Formation of D Loops by the UvsX Protein of T4 Bacteriophage: A Comparison of the Reaction Catalyzed in the Presence or Absence of Gene 32 Protein†

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ABSTRACT: The UvsX protein of T4 bacteriophage will catalyze the formation of D loops between linear single-stranded DNA (ssDNA) and homologous supercoiled double-stranded DNA (dsDNA) in the absence of T4 gene 32 protein (gp32). This reaction requires one monomer of UvsX protein per three nucleotides of ssDNA so that the ssDNA is completely covered with UvsX protein. Under these conditions, high rates of ATP hydrolysis are observed, and one-third of the products are joined paranemically. The reaction proceeds through a mechanism that creates homology-independent coaggregates of UvsX protein, dsDNA, and ssDNA. When UvsX protein is added to only 1 monomer per 8 nucleotides, but with 1 monomer of gp32 per 12 nucleotides, the rate of ATP hydrolysis is depressed, but D-loop formation is enhanced. Nearly all of the product is bound in plectonemic joints, and no coaggregated intermediates are formed. Coaggregate formation at high concentrations of UvsX protein is not inhibited by the presence of gp32; gp32 simply allows for efficient formation of D loops at such low concentrations of UvsX protein that coaggregates are not constructed. Electron microscopic visualization of the joint structures in this reaction reveals that both gp32 and UvsX protein are bound to the ssDNA. The single-stranded DNA binding (SSB) protein of *Escherichia coli* will substitute only partially for gp32: in the presence of SSB protein, D-loop formation can be catalyzed at one UvsX protein monomer per eight nucleotides, and it is accomplished without the formation of coaggregates, but a major portion of the product is joined paranemically.

The UvsX protein of T4 bacteriophage has been shown to be analogous to the RecA protein of *Escherichia coli* in its catalysis of the simple in vitro reactions of homologous recombination (Radding, 1982; Yonesaki & Minagawa, 1985; Yonesaki et al., 1985; Formosa & Alberts, 1986; Hinton & Nossal, 1986). Both proteins arrange single-stranded DNA (ssDNA)[1] into extended helical nucleoprotein filaments that provide the scaffolds upon which synapsis and strand exchange with homologous dsDNA occur. Both UvsX protein and RecA protein function with the assistance of a helix-destabilizing protein: gene 32 protein (gp32) with UvsX protein and single-stranded DNA binding protein (SSB protein) with RecA protein (McEntee et al., 1980; Radding, 1982; Yonesaki & Minagawa, 1985; Formosa & Alberts, 1986; Hinton & Nossal, 1986). Under conditions that are optimal for catalysis of strand exchange by RecA protein in vitro, SSB protein is required to assist in the removal of the secondary structure in ssDNA, allowing for the complete assembly of RecA protein along the ssDNA (Muniyappa et al., 1984; Griffith et al., 1984; Kowalczykowski et al., 1987a,b; Thresher et al., 1988). In contrast, the analogous in vitro conditions for the UvsX protein are such that UvsX protein will disrupt ssDNA secondary structure without the aid of gp32 (Griffith & Formosa, 1985). Nonetheless, gp32 has been shown to enhance the catalysis of homologous strand exchange by UvsX protein (Yonesaki & Minagawa, 1985; Formosa & Alberts, 1986; Hinton & Nossal, 1986).

The role of gp32 in these reactions is poorly understood. In high concentrations, UvsX protein alone will catalyze strand exchange and D-loop formation, but in lower concentrations, it will not, unless the reactions are supplemented with gp32 (Yonesaki & Minagawa, 1985; Formosa & Alberts, 1986; Hinton & Nossal, 1986). The formation of D loops in the absence of gp32 has been characterized (Harris & Griffith, 1987) and was seen to depend on a critical concentration of UvsX protein. The reaction required enough UvsX protein to completely cover the ssDNA and form regular nucleoprotein filaments, but was inhibited when that amount was exceeded. The inhibition was seen to result from the binding of the UvsX protein to the dsDNA, which was initiated once the ssDNA was fully covered.

These findings suggest a simple model in which gp32 functions only to sequester excess ssDNA. This model is supported by evidence that the two proteins compete for ssDNA binding sites: EM studies of Griffith and Formosa (1985) suggested that, under simple binding conditions, the two proteins bind in independent, separate tracts along ssDNA, and Formosa and Alberts (1986) observed that the addition of gp32 lowered the rate of ssDNA-dependent ATP hydrolysis catalyzed by UvsX protein. However, if this simple model were valid, other helix-destabilizing proteins should substitute for gp32, and this appears not to be the case. Formosa and Alberts (1986) have shown that the SSB protein substitutes poorly for gp32 in UvsX protein-catalyzed strand exchange; a higher concentration of SSB protein is necessary, and the rate of the reaction is significantly slower than in the presence of gp32. Furthermore, genetic and biochemical evidence implies a more direct interaction between gp32 and UvsX protein.

