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Biochemistry

Intercalators promote the binding of RecA protein to double-stranded DNA

(electron microscopy/DNA intercalating drugs)

RANDY J. THRESHER AND JACK D. GRIFFITH*

Lineberger Cancer Research Center and Department of Microbiology and Immunology, University of North Carolina Medical School, Chapel Hill, NC 27514

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ABSTRACT **Ethidium bromide, acridine orange, 4′-(9-acridinylamino)methanesulfon-*o*-anisidide (*o*-AMSA), and *m*-AMSA induce the rapid binding of RecA protein to double-stranded (ds) DNA. The filaments formed appear to retain the drug and are 12.8 nm in diameter with an 8.0-nm pitch. Two classes of drugs have been distinguished: (*i*) those that bind to RecA protein and induce assembly at low relative concentrations (e.g., ethidium bromide) and (*ii*) those that do not appear to interact directly with RecA protein and must be present at relatively high drug concentrations to stimulate assembly (e.g., *m*-AMSA). Ethidium bromide, acridine orange, and quinacrine inhibit RecA protein binding to single-stranded DNA. Addition of ATP to the drug-induced filaments causes the protein to rapidly dissociate from dsDNA, and protein binding to dsDNA diminishes upon extended exposure to room light. We suggest that the structure of the drug-induced filaments may be more typical of the complex that initiates RecA protein assembly along DNA rather than the product of extensive polymerization as induced by adenosine 5′-[γ-thio]triphosphate.**

RecA protein is a recombinational scaffolding protein that catalyzes homologous recombination reactions in *Escherichia coli*. Unlike the synthetic reactions of replication and transcription, strand exchange occurs within large filaments composed of RecA protein assembled onto DNA (reviewed in ref. 1). Thus, an understanding of the molecular details of DNA strand exchange will require elucidation of the structure, physical properties, and biological functions of active nucleoprotein filaments. Structural studies of the complexes that RecA protein forms upon binding to DNA have focused on the helical filament formed along double-stranded (ds) DNA in the presence of the ATP analog adenosine 5′-[γ-thio]triphosphate (ATP[γS]). The right-handed filaments formed are extremely stable, have a 9.5-nm pitch and 10-nm diameter (2–7), and extend the dsDNA to 1.5 times its original length (3). When binding occurs on negatively supertwisted DNA, the DNA becomes progressively unwound as RecA protein coverage increases, and at a rate equivalent to an unwinding of 11.5°–13° per base pair of RecA protein-bound DNA (4, 5). These average values do not necessarily define the local structure of dsDNA within the filaments (7). Nonetheless, the analogy with DNA intercalated with ethidium bromide was evident. Intercalation of ethidium bromide into dsDNA unwinds duplex DNA by 26° per intercalated molecule (8–11). As indicated by model building and x-ray diffraction analyses (12–14), the flat chromophores can maximally occupy sites between every other base pair with adjacent sites unoccupied (neighbor exclusion). Since an intercalating chromophore extends duplex DNA by approximately the width of a base pair (3.4 Å), saturation of linear dsDNA by ethidium must cause a net unwinding of 13° per base pair and a 1.5-fold linear extension. These helical parameters are thus the same as the average extension and unwinding of dsDNA in the RecA protein dsDNA filaments formed with ATP[γS]. This parallel led us to investigate how a number of simple DNA intercalators might promote or inhibit RecA protein binding to DNA.

MATERIALS AND METHODS

Protein and DNA. RecA protein and M13mp7 ds and single-stranded (ss) DNAs were purified as described (15, 16). M13mp7 dsDNA was cleaved with *Pst* I endonuclease (Bethesda Research Laboratories).

Drugs. Ethidium bromide, acridine orange, doxorubicin, quinacrine, naladixic acid, distamycin, and caffeine were purchased from Sigma. Ethidium homodimer was purchased from Molecular Probes; [^{14}C]ethidium bromide was purchased from Amersham; and ATP[γS] was purchased from Boehringer Mannheim. The aminoacridine derivatives, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) and its ortho isomer (*o*-AMSA), were obtained as a gift from Tao Hsieh (Duke University); [^{14}C]*m*-AMSA was the gift of Richard Cysyck (National Cancer Institute). Actinomycin D was a gift from Ryszard Kole at this university.

RecA Protein Binding Assays. In the RecA protein-binding assays, linear dsDNA (2 μg/ml or 3 μM in nucleotide pairs) was incubated with or without drug for 10 min at 37°C in 20 mM Hepes, pH 7.3/30 mM NaCl/0.1 mM EDTA (HNE buffer). RecA protein was then added to 80 μg/ml (2.1 μM) and incubation continued. Reaction vessels were sequestered from light unless otherwise specified. To examine the effects of light, reaction vessels without foil were incubated at 37°C and exposed to fluorescent room light. ATP[γS] filaments were formed by incubating identical concentrations of RecA protein and DNA as described above with 0.6 mM ATP[γS] and 2 mM $MgCl_2$ in HNE buffer.

