Kinetics of Transfer of *Escherichia coli* Single Strand Deoxyribonucleic Acid Binding Protein between Single-Stranded Deoxyribonucleic Acid Molecules†

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ABSTRACT: The binding of homogeneous *Escherichia coli* K12 single strand DNA binding protein to a ^{3}H-labeled 375-nucleotide single-stranded DNA of known sequence was detected with a filter-binding assay. The binding of protein is sufficiently cooperative that an all-or-nothing mechanism governs and DNA is either free of or saturated with protein. The stoichiometry of filter binding agrees with this model. Also, the first-order kinetics of transfer of protein from donor DNA–protein complexes to recipient DNA of the same size is the same whether ^{3}H-labeled donor or recipient DNA is used. The rate of transfer of protein from donor to recipient is found to be weakly temperature dependent, independent of salt concentration over a defined range, and inversely proportional to solvent viscosity. These results are consistent with a diffusion-controlled reaction mechanism. When much larger recipient DNA is used, the rate of transfer of protein is greatly reduced. The magnitude of and the length dependence of the rate constants for protein tranfer are incompatible with a mechanism involving uptake of free protein from solution. A model is proposed involving direct transfer of cooperative units of protein from donor to recipient strands. Electron micrographs consistent with this model are presented.

Many proteins have been purified that bind single-stranded DNA stoichiometrically, cooperatively, and with little regard to nucleotide sequence. The first such protein to be extensively characterized was isolated from bacteriophage T4 infected *Escherichia coli* (*E. coli*) and was found to be the product of T4 gene 32 (Alberts & Frey, 1970). Subsequently, with the technique of DNA–cellulose chromatography, developed by Alberts et al. (1968) and Alberts & Herrick (1971), proteins qualitatively similar to T4 gene 32 protein have been isolated from a variety of prokaryotic sources, including *E. coli* (Sigal et al., 1972) as well as eukaryotic sources. The ability of some of these proteins to denature poly(deoxyadenylic–thymidylic acid) led to the designation "DNA-unwinding proteins" (Sigal et al., 1972). Many other names have been attached to these proteins, including helix-destabilizing proteins, DNA-melting proteins, DNA-extending proteins, and DNA-binding proteins. Because of its association with the designation for mutants of the *E. coli* protein (Meyer et al., 1979), we will use the name single strand binding protein, SSB,[1] first suggested by Geider (1978). In vitro evidence has been obtained, suggesting that *E. coli* SSB may play an important role in DNA replication, recombination, and repair. *E. coli* SSB has been found to play such a role in the in vitro conversion of single-stranded circular bacteriophage DNA to the duplex replicative form (Weiner et al., 1975; Wickner & Hurwitz, 1974), as well as the in vitro synthesis of single strands from the replicative form (Scott et al., 1977). *E. coli* SSB forms a weak complex with *E. coli* DNA polymerase II and exonuclease I (Molineux & Gefter, 1975) and stimulates the activity of *E. coli* DNA polymerases II and III* (Sigal et al., 1972; Molineux & Gefter, 1974; Weiner et al., 1975). *E. coli* SSB has also been shown to promote DNA strand reassociation about 5000-fold under somewhat physiological conditions at 37 °C with 2 mM spermidine (Christiansen & Baldwin, 1977). Recently, temperature-sensitive mutants of the *E. coli* SSB have been isolated and mapped at 91 min on the *E. coli* genome (Meyer et al., 1979). These mutants have provided in vivo evidence for the role of SSB in DNA replication, recombination, and repair. The mutants, SSB mutants, possess a fast-stop phenotype for DNA replication when shifted to the nonpermissive temperature of 42 °C. They are extremely sensitive to ultraviolet irradiation, especially at 42 °C (Johnson, 1977), and are less efficient at promoting recombination when acting as recipient strains (Glassberg et al., 1979). The mutant protein is also temperature sensitive in the in vitro assay system for SSB involving the conversion of single-stranded circular bacteriophage G4 DNA to the duplex replicative forms.

Many studies have been carried out on the physical properties of *E. coli* SSB and *E. coli* SSB–DNA complexes. *E. coli* SSB exists in solution as a tetramer composed of four identical polypeptides of $M_r \approx 19000$ (Molineux et al., 1974; Weiner et al., 1975). It binds single-stranded DNA cooperatively and holds the DNA in an extended configuration, although the complex has a reduced internucleotide spacing (Sigal et al., 1972). SSB bound to DNA protects the DNA from attack by a variety of nucleases (Molineux & Gefter, 1975). Mutant SSB is less efficient in nuclease-protection assays (Meyer et al., 1980). With nuclease-protection assays as well as other methods, the stoichiometry of complex formation has been determined to be 30–36 nucleotides per tetramer (Sigal et al., 1972; Weiner et al., 1975; Ruyechan & Wetmur, 1975; Molineux et al., 1975). Bandyopadhyay & Wu (1978) have reported that *E. coli* SSB–DNA complexes have a surprising degree of local flexibility. Whether or not this flexibility reflects a variable stoichiometry has yet to be determined.

The cooperative binding of *E. coli* SSB to single-stranded DNA is so strong that the melting temperature of DNA may be lowered as much as 20 °C (Sigal et al., 1972). Quantitative measurements of the binding of *E. coli* SSB to single-stranded DNA have been obtained by Ruyechan & Wetmur (1975,

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[1] Abbreviations: SSB, single strand binding protein; EDTA, ethylenediaminetetraacetate; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; NaDodSO$_4$, sodium dodecyl sulfate.

0006-2960/82/0421-0608$01.25/0

1976). One SSB molecule interacts with four bases. The association constant in physiological salt to a site of 30–36 bases is about 2×10^6 M^{-1} and is nearly independent of base composition. Cooperative binding next to occupied sites occurs with an association constant approximately 10^5 times greater than the noncooperative binding constant. Analysis of oligonucleotide binding indicates that there were two possible DNA binding sites per SSB. Only one of these sites may be occupied in the cooperative binding mode.

Very little is known about the kinetics and mechanism of exchange of SSB among single-stranded DNA molecules. Weiner et al. (1975) measured exchange of SSB among full-length ϕX174 viral DNAs and found an extremely slow rate of transfer. Peterman & Wu (1978) have used salt-jump kinetics to study the DNA–T4 gene 32 protein complex, again with the same size DNA. We report in this paper studies of *E. coli* SSB exchange between shorter DNAs of known length. An all-or-none mechanism may be used to interpret the kinetics and mechanism of these exchange reactions.