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[1] Abbreviations: gp32, gene 32 protein; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; EM, electron microscopy; SSB protein, single-stranded DNA binding protein; Gdn·HCl, guanidine hydrochloride; ATP, adenosine 5′-triphosphate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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gp32 binds to several proteins in the *uvsX* pathway, in particular UvsX protein and the *dda* helicase (Formosa et al., 1983). Its interaction with the *dda* helicase may be important in branch migration (Kodadek & Alberts, 1987), and its specific binding to UvsX protein implies a direct interaction between UvsX protein and gp32.

A resolution of these conflicting data is made difficult by the fact that the reaction conditions used in different studies have not been the same. This is a particular problem since the efficiencies of D-loop formation and strand exchange are strongly dependent on the concentrations of UvsX protein, gp32, ssDNA, ATP, salts, and various anions used (Yonesaki & Minagawa, 1985; Formosa & Alberts, 1986; Hinton & Nossal, 1986; Harris & Griffith, 1987). In this paper, we explore the role of gp32 in D-loop formation catalyzed by UvsX protein by making direct comparisons between data obtained through EM, nitrocellulose filter binding, coaggregation of proteins and DNA, and studies of ATP hydrolysis rates. To elucidate the function of gp32, we have posed two specific questions. (1) How does the reaction catalyzed in the presence of gp32 proceed differently from that catalyzed by UvsX protein alone? (2) In what ways will SSB protein substitute for gp32?

Materials and Methods

Proteins and DNA. SSB and UvsX proteins were prepared as described (Chase et al., 1980; Formosa & Alberts, 1986). gp32 was the gift of Kathleen Keating, Yale University. M13 ssDNA and dsDNA were prepared as described (Modrich & Zabel, 1976) including linearization of the ssDNA with *Bam*HI enzyme. dsDNA was radiolabeled with [^{3}H]thymidine from Amersham Laboratories.

Joint Formation. Two basic procedures were used, unless otherwise described.

(*1*) *Joining Catalyzed by UvsX Protein Alone.* Linear M13mp7 ssDNA and M13mp7 RFI were incubated at concentrations of 7 and 14 μM, respectively, in a buffer containing 5 mM ATP, 7 mM magnesium acetate, 75 mM potassium acetate, and 20 mM HEPES, pH 7.5. UvsX protein was added to 2.3 μM (three nuleotides of ssDNA per UvsX protein monomer), and the mixture was incubated at 37 °C.

(*2*) *Joining Catalyzed by UvsX Protein with gp32.* The same conditions as above were used, but UvsX protein was added to 0.9 μM (8 nucleotides of ssDNA per UvsX protein monomer) and gp32 was added to 0.6 μM (12 nucleotides of ssDNA per gp32 monomer). When SSB protein replaced gp32, it was added to 1.2 μM (six nucleotides of ssDNA per SSB protein monomer).

Nitrocellulose Filter Binding Assays. Joining reactions described above were terminated by adding an equal volume of 20 × SSC (3 M NaCl and 0.3 M sodium citrate) and immediately applying the samples to a nitrocellulose membrane (Schleicher & Schuell BA85). Membranes were rinsed with 5 mL of 10 x SSC, dried under a heat lamp, and placed in scintillation fluid to determine the amount of radioactivity. Alternatively, the samples were deproteinized first with a solution containing 5.2 M Gdn·HCl, following the method used by Riddles and Lehman (1985) for reactions with RecA protein.

Aggregation Assays. The methods developed by Honigberg et al. (1986) for RecA protein were used. Joint formation reactions were conducted with radiolabeled dsDNA in a 50-μL volume, and the sample was then centrifuged in an Eppendorf microfuge for 3 min. Three 15-μL supernatants were drawn from the top, and the remaining 5 μL and pellet were resuspended in 200 μL of water. Each of the three supernatants and the pellet were mixed with scintillation fluid to determine radioactivity.