Electron Microscopy (EM). Unless otherwise indicated, RecA protein–DNA complexes were prepared for EM by removing aliquots from the incubation mixture, fixing the samples with glutaraldehyde, and then mounting onto glow-charged thin carbon films (17, 18). Rapid freezing and freeze-drying were carried out by briefly adsorbing the samples (unfixed) to a thin carbon film supported by a copper mesh grid. Excess liquid was blotted away and the samples were plunged into liquid ethane chilled with liquid nitrogen. The frozen samples were transferred into a Wiltek-modified Balzers 300 freeze-etch machine and freeze-dried for 2 hr at −85°C. Samples were then rotary shadowcast with tantalum at −170°C and 10^{-7} torr (1 torr = 133.3 Pa). Molecule length measurements were carried out by projecting the EM nega-

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Abbreviations: ds, double stranded; ss, single stranded; ATP[γS], adenosine 5′-[γ-thio]triphosphate; EM, electron microscopy; AMSA, 4′-(9-acridinylamino)methanesulfonanisidide.

*To whom reprint requests should be addressed.

tives onto a Summagraphics digitizer tablet coupled to an IBM PC-AT computer programmed with software developed in this laboratory.

RESULTS

Several Simple Intercalators Cause RecA Protein to Bind Tightly to dsDNA. Filter binding (19) and EM studies (3) have demonstrated that RecA protein will not bind to dsDNA in low salt buffers lacking nucleotides. To illustrate this, RecA protein (2.1 μM) was incubated in the dark with linear M13mp7 dsDNA (3 μM nucleotide pairs) in HNE buffer at 37°C for 30 min. DNA–protein complexes were fixed in 1% glutaraldehyde and chromatographed over Sepharose 4B. When the fractions containing DNA were examined by EM, only protein-free DNA was observed (Fig. 1*A*). However, when 15 μM ethidium bromide was included in an otherwise identical incubation, much of the dsDNA was enveloped in a protein sheath (Fig. 1*B*). Under these conditions, acridine orange, *m*-AMSA, and *o*-AMSA were also found to stimulate extensive RecA protein assembly onto dsDNA. Drugs that failed to or only marginally stimulated RecA protein assembly onto dsDNA (over a range of 0.15 to 60 μM) included quinacrine, doxorubicin, actinomycin D, nalidixic acid, distamycin, caffeine, and ethidium homodimer.

Structure of the Drug-Induced Filaments. The length of the RecA protein filaments formed on linear dsDNA in the presence of intercalating drugs did not vary significantly for the four drugs that stimulated protein binding. When prepared by negative staining (Fig. 1*E*) or rapid freezing and freeze-drying (Fig. 1*C*), unfixed filaments measured 1.45 ± 0.07 (ethidium bromide; $n = 18$), 1.40 ± 0.07 (acridine orange; $n = 17$), 1.46 ± 0.09 (*o*-AMSA; $n = 21$), and 1.44 ± 0.09 (*m*-AMSA; $n = 12$) times the length of protein-free DNA (1.00). For all drugs, the filament diameter measured 12.8 ± 0.8 nm, and the helical pitch measured 8.0 ± 0.8 nm. Filaments formed by incubating RecA protein with dsDNA in the presence of ATP[γS] and magnesium (Fig. 1*D*) were extended 1.5-fold but had a 10-nm diameter and 9- to 9.5-nm pitch ($n = 16$), in agreement with previous studies (3, 4, 20).

When the NaCl concentration in the assembly buffer was increased from 30 to 150 mM, no RecA protein assembly occurred with any drug. The concentration of NaCl inducing at least 50% protein loss from preassembled filaments (in HNE buffer) was determined by adding NaCl to various concentrations for 2 min (at 37°C) prior to fixation. By this protocol, filaments were found to be stable up to 250 mM NaCl in ethidium bromide, 200 mM NaCl in acridine orange, 150 mM NaCl in *o*-AMSA, and 100 mM NaCl in *m*-AMSA. In comparison, RecA protein dsDNA filaments formed in the

