Localized Interaction of the Polyamine Methylspermidine with Double-Helical DNA As Monitored by ^{1}H NMR Self-Diffusion Measurements†

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ABSTRACT: The ^{1}H NMR pulsed field gradient self-diffusion method has been used to measure the diffusion coefficient of the polyamine analogue methylspermidine (completely *N*-methylated spermidine) in DNA solution, as a function of the concentration ratio of methylspermidine to DNA phosphate. Three different DNA's have been investigated: d(GC)$_4$ (8 base pairs), core length calf thymus DNA (~120 base pairs), and sonicated high molecular weight calf thymus DNA (average 7500 base pairs). For a constant ratio of methylspermidine to DNA phosphate, the diffusion coefficient decreases with increasing DNA length. Moreover, at low concentration ratios the diffusion coefficient of methylspermidine approaches a limiting value that is close to that of the DNA molecule. The experimental data are well reproduced by a two-state diffusion model. In this model the diffusion coefficient of the polyamine is a population-weighted average of polyamine associated with DNA (with a diffusion coefficient given by that of the DNA molecule) and polyamine free in solution.

The polyamines are abundant in living cells and are essential for normal cell growth (Cohen, 1978; Tabor & Tabor, 1984). In vitro, polyamines induce and stabilize compact ordered forms of nucleic acids. It is quite likely that polyamines also stabilize ordered nucleic acid structures in vivo (Record et al., 1981; Wilson & Bloomfield, 1979). Consequently, it is of some interest to examine the structure and dynamics of polyamine–DNA interactions in well-defined model systems. DNA is a highly negatively charged polyion. According to polyelectrolyte theory (Anderson & Record, 1982), strong electrostatic interactions with positively charged multivalent cations will result in a steep radial distribution of these counterions around the DNA. The resulting accumulation of polyamines close to the DNA surface may in turn enable more specific and localized interactions ("site-binding").

There is a widely held notion (Saenger, 1984) that polyamines bind in a highly specific manner to well-defined sites on DNA. This idea derives primarily from X-ray studies (Liquori et al., 1967; Tsuboi, 1964), which have shown several distinct types of polyamine binding sites on nucleic acids (Quigley et al., 1978; Drew & Dickerson, 1981; Kopka et al., 1983; Jain et al., 1989). DNA–polyamine interactions in dilute isotropic solutions have been studied by equilibrium dialysis (Hirschman et al., 1968; Shapiro et al., 1969; Braunlin et al., 1982), DNA melting studies (Plum & Bloomfield, 1990), ^{23}Na NMR (Burton et al., 1981; Braunlin et al., 1986; Padmanabhan et al., 1988, 1991; Besley et al., 1990), ^{14}N NMR (Padmanabhan et al., 1988), and by ^{1}H NMR NOE measurements (Wemmer et al., 1985). In contrast to the X-ray work, these solution studies are most simply interpreted as indicating that polyamines bind to DNA in a loose, nonspecific manner. Thus, Wemmer et al. (1985) found that proton NOE's of spermine bound to d(CGCGAATTCGCG) were positive, indicating that the motional flexibility of this polyamine is largely independent of the overall tumbling of the oligonucleotide. Besley et al. (1990) proposed that the translational motion of polyamines associated with DNA must be extremely rapid and essentially diffusion controlled.

Recently, Gibbs and Johnson (1991) studied the ^{1}H NMR self-diffusion and spin–lattice relaxation times of one methylated divalent polyamine analogue and one partly methylated trivalent polyamine analogue, in high molecular weight (unsonicated) calf thymus DNA solution as a function of NaCl concentration, but at constant polyamine concentration. At a ratio of polyamine to DNA phosphate concentration of 0.06, these authors observed that the polyamine diffusion coefficient is considerably retarded at low amounts of added NaCl.

We have previously studied the interactions between counterions and DNA (Nilsson et al., 1985; Einarsson et al., 1990) using the FT NMR pulsed gradient spin-echo (PGSE) diffusion method (Stilbs, 1987). This experiment provides a convenient means of determining the diffusion coefficient, a quantity that is very sensitive to ion binding in polyelectrolyte systems (Stilbs, 1987). In previous work, we found that a nonspecific electrostatic model suffices to describe the salt dependence of ^{7}L$^+$ diffusion in DNA solution (Nilsson et al., 1985).

