We have calculated $G_t(R)$ (Figure 7) as a function of R, r, and n with the following values: $T = 297$ K, $\epsilon = 79$, $h = 50$ Å, $S = 85$ Å^2 for a small vesicle ($2r = 300$ Å), and 70 Å^2 for two large vesicles of different sizes ($2r = 1500$ and 3000 Å) (Brouillette et al., 1982), $K = 0.7$ M^{-1} [the average value of those obtained by Nir et al. (1978) and Ohki & Kurland (1981)], and $A = 4 \times 10^{-14}$ erg (Gingell & Parsegian, 1972; Brooks et al., 1975; Requena & Haydon, 1975; Ohshima et al., 1982).

Registry No. Na, 7440-23-5; Li, 7439-93-2; K, 7440-09-7; Cs, 7440-46-2.

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Escherichia coli Single-Stranded DNA Binding Protein Is Mobile on DNA: ^{1}H NMR Study of Its Interaction with Oligo- and Polynucleotides†

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ABSTRACT: The interaction of the *Escherichia coli* single-stranded DNA binding protein (SSB) with oligo- and polynucleotides has been studied by 270-MHz ^{1}H NMR spectroscopy and fast kinetic techniques. $d(pT)_8$ and poly(dT) were used to study noncooperative and cooperative binding, respectively. The H6, H1′, and CH_3 resonances of $d(pT)_8$ are high-field shifted by less than 0.05 ppm, and H8 and H2 of poly(dA) are low-field shifted upon complexation. The protein resonances remain virtually unshifted. The small shifts upon complexation provide no evidence for extensive stacking interactions between the nucleotide bases and aromatic amino acid side chains of SSB. The $d(pT)_8$ and poly(dA) signals are broadened to about 30 Hz whereas the resonances of poly(dT) are broadened beyond detection upon stoichiometric complexation. Continuous broadening of all poly(dT) signals even at a 10-fold excess of poly(dT) indicates fast exchange of SSB between different binding sites. Dissociation and reassociation rates determined from stopped-flow experiments are too slow by at least 2 orders of magnitude to account for the experimental line widths. Therefore, we conclude that SSB translocates without dissociation from the DNA template. A model for the translocation is outlined. It is based on partial dissociation of octamer sections of poly(dT) from the complex with a rate constant as previously published for the dissociation of $d(pT)_8$ from SSB.

Single-stranded DNA binding proteins perform specific functions during the replication of DNA [for reviews, see Coleman & Oakley (1980), Kornberg (1980), Kowalczykowski et al. (1981), and Kornberg (1982)]. They bind preferentially to single-stranded DNA and much more weakly to double-stranded DNA, thereby destabilizing double-stranded DNA. This property facilitates the removal of secondary structures in the template (LaDuca et al., 1983). Furthermore, single-stranded DNA binding proteins promote the renaturation of DNA (Christiansen & Baldwin, 1977) and protect single-stranded DNA against attack of nucleases (Geider, 1978).

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The great variety of biochemical functions that can be influenced by single-stranded DNA binding proteins is best illustrated by the protein from *Escherichia coli* (SSB). SSB has been shown to be involved in DNA replication, recombination, and repair processes. Although the biochemical and physical properties of SSB and of the complexes between SSB and DNA have been studied in some detail, important aspects of the function of SSB are not yet understood. First of all, the structure of the complexes of SSB with DNA is unknown. The magnitude of the contribution of cooperative protein–protein interactions to the stability of the complexes is a matter of debate (Molineux et al., 1975; Ruyechan & Wetmur, 1975; Williams et al., 1983). Furthermore, the nature of the forces stabilizing the complexes is unknown. Finally, a problem arises from the slow dissociation rate of SSB from single-stranded nucleic acids, which seems incompatible with the high rate of DNA replication by *E. coli* DNA polymerase III holoenzyme. Rates of 500–1000 nucleotides per second have been reported in vivo and in vitro [see p S35 of Kornberg (1982)]. In contrast, the dissociation rate constant of SSB and $d(pT)_{30-40}$ has been shown to be smaller than 1 s^{-1} (Krauss et al., 1981).

In the present paper we have investigated the mobility of SSB on single-stranded homopolymers using NMR. Our data also provide insight into some structural aspects of SSB·DNA complexes.