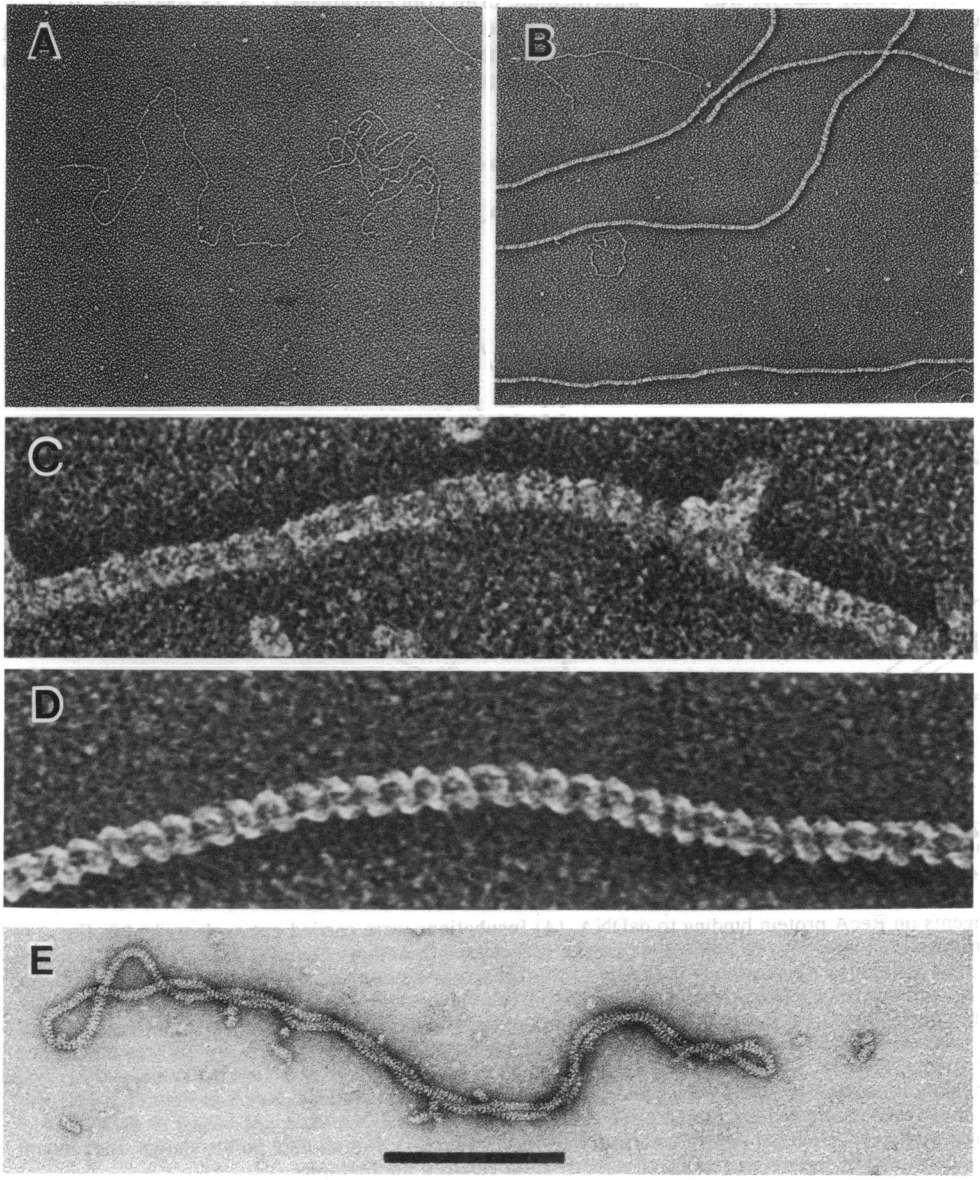

FIG. 1. Visualization of the drug-induced assembly of RecA protein onto duplex DNA. When linear M13mp7 DNA was incubated with RecA protein in 20 mM Hepes/30 mM NaCl/0.1 mM EDTA for 10 min at 37°C, no protein binding was detected (*A*). However, addition of 15 μM ethidium bromide to the incubation buffer resulted in the formation of helical nucleoprotein filaments (*B*) that extended the duplex DNA to ≈1.5 times its original length. Preparation of the ethidium-induced filaments for EM without fixation by rapid freezing and freeze-drying revealed a compact right-handed helical substructure (*C*). Filaments formed in the presence of ATP[γS] (*D*) prepared for EM as in *C* revealed a more extended helical pitch. Negative staining of unfixed complexes of RecA protein and relaxed circular M13mp7 dsDNA induced by ethidium bromide as in *B* with 2% aqueous uranyl acetate (*E*) revealed a filament diameter of 12.8 nm and a helical repeat of 8.0 nm. (*A* and *B*, bar = 0.5 μm; *C* and *D*, bar = 0.05 μm; *E*, bar = 0.2 μm.)

presence of ATP[γS] were stable to challenge by at least 700 mM NaCl. When the filaments were formed in the presence of the drugs and then 1–5 mM ADP, ATP, or ATP[γS] (with or without magnesium) was added for 2 min, no binding of RecA protein to the dsDNA was observed by EM.