Materials and Methods

Preparation of E. coli Single Strand DNA Binding Protein (SSB). *E. coli* K12 frozen cell paste was purchased from Grain Processing Corp., Muscatine, IA. SSB was isolated by a modification of the procedure of Sigal et al. (1972). All operations were carried out at 4 °C unless otherwise specified. Cell paste (250 g) was added to 1200 mL of 0.05 M NaCl, 0.01 M $MgCl_2$, 0.002 M EDTA, 0.001 M 2-mercaptoethanol, 0.0001 M dithiothreitol, 0.05 M Tris-HCl, pH 7.6, 130 μg/mL lysozyme (Worthington), 23 μg/mL phenylmethanesulfonyl fluoride, 20 μg/mL DNase I (Sigma), and 0.4% sodium deoxycholate. After the mixture was blended in a Waring blender, the suspension was allowed to stand at least 15 min and was sonicated for 3 min in 100-mL aliquots with a Bronson power sonifier. The extract was incubated for 4 h at 10 °C before clarification by low-speed centrifugation, followed by centrifugation at 54000*g* for 45 min. The supernatant was dialyzed against buffer A of Sigal et al. (1972): 0.05 M NaCl, 0.001 M EDTA, 0.001 M 2-mercaptoethanol, and 0.05 M Tris-HCl, pH 7.6.

A 100-mL single-strand DNA-cellulose column (Alberts & Herrick, 1971) with 1.5 mg/mL DNA was prepared with denatured calf-thymus DNA and highly purified cellulose powder (Whatman) and was poured in buffer A plus 10% glycerol. The dialyzed extract was made 10% in glycerol and pumped through the DNA–cellulose column at 30 mL/h. The column was eluted with buffer A containing 0.46 mg/mL dextran sulfate (Pharmacia), followed by buffer A containing 0.15 M NaCl until the effluent contained no detectable absorbance at 280 nm. The SSB and other contaminating proteins were obtained by elution with buffer A containing 2 M NaCl and were dialyzed into 0.2 M NaCl, 0.02 M Tris-HCl, pH 7.6, 0.001 M EDTA, 0.001 M 2-mercaptoethanol, and 20% glycerol.

Blue Sepharose CL6B (Pharmacia) was prepared as recommended by the manufacturer. The SSB-containing sample was pumped through a 20-mL Blue Sepharose column at 20 mL/h. A blue dextran column has been used by Meyer et al. (1979) for SSB purification. The elution properties differ from those found with Blue Sepharose CL6B. The column was washed with the same buffer containing 2 M NaCl instead of 0.2 M NaCl to remove all of the bound protein except SSB protein. The SSB protein was eluted with 4 M guanidine hydrochloride (Schwarz/Mann, ultrapure) in 0.02 M sodium phosphate buffer, pH 6.86, 0.001 M EDTA, 0.001 M 2-mercaptoethanol, and 20% glycerol and dialyzed exhaustively at 4 °C at 30–200 μg/mL protein into 0.05 M NaCl, 0.02 M Tris-HCl, pH 7.6, 0.001 M EDTA, and 10% glycerol to remove the guanidine and renature the SSB protein. The protein may be stored at 4 °C over $CHCl_3$ or frozen once at greater than 100 μg/mL protein and stored at −20 °C. From 250 g of *E. coli* K12 cell paste, approximately 3 mg of homogeneous SSB protein was obtained.

Characterization of SSB Protein. The protein concentration was determined by the method of Lowry et al. (1951). The polypeptide molecular weight was determined by NaDodSO$_4$–polyacrylamide gel electrophoresis (Weber & Osborn, 1969), staining with Coomassie Brilliant Blue and destaining with a mixture of 7% glacial acetic acid and 10% methanol in water. The native molecular weight was estimated by gel-filtration chromatography on Sephadex G-100 (Pharmacia) using SSB labeled with ^{125}I by the method of Greenwood et al. (1963). The elution profile of SSB was compared with the elution profiles of standard proteins. The amount of active SSB protein was determined by the nuclease-protection assay of Ruyechan & Wetmur (1976) using radioiodinated DNA (Orosz & Wetmur, 1974). Separation of SSB-protected ^{125}I-labeled single-stranded DNA from digested material was achieved by thin-layer chromatography on poly(ethylenimine)–cellulose strips (Brinkmann Industries) with 1 M HCl. The ability of SSB protein to extend single-stranded DNA was determined as reported below. In every assay, the renatured SSB protein behaved in a manner indistinguishable from SSB protein that had never been denatured by heating or treatment with guanidine hydrochloride.

Preparation of DNAs. A thymidine-requiring mutant (CR34) of *E. coli* K12 transformed with pBR322 was a gift of Dr. J. Young. These bacteria were grown in M9 minimal medium plus 0.4% casein hydrolysate, 0.4% glucose, 2 μg/mL thymidine, and 25 μg/mL each of ampicillin and tetracycline. The plasmid was selectively replicated and labeled with 4 μCi/mL of [6-^{3}H]thymidine (New England Nuclear) and chloramphenicol according to the procedure of Clewell (1972). ^{3}H-Labeled DNA was isolated by the method of Birnboim & Doly (1979) and purified by CsCl density-gradient sedimentation. Specific activities of 10^5 cpm/μg were routinely obtained. Various restriction fragments of pBR322 were isolated on 1% agarose gels containing ethidium bromide and removed from the gels by a variety of methods (Yang et al., 1979). DNA concentrations were determined from the absorbance at 260 nm.

Single-stranded DNA circles from bacteriophage G4, isolated by Dr. N. Godson, were a gift of Dr. J. Young. The molecules were found to be almost entirely circles when visualized in the electron microscope (see below). Calf-thymus DNA was sonicated with a Bronson power sonifier and examined by agarose gel electrophoresis with marker DNAs. The calf-thymus DNA was 300–500 base pairs long. All DNAs used for transfer studies were denatured at 100 °C and ice quenched immediately before use.

Protein-Transfer Experiments. A 375 base pair fragment of ^{3}H-labeled pBR322 DNA resulting from digestion with both *Eco*RI and *Bam*HI restriction endonucleases (New England Biolabs) was isolated and used for the donor strands for all the transfer experiments unless otherwise specified. The donor DNA fragments were present at 10^{-9} M (3.75×10^{-7} M nucleotide) prior to mixing. SSB was added to the denatured donor DNA to produce 80% coverage corresponding to a protein to DNA weight ratio of almost 8:1. All buffers and concentrations are given under Results. Each donor DNA–SSB mixture was incubated at 20 °C for 15 min and then

equilibrated in a 37 °C water bath. A donor DNA–SSB sample was mixed by vortexing with an equal volume and concentration of a recipient single-stranded DNA sample at 37 °C. Aliquots were removed as a function of time to detect transfer of SSB from the ^{3}H-labeled donor strands. Nitrocellulose filtration was sufficiently rapid compared to the transfer reaction to serve to terminate the SSB protein transfer reaction.