Materials and Methods

Nucleic Acids. $d(pT)_8$, poly(dT), and poly(dA) were obtained from P-L Biochemicals. Concentrations of polynucleotides were determined by UV spectrometry using the following extinction coefficients (per mole of nucleotide at 260 nm): 7.8×10^3 M^{-1} cm^{-1} for poly(dT) and $d(pT)_8$ and 8.1×10^3 M^{-1} cm^{-1} for poly(dA). The average chain lengths of poly(dT) and poly(dA) were 800 and 500, respectively, as determined by gel electrophoresis. The concentrations of polymers refer to nucleotides if not stated otherwise.

Preparation of SSB. SSB was prepared from an overproducing strain containing a plasmid carrying the *E. coli* ssbA$^+$ gene (Chase et al., 1980). A slightly modified version of the procedure described by Krauss et al. (1981) was used. SSB was stored at −20 °C in a buffer containing 20 mM potassium phosphate, pH 7.8, 300 mM NaCl, and 60% glycerol. Concentrations of SSB tetramer were determined by UV spectrometry using an extinction coefficient of 94 800 M^{-1} cm^{-1} per tetramer at 280 nm (Krauss et al., 1981).

NMR Spectra. For the NMR measurements the stock solutions were diluted 10-fold with distilled water and applied to a small heparin–Sepharose column, followed by extensive washing with 20 mM potassium phosphate buffer, pH 7.8, to remove glycerol. Elution of SSB protein with standard buffer [20 mM potassium phosphate, pH 7.8, 500 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA)] yielded SSB tetramer in concentrations up to 80 μM.

For measurements in D_2O, SSB and polynucleotides dissolved in standard buffer were lyophilized and redissolved in D_2O. This procedure was repeated 3 times. Lyophilization did not change the binding properties of SSB, as judged from fluorescence titrations and from line broadening of the poly(dT) signals (Figure 5). After transfer to D_2O, however, at high concentrations some precipitation of free SSB usually occurred. The precipitation decreased the intensity of the NMR signals of free SSB. The decrease in intensity was used to correct the protein concentration for precipitation.

The spectra were accumulated for 10–30 h on a Bruker WH 270 instrument and referenced downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate with the water signal in standard buffer as a secondary standard. In H_2O, either the correlation or pulsed Fourier transformation technique with solvent presaturation was applied. Spectra of the same sample were well reproducible within 1 week. The same SSB protein sample was used for the interaction difference spectra in order to minimize the influence of deviations among the spectra of SSB from different sample preparations.

Stopped-Flow Kinetics. Stopped-flow experiments were performed as described by Pingoud et al. (1973).

Results

Stopped-Flow Kinetics. In the mechanism of the association reaction between SSB and DNA, three steps may be considered: (i) binding to isolated positions, (ii) cooperative binding in contiguous positions, and (iii) gap closure by sliding mechanisms (Lohman & Kowalczykowski, 1981). Contributions from these steps may result in a rather complex reaction behavior. However, at a large excess of free sites on the polynucleotide over SSB, it can be expected that the association kinetics are determined by step i only. All stopped-flow experiments were carried out under these conditions. After poly(dT) was mixed with SSB, their reaction was followed by monitoring the quenching of the intrinsic SSB fluorescence. The time course of the complex formation could be described by a single exponential independent of the SSB concentration. In the concentration range studied, the reciprocal time constant depends linearly on the poly(dT) concentration (data not shown). This behavior can be described by a quasi-monomolecular association mechanism, in which the concentration of poly(dT) c_{pdT} is constant because of the large excess of poly(dT):

$$\text{SSB} + \text{poly(dT)} \rightleftharpoons \text{SSB·poly(dT)}$$

with $\tau^{-1} = k_r c_{pdT} + k_d$. From the slopes of the concentration dependence on τ^{-1} we evaluate the following values for k_r: 2.4×10^9 M^{-1} s^{-1} at 200 mM NaCl and 1.1×10^9 M^{-1} s^{-1} at 950 mM NaCl. These values are in good agreement with those estimated for diffusion-controlled reactions on the basis of a modified von Smoluchowsky expression (Berg et al., 1981). The small decrease in k_r with increasing ionic strength can be attributed to screening effects. Under similar ionic concentrations a comparable behavior was reported for the kinetics of association between poly(dT) and gene 32 protein (Lohman & Kowalczykowski, 1981) and gene 5 protein (Poerschke & Rauh, 1983).