The drug-induced binding of RecA protein to supertwisted M13mp7 DNA resulted in highly twisted and aggregated complexes whose structure was difficult to interpret. This was likely due to unwinding of the dsDNA from both drug intercalation and the binding of RecA protein.

Drug-Induced RecA Protein dsDNA Filaments Form Rapidly. The kinetics of RecA protein assembly onto dsDNA for each drug was followed. The dsDNA (3 μM nucleotide pairs) and drug (15 μM) were incubated for 10 min at 37°C, RecA protein (2.1 μM) was added, and aliquots were withdrawn over 60 min, fixed, and prepared for EM as described in Fig. 1. For each time point, low magnification fields containing many molecules were photographed and the total amount of protein-free and protein-covered DNA was determined. The latter value was corrected for the 1.5-fold greater length of the protein-covered regions. Nearly all of the dsDNA was covered by RecA protein within 5–10 min (Fig. 2*A*). Numerous partially covered dsDNA molecules were found over the first 5 min and were characterized by multiple protein tracts. The assembly kinetics for ethidium bromide, acridine orange, and *o*-AMSA were similar: by 10 min, >90% of the dsDNA was covered by RecA protein. This coverage was sustained over the 60 min of incubation. While the early assembly kinetics with *m*-AMSA were similar, protein coverage was found to decline at later times. In contrast to the very rapid rate of assembly induced by these drugs, the assembly induced by ATP[γS] was much slower (Fig. 2*A*). Here, there were few partially covered DNAs, and they contained only single RecA protein tracts, indicating that assembly was nucleation limited.

Dose–Response Curves Suggest Two Different Modes by Which These Drugs Stimulate RecA Protein Binding. The dependence of RecA protein assembly on drug concentration was followed by EM. Drug concentrations ranged from 0.5 to 60 mM, corresponding to 0.1–40 times the available intercalation sites in the reaction, assuming a maximum number of intercalation sites based on neighbor-exclusion stoichiometry. The drug and dsDNA were incubated for 10 min at 37°C, RecA protein (2.1 μM) was added, and incubation continued for 10 min. As shown in Fig. 2*C*, 0.15–1.5 μM ethidium bromide or acridine orange produced only low levels of protein binding. However, at 3 μM, >60% of the dsDNA was fully covered by RecA protein. Increasing concentrations of ethidium bromide (up to 60 μM) produced high levels of DNA coverage, but concentrations of acridine orange >15 μM yielded lower levels of coverage. In contrast to ethidium bromide and acridine orange, the AMSAs produced a more gradual dose-dependent increase in protein coverage, with *o*-AMSA being somewhat more efficient than *m*-AMSA at intermediate drug concentrations (7.5–15 μM; Fig. 2*C*).

In the experiments described in Fig. 2*C*, the greatest incremental increase in RecA protein binding with ethidium bromide and acridine orange occurred when the drug con-