Millipore nitrocellulose filters (type HA, 0.45 μm, HAWP 024-00) were soaked in 0.01 M Tris-HCl and 0.001 M EDTA, pH 7.6, and rinsed with the same buffer in a Hoefer Scientific stainless steel filtration assembly equipped with a vacuum regulator to assure a flow rate of 2 mL/min. After application of a sample, filters were again rinsed with 2 mL of the same buffer before drying. The filters were counted in a toluene-based scintillation mixture in a Beckman LS9000 scintillation counter.

SSB-Transfer Experiments Visualized by Electron Microscopy. Highly sonicated and denatured calf-thymus DNA with an average length of 400 bases was used as the SSB donor. Donor DNA was incubated with SSB at 80% site saturation in 0.15 M NaCl, 0.01 M Tris, pH 7.6, and 0.001 M EDTA for 20 min at 25 °C. In one set of transfer experiments, 0.01 M NaCl was substituted for 0.15 M NaCl. An equal concentration of intact bacteriophage G4 single-stranded DNA circles was added and incubated for 30 min at 25 °C. The final nucleotide concentration was 9×10^{-6} M. As a control, SSB was incubated only with G4 circles at 40% site saturation under the same conditions. Cytochrome *c* was added to both reaction mixtures to a final concentration of 0.1 mg/mL. Each mixture was spread on a 0.2 M ammonium acetate aqueous hypophase with the aqueous technique described by Davis et al. (1971). Photographs of uranyl acetate stained preparations were taken with an AEI EM801 at a magnification of 6300× and further enlarged during printing.

Results

Characterization of SSB Protein. The SSB protein used in this work was purified by chromatography on single strand DNA–cellulose and Blue Sepharose CL6B. The SSB protein is eluted from Blue Sepharose in 4 M guanidine hydrochloride and dialyzed to remove guanidine and renature the protein. The ability to withstand treatment with guanidine is consistent with the ability of this protein to withstand boiling (Weiner et al., 1975). The SSB protein had the following characteristics: (1) Only one band was seen at 19 000 daltons following $NaDodSO_4$ gel electrophoresis of 50 μg of protein. The polypeptide molecular weight is in good agreement with previously reported values (Sigal et al., 1972; Molineux et al., 1974; Weiner et al., 1975; Ruyechan & Wetmur, 1975). This 19 000-dalton band was nearly absent from an $NaDodSO_4$ gel of the proteins eluted from Blue Sepharose with 2 M NaCl. SSB protein is apparently more difficult to remove from Blue Sepharose than from blue dextran (Meyer et al., 1979). (2) The determinations of protein concentration by the Lowry procedure and absorbance at 280 nm (Ruyechan & Wetmur, 1976) were in agreement. (3) The native molecular weight of ^{125}I-labeled SSB was estimated by gel-filtration chromatography to be 70 000–80 000 daltons, in agreement with the known native tetramer structure. (4) The specific activity of the SSB protein isolated by Blue Sepharose chromatography and assayed with the nuclease-protection assay was identical with that of the best isolates from other chromatographic systems. This specific activity was not affected by boiling and cooling the protein. When SSB protein is stored at 4 °C, it loses activity over a period of several months. This loss in activity is accompanied by the appearance of degradation products of lower than 19 000-dalton polypeptide mass. No loss of activity has been detected for covalently intact SSB protein. (5) SSB protein extends single-stranded G4 DNA circles to the extent reported in the literature (Ruyechan & Wetmur, 1975).

Detection of SSB–Single-Stranded DNA Complexes. We have used a nitrocellulose filter binding assay to detect single-stranded DNA–SSB complexes. In this assay, single-stranded DNA only binds to the filter when complexed with SSB. Similar assays have been used by Oey & Knippers (1972) to study the binding of bacteriophage fd gene 5 SSB to single-stranded DNA and by Weiner et al. (1975) to determine the stoichiometry of *E. coli* SSB binding to single-stranded DNA. The assay was calibrated with a denatured 375 nucleotide long *Eco*RI plus *Bam*HI restriction fragment of pBR322 ^{3}H-labeled DNA. Only small and reproducible amounts of this DNA bound to the filters. When SSB was added to the DNA, the amount of [^{3}H]DNA retained on the filters was proportional to the saturation of sites on the DNA as determined by the nuclease-protection assay. The low background, linearity, and stoichiometry of the assay were confirmed in 0.003, 0.1, 0.2, 0.3, and 0.4 M NaCl plus 0.01 M Tris and 0.001 M EDTA, pH 7.8, and in buffers containing glycerol. The assay was also found to be independent of DNA–SSB complex concentration over a 4-fold range around the concentration of 4×10^{-7} M nucleotides used for the experiments reported in this paper. SSB binds cooperatively to single-stranded DNA. The linear relationship between the nuclease-protection assay and the filter-binding assay provides additional evidence for cooperativity. Transfer reaction rates obtained with various DNAs of different complexities, as well as lengths, at many ionic strengths and temperatures were found to be independent of complexity. These results confirmed those of Christiansen & Baldwin (1977) that DNA renaturation should not occur under any of the conditions investigated in this paper. The only structures present in significant concentrations in these DNA plus SSB mixtures are free DNA and DNA fully complexed with SSB.

Reversible SSB-Transfer Reactions—An All-or-None Mechanism. When a labeled DNA–SSB complex is mixed with an equal amount of unlabeled DNA of the same length, SSB molecules may exchange until the SSB is equally partitioned between the labeled and unlabeled DNAs. The progress of the reaction may be followed by detecting labeled DNA–SSB complexes by filter binding at various times after mixing. The reverse reaction may be followed by first mixing the SSB with unlabeled DNA. If the only significant species in solution at all times are free DNA and fully complexed DNA, these reactions may be said to follow an all-or-none mechanism.