From the intercepts of the concentration dependence of τ^{-1}, which are indistinguishable from zero, an upper limit of 1 s^{-1} for the dissociation rate constant of SSB and poly(dT) can be established, even at the highest salt concentrations applied. The same limit has been previously reported for the dissociation rate constant of SSB and $d(pT)_{30-40}$ (Krauss et al., 1981).

NMR Spectra. SSB interacts with $d(pT)_8$ by simple noncooperative complex formation (Krauss et al., 1981). The difference spectra between solutions of $d(pT)_8$ with increasing amounts of SSB and the free protein are dominated by the oligonucleotide resonances (Figure 1). The integrated intensity of the complex and of free SSB between 6.5 and 8.0 ppm corresponds to about 85% of the protons expected in this range from the amino acid sequence (Sancar et al., 1981). The protein spectrum shows only minor changes in proportion to $d(pT)_8$ binding, especially in the low-field region: Some positive intensity difference appears at the positions of random-coil phenylalanine around 7.3 ppm (Figure 1). Analogous difference spectra have been reported previously for the interaction of $d(pT)_8$ with gene 5 protein from bacteriophage fd (Coleman et al., 1976). In this case most of the intensity

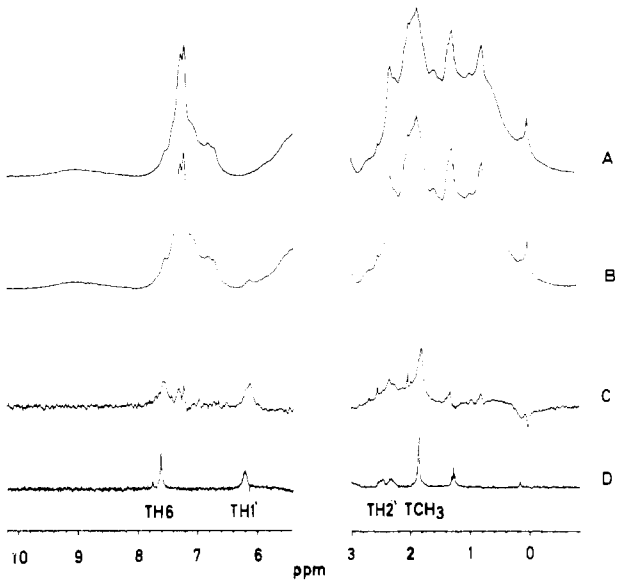

FIGURE 1: Interaction of SSB with $d(pT)_8$. Standard D_2O buffer, 25 °C. (A) 82 μM SSB; (B) 82 μM SSB and 61 μM $d(pT)_8$; (C) difference spectrum of (B) minus (A); (D) 61 μM $d(pT)_8$. Vertical scales: low field is enhanced 2-fold with respect to high field and difference spectrum C is enhanced 4-fold relative to full spectra A and B.

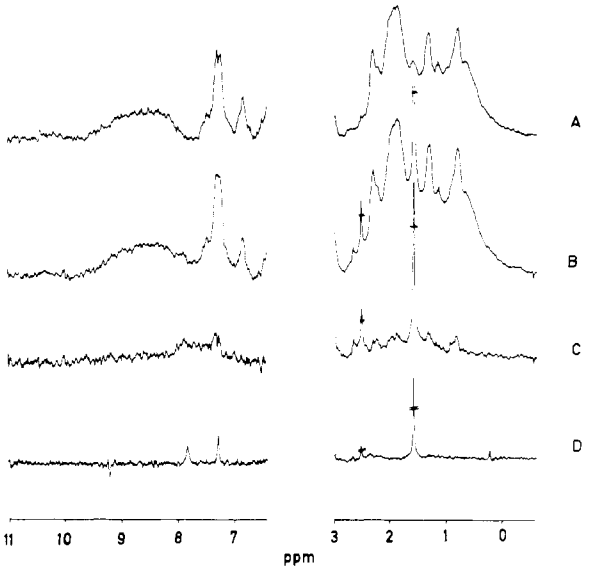

FIGURE 2: Interaction of SSB with poly(dA). Standard H_2O buffer with H_2O presaturation, 25 °C. (A) 20 μM SSB; (B) 20 μM SSB and 403 μM poly(dA); (C) difference spectrum of (B) minus (A); (D) 825 μM poly(dA). Vertical scale of low-field sections is enhanced 2-fold. Signals of buffer impurities are marked by double slashes.