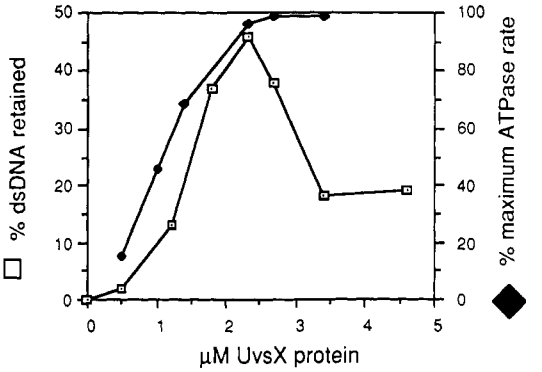

FIGURE 1: Optimal joint formation is reached at the maximum rate of ATP hydrolysis. UvsX protein was incubated in increasing concentrations with 7 μM M13mp7 ssDNA under the reaction conditions specified in the text for 20 min at 37 °C, while the rate of ATP hydrolysis was measured (closed diamonds). The maximum rate of ATP hydrolysis (100%) corresponds to 330 ATP molecules hydrolyzed per minute per UvsX protein monomer. In a parallel experiment, 14 μM M13mp7 RFI was added to the reaction. After 20 min, the sample was passed over nitrocellulose filter membranes to determine the amount of joint formation (open squares).

The spectrophotometric assay for ATP hydrolysis (Panuska & Goldthwait, 1980; Kreuzer & Jongeneel, 1983) has been described. Preparation for electron microscopy by mounting directly onto carbon-coated grids and rotary-shadowing with tungsten was done as described earlier (Griffith & Christiansen, 1978; Harris & Griffith, 1987), except that, following a 10-min wash of the samples in water, the excess water was removed and the grids were plunged into liquid ethane chilled in liquid nitrogen. The grids were then transferred to a freeze–etch machine (Wiltek Industries) for freeze-drying, were rotary-shadowcast with tungsten, and were examined in a Philips EM400.

Results

Properties of D-Loop Reactions Catalyzed by UvsX Protein Alone. To follow the joining of ssDNA and homologous dsDNA catalyzed by UvsX protein alone, radiolabeled M13mp7 RFI and linear M13mp7 ssDNA were incubated with increasing concentrations of UvsX protein (Materials and Methods). The formation of joint molecules was detected by passing the sample over nitrocellulose filters under conditions in which dsDNA is retained only if it is joined to ssDNA. Figure 1 shows that, after a 20-min incubation with varying amounts of UvsX protein, a maximum of about 45% of the dsDNA was joined to ssDNA when UvsX protein was present at three nucleotides of ssDNA per UvsX protein monomer. At higher levels of UvsX protein, the total joint formation decreased significantly.

In parallel, the rate of ATP hydrolysis was measured. UvsX protein was incubated under the same conditions with M13 ssDNA. The rate increased linearly with the increase in protein concentration until it reached a maximum at a concentration of 3 nucleotides per UvsX protein monomer (Figure 1), where the rate of ATP hydrolysis was 330 ATP molecules hydrolyzed per UvsX protein monomer per minute. This is comparable to the turnover rate of 240 per minute previously reported (Formosa & Alberts, 1986).

Under the conditions which produced the highest rate of D-loop formation catalyzed by UvsX protein alone, the reactants were organized into homology-independent coaggregates of ssDNA, dsDNA, and protein. These coaggregates were detected by incubating increasing concentrations of UvsX

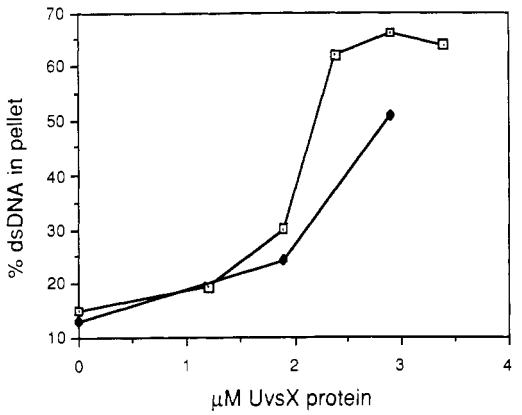

FIGURE 2: UvsX protein promotes the formation of homology-independent coaggregates of protein, ssDNA, and dsDNA. 7 μM linear M13 mp7 ssDNA and 14 μM ^{3}H M13mp7 RFI (open squares) or 14 μM pBR322 (closed diamonds) were incubated with the amount of UvsX protein shown and then were assayed for the presence of DNA–protein coaggregates as described under Materials and Methods. The reactions begain with the addition of UvsX protein to the reaction mixture and were incubated for 10 min at 37 °C.