Consider the reaction

$$\text{D–SSB} + \text{R} \underset{k_2}{\overset{k_2}{\rightleftharpoons}} \text{D} + \text{R–SSB} \qquad (1)$$

where D and R stand for donor and recipient strands and k_2 is the rate constant for transfer of all the SSB molecules from one strand to another. Let C_0 be the total DNA strand concentration. Let Δ be the absolute value of the difference between the labeled DNA–SSB complex bound to a filter at time t and the labeled DNA–SSB complex bound at infinite time (equilibrium). Then

$$-\mathrm{d} \ln \Delta/\mathrm{d}t = k_1 = 2k_2C_0 \qquad (2)$$

where k_1 is the apparent first-order rate constant. The reverse

Table I: Reversibility of SSB-Transfer Reactions

donor DNA	recipient DNA	k_1 (s^{-1})	$t_{1/2} = \ln 2/k_1$ (s)	k_2 (M^{-1} s^{-1})
375-nucleotide [^{3}H]DNA	400-nucleotide sheared DNA	$(7.0 \pm 0.3) \times 10^{-3}$	98	7×10^6
400-nucleotide sheared DNA	375-nucleotide [^{3}H]DNA	$(8.0 \pm 0.3) \times 10^{-3}$	87	8×10^6

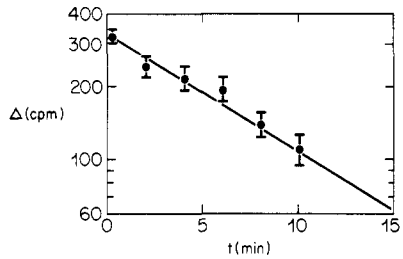

FIGURE 1: A first-order, all-or-none model, rate plot. Donor DNA is a denatured, 80% site saturated 375-nucleotide ^{3}H-labeled DNA plus SSB. Recipient DNA is a 2180-nucleotide DNA at the same nucleotide concentration. The final nucleotide concentration is 4×10^{-7} M. The reaction was followed at 37 °C in 0.15 M NaCl, 0.01 M Tris, 0.001 M EDTA, and 50% glycerol, pH 7.6.

reaction will give the same rate constant as the forward reaction. If an all-or-none mechanism is not followed, the prediction of identical first-order reactions in both directions would not be expected to be fulfilled because partially complexed DNA with SSB would bind to a nitrocellulose filter. The results we now present are consistent with an all-or-none mechanism.

The ^{3}H-labeled denatured 375-nucleotide *Eco*RI plus *Bam*HI restriction fragment of pBR322 was complexed with SSB at 80% site saturation. This DNA can bind 11–12 SSB proteins at about 32 nucleotides per tetramer. The competing unlabeled DNA was extensively sonicated denatured calf-thymus DNA, which had an average length of 400 nucleotides as determined by gel electrophoresis. For the reverse experiment, SSB was complexed to single-stranded calf-thymus DNA at 80% site saturation prior to addition of the equal concentration of competing ^{3}H-labeled 375-nucleotide DNA. The solvents were 0.15 M NaCl, 50% glycerol, 0.01 M Tris, and 0.001 M EDTA, pH 7.6, at 37 °C. The DNA fragments were present at 10^{-9} M (about 4×10^{-7} M nucleotide) prior to mixing. First-order rate profiles were observed for both reactions. As given in Table I, the forward and reverse rate constants were the same within experimental error. We conclude that the kinetics of the exchange of SSB between short DNA molecules may be treated with an all-or-none model. We also conclude that the 375-nucleotide donor behaves like the random-sequence calf-thymus DNA.

Surprisingly, the same type of first-order kinetic behavior is observed with reactions between ^{3}H-labeled 375-nucleotide fragment–SSB complexes and longer recipient strands. Figure 1 shows the kinetic analysis of a reaction with a 2180-nucleotide denatured *Ava*I plus *Pst*I fragment of pBR322 used as the recipient strand. The recipient strand is always added at the same SSB site (nucleotide) concentration as the donor strand. The reaction is followed until ^{3}H-binding results are constant at several widely spaced time points. Not only does the reaction between the short and long DNA strands appear to be first order but also the amount of [^{3}H]DNA retained on the nitrocellulose filters at equilibrium accounts for approximately half of the [^{3}H]DNA retained at the start of the reaction. Possible explanations for this kinetic behavior will be given under Discussion. All of the experiments reported

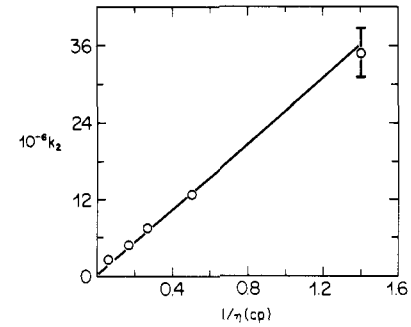

FIGURE 2: The effect of viscosity on the rate constant for SSB transfer between 375 nucleotide donor and 400 nucleotide recipient strands. The reactions were followed at 37 °C in 0.15 M NaCl, 0.01 M Tris, and 0.001 M EDTA, pH 7.6.

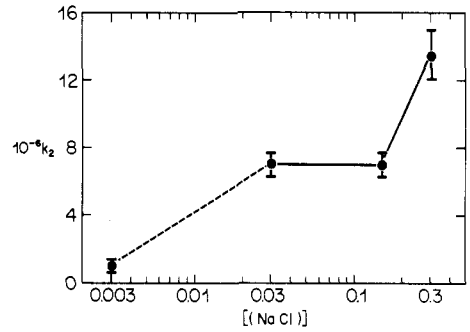

FIGURE 3: The effect of NaCl concentration on the rate constant for SSB transfer between strands. The same materials were used as in Figure 2. The glycerol concentration was 50%.

in the remainder of this paper take advantage of the apparent all-or-none mechanism for reactions with ^{3}H-labeled 375-nucleotide fragment–SSB complex as donor. C_0 is taken to be twice the 375 nucleotide fragment concentration.

Viscosity and Temperature Dependence of the Rate of SSB Transfer. Viscosity and temperature effects on SSB transfer were studied by using ^{3}H-labeled 375-nucleotide DNA plus SSB at 80% site saturation as donor. The recipient DNA was unlabeled sonicated calf-thymus DNA. Equal single-stranded DNA concentrations ($\sim 10^{-9}$ M fragments) were employed. The solvent contained 0.15 M NaCl, 0.01 M Tris, and 0.001 M EDTA, pH 7.6, plus various amounts of glycerol. The glycerol was added w/v and viscosities (η) are stated in centipoise [cP; from Wolf et al. (1976)]. Reactions were performed at 37 °C except where noted.

The effect of viscosity on the second-order rate constant for SSB transfer is depicted in Figure 2. Studies were performed in solutions with 2.7, 5.3, 11, and 29 times the viscosity of the solvent without glycerol. There is a reciprocal relationship between solution viscosity and the rate constant for SSB transfer. This inverse relationship is observed over the extremes of solution viscosity from 0 to 75.6% w/v glycerol.