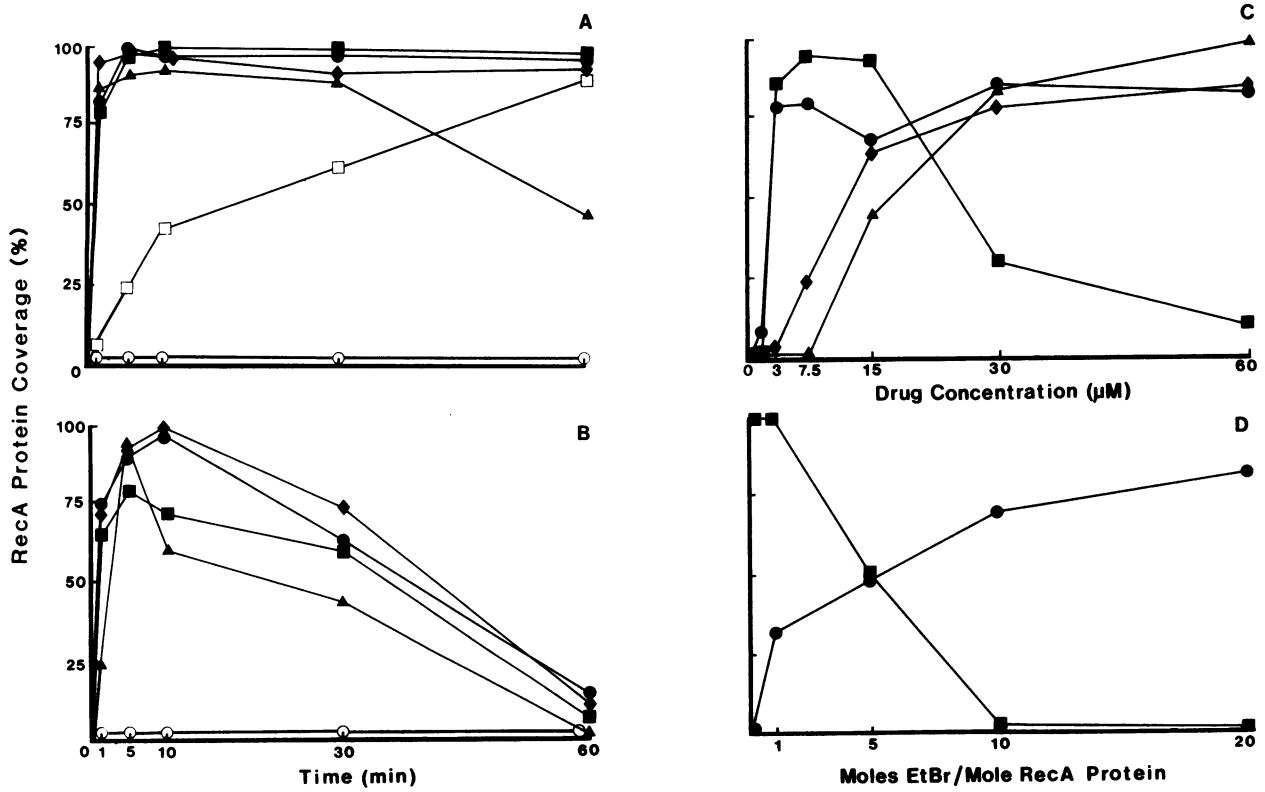

FIG. 2. The effects of intercalating agents on RecA protein binding to dsDNA. (*A*) Incubations were carried out as described in Fig. 1 (3 μM dsDNA, 2.1 μM RecA protein, ± drug) and followed over 60 min with reactions sequestered from light. Samples were fixed and prepared for EM as described in Fig. 1 *A* and *B*. The amount of RecA protein coverage of the DNA was determined directly from the micrographs. Incubations included ethidium bromide (●), acridine orange (■), *o*-AMSA (◆), *m*-AMSA (▲), magnesium and ATP[γS] (□), or buffer alone (○). (*B*) Identical reactions were carried out in room light. (*C*) The concentration dependence of the drug-induced binding of RecA protein to duplex DNA is shown. Linear M13mp7 dsDNA was incubated with various concentrations of drugs for 10 min at 37°C; RecA protein was then added to 2.1 μM, and incubation continued for 10 min. Samples were fixed and prepared for EM as in *A* and *B*. Symbols are the same as described above. (*D*) Inhibition of RecA protein binding to ssDNA by ethidium bromide is shown. In separate reactions, RecA protein (2.1 μM) was incubated with ethidium bromide at the molar ratios indicated for 10 min prior to addition of either ssDNA (■; 3 μM bases) or dsDNA (●; 3 μM base pairs). After 10 min of incubation, samples were fixed and spread from 40% formamide (21). For all data points shown, the amount of DNA coverage by RecA protein was determined by measurements taken directly from electron micrographs.

centrations were raised from 1.5 to 3 μM. Since the assembly reaction mixtures contained 2.1 μM RecA protein, this suggested that drug-induced binding might be stimulated by a direct interaction of ethidium bromide or acridine orange with RecA protein, independent of the DNA concentration. To examine this, 3 μM RecA protein was incubated with 3 μM drug for 10 min at 37°C, and then dsDNA was added to 30 μM (in nucleotide pairs). This concentration of drug could fill, at most, one-fifth of the available intercalation sites on the linear dsDNA. Both ethidium bromide and acridine orange efficiently stimulated RecA protein assembly, and it appeared that nearly all of the RecA protein was bound to the dsDNA, since inspection of the reaction mixtures by EM after 10 min (without Sepharose 4B chromatography) revealed little free protein. Little or no protein binding was seen under these conditions with the AMSAs or any of the other drugs tested. These observations suggested that DNA saturation by the AMSAs was required before protein binding could occur, but RecA protein may directly bind ethidium bromide or acridine orange and then bind dsDNA to form a ternary complex.