differences were assigned to protein resonances, implying extensive high-field shifts of aromatic amino acid residues upon complex formation. Our spectra show considerably smaller changes of the protein signals upon complexation with no evidence of intensive high-field shifts of aromatic amino acid resonances. A detailed report on the spectra of SSB and its cooperative and noncooperative complexes will be presented elsewhere.

The oligonucleotide signals in the noncooperative SSB·$d(pT)_8$ complex are broadened to about 30 Hz, suggesting an average immobilization by more than 1 order of magnitude (Figure 1). In the cooperative SSB·poly(dT) complex the poly(dT) resonances are broadened beyond detection (Figures 3 and 4).

The H6, CH_3, and H1′ signals of $d(pT)_8$ are only very slightly high-field shifted, by about 0.05 ppm, upon noncooperative complexation with SSB. The protein resonances remain virtually unshifted (Figure 1). These shifts again are considerably smaller than the 0.1–0.2 ppm proposed for $d(pT)_8$ complexed by gene 5 protein from bacteriophage fd (Coleman et al., 1976).

Mixtures of SSB and $d(pT)_8$ do not show any signals of free $d(pT)_8$ even when $d(pT)_8$ is in excess (not shown). Instead, we observe average signals of free and bound $d(pT)_8$, e.g., the resonance of thymidine H6 at 7.6 ppm. Obviously, complex formation is fast with respect to the small experimentally observed binding shifts. This result was expected from earlier temperature-jump experiments (Krauss et al., 1981). Since none of the eight residues of bound $d(pT)_8$ gives signals with width and position as in free $d(pT)_8$, all thymidines must be influenced by complex formation, either in a permanent state or by exchanging position with the directly affected nucleotides. In the latter case there could not be any strict positioning of the oligonucleotide on its binding site as observed for gene 5 protein (O'Connor & Coleman, 1983).

The shifts of the poly(dT) signals due to cooperative complexation can only be detected at an excess of nucleotide since the poly(dT) resonances in the stoichiometric cooperative complex are broadened beyond detection. The small shifts of the thymidine signals at nucleotide excess extrapolate well to the shifts induced by stoichiometric noncooperative complexation with $d(pT)_8$ (Figure 6). An approximately linear relationship was established up to 0.4 SSB tetramer per 32 nucleotides. At this degree of binding, the extensive broadening of the poly(dT) signals indicates cooperative complexation because it is already several times larger than expected for noncooperative binding (Figure 5). The contribution of the noncooperative complexation was interpolated from the binding experiments at a high excess of poly(dT) and stoichiometric noncooperative binding of $d(pT)_8$. Apparently, cooperative binding and noncooperative binding differ largely in the effect on the line width of the thymidine residues, whereas they both induce similar small changes in the chemical shifts. The interaction of SSB with poly(dA) leaves the protein resonances largely unchanged. The nucleotide signals are slightly shifted to lower field and broadened to about the same extent as in the noncooperative SSB·$d(pT)_8$ complex (Figure 2). These results suggest noncooperative binding and some decrease of the stacking interactions present in dA homopolymers at low temperatures.

Mobility of SSB on Nucleic Acids. Upon titration of poly(dT) with SSB, the thymidine resonances continuously broaden before they become undetectable (Figures 3 and 4). The broadening does not change at increased EDTA concentrations (5 mM; not shown), suggesting that it is not due to paramagnetic impurities. The broadening is evident at degrees of binding, θ = SSB per 32 nucleotides, as low as 0.1. In the limit of extremely slow exchange between free and bound nucleotides, the contributions of both species to the total NMR signal should be additive. At $\theta = 0.1$, the small and at least 10 times (cf. below) broadened signal of the bound nucleotides would be hardly detectable and the total signal would virtually retain the narrow line width of free poly(dT) with an intensity reduced by 10%. Obviously, the observed line broadening rules out extremely slow exchange. The exchange between free and bound nucleotides has to be fast enough to produce either lifetime broadening of the signal of the free thymidines or even averaged signals of free and bound nucleotides. The latter possibility is suggested by the finding that both the fractional broadening of the poly(dT) signals at $\theta \leq 0.15$ and the small upfield shifts extrapolate well to