The dependence of SSB-transfer rates on temperature was tested by comparing reaction rates at 37 and 15 °C. The rate constant was reduced about 1.4-fold at 15 °C. The decrease in solution viscosity from 37 to 15 °C is 1.6-fold. Thus, within experimental error, there appears to be no temperature dependence of the SSB-transfer rate except that which is accounted for by the viscosity dependence of the reaction. The inverse linear relationship between solution viscosity and SSB-transfer rate is consistent with the hypothesis that SSB exchange is a diffusion-controlled reaction.

Effect of Salt Concentration on the Rate of SSB Transfer. The effect of salt concentration on SSB-transfer rates was studied with the same DNAs and SSB site saturation and at

Table II: Effect of DNA Length on SSB-Transfer Reactions

length of recipient	recipient/donor length ratio	NaCl concn (M)	k_1 (s^{-1})	k_2 (M^{-1} s^{-1})
400	1	0.15	$(7.0 \pm 0.3) \times 10^{-3}$	7×10^6
		0.03	$(7.0 \pm 0.3) \times 10^{-3}$	7×10^6
2180	6	0.15	$(1.9 \pm 0.2) \times 10^{-3}$	1.9×10^6
4361	12	0.15	$(1.0 \pm 0.15) \times 10^{-3}$	1.0×10^6
		0.03	$(5.9 \pm 0.9) \times 10^{-4}$	5.9×10^5
5577 circle	15	0.15	$(1.3 \pm 0.2) \times 10^{-3}$	1.3×10^6

37 °C. The solvent contained 0.01 M Tris-HCl, 0.001 M EDTA, pH 7.6, 50% glycerol, and various concentrations of NaCl. Figure 3 shows the effect of NaCl concentration on the second-order rate constant for SSB transfer. There is a broad plateau from 0.03 to 0.15 M NaCl where the rate of SSB transfer is constant. The rate of SSB-strand transfer increases 2-fold upon increasing the NaCl concentration from 0.15 to 0.3 M. The plateau range for SSB-transfer rates occurs in the same ionic strength region where the association constants for SSB binding to DNA are independent of NaCl concentration (Ruyechan & Wetmur, 1975, 1976). It is not clear why the transfer rate in 0.003 M NaCl is 7-fold lower than it is above 0.03 M. No attempt has been made to explain this result. The dotted line in Figure 3 is used to indicate uncertainty concerning the actual lower salt limit of the plateau region.

Effect of Recipient DNA Strand Length or Circularity on the Rate of SSB Transfer. All of the studies of DNA strand length effects on SSB-transfer rates were carried out at 37 °C in 0.15 M NaCl, 0.01 M Tris-HCl, 0.001 M EDTA, pH 7.6, and 50% glycerol. The donor DNA was ^{3}H-labeled 375-nucleotide DNA complexed with SSB at 80% site saturation. The DNA fragment concentration was 10^{-9} M before 2-fold dilution with recipient DNA. The recipient DNA was present at the same SSB site (nucleotide) concentration as the donor DNA.

The recipient single-stranded DNA molecules were either 400-nucleotide (average) calf-thymus DNA, 2180-nucleotide *Ava*I plus *Pst*I digest of pBR322, 4361-nucleotide *Eco*RI digest of pBR322, or circular 5577-nucleotide bacteriophage G4 DNA (Godson et al., 1978). All linear double-stranded restriction fragments of pBR322 were purified by agarose gel electrophoresis and elution before denaturation and use. The single-stranded circular DNA was purified by CsCl density-gradient sedimentation. The various DNA sizes were confirmed by observation of SSB–DNA complexes in the electron microscope. The G4 DNA was more than 90% circular.

First-order, as well as second-order, rate constants based on the all-or-none model for SSB-transfer reactions involving DNAs of different length or shape are given in Table II. These reactions proceeded approximately 50% to completion, an equilibrium concentration required for the all-or-none model. Some small downward curvature was observed at the start of the rate plots with 4361- and 5577-nucleotide recipient DNAs. This indicates that incomplete long DNA–SSB complexes might be better recipients than long DNA alone. The rate constants were calculated from the entire rate plot. The reported values include the range of systematic error that could have been introduced by fitting a first-order reaction.

The first-order rate constant for 400-nucleotide DNA is 7×10^{-3} s^{-1}. We have already observed that this result is independent of NaCl concentration in the range of 0.03–0.15 M. When the recipient DNA is increased in length by 6-fold,

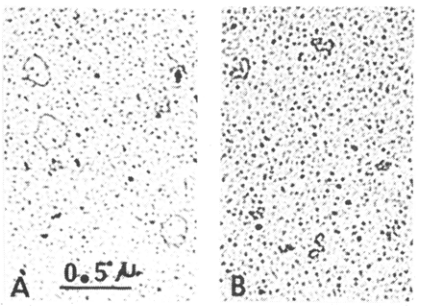

FIGURE 4: Electron micrographs of bacteriophage G4 DNA at 40% coverage by SSB. (A) DNA–SSB complexes prepared by mixing SSB and G4 DNA. (B) DNA–SSB complexes prepared by transfer of SSB from 400-nucleotide DNA–SSB complexes to G4 DNA.

Table III: Electron Microscopic Analysis of SSB-Transfer Reactions

	fully open circles (%)	fully closed circles (%)	partially open circles[a] (%)
control[b] (405 molecules scored)	45	44	11
SSB transfer to G4 DNA[c] (260 molecules scored)	3	61	36
SSB transfer from G4 DNA[d] (350 molecules scored)	70	24	6

[a] Defined as less than one-half the contour length of fully open circles. [b] G4 ssDNA circles + SSB at 40% saturation in 0.15 M NaCl. [c] Sonicated DNA–SSB complex at 80% saturation + G4 DNA in 0.15 M NaCl. [d] G4 DNA–SSB complex at 80% saturation + sonicated DNA in 0.15 M NaCl.

the rate constant drops 3.5-fold. Further increase of the recipient DNA length to 12-fold leads to a total rate constant decrease of 7-fold. When this 4361-nucleotide DNA was used as a recipient in 0.03 M NaCl, the total rate constant decrease was 12-fold. The rate of SSB exchange becomes somewhat dependent upon ionic strength in the 0.03–0.15 M NaCl range when the recipient DNA becomes long. Finally, we find that recipient circular DNA of 5577 nucleotides reacts as rapidly as linear DNA of 4361 nucleotides. This result eliminates any mechanism requiring nucleation of SSB–DNA complexes at the end of DNA molecules.