Somewhat lower levels of RecA protein binding were observed in the 10-min assembly reactions containing the AMSAs than seen previously (compare Fig. 2 *A* with *C*). We cannot fully explain this discrepancy, although some loss of solubility of the stock drug (dissolved in dimethyl sulfoxide) over time or the relatively high sensitivity of *m*-AMSA-induced RecA protein assembly to light (see Fig. 2*B*) may have been contributing factors.

RecA Protein Binds Ethidium Bromide in Solution. Direct drug–protein interactions were examined by incubating RecA protein (13 μM) with either [^{14}C]ethidium bromide or *m*-AMSA (13 μM) for 10 min at 37°C and applying the sample (unfixed) to Sepharose 4B columns equilibrated with buffer containing 13 μM labeled drug according to the method of Hummel and Dreyer (22). Fractions were collected and the amount of protein-associated ^{14}C label was determined by liquid scintillation counting. The emergence of RecA protein was accompanied by an increase in ^{14}C above background (Fig. 3) that corresponded to a stoichiometry of 1 ethidium molecule per 1.6–3 RecA protein monomers (separate experiments). The protein-associated peak of label was not observed when [^{14}C]*m*-AMSA was used or when labeled ethidium was incubated with other proteins, such as proteinase K. The addition of 2 mM ATP[γS] to a mixture of RecA protein and [^{14}C]ethidium bromide 2 min prior to loading onto the column abolished the protein-associated ethidium label.

Some Drugs Inhibit the Binding of RecA Protein to ssDNA. In simple, low-salt buffers, RecA protein readily binds to ssDNA (3). To determine the effects of intercalating agents on this interaction, RecA protein (2.1 μM) was incubated with each drug tested (2.1–40 μM) for 10 min at 37°C and then ssDNA or dsDNA was added (3 μM bases or base pairs). After 10 min further incubation, the samples were fixed with 1% glutaraldehyde and then prepared for EM by surface spreading from 40% formamide (21). This EM preparative method was required to spread out protein-free ssDNA. The fractional coverage of ssDNA and dsDNA was then measured. A 5-fold molar excess of ethidium bromide, acridine orange, quinacrine, or doxorubicin (relative to protein monomers) inhibited RecA protein binding to ssDNA by at least 50% and assembly was completely blocked when a 10-fold molar excess was used (Fig. 2*D*). In contrast, a 10-fold molar excess of *m*-AMSA or *o*-AMSA did not inhibit RecA protein assembly onto ssDNA.

Drug-Induced RecA Protein–DNA Filaments Are Light Sensitive. Intercalating agents contain chromophores that can absorb visible light and damage proteins through the generation of reactive oxygen (23). When the kinetics of RecA protein binding to dsDNA were followed as described in Fig. 2*A* with the reactions exposed to fluorescent room light, a time-dependent loss of RecA protein binding was observed (Fig. 2*B*). Light inactivation was not reversible, since incubation of the dsDNA and RecA protein with 15 μM ethidium bromide for 60 min (as in Fig. 2*A*) followed by further incubation for 60 min in the dark did not restimulate binding. The involvement of free radicals in this process was indicated by the finding that inclusion of 1 mM ascorbic acid inhibited the light-dependent protein loss by ≈50% (data not shown).