protein with linear M13 mp7 ssDNA and radiolabeled M13 mp7 RFI for 10 min under the conditions described and then centrifuging the reaction mixture (Materials and Methods). Figure 2 shows that an increase in the amount of DNA in coaggregates paralleled the increase in UvsX protein concentration. This was true whether or not the dsDNA was homologous to the ssDNA. The same results were seen when the ssDNA was radiolabeled (data not shown). At the protein concentrations which best supported D-loop formation (three nucleotides per UvsX protein monomer), as much as 60% of the dsDNA was organized into coaggregates within 2 min after the addition of UvsX protein to the reaction. These coaggregates were stable for over 35 min of incubation (data not shown).

The formation of both plectonemic and paranemic joints can be followed in these reactions by filter binding assays. Here, we use the definitions for paranemic and plectonemic joints given by Riddles and Lehman (1985), which describe the two types of joints in terms of their behavior on nitrocellulose filters. If the reaction sample is applied directly to the filter, both plectonemic and paranemic joints are retained. If, however, the DNA is deproteinized with 5.2 M Gdn·HCl prior to being applied to the filter (to disrupt paranemic joints), only the dsDNA bound in plectonemic joints is retained on the filter. The number of paranemic joints, therefore, is derived from the difference between the measurements of dsDNA retained with and without treatment with Gdn·HCl. Such analysis revealed that the maximal joint formation was observed after only 5-min incubation. One-third of these joints were removed by Gdn·HCl treatment and were, therefore, paranemic. This fraction remained constant throughout a 40-min incubation.

Properties of D-Loop Reactions Catalyzed by UvsX Protein in the Presence of gp32. When the D-loop reactions above were carried at a stoichiometry of one UvsX protein monomer per eight nucleotides of ssDNA, less than 12% of the dsDNA was driven into D loops; even the optimal concentration of one protein monomer per three nucleotides yielded only 45% D loops (Figure 1). However, when a reaction containing one UvsX protein monomer per eight nucleotides was supplemented with increasing amounts of gp32, nitrocellulose filter binding assays revealed that as much 95% of the dsDNA was joined in D-loop complexes (Figure 4). This was achieved at a ratio of 1 gp32 monomer per 12 nucleotides.

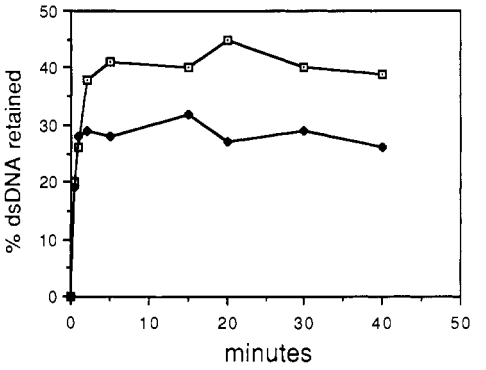

FIGURE 3: In the presence of UvsX protein only, both paranemic and plectonemic joints are stably formed. 2.3 μM UvsX protein was incubated with 7 μM linear M13mp7 ssDNA and 14 μM ^{3}H M13mp7 RFI under the reaction conditions described under Materials and Methods. The 50-μL reactions were started by adding the protein to the DNAs, were allowed to continue for the time indicated, and then were stopped by either (1) adding an equal volume 20 × SSC and applying to a nitrocellulose filter (open squares) or (2) mixing with a 1-mL solution containing 5.2 M guanidine hydrochloride and incubating for an additional 10 min at 37 °C and then applying to a nitrocellulose filter as described by Riddles and Lehman (1985) (closed diamonds).

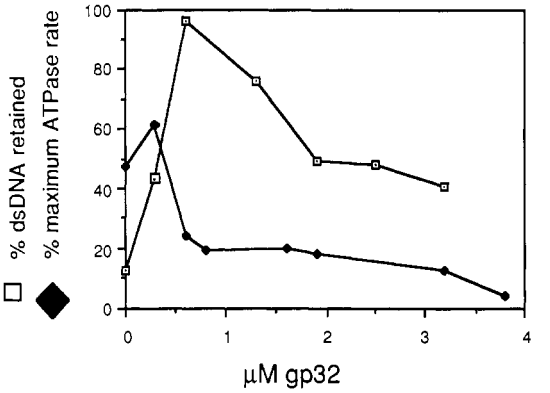

FIGURE 4: ATP hydrolysis is depressed under conditions which optimally promote D-loop formation in the presence of gp32. 0.9 μM UvsX protein was incubated with increasing concentrations of gp32, 7 μM M13mp7 ssDNA, and 14 μM M13mp7 RFI under the reaction conditions specified in the text for 20 min at 37 °C. The sample was then passed over nitrocellulose filter membranes to determine the amount of joint formation (open squares). In a parallel experiment, ssDNA was incubated under the same conditions with UvsX protein for 20 min while the rate of ATP hydrolysis was measured (closed diamonds).