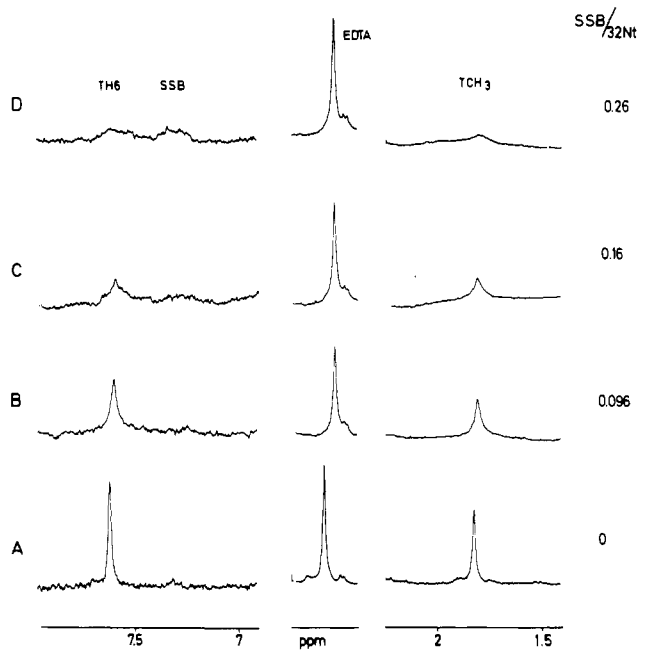

FIGURE 3: Broadening of poly(dT) signals upon titration with SSB. Standard H_2O buffer with H_2O presaturation, 25 °C. (A) 950 μM poly(dT); (B) 950 μM poly(dT) and 2.9 μM SSB; (C) 897 μM poly(dT) and 4.5 μM SSB; (D) 822 μM poly(dT) and 6.6 μM SSB.

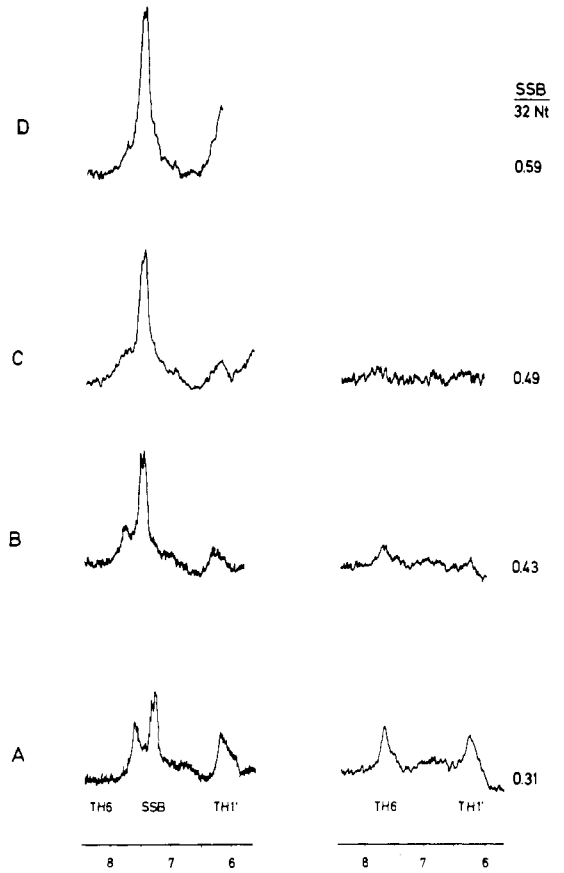

FIGURE 4: Broadening of poly(dT) signals upon titration with SSB. Standard D_2O buffer, 25 °C. (Left) Spectrum of SSB·poly(dT); (right) difference spectrum of SSB·poly(dT) minus (D), in which the T signals have been broadened beyond detection. (A) 682 μM poly(dT) and 6.7 μM SSB; (B) 558 μM poly(dT) and 7.65 μM SSB; (C) 412 μM poly(dT) and 6.3 μM SSB; (D) 243 μM poly(dT) and 4.5 μM SSB.

the effects of stoichiometric noncooperative binding to $d(pT)_8$ (Figures 5 and 6). At $\Theta > 0.15$, the binding of SSB to poly(dT) induces much stronger broadening, suggesting enhanced immobilization as a consequence of cooperative interaction. The broadening depends not only on Θ but also on the poly(dT) concentration. The experiments with poly(dT) summarized in Figure 5 were performed at concentrations between 0.4 and 0.9 mM nucleotides. A systematic investigation of the influence of the poly(dT) concentration on the line broadening is under way.