The purpose of the present work is to apply the NMR self-diffusion method to study polyamine–DNA interactions, particularly with respect to the dynamics of the association. We have measured the diffusion coefficient of the trivalent, completely *N*-methylated polyamine analogue $N^+(CH_3)_3$–$(CH_2)_3$–$N^+(CH_3)_2$–$(CH_2)_4$–$N^+(CH_3)_3$, methylspermidine (Me_8Spd),[1] in solutions of NaDNA/NaCl, as a function of added Me_8Spd. We have chosen the methylated spermidine because at the present magnetic field the 24 methyl protons give a single resonance. Thus, the sensitivity is very high, which has enabled us to make diffusion measurements in the

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[1] Abbreviation: Me_8Spd, *N*-methylspermidine [$N^+(CH_3)_3$–$(CH_2)_3$–$N^+(CH_3)_2$–$(CH_2)_4$–$N^+(CH_3)_3$].

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interesting region at very low values of the concentration ratio Me_8Spd/P.

Diffusion in solutions containing three different types of DNA have been studied: (1) the oligomeric 8-mer $d(GC)_4$; (2) core length DNA from calf thymus (~120 base pairs); (3) high molecular weight sonicated calf thymus DNA with an average length of 7500 base pairs.

Measured diffusion coefficients were compared to the theoretical predictions of two different models: the Poisson–Boltzmann–Smoluchowski cylindrical cell model (PBS model) and a two-state diffusion model (see below). The results obtained indicate a surprising degree of retardation of the macroscopic translational diffusion of the polyamine upon association to DNA. In fact, our observations demonstrate that it is the diffusion of the DNA molecule itself which determines the diffusion of DNA-associated methylspermidine molecules.

MATERIALS AND METHODS

Core length DNA was prepared from calf thymus as described by Rill et al. (1983). The DNA was phenol extracted, ethanol precipitated, and dialyzed against NaCl solutions as previously described (Braunlin et al., 1989). The average length of the DNA was 120 base pairs, determined by polyacrylamide gel electrophoresis. On the basis of densitometric determinations of the gels, 90% of the sample was in the range 110–150 base pairs. The DNA phosphate concentration was 18.8 mM and the ratio of total sodium to DNA phosphate, Na/P, was 1.20. Sonicated calf-thymus DNA (Worthington) was purified and prepared for NMR measurements in the same way as the core length sample. The average molecular weight of the DNA molecules was approximately 7500 base pairs, determined by agarose gel electrophoresis. The phosphate and total sodium concentrations were 26.6 and 32 mM, respectively. The oligonucleotide GCGCGCGC was synthesized on an Applied Biosystems DNA synthesizer, filtered, and dialyzed into low salt. From ^{23}Na intensity measurements (compared to a NaCl standard curve), the sodium to DNA phosphate ratio was nearly 1:1. The nucleotide concentration of the oligonucleotide was 30.8 mM. This concentration was determined using an extinction coefficient of 9000 M^{-1} cm^{-1}, as calculated by the nearest neighbor method (Fasman, 1975). It should be noted that since there are seven phosphate groups for each oligonucleotide strand, the nucleotide concentration is not identical to the phosphate concentration. The total sodium concentration of the oligonucleotide sample was 30 mM, as determined by atomic absorption. The purity of the oligonucleotide sample was checked by gel electrophoresis. All DNA samples were lyophilized and redissolved in D_2O. Methylated spermidine was synthesized according to the method of Sommer et al. (1971) and analyzed by elemental analysis and ^{13}C NMR.

^1H NMR pulsed gradient spin-echo (PGSE) diffusion measurements were performed for the nucleosomal DNA samples on a JEOL FX-100 spectrometer. For these experiments, the temperature was maintained constant at 24 ± 0.5 °C. For the $d(GC)_4$ and the sonicated DNA samples, the measurements were performed on an FX-90Q spectrometer, at 25 ± 0.5 °C. All measurements were performed using 5-mm NMR tubes on DNA samples dissolved in D_2O to a total volume of 0.5 mL. The diffusion measurements were performed according to the scheme developed by Stilbs and Moseley (1980). In this experiment a series of absorption spectra is generated with the amplitude, A, as a function of gradient pulse length, δ. Under suitable conditions (Stilbs & Moseley, 1980) the amplitude follows the relation