Visualization of SSB Transfer by Electron Microscopy. Complexes of single-stranded G4 DNA circles incubated with SSB at 40% site saturation are shown in Figure 4A. Uncomplexed circles appear as folded and compact structures. Fully complexed circles appear round and large, whereas partially complexed circles appear folded or twisted with one or more extended loops. Uptake of SSB from solution by G4 DNA results predominantly in structures that are fully extended circles (Figure 4A), indicating cooperative binding of protein throughout the entire length of the DNA. A statistical analysis of the types of molecules observed appears in Table III, line 1. Collapsed circles are present in roughly an equal concentration and only occasionally are partially complexed circles visible. When this solution is treated with endonuclease S1 or endonuclease I before mounting, the extended DNA molecules remain full length.

Transfer experiments were performed in which a short 400-base DNA was incubated with SSB at 80% site saturation and then an equal concentration of G4 DNA circles was added. The final nucleotide concentration was 9×10^{-6} M. This is roughly 20 times more concentrated than the reactions assayed

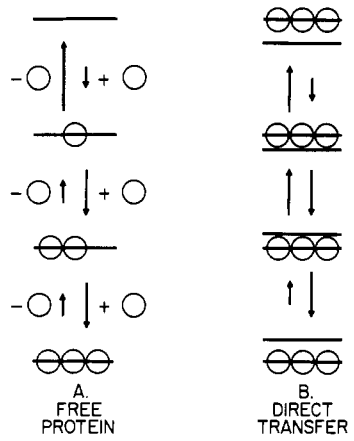

FIGURE 5: Models for SSB transfer between DNA molecules.

by nitrocellulose filter binding, ensuring that only equilibrium products would be visualized. The results of the transfer reactions are given in Table III, line 2. Most DNA structures found in transfer reactions were partially collapsed with extended loops (Figure 4B). A few fully collapsed uncomplexed circles were also found. Well-extended circles were extremely rare, being present in only a few cases out of many hundreds of molecules photographed. Transfer reactions were also performed in 0.01 and 0.2 M NaCl, as previously described, and the same results were obtained (data not shown). Finally, the reverse reaction with 80% site-saturated G4 DNA circles plus 400-base recipient DNA was observed. The extended G4 DNA circles remained for the most part extended (Table III, line 3). The lack of fully extended DNA circles in transfer reactions onto circles suggests that a mechanism other than uptake of free SSB from solution might be involved.

Discussion

There are two ways by which SSB could be transferred from one strand of DNA to another. These mechanisms are illustrated in Figure 5. SSB could dissociate from the first DNA to join a pool of free molecules. SSB from this pool could then bind to another DNA strand. This mechanism is illustrated in Figure 5A. The second possibility would involve direct and cooperative transfer of a chain of SSB molecules from one strand of DNA to another. This mechanism is illustrated in Figure 5B.

If free SSB and free single-stranded DNA are mixed together, a DNA–SSB complex will be formed. This uptake of free SSB by DNA, illustrated in Figure 5A, will involve a nucleation reaction and a propagation reaction analogous to those occurring in DNA-renaturation (reassociation) kinetics. First, a noncooperative reversible binding may occur between one SSB and a DNA molecule. This step, like the formation of the first base pair in DNA reassociation, rarely results in complex formation. The preequilibrium is described by the noncooperative binding constant $K_1^0 = k_n/k_{-n}$, where k_n and k_{-n} are the rates of formation and dissociation of a single SSB–DNA complex. Using equilibrium dialysis of oligonucleotides, Ruyechan & Wetmur (1976) determined K_1^0 to be about 5×10^4 per specific nucleotide. If the specific four nucleotide–SSB interaction may occur at any position along an $S' = 32$-nucleotide binding site for an SSB tetramer, an observed association constant to such a site would be $K_1 = S'K_1^0 = 1.6 \times 10^6$. The next step in a nucleation reaction involves binding of an SSB molecule next to a noncooperatively bound SSB. The cooperative binding constant is $K_2 = k_c/k_{-c}$, where k_c and k_{-c} are rate constants for association of SSB from the end of a group of bound SSB molecules. This cooperative binding step is effectively irreversible. The cooperative binding of an SSB, following the reversible noncooperative binding, is the rate-determining step for nucleation. Propagation involves addition of more SSB molecules to fill the remaining sites.

The cooperative binding of SSB to single-stranded DNA may be shown in many ways. When SSB is mixed with bacteriophage G4 DNA, even at 40% coverage, highly extended molecules are seen in the electron microscope (Figure 4A). When these molecules are subjected to extensive digestion with endonuclease I or endonuclease S1, these molecules remain full length. Ruyechan & Wetmur (1975) showed that the cooperativity, $\omega = K_2/K_1 = K_2/(S'K_1^0)$, at 50% site saturation, must be equal to the square of the average number of sites covered by SSB on an infinitely long DNA strand. The nuclease data with bacteriophage G4 DNA means that $\omega > 3 \times 10^4$ or $K_2 > 5 \times 10^{10}$ M^{-1}. Using longer DNA molecules, Ruyechan & Wetmur (1975) also obtained $K_2 > 5 \times 10^{10}$ M^{-1}. Sigal et al. (1972) showed that SSB could lower the melting temperature of T_4 DNA from $T = 328$ K to below $T_m' = 310$ K when present at a concentration less than $S = 1.7 \times 10^{-6}$ M. We may calculate a minimum association constant to be (Crothers, 1971)

$$K_2 = (1/S)e^{-(T_m - T_m')\Delta H/(B_c R T_m T_m')} \quad (3)$$

where ΔH for DNA melting is about −8000 cal/mol of base pairs, R is the gas constant, and $B_c = 1/16$ is the density of sites for SSB (1 per 16 base pairs). The melting temperature depression data also imply that $K_2 > 5 \times 10^{10}$ M^{-1}.