The addition of gp32 lowered the rate of ATP hydrolysis catalyzed by UvsX protein (Figure 4). At the concentration of gp32 which supported maximal joint formation, the rate of ATP hydrolysis by UvsX protein was slowed to less than half of the rate measured in the absence of gp32.

EM visualization of the DNA–protein complexes present in the reactions containing both proteins revealed no complexes which contained either UvsX protein or gp32 exclusively (Figure 5). As in the reactions catalyzed by UvsX protein alone (Harris & Griffith, 1987), the dsDNA remained protein free, except for the region included in the joint. The organization of the two proteins along the ssDNA, however, was unclear. UvsX protein alone forms a very distinct 12.5-nm helical filament on DNA (Griffith & Formosa, 1985). In some of the joints, long regions of the filament displayed this characteristic architecture (Figure 5, top panel). In other joints, however, such clearly defined helical filaments were not present (Figure 5, bottom panel), while still others displayed an intermediate structure, where this helical pattern was visible within short regions, but not over the length of the entire UvsX

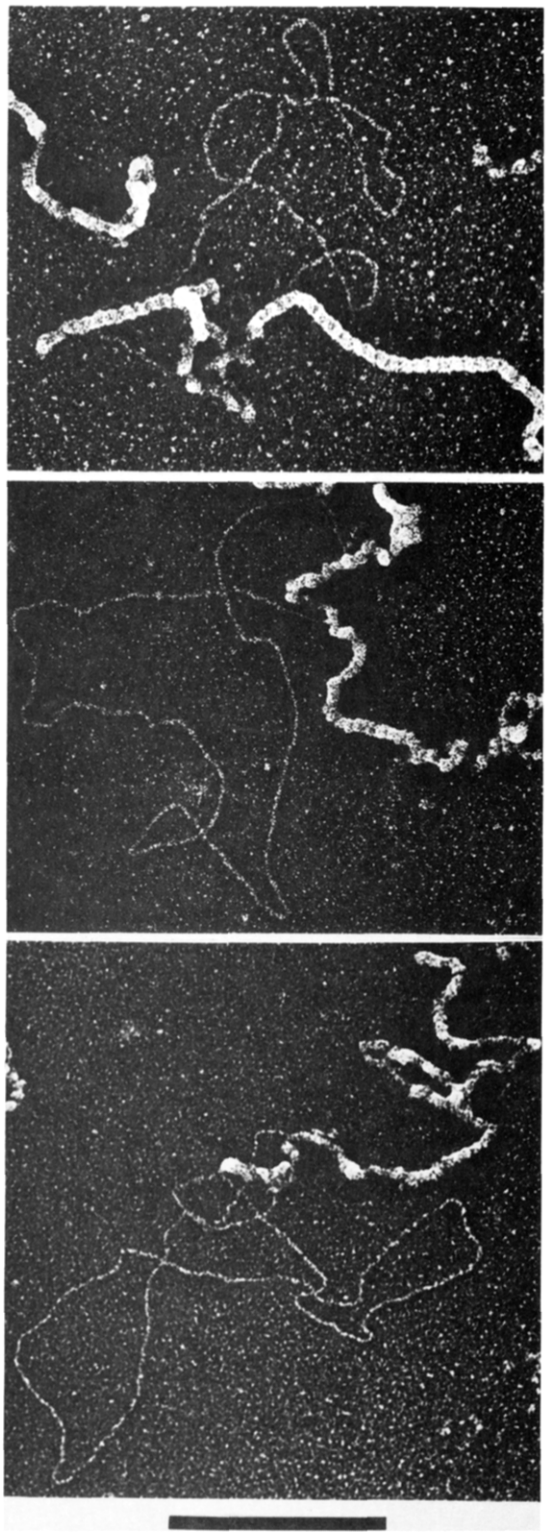

FIGURE 5: Active D loops include ssDNAs which are complexed to both UvsX protein and gp32. Joint formation, in the presence of UvsX protein and gp32, and preparation for electron microscopy were carried out as described under Materials and Methods. Linear ssDNA is bound by both gp32 and UvsX protein, while circular dsDNA is free of proteins. The protein-covered ssDNA here shows a different architecture from that seen with UvsX protein alone (Harris & Griffith, 1987) and from that seen when gp32 and UvsX protein bind along ssDNA in the absence of homologous dsDNA (Griffith & Formosa, 1985). Bar equals 0.2 μm.

protein/ssDNA filament (Figure 5, center panel). In any case, this clear, helical pattern was never observed in the region where the dsDNA joined the ssDNA, so it is unlikely that the joint itself was composed of uniform tracts of UvsX protein.