$$A \sim \exp[-\gamma^2 G^2 \delta^2 D(\Delta - \delta/3)] \quad (1)$$

where γ is the magnetogyric ratio of the observed nucleus, G is the gradient strength (which was applied in the direction parallel to the NMR sample tube and thus perpendicular to the static magnetic field), δ is the gradient duration, D is the self-diffusion constant, and Δ is the common rf frequency and field gradient pulse interval. Each experiment was performed by holding G and Δ constant, varying δ, and fitting to eq 1. The value of Δ was 140 ms for all measurements on Me_8Spd and 70 ms for the measurements on $d(GC)_4$. The magnitude of the gradient G varied around 1 G cm^{-1}, depending on the diffusion coefficient to be measured in the given experiment. The choice of δ values depended on the gradient strength G and also on the diffusion coefficient to be measured. Some typical parameters are given below. The calibration of G was performed by measuring the self-diffusion of water. The diffusion coefficient of Me_8Spd was obtained from the spin-echo decay of the signal from the 24 methyl protons, which at the present magnetic field give one unresolved signal. The "free" diffusion constant of Me_8Spd in a dilute DNA-free solution, D_0, was measured for a 0.137 M solution as 0.454 ± 0.005 × 10^{-9} m^2 s^{-1} at 25 °C and 0.448 ± 0.005 × 10^{-9} m^2 s^{-1} at 24 °C. In the experiment at 25 °C, G had the value 1.10 G cm^{-1}, and δ varied between 30 and 90 ms. The diffusion coefficient of the oligonucleotide $d(GC)_4$ itself was independently obtained by measuring the echo decay of the peak corresponding to the unresolved signals from the aromatic protons. The value obtained did not vary with the amount of added Me_8Spd. The value determined at 25 °C was 0.13 ± 0.02 × 10^{-9} m^2 s^{-1}. The measurements in DNA solutions were performed as titration experiments, in which microliter amounts of a stock solution of Me_8Spd (0.052 M) were added to the DNA sample. The ratio of Me_8Spd to DNA phosphate was varied between 0.01 and 5. The error in the diffusion measurements is estimated at ±5%, based on reproducibility.

The effect on the ^1H NMR line widths of addition of methylspermidine to the different DNA solutions was in all cases modest and of similar magnitude to previous observations (Wemmer et al., 1985; Besley et al., 1990).

The theoretical diffusion coefficients of Me_8Spd were calculated from the Poisson–Boltzmann–Smoluchowski cylindrical cell model (PBS model) as previously described (Nilsson et al., 1985). The fraction of bound methylspermidine within the two-state model, P_B, was obtained from solution of the Poisson–Boltzmann equation within the cylindrical cell model as previously described (see below) (Braunlin et al., 1987; Nilsson et al., 1985). The values thus obtained from calculations corresponding to the core length DNA system are given in Table I.

RESULTS

The experimental Me_8Spd diffusion coefficients obtained for titrations of core length NaDNA/NaCl are given in Table I. At the first point of the titration, the value of the concentration ratio Me_8Spd/P is 0.011. The value of the diffusion coefficient at this point is 0.027 × 10^{-9} m^2 s^{-1}, as compared to 0.448 × 10^{-9} m^2 s^{-1} for Me_8Spd in a DNA free solution. Hence, there is a considerable retardation of the diffusion of Me_8Spd due to interaction with DNA. One particularly striking feature of the data is that, at low concentrations of Me_8Spd, the diffusion coefficient is independent of the amount of added Me_8Spd. This observation is valid for the first four points in the titration, corresponding

Table I: Values of P_B Calculated from the Poisson–Boltzmann Theory (See Text) and of Self-Diffusion Coefficients for Methylspermidine in Core Length DNA Solutions at Various Contents of Methylspermidine