What is the possibility that the mechanism involved in formation of DNA–SSB complexes from DNA and SSB is the same as the mechanism involved in the transfer of SSB from one DNA molecule to another? For the transfer between 375- and 400-nucleotide DNA strands, the all-or-none reaction observed is consistent with this mechanism involving uptake of free SSB. However, we will show below that the rate of transfer is too great to be explained by uptake of the exceedingly low concentration of free SSB. The dependence of the rate of SSB transfer on inverse viscosity suggests the calculation of a maximum possible rate of transfer based on a diffusion-limited reaction. Consider the reaction

$$R + S \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} RS \quad (4a)$$

$$RS + S \xrightarrow{k_c} RS_2 \quad (4b)$$

where R is recipient DNA, S is free SSB, RS is a noncooperative DNA–SSB complex, and RS_2 is the first cooperative DNA–SSB complex. The rate equation for this reaction is

$$-d[R]/dt = 2Nk_nk_c[R][S]^2/(k_{-n} + 2k_c[S]) \quad (5)$$

where N is the number of nucleotides minus 3 (372 for a 375-nucleotide oligomer) and the 2 comes from the two ways of obtaining a cooperative binding interaction. We know $k_n/k_{-n} = 6 \times 10^4$, and we may assume $k_n = k_c$ = rate constant for diffusion of SSB to a DNA site (nucleotide). For the 375-nucleotide molecules

$$-d \ln[R]/dt = 744k_n[S]^2/[(6 \times 10^4)^{-1} + 2[S]] = (4.5 \times 10^7)k_n[S]^2 \quad (6)$$

for the small values of [S] used in the experiments. Relating this rate equation to the all-or-none reactions (eq 1 and 2), we find an upper limit for k_1 to be specified to be

$$k_1 \leq (4.5 \times 10^7)k_n[S]^2 \quad (7)$$

if the free-protein mechanism is correct. The total protein concentration in the experiments is 4.5×10^{-9} M, and most of it is bound. In fact, it may be shown that [S] must be of the order of $3/K_2$ or 6×10^{-11} M. k_n for a diffusion-controlled reaction involving SSB and single-stranded 375-nucleotide DNA may be estimated by the Smoluchowski equation to be

$$k_n \leq 4\pi D_{1,2}(N_0/1000)e \quad (8)$$

where N_0 is Avogadro's number, $D_{1,2}$ is the mutual diffusion coefficient, and e is the required approach distance. Letting e be 3 Å and using sedimentation data to obtain $D_{1,2}$

$$k_n \leq 2.3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1} \quad (9)$$

in water. This leads to a prediction of

$$k_1 \text{ (theory)} \leq 3.7 \times 10^{-5} \text{ s}^{-1} \quad (10a)$$

whereas

$$k_1 \text{ (expt)} = 3.7 \times 10^{-2} \text{ s}^{-1} \quad (10b)$$

The theory is unable to account for the rapid observed reaction rates even after taking all statistical factors into account. The mechanism involving uptake of free SSB from solution also fails to account for the observed DNA-length dependence of SSB uptake. In fact, the yield of SSB molecules per nucleation event should rise with increasing recipient DNA length, leading to a length dependence of the transfer rates opposite to that observed. Furthermore, the products of the transfer reactions should be no different from those seen when SSB and recipient DNA are mixed in the same ratios. A comparison of Table III, lines 1 and 2, shows that this is not the case.

To account for the relatively rapid rate of transfer of SSB from one strand to another, one needs to invoke a mechanism where the formation of a cooperative complex takes place in a single step and is diffusion controlled. One such mechanism would involve uptake of polymers (dimers or higher) of SSB by a recipient strand. Sedimentation studies of SSB (Ruyechan & Wetmur, 1976) failed to indicate such aggregates even at 10^5 greater SSB concentration than the free SSB in the experiments. Furthermore, invoking a mechanism involving association and dissociation of SSB–SSB complexes does not help to explain the DNA-length dependence of SSB transfer rates or the dependence of reaction products on the order of addition of reactants.

We believe the most likely explanation of the kinetics is that transfer of SSB between strands, unlike addition of SSB to DNA where the free SSB concentration is much greater, follows the mechanism outlined in Figure 5B. In this case, the reaction involves the mutual diffusion of donor DNA–SSB complex and recipient DNA into the proper orientation, followed by direct and cooperative transfer of bound SSB from one strand to the other. We do not know whether the 2 binding sites per SSB tetramer (Ruyechan & Wetmur, 1976) are involved in this process. The rate constant for this process, the same as k_2 for the all-or-none mechanism, would be limited by diffusion to

$$k_2 \leq 4\pi D_{1,2}(N_0/1000)eN \quad (11)$$

where $D_{1,2}$ is now the mutual diffusion coefficient of a DNA–SSB complex and a recipient DNA and N is the number of ways the bound SSBs could interact with the recipient strand to produce a productive transfer. For all reasonable calculations of $D_{1,2}$, the theoretical k_2 is much larger than the experimental k_2. Thus, there is no a priori reason to exclude a direct-transfer mechanism.

The direct-transfer mechanism also helps to explain the length effect of recipient DNAs. The yield per transfer reaction is limited to the number of SSB molecules complexed to a donor strand. Thus, unlike the rate of uptake of SSB from solution, the rate of uptake of SSB by direct transfer will not be expected to increase with increasing recipient DNA length when the recipient DNA is longer than the donor DNA. In fact, the rates of transfer are found to decrease with increasing recipient DNA strand length. This decrease could be the result of some combination of two effects. First, the mutual diffusion coefficient will decrease when the recipient strand becomes longer. Second, the availability of sites on the recipient DNA may decrease when the recipient DNA length increases. Single-stranded DNA at 37 °C in 0.03–0.15 M NaCl is known to have a significant amount of secondary structure. No data are available concerning the magnitude of these two effects.

The products of SSB transfer from short DNA–SSB complexes to recipient G4 DNA, seen in Figure 4B and compiled in Table III, line 2, appear to be the result of all of the G4 DNA molecules acquiring clusters of SSB rather than the highly cooperative product distribution seen in Figure 4A and compiled in Table III, line 1. This product of G4 DNA–SSB clusters explains the continued adherence of the kinetics to reversible all-or-none behavior even with long DNA recipients. The observed product distribution is not an equilibrium distribution. If G4 DNA–SSB complexes are formed first and the recipient 400-nucleotide DNA added later, the open circular G4 DNA molecules retain that conformation (Table III, line 3). Eventually, the observed product distribution (Table III, line 2) will convert to the expected cooperative distribution (Table III, line 1) by intramolecular and intermolecular redistribution of the SSB molecules. Weiner et al. (1975) and we (unpublished observations) have found, however, that SSB transfer between such long DNA molecules is an extremely slow process, even in the presence of facilitating short single-stranded DNA.

We conclude that there are two possible mechanisms that may facilitate the transfer of SSB from a single-stranded DNA–SSB mixture onto newly added single-stranded DNA. If there is excess SSB, then free SSB from solution will simply add on to the newly added single-stranded DNA. If there is more single-stranded DNA than SSB in the mixture, then direct transfer of clusters of SSB from DNA–SSB complexes onto the added DNA may occur according to the mechanism in Figure 5B. We can only speculate on the relative importance of these two mechanisms in governing SSB redistribution in such processes as DNA replication.