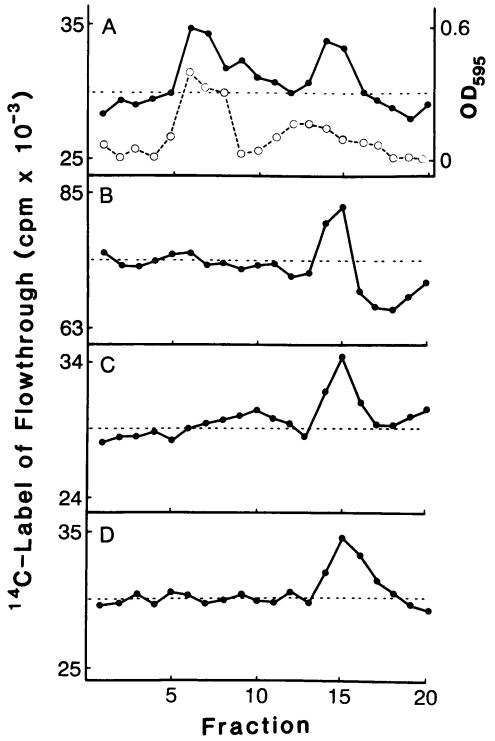

FIG. 3. Analysis of the binding of ethidium bromide and *m*-AMSA to RecA protein. Following the procedure of Hummel and Dreyer (22), RecA protein (13 μM) was incubated with either [^{14}C]ethidium bromide (13 μM) or [^{14}C]*m*-AMSA (13 μM) for 10 min in HNE buffer and applied to 2-ml Sepharose 4B columns equilibrated with HNE buffer containing 13 μM labeled drug. A peak of ethidium bromide emerged with the RecA protein that corresponded to a stoichiometry of 1 ethidium molecule per 1.6 molecules of RecA protein (*A*). A protein-associated drug peak was not found when labeled *m*-AMSA was used (*B*) or when labeled ethidium bromide was incubated with 13 μM proteinase K (*D*). In addition, the protein-associated [^{14}C]ethidium bromide peak could be abolished by adding 2 mM ATP[γS] to the reaction mixture just prior to loading the sample onto the column (*C*). In *A*, the RecA protein profile of the column fractions as determined by the Bio-Rad protein assay is shown (○).

DISCUSSION

In this paper, we report that ethidium bromide, acridine orange, *o*-AMSA, and *m*-AMSA induce the rapid binding of RecA protein to dsDNA. This binding produces helical filaments that are similar to, but measurably different from, any RecA protein dsDNA filaments previously described. Two classes of drugs can be distinguished: ethidium bromide (and likely acridine orange) bind to RecA protein in the absence of DNA, while *m*-AMSA (and presumably *o*-AMSA) do not. The drug-induced filaments are rapidly dissociated by the addition of ATP, and filament formation is sensitive to room light. Finally, ethidium bromide and acridine orange, but not the AMSAs, inhibit RecA protein binding to ssDNA.

These studies used a quantitative EM assay to measure the coverage of DNA by RecA protein. While the assay involved

chemical fixation and gel-exclusion chromatography, samples were routinely imaged without fixation or filtration by negative staining, and in no cases were significant differences observed.

Evidence that the drug-induced filaments retain the drugs within them stems primarily from experiments (data not shown) in which RecA protein dsDNA filaments were formed by using labeled ethidium bromide or *m*-AMSA. It was found that labeled drug was retained in the unfixed filaments after chromatography over Sepharose 4B. For both drugs, we estimated that 1 drug molecule remained bound per 6–9 base pairs in the DNA–protein complexes, arguing against a strict exclusion of the drug from the filaments. The lack of a drug-associated peak with [^{14}C]*m*-AMSA by Hummel–Dreyer analysis could have been indicative of a very tight association between the drug and RecA protein. Without exchange, no peak would be seen. This is unlikely, however, since the association between ethidium bromide and RecA protein correlated well with protein binding, yet high concentrations of the AMSAs were required to stimulate RecA protein assembly. In addition, the inhibition of RecA protein binding to ssDNA by ethidium bromide, acridine orange, and quinacrine, but not by the AMSAs, may reflect the differences between drugs in their affinities for RecA protein.

Of the drugs that promoted RecA protein assembly, only acridine orange showed a reduced level of protein assembly as the drug concentration increased. A trivial explanation is that this drug was supplied as a zinc salt and that the metal may have interfered with the protein DNA–drug interaction. A more interesting analogy exists with dsDNA and topoisomerase II activity as stimulated by intercalating agents, where the formation of a cleavable complex is observed to peak and then decline as the drug concentration is increased (24).

The sensitivity of the drug-induced filaments to room light raises an important concern. The AMSAs and other intercalators are used extensively to interrupt the cycle of cleavage and religation of dsDNA by topoisomerase II (25). We have found that these drugs show dramatic differences in the extent and longevity of RecA protein binding that is dependent on the presence or absence of light. Clearly, the effects of light on drug inactivation of topoisomerase II should be determined.

The drug binding site on RecA protein has not been defined. Our data can be interpreted as being consistent with binding at or near the ATP binding site, the ssDNA binding site, or possibly another site(s). The observations that drug-induced filaments were rapidly disrupted by nucleotides and that ATP[γS] released ethidium bromide from RecA protein in the absence of DNA argue that the drugs compete for the ATP binding site on RecA protein. However, ATP binding could induce a conformational change in RecA protein that facilitates the release of the drug molecule bound to RecA protein at some other site, such as the ssDNA binding site, as suggested by the inhibition of RecA protein binding to ssDNA exhibited by several drugs.

We know little about the structure of the dsDNA within the drug-induced filaments. Models describing the nature of DNA near sites of actinomycin D intercalation (26) may provide insights, but in our studies this drug did not induce RecA protein binding. Before a model of the DNA within the drug-induced filaments can be presented, it will be essential to determine the exact number of drug molecules bound per base pair, whether the drug is bound to the DNA or to the protein, and, if bound to the protein, whether it is near the ATP binding site.