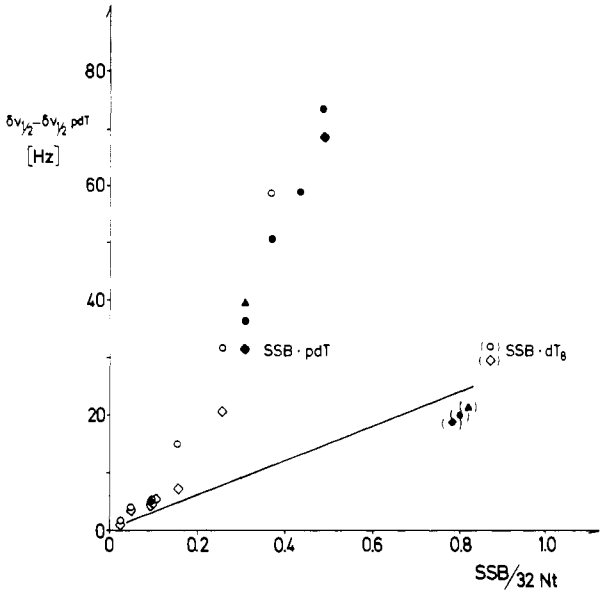

FIGURE 5: Broadening of signals of poly(dT) and $d(pT)_8$ upon titration with SSB. Standard H_2O buffer, open symbols; standard D_2O buffer, filled symbols; SSB·$d(pT)_8$, symbols in parentheses. For temperature and concentrations cf. Figures 1, 3, and 4. The abscissa refers to bound SSB. In the presence of excess poly(dT) this is equal to total SSB. In the presence of excess $d(pT)_8$, bound SSB was calculated with a binding constant of 1.4×10^4 M^{-1} (Krauss et al., 1981). Symbols: ○ and ●, TH6; ◇ and ◆, TCH_3; △ and ▲, TH1'.

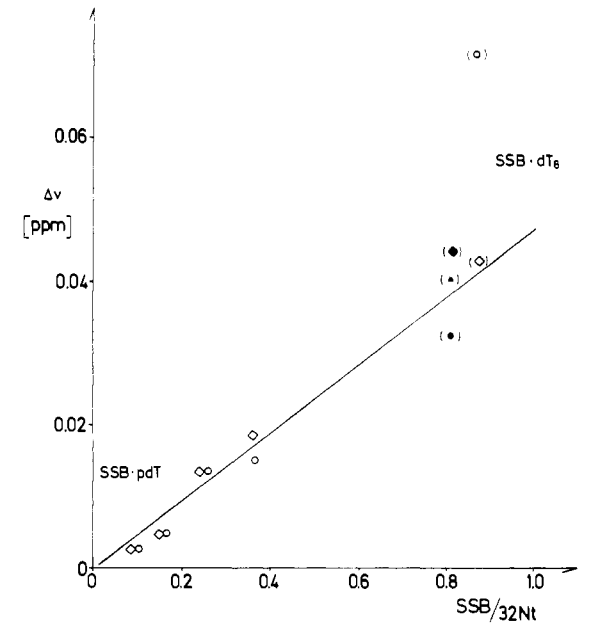

FIGURE 6: High-field shifts of signals of poly(dT) and $d(pT)_8$ upon titration with SSB. Symbols and conditions as in Figure 5.