References

Alberts, B. M., & Frey, L. (1970) *Nature (London) 227*, 1313–1318.

Alberts, B. M., & Herrick, G. (1971) *Methods Enzymol. 21D*, 198–217.

Alberts, B. M., Amodio, F. J., Jenkins, M., Gutman, E. D., & Ferris, F. L. (1968) *Cold Spring Harbor Symp. Quant. Biol. 33*, 289–305.

Bandyopadhyay, P. K., & Wu, C.-W. (1978) *Biochemistry 17*, 4078–4085.

Birnboim, H. C., & Doly, J. (1979) *Nucleic Acids Res. 7*, 1513–1523.

Christiansen, C., & Baldwin, R. L. (1977) *J. Mol. Biol. 115*, 441–454.

Clewell, D. B. (1972) *J. Bacteriol. 110*, 667–676.

Crothers, P. M. (1971) *Biopolymers 10*, 2147–2160.

Davis, R. W., Simon, M., & Davidson, N. (1971) *Methods Enzymol. 21D*, 413–428.

Geider, K. (1978) *Eur. J. Biochem. 87*, 617–622.
Glassberg, J., Meyer, R. R., & Kornberg, A. (1979) *J. Bacteriol. 140*, 14–19.
Godson, G. N., Barrell, B. G., Staden, P., & Fiddes, J. C. (1978) *Nature (London) 276*, 236–247.
Greenwood, F. C., Hunter, W. M., & Glover, J. S. (1963) *Biochem. J. 89*, 114–123.
Johnson, B. F. (1977) *Mol. Gen. Genet. 157*, 91–97.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Meyer, R. R., Glassberg, J., & Kornberg, A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1702–1705.
Meyer, R. R., Glassberg, J., Scott, J. U., & Kornberg, A. (1980) *J. Biol. Chem. 255*, 2897–2901.
Molineux, I. J., & Gefter, M. L. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3858–3862.
Molineux, I. J., & Gefter, M. L. (1975) *J. Mol. Biol. 98*, 811–825.
Molineux, I. J., Friedman, S., & Gefter, M. L. (1974) *J. Biol. Chem. 249*, 6090–6098.
Molineux, I. J., Pauli, A., & Gefter, M. L. (1975) *Nucleic Acids Res. 2*, 1821–1837.
Oey, J. L., & Knippers, R. (1972) *J. Mol. Biol. 68*, 125–138.
Orosz, J. M., & Wetmur, J. G. (1974) *Biochemistry 13*, 5467–5473.
Peterman, B. F., & Wu, C.-W. (1978) *Biochemistry 17*, 3889–3892.
Ruyechan, W. T., & Wetmur, J. G. (1975) *Biochemistry 14*, 5529–5534.
Ruyechan, W. T., & Wetmur, J. G. (1976) *Biochemistry 15*, 5057–5063.
Scott, J. E., Eisenberg, S., Bertsch, L. L., & Kornberg, A. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 133–137.
Sigal, N., Delius, H., Kornberg, T., Gefter, M. L., & Alberts, B. M. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 3537–3541.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.
Weiner, J. H., Bertsch, L. L., & Kornberg, A. (1975) *J. Biol. Chem. 250*, 1972–1980.
Wickner, S., & Hurwitz, J. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4120–4124.
Wolf, A. V., Brown, M. G., & Prentiss, P. G. (1976) in *CRC Handbook of Chemistry and Physics* (Weast, R. C., Ed.) 57th ed., CRC Press, Cleveland, OH.
Yang, R. C.-A., Lis, R., & Wu, R. (1979) *Methods Enzymol. 68*, 176–182.

Binding of 5-Fluorotryptamine to Polynucleotides as a Model for Protein–Nucleic Acid Interactions: Fluorine-19 Nuclear Magnetic Resonance, Absorption, and Fluorescence Studies†

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ABSTRACT: Fluorine-19 nuclear magnetic resonance (^{19}F NMR), optical absorption, and fluorescence spectroscopy have been used to study the interaction of 5-fluorotryptamine (5FTA) with polynucleotides as a model for protein–nucleic acid interactions. In the presence of DNA, denatured DNA, poly(A), and poly(A)·poly(U), the ^{19}F resonance of 5FTA shifted 0.3–0.6 ppm upfield while the presence of poly(I)·poly(C) had little effect on the chemical shift. Differences in the ^{19}F chemical shift induced upon changing from H_2O to ^2H_2O indicate differences in the solvent accessibility of 5FTA bound to the various polynucleotides. ^{19}F NMR relaxation experiments were carried out for free 5FTA and in its nucleic acid complexes, and the results were interpreted by using a two correlation time model that included contributions to relaxation from dipolar coupling and chemical shift anisotropy. Values for the internal motion correlation time and the overall motion correlation time are reported. The effect of 5FTA on the melting transition of the double-stranded polynucleotides and on the quenching of 5FTA fluorescence was also studied. The ^{19}F NMR results support the model of partial intercalation of the 5FTA chromophore into the polynucleotides, and the implications for protein–nucleic acid interactions are discussed.

Over the past 2 decades a great deal of experimental work has been focused on the binding of proteins to nucleic acids. These studies have examined the factors involved in the recognition process through the use of model protein systems, such as mono-, di-, and tripeptides (Dimicoli & Hélène, 1974a,b), as well as more complex systems such as the *lac* operon (Caruthers, 1980) and gene 32 protein (Kelly & von Hippel, 1976; Kelly et al., 1976). The forces involved may include electrostatic interactions, hydrogen bonding, and hydrophobic interactions. Of particular interest is the interaction of planar aromatic amino acids, tryptophan, phenylalanine, and tyrosine, with nucleic acids. It is thought that these amino acids may bind DNA in a manner similar to the classical intercalators ethidium bromide and proflavin.

As a probe of the interaction of aromatic amino acids with polynucleotides, we have used optimal absorption, fluorescence, and fluorine-19 nuclear magnetic resonance (^{19}F NMR) spectroscopy to study the interaction of 5-fluorotryptamine (5FTA), Figure 1, with single- and double-stranded DNA and RNA. ^{19}F NMR is a convenient probe of noncovalent in-

† From the Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143. *Received February 10, 1981; revised manuscript received August 3, 1981.* This work was supported by the National Institutes of Health through Grants CA 27343, GM 25018, and RR 00892 for maintenance of the UCSF Magnetic Resonance Laboratory.
‡ Supported by Training Grant GM 07175 from the National Institutes of Health.
§ Recipient of Career Development Award AM 00291 from the National Institutes of Health.

0006-2960/82/0421-0615$01.25/0