stoichiometries in the two systems: UvsX and UvsY are required in a 1:1 stoichiometry for optimal activity, while SEP1 and SF1 are required in a 1:30 stoichiometry for optimal activity. Moreover, if this model accounted for the SF1-mediated stimulation, then SEP1 would have to function in a highly catalytic mode since; in the presence of SF1, only one to two SEP1 molecules are required per joint molecule formed. This, in turn, would imply either that a small number of SEP1 molecules could recycle within or between substrate molecules to promote heteroduplex joint formation or that a large number of SEP1 molecules would distribute to one substrate molecule, catalyze the formation of a joint molecule, and then recycle to another substrate.

The second possibility is that SF1 acts directly in the strand exchange reaction. For instance, SF1 could initiate synapsis, and SEP1 could promote branch migration. As discussed above, this type of mechanism would require SEP1 molecules either to recycle within a substrate molecule as the heteroduplex DNA joint grows or to recycle to new substrates as each product molecule is completed. Alternatively, SEP1 could initiate synapsis, and SF1 could promote branch migration. In this case, the initiation of homologous pairing might only require a small number of SEP1 molecules, while the requirement for a high stoichiometry of SF1 could reflect its role in promoting subsequent branch migration. At present, we favor this type of model because none of the participating proteins needs a priori to recycle between or within substrates, a characteristic which might be imagined to require ATP hydrolysis, as seen with RecA protein (Menetski et al., 1990). However, there is little experimental evidence at the present time that allows us to distinguish between any of these different possibilities.

Regardless of the molecular details of the stimulated strand exchange reaction, the unprecedented levels of stimulation seen in this and the preceding paper (Norris & Kolodner, 1990) suggest that the mechanism of heteroduplex DNA formation may differ in yeast and prokaryotes. Specifically, the observation that joint molecule formation, in the presence of SF1, occurs at low concentrations of strand exchange protein suggests that a presynaptic filament composed solely of SEP1, analogous to one composed solely of RecA or UvsX, may not catalyze strand exchange in yeast. Extensive experimental analysis will be required to determine how these proteins promote joint molecule formation in vivo and to determine their specific role in genetic recombination.

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Phospholipid Interactions of Synthetic Peptides Representing the N-Terminus of HIV gp41

M. Rafalski, J. D. Lear,* and W. F. DeGrado*
Central Research and Development Department, E. I. du Pont de Nemours & Company, Inc., Wilmington, Delaware 19880-0328

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ABSTRACT: Peptides representing the N-terminal 23 residues of the surface protein gp41 of LAV_{1a} and LAV_{mal} strains of the human immunodeficiency virus were synthesized and their interactions with phospholipid vesicles studied. The peptides are surface-active and penetrate lipid monolayers composed of negatively charged but not neutral lipids. Similarly, the peptides induce lipid mixing and solute (6-carboxyfluorescein) leakage of negatively charged, but not neutral, vesicles. Circular dichroism and infrared spectroscopy show that at low peptide:lipid ratios (approximately 1:200), the peptides bind to negatively charged vesicles as α-helices. At higher peptide:lipid ratios (1:30), a β conformation is observed for the LAV_{1a} peptide, accompanied by a large increase in light scattering. The LAV_{mal} peptide showed less β-structure and induced less light scattering. With neutral vesicles, only the β conformation and a peptide:lipid ratio-dependent increase in vesicle suspension light scattering were observed for both peptides. We hypothesize that the inserted α-helical form causes vesicle membrane disruption whereas the surface-bound β form induces aggregation.

Enveloped viruses such as influenza and human immunodeficiency virus (HIV) infect their target cells in a process involving cell-specific binding followed by fusion of the viral envelope membrane with the appropriate cellular membranes (White, 1990; Blumenthal, 1984). Influenza viruses bind to cells expressing surface sialic acid residues, are endocytosed,

* To whom correspondence should be addressed.

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