[DNA-P] (mM)	[Me_8Spd] (mM)	P_B	D ($\times 10^9$ $m^2 s^{-1}$)
18.8	0.207	0.996	0.027
18.7	0.413	0.995	0.024
18.6	0.819	0.994	0.027
18.3	1.61	0.989	0.032
18.0	2.48	0.979	0.041
17.7	3.40	0.948	0.057
17.3	4.29	0.871	0.081
16.9	5.57	0.714	0.15
16.5	6.78	0.592	0.20
16.1	7.56	0.529	0.22
15.7	8.67	0.457	0.24
15.5	9.38	0.419	0.27
15.1	10.4	0.372	0.29
14.8	11.3	0.335	0.29
14.4	12.3	0.304	0.30
14.0	13.5	0.272	0.32
13.5	14.9	0.239	0.34
13.0	16.1	0.214	0.36
12.6	17.3	0.194	0.37
11.8	19.5	0.164	0.39
11.1	21.4	0.141	0.40
9.9	24.6	0.111	0.41
9.0	27.2	0.087	0.43
6.7	33.4	0.055	0.43

Table II: Self-Diffusion Coefficients of Methylspermidine in Solutions of $d(GC)_4$ at Various Contents of Methylspermidine

[nucleotide] (mM)	[Me_8Spd] (mM)	D ($\times 10^9$ $m^2 s^{-1}$)	[nucleotide] (mM)	[Me_8Spd] (mM)	D ($\times 10^9$ $m^2 s^{-1}$)
30.6	0.328	0.16	24.2	11.2	0.35
30.5	0.544	0.16	23.6	12.2	0.36
30.4	0.758	0.14	22.8	13.4	0.36
30.1	1.29	0.15	22.2	14.6	0.37
29.7	1.80	0.18	21.3	16.0	0.37
29.4	2.31	0.18	20.6	17.3	0.38
28.9	3.30	0.23	19.6	18.9	0.39
28.3	4.24	0.23	18.7	20.4	0.40
27.8	5.15	0.24	17.9	21.7	0.41
27.2	6.03	0.28	16.9	23.5	0.41
26.7	6.87	0.30	16.0	25.1	0.40
26.1	7.92	0.30	14.4	27.7	0.42
25.4	9.08	0.32	11.1	33.3	0.42
24.8	10.2	0.34			

to a nearly 10-fold increase in the Me_8Spd/P ratio. The diffusion coefficient then increases with increasing Me_8Spd concentration and gradually approaches D_0, the value for the "free" diffusion constant, obtained in the absence of DNA. On the basis of the calculated values of P_B, the fraction of trivalent counterions residing within a 0.5-nm shell around DNA is nearly constant at about 99% during the first four titration points. Thus, the measurements at low Me_8Spd/P ratios clearly probe a very localized interaction with the nucleic acid.

In Table II, diffusion results for Me_8Spd in solutions of the oligonucleotide $d(GC)_4$ are presented. Again, the diffusion coefficient is constant at low Me_8Spd/P ratios and then increases with increasing Me_8Spd/P to approach the value of D_0. Moreover, the diffusion coefficient values at low Me_8Spd/P are considerably higher than those found in core length DNA solution, demonstrating more rapid diffusion of Me_8Spd in the 8-mer DNA solution.

Finally, the diffusion data for the sonicated calf thymus DNA is presented in Table III. For this system, at the given DNA concentration (25 mM), it was not possible to measure the diffusion coefficient for a Me_8Spd concentration below 2 mM, corresponding to a Me_8Spd/P ratio of 0.08. This limitation reflects the combined effects of a short transverse relaxation rate and slow diffusion. In order to achieve reliable diffusion measurements at such low ratios, a larger magnetic field gradient would be required than can be achieved by our present spectrometer system (Stilbs, 1987). Nonetheless, the initial diffusion coefficient measured for the sonicated DNA titration is significantly smaller than the initial diffusion coefficient measured in the core length DNA solution. Moreover, for the sonicated DNA titration, no plateau in D is observed at low ratios, in contrast to the observations for the two lower molecular weight DNA's.