The binding of SSB to poly(dT) is characterized by a large increase in line width but only minor changes in the chemical shifts of the nucleotide resonances. The appearance of NMR signals from nuclei exchanging between two sites with different intrinsic line widths but the same chemical shift has been discussed in the literature (Fischer & Jardetzky, 1965). The results are analogous to the more familiar case of chemical

exchange between sites with different chemical shifts. Instead of the chemical shift differences, the spin–spin relaxation rate T_{2b}^{-1} corresponding to the larger of the two line widths determines the limit between slow and fast exchange in our case:

slow exchange

$$k_f + k_b < T_{2b}^{-1} \quad (1)$$

$$\pi\Delta\delta\nu_{1/2f} = k_f \quad (2)$$

fast exchange

$$k_f + k_b > T_{2b}^{-1} \quad (3)$$

$$\delta\nu_{1/2fb} = (1 - \Theta)\delta\nu_{1/2f} + \Theta\delta\nu_{1/2b} \quad (4)$$

$\delta\nu_{1/2b}$ and $\delta\nu_{1/2f}$ are the line widths of completely bound and free poly(dT), respectively; $\Delta\delta\nu_{1/2f}$ is the lifetime broadening of the signal of the free nucleotide residues and $\delta\nu_{1/2fb}$ is the half-width of the average signal of free and bound residues, $k_b/k_f = (1 - \Theta)/\Theta$.

With the experimental line broadenings and degrees of binding from Figure 5, eq 1–4 yield an upper limit for k_b:

$$k_b > 100\ s^{-1}$$

This limit turns out to be independent on the assumption of noncooperative binding ($\Theta \leq 0.1$, $T_{2b}^{-1} = 100\ s^{-1}$, fast exchange) and cooperative binding ($\Theta \leq 0.3$, $T_{2b}^{-1} > 300\ s^{-1}$, slow exchange).

The simplest mechanism to describe the observed exchange between the bound and free state of the nucleotides would proceed by dissociation and reassociation of SSB. If this were the case, the average transition rates determined by NMR line broadening should be within the limits of the corresponding rates measured by stopped-flow kinetics. However, the dissociation of SSB from poly(dT) as measured by stopped-flow kinetics, $k_d < 1\ s^{-1}$, is several orders of magnitude too slow to explain the NMR line broadening data, $k_b > 100\ s^{-1}$. Evidently, cooperatively as well as noncooperatively bound SSB can change its site more than 100 times faster than expected from its dissociation rate.

Discussion

Structural Aspects. Stacking interactions between the bases of the nucleic acid and the aromatic amino acid side chains have often been considered to be involved in protein–nucleic acid recognition processes [for a review, see Hélène & Lancelot (1982)]. For the complexes of oligonucleotides with gene 5 protein, the presence of stacking interactions has been deduced from high-field shifts of both the protein and nucleic acid resonances (Coleman et al., 1976; Garssen et al., 1977; Alma et al., 1981; O'Connor & Coleman, 1983). In the SSB complexes we found much less evidence for stacking. The signals of $d(pT)_8$ are considerably less high-field shifted than with gene 5 protein, and there are also much less changes in the protein spectrum. The spectra of the poly(dA)·SSB complexes even indicate some destacking of the adenine residues upon binding. This supports both earlier CD data (Anderson & Coleman, 1975) and the observed increase of SSB·poly(dA) binding upon thermal unstacking of poly(dA) around 15 °C (Krauss et al., 1981).

The NMR spectra also allow us to distinguish between cooperative and noncooperative binding. In the noncooperative binding to $d(pT)_8$, the nucleotide signals are readily detectable, whereas they are broadened beyond detection by cooperative binding of SSB to poly(dT). In this respect the binding of poly(dA) is very similar to that of $d(pT)_8$, suggesting noncooperative interaction of SSB with poly(dA). The comparatively small affinity between SSB and dA homopolymers has been reported previously (Krauss et al., 1981). Although the flexibility of the nucleotides seems very different in the cooperative and noncooperative binding mode, their chemical shifts hardly differ in both complexes. Apparently, the local environments of the cooperatively and noncooperatively complexed nucleotides are similar. The same conclusion has been derived from the stoichiometry and the tryptophan fluorescence intensity of those complexes (Krauss et al., 1981).