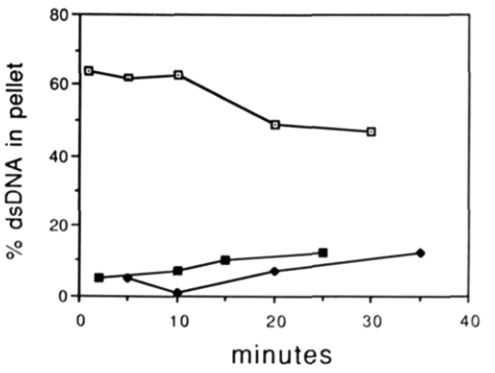

FIGURE 6: Coaggregates are not formed at the concentrations of UvsX protein and gp32 or SSB protein which best catalyze D-loop formation. 7 μM linear M13mp7 ssDNA was incubated at 37 °C under the reaction conditions described under Materials and Methods and with the following coreactants: closed squares, 14 μM ^{3}H M13mp7 RFI, 0.9 μM UvsX protein, and 0.6 μM gp32; closed diamonds, 14 μM ^{3}H M13mp7 RFI, 0.9 μM UvsX protein, and 1.2 μM SSB; open squares, 14 μM ^{3}H M13mp7 RFI, 2.3 μM UvsX protein, and 0.6 μM gp32. Coaggregate assays were performed as described.

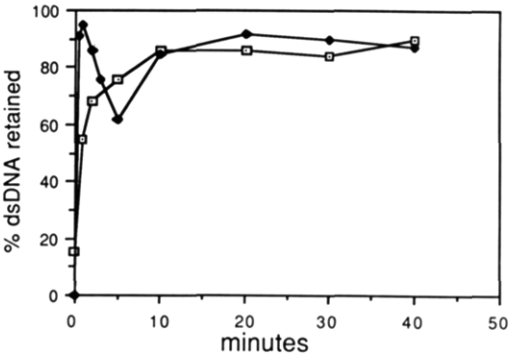

FIGURE 7: In the presence of both gp32 and UvsX protein, the product is primarily plectonemic joints. 0.9 μM UvsX protein was incubated with 0.6 μM gp32, 7 μM linear M13mp7 ssDNA, and 14 μM ^{3}H M13mp7 RFI under the reaction conditions described under Materials and Methods. 50-μL reactions were started by adding the protein to the DNAs, were allowed to continue for the time indicated, and then were stopped by either (1) adding in equal volume of 20 × SSC and applying to a nitrocellulose filter (closed diamonds) or (2) mixing with a 1-mL solution containing 5.2 M guanidine hydrochloride and incubating for an additional 10 min at 37 °C and then applying to a nitrocellulose filter as described by Riddles and Lehman (1985) (open squares).

Thus, the organization of the proteins within the joint was difficult to assess.

The assay for coaggregation used above was applied to reactions containing both 1 UvsX protein monomer per 8 nucleotides and 1 gp32 monomer per 12 nucleotides ssDNA (protein concentrations which were optimal for D-loop formation). In these reactions, unlike those catalyzed by UvsX protein alone, no coaggregates were detected (Figure 6). On the other hand, the addition of gp32 did not block the formation of coaggregates which formed in the presence of high concentrations of UvsX protein (one UvsX monomer per three nucleotides of ssDNA) (Figure 6). This indicates that the lack of coaggregation in the gp32-supplemented reaction resulted because a lower concentration of UvsX protein was used, and not because of any ability of gp32 to block coaggregation.