The binding of RecA protein to ss- and dsDNA occurs in two steps: nucleation followed by polymerization. RecA protein will nucleate on ssDNA with relative ease; this can be enhanced by high levels of ATP and depressed by excess amounts of SSB protein (18). Polymerization then proceeds 5′ to 3′ (27) to produce the active filaments that catalyze strand exchange and enhance the cleavage of the repressors of the SOS response. On dsDNA, nucleation is rare, but it can be promoted by discontinuities in the helix such as thymine dimers (28), psoralen crosslinks (29), B–Z junctions (30), and extra base bulges (C. Bortner, Y.-W. Wang, and J.D.G., unpublished observations). Thus, understanding the nature of the nucleation step and structure of the DNA–protein complexes that initiate the assembly of RecA protein filaments is central to defining the biological roles of this protein. Here, we have shown that intercalating drugs are highly efficient at inducing RecA protein binding. Our presumption is that they do so by creating nucleation sites. Since the number of drug molecules per DNA length is high, these filaments may be thought of as a continuous nucleation complex. In contrast, the ATP[γS]-induced filaments represent the product of extensive polymerization. If this model is true, it could explain the structural differences seen between drug-induced and ATP[γS]-induced filaments. It also suggests that the drug-induced filaments should provide a valuable tool for probing the early events in which RecA protein binds to DNA.

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1. Griffith, J. D. & Harris, L. D. (1988) *CRC Crit. Rev. Biochem.* **23,** S43–S86.
2. DiCapua, E., Engel, A., Stasiak, A. & Koller, T. (1982) *J. Mol. Biol.* **157,** 87–103.
3. Dunn, K., Chrysogelos, S. & Griffith, J. (1982) *Cell* **28,** 757–765.
4. Stasiak, A. & DiCapua, E. (1982) *Nature (London)* **299,** 185–186.
5. Chrysogelos, S., Register, J. C. & Griffith, J. (1983) *J. Biol. Chem.* **258,** 12624–12631.
6. Heuser, J. & Griffith, J. (1989) *J. Mol. Biol.* **201,** 473–483.
7. Egelman, E. H. & Yu, X. (1989) *Science* **245,** 404–406.
8. Wang, J. C. (1974) *J. Mol. Biol.* **89,** 783–801.
9. Keller, W. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4876–4880.
10. Pulleybank, D. E. & Morgan, A. R. (1975) *J. Mol. Biol.* **91,** 1–13.
11. Stasiak, A., DiCapua, E. & Koller, T. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **47,** 811–820.
12. Lerman, L. S. (1961) *J. Mol. Biol.* **3,** 18–30.
13. Crothers, D. M. (1968) *Biopolymers* **6,** 575–584.
14. Bond, P. J., Langridge, R., Jennette, K. W. & Lippard, S. J. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4825–4829.
15. Griffith, J. D. & Shores, C. G. (1985) *Biochemistry* **24,** 158–162.
16. Register, J. C. & Griffith, J. D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 624–628.
17. Griffith, J. D. & Christiansen, G. (1978) *Annu. Rev. Biophys. Bioeng.* **7,** 19–35.
18. Thresher, R. J., Christiansen, G. & Griffith, J. D. (1988) *J. Mol. Biol.* **201,** 101–113.
19. McEntee, K., Weinstock, G. M. & Lehman, I. R. (1981) *J. Biol. Chem.* **256,** 8835–8844.
20. Stasiak, A. & Egelman, E. H. (1987) in *DNA Replication and Recombination*, eds. McMacken, R. & Kelly, T. J. (Liss, New York), pp. 619–628.
21. Chow, L. T. & Broker, T. R. (1981) in *Electron Microscopy in Biology*, ed. Griffith, J. D. (Wiley, New York), Vol. 1, pp. 139–188.
22. Hummel, J. P. & Dreyer, W. J. (1962) *Biochim. Biophys. Acta* **63,** 530–532.
23. Bryant, J. L. & King, J. (1984) *J. Mol. Biol.* **180,** 837–863.
24. Tewey, K. M., Rowe, T. C., Yang, L., Halligan, B. D. & Liu, L. F. (1984) *Science* **226,** 466–468.
25. Liu, L. F. (1989) *Annu. Rev. Biochem.* **58,** 351–375.
26. Sobell, H. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5328–5331.
27. Register, J. C. & Griffith, J. (1985) *J. Biol. Chem.* **260,** 12308–12312.
28. Lu, C., Scheuermann, R. H. & Echols, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 619–623.
29. Shi, Y.-B., Griffith, J., Gamper, H. & Hearst, J. E. (1988) *Nucleic Acids Res.* **16,** 8945–8952.
30. Blaho, J. A. & Wells, R. D. (1987) *J. Biol. Chem.* **262,** 6082–6088.