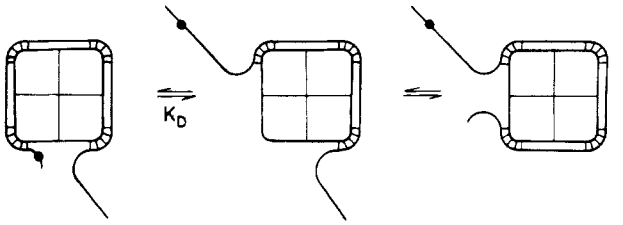

FIGURE 7: Schematic representation of the basic steps of the translocation of SSB on DNA.

Mobility of SSB on DNA. The observed line width of the NMR signals in the SSB·poly(dT) titrations allows us to estimate the lifetimes of the bound and free states of the thymidine residues. The values obtained indicate a high rate of translocation of SSB on poly(dT). Association and dissociation events cannot account for this phenomenon. They are at least 2 orders of magnitude too slow. We conclude that the protein translocates on the DNA without dissociating from it. Translocation of bacteriophage T4 coded gene 32 protein on single-stranded polynucleotides has been inferred from a comparison of experimental and theoretical association rates (Lohman & Kowalczykowski, 1981). From filter binding studies, interstrand translocation of *E. coli* SSB has been proposed to explain the transfer of cooperative units of the protein between different DNA strands (Schneider & Wetmur, 1982).

We propose a mechanism for the migration of SSB on poly(dT) based on partial dissociation of poly(dT) from one subunit binding site according to kinetic data for the binding of SSB to oligo- and poly(dT). In addition, we assume that the subunits in the tetrameric protein and its DNA complex are equivalent, each of them providing an independent subsite (Krauss et al., 1981). Subunit equivalence implies that the incoming and outgoing ends of the bound polynucleotide are located at adjacent subunit binding sites not farther apart than two contiguous octamer sections of poly(dT) bound to two subunits of the tetramer (Figure 7). The vicinity of the incoming and outgoing ends is also supported by the short contour length of DNA completely complexed by tetrameric *E. coli* SSB, as compared with DNA bound by the monomeric gene 32 protein of bacteriophage T4 (Sigal et al., 1972; Delius et al., 1972). The proximity of the incoming and outgoing ends suggests that they may easily compete for the same subunit binding site. The kinetics of this process may be estimated from published stopped-flow and temperature-jump data (Krauss et al., 1981): The dissociation rate constant of $d(pT)_8$ and SSB is $1700\ s^{-1}$. Because of the flexibility of poly(dT) the dissociation of an octamer section of the polynucleotide from its subunit binding site might proceed with a similar rate constant k_d. The empty subsite will then quickly reassociate with an octamer section from the same or the opposite end of the polynucleotide with similar probablity so that, on an average, one out of two dissociations of an octamer section leads to a translocation of SSB by eight nucleotides. In this

basic version the model predicts a random walk in steps of $n = 8$ nucleotides at a rate of $k_d \approx 1700\ s^{-1}$. For a quantitative comparison with the experimental results, however, the model has to be extended to include the formation of larger poly(dT) loops between the subsites, cooperative protein–protein interactions, and intermolecular exchange of SSB between different poly(dT) strands. Corresponding detailed calculations will be published in a separate paper. These calculations support the conclusions drawn from the basic model.

NMR Detection of Protein Mobility on DNA. Although there has been considerable interest in the translocation of proteins bound to DNA [see references cited in Berg et al. (1981)], experimental evidence to support this mobility has been rather indirect. In this paper we show that NMR line broadening is a convenient method to study the mobility of proteins on single-stranded DNA. This approach was based on two prerequisites: (1) a slow exchange between different binding sites by dissociation and reassociation events and (2) a large difference in line widths of free and bound DNA as a spectroscopic indicator for the interaction.

The second condition is not met with double-stranded DNA. In complexes with double-stranded DNA, the binding to a specific site cannot be monitored by line broadening, because the signals from neighboring unoccupied sites will also be broadened as a consequence of the stiffness of double-stranded DNA. In this case a specific difference in chemical shift upon binding might be used as a spectroscopic indicator. With a chemical shift difference $\Delta\nu$ between the NMR signals of free and bound sites, the exchange rate could be estimated if it is not too far from the range between $\Delta\nu$ and the line width, $\delta\nu_{1/2}$, of some signal from the bound double helix. This approach requires short DNA fragments to allow the resolution of individual NMR signals. Under these conditions our approach should also be applicable to the detection of translocation processes of proteins on double-stranded DNA.

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