The formation of joints in the presence of gp32 over time is shown in Figure 7. Two peaks were observed; the first was transient and appeared in the period from 30 s to 2 min after the addition of UvsX protein; the second rose after 10-min incubation. The Gdn·HCl-deproteinized samples lacked the early peak, but instead, the curve proceeded smoothly to meet the second peak, revealing that, by 10 min, all of the ho-

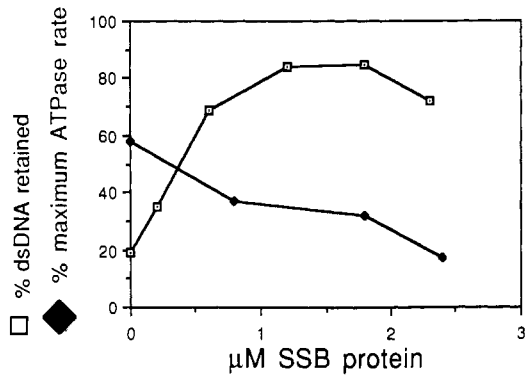

FIGURE 8: ATP hydrolysis is depressed at the concentration of SSB protein which optimally promotes D-loop formation. 0.9 μM UvsX protein was incubated with increasing concentrations of SSB protein and 7 μM M13mp7 ssDNA under the reaction conditions specified in the text for 20 min at 37 °C, while the rate of ATP hydrolysis was measured (closed diamonds). In a parallel experiment, 14 μM M13mp7 RFI was added to the reaction. After 20 min, the sample was passed over nitrocellulose filter membranes to determine the amount of joint formation (open squares).

mologously paired products of this reaction were joined plectonemically. This pattern of joint formation was consistent and repeatable.

Properties of D-Loop Reactions Catalyzed by UvsX Protein in the Presence of SSB Protein. In a reaction similar to that depicted in Figure 4, homologous DNAs were incubated in a series of reactions with a concentration of UvsX protein (one UvsX protein monomer per eight nucleotides) too low to support D-loop formation, and with increasing amounts of SSB protein. Figure 8 reveals that the peak of D-loop formation stimulated by SSB protein occurred when the reaction contained 1 SSB monomer per 6 nucleotides (as opposed to 1 per 12 for gp32). Again, the addition of SSB protein, like gp32, lowered the rate of ATP hydrolysis catalyzed by UvsX protein (Figure 8). These results demonstrate that SSB protein shares the ability of gp32 to stimulate the formation of D loops at lower concentrations of UvsX protein. The SSB protein-supplemented reaction also proceeded without the formation of stable coaggregates (Figure 6).

Using the optimal concentration of SSB protein as defined above, we followed the formation of D loops over time (Figure 9). Here, a distinction between gp32 and SSB protein became clear. The early, transient peak observed before with gp32 (Figure 7) was not seen. Rather, the pattern was more similar to that generated in the reaction conducted in the presence of UvsX protein alone (Figure 3). Gdn·HCl deproteinization of the sample revealed that at least one-fourth of the complexes remained paranemically joined, unlike the reaction with gp32, in which all dsDNAs were eventually joined plectonemically.

DISCUSSION

We have examined the interaction between UvsX protein and gp32 of T4 bacteriophage during D-loop formation. UvsX protein, in the absence of gp32, catalyzed the formation of D loops when the concentration of protein was sufficient to allow formation of regularly ordered nucleoprotein filaments. Under these conditions, ssDNA and dsDNA were organized into stable coaggregates, which were maintained for at least 35 min. One-third of the paired products of this reaction were paranemic joints. When the reaction was supplemented with gp32, D-loop formation increased, while the rate of ATP hydrolysis decreased. No coaggregates were detected. Within 5 min, all of the joints were plectonemic. When SSB protein replaced gp32, D loops were formed at concentrations of UvsX protein too low to catalyze the reaction alone, but a large portion of

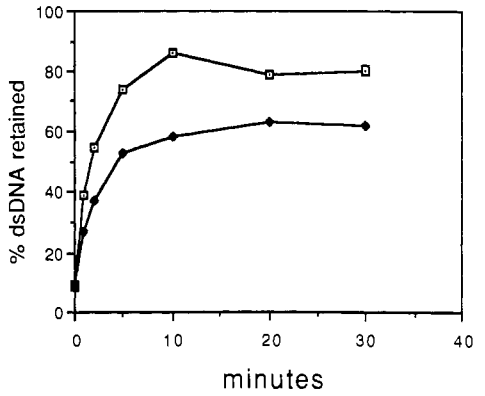

FIGURE 9: In the presence of both UvsX and SSB proteins, both paranemic and plectonemic joints are stably formed. 0.9 μM UvsX protein and 1.2 μM SSB protein were incubated with 7 μM linear M13mp7 ssDNA and 14 μM ^{3}H M13mp7 RFI under the reaction conditions described under Materials and Methods. 50-μL reactions were started by adding the protein to the DNAs, were allowed to continue for the time indicated, and then were stopped by either (1) adding an equal volume of 20 × SSC and applying to a nitrocellulose filter (open squares) or (2) mixing with a 1-mL solution containing 5.2 M guanidine hydrochloride and incubating for an additional 10 min at 37 °C and then applying to a nitrocellulose filter as described by Riddles and Lehman (1985) (closed diamonds).

the products remained paranemically joined.

We have used electron microscopy to examine the structure of the nucleoprotein filaments which catalyze D-loop formation. Previous studies have shown that, while UvsX protein binds readily to ssDNA at low concentrations, it is only at concentrations of UvsX protein which completely saturate ssDNA binding sites that ordered, helical filaments are seen and D-loop formation is catalyzed (Griffith & Formosa, 1985; Harris & Griffith, 1987). When gp32 was added to the reaction in optimal concentrations to allow for D-loop formation, ssDNAs were bound by both gp32 and UvsX protein. Much of the ssDNA was bound by UvsX protein in ordered helical tracts, but not all of the ssDNA was in this form. Earlier studies had shown gp32 and UvsX protein bound to ssDNA in separate, distinct tracts, in the absence of any homologous dsDNA (Griffith & Formosa, 1985). The micrographs shown here, which depict nucleoprotein filaments actively undergoing strand exchange, are more difficult to interpret, particularly within the joint region itself. therefore, while the results above reveal that both gp32 and UvsX protein are bound to ssDNA under optimal conditions for D-loop formation, they do not define the organization of the two proteins along the ssDNA. The possibility that UvsX protein and gp32 interact directly during D-loop formation cannot be ruled out. Future studies using tagged antibodies to gp32 or UvsX protein will hopefully resolve this question.

It is also demonstrated that both paranemic and plectonemic joints are formed, whether the reaction is catalyzed by UvsX protein alone or by UvsX protein supplemented with gp32 or SSB protein. It is only in the presence of gp32, however, that paranemic joints are "converted" to plectonemic joints. The nature of this conversion is unclear, but one interpretation is implied by the presence of the two separate peaks in Figure 7. The DNA in the first peak is largely joined paranemically. These paranemic joints quickly disperse, while the number of plectonemic joints steadily increases throughout the reaction. This may indicate that both plectonemic and paranemic joints are formed rapidly (as with UvsX protein alone) but, within 5 min, the paranemic joints dissociate and then re-form, yielding either plectonemic or paranemic joints, while the plectonemic joints remain stable. Several rounds of formation

and dissociation of paranemic joints yield a product which is mostly composed of plectonemic jounts. Riddles and Lehman (1985) propose such a model as one alternative for the conversion of paranemic to plectonemic joints by RecA protein, though such a mechanism has not been directly demonstrated.

The "coaggregates" described in this paper should not be confused with the "networks" described by Formosa and Alberts (1986) or with the "protein-independent networks" described by Chow et al. (1988). Networks and protein-independent networks formed as a result of multiple synaptic events between linear dsDNAs and homologous, circular, ssDNAs. As a result, these networks were held together by multiple intermolecular base-pairing interactions and were stable to deproteinization by SDS. However, the DNA templates used here to form D loops (circular dsDNA with linear ssDNA) do not undergo such multiple synaptic events. Deproteinization yields individual D loops, each composed of a single dsDNA and a single ssDNA (Harris & Griffith, 1986). This set of templates, therefore, can undergo protein-dependent, but not protein-independent, aggregation.

The coaggregates described in this study are comparable to what have been called coaggregates, nucleoprotein networks, or protein-dependent networks in previous studies (Tsang et al., 1985; Gonda & Radding, 1986; Chow et al., 1988). Formation of these coaggregates is dependent on the concentration of RecA or UvsX protein, and formation is maximal when the ssDNA is completely saturated with protein. These coaggregates form independently of homology and are protein dependent. However, the coaggregates which have been described in studies with RecA protein are formed in the presence of SSB protein and under reaction conditions which are optimal for strand exchange. In our studies with UvsX protein, coaggregates did not form under conditions which were optimal for D-loop formation, i.e., when gp32 was present and the concentration of UvsX protein was low. RecA protein excludes dsDNAs outside of the coaggregates from joining in strand exchange, while preliminary work indicates this is not true with UvsX protein (data not shown). In light of these differences, it is unlikely that the coaggregates observed here to be formed by UvsX protein play the same role in the search for homology which has been hypothesized previously with RecA protein (Tsang et al., 1985; Gonda & Radding, 1986; Chow et al., 1988).

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