Table III: Self-Diffusion Coefficients of Methylspermidine in Solutions of Sonicated Calf Thymus DNA at Various Contents of Methylspermidine

[DNA-P] (mM)	[Me_8Spd] (mM)	D ($\times 10^9$ $m^2 s^{-1}$)	[DNA-P] (mM)	[Me_8Spd] (mM)	D ($\times 10^9$ $m^2 s^{-1}$)
25.0	2.00	0.016	17.6	16.9	0.30
24.5	2.94	0.024	16.5	19.1	0.31
24.1	3.85	0.037	15.5	21.0	0.35
23.6	4.73	0.046	14.6	22.8	0.38
22.8	6.39	0.099	13.8	24.3	0.38
21.7	8.67	0.117	11.9	28.1	0.40
20.3	11.4	0.22	10.9	30.1	0.40
18.8	14.32	0.28			

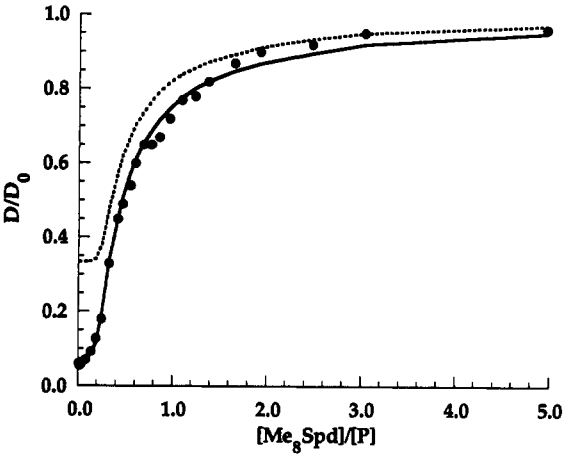

FIGURE 1: Experimental and calculated diffusion quotients, D/D_0, for methylspermidine (Me_8Spd), as a function of the ratio of methylspermidine to DNA phosphate, Me_8Spd/P, during titration of a solution of core length (120 base pair) calf thymus NaDNA, corresponding to the data in Table I. Filled circles are experimental results, and the dotted line is the theoretical curve calculated from the Poisson–Boltzmann–Smoluchowski cylindrical cell model. The closed line is obtained from the two-state model.

DISCUSSION

Diffusion in Solutions of Core Length DNA. We have determined the diffusion quotient, D/D_0, as a function of the concentration ratio Me_8Spd/P. In Figure 1 these data are given for the core length DNA experiment. For comparison, theoretical calculations based on the PBS model and the two-state model are also shown. The experimental curve clearly has a sigmoidal appearance, with a nearly constant diffusion quotient at low Me_8Spd/P ratios. At higher Me_8Spd/P ratios, D/D_0 increases rapidly at first, before approaching a plateau at its limiting value of one.

In the PBS model, DNA is assumed to be an infinitely long cylinder with a uniform surface charge density. The solvent water is treated as a dielectric continuum, and all mobile charged ions are treated as point charges. The Poisson–Boltzmann (PB) cylindrical cell model equation is solved,

giving the electrostatic potential. The diffusion quotient, D/D_0, is given by the relation (Nilsson et al., 1985)

$$D/D_0 = (1/3)D_{\parallel}/D_0 + (2/3)D_{\perp}/D_0 \quad (2)$$

Here $D_{\parallel}$ and $D_{\perp}$ are, respectively, the diffusion coefficients in directions parallel and perpendicular to the DNA cylinder axis. Since DNA is treated as an infinitely long, uniformly charged cylinder, there is no potential gradient along the DNA cylinder axis, and $D_{\parallel}$ is equal to D_0. The first term in eq 2 thereby contributes a constant value of $^1/_3$ as the lower limiting value of D/D_0. $D_{\perp}$ is calculated from the cylindrical stationary state Smoluchowski equation (Nilsson et al., 1985). It is clear from Figure 1 that the PBS model does not give a realistic description of our data.

It is worth noting that eq 2 is a general result for cylindrical symmetry and can therefore be used as a starting point for a more refined electrostatic model where the DNA charge distribution may be discrete. Such a procedure would enable a more realistic calculation of $D_{\parallel}$ (Guldbrand & Nordenskiöld, 1987).

It is apparent that, for Me_8Spd/P ratios greater than 0.25, the PBS curve lies above the experimental points and approaches the same limiting value at very high ratios. At lower values of Me_8Spd/P, $D_{\perp}/D_0$ approaches zero and the theoretical curve approaches the limiting value of $^1/_3$. The experimental curve, on the other hand, has a limiting value of approximately 0.03.

In attempting to understand the discrepancy between the experimental and theoretical curves, it is useful to examine the approximations of the PBS model. For multivalent ions, the point charge approximation, while physically unrealistic, has the effect of underestimating the calculated values of D/D_0 and thus cannot explain the observed discrepancy. The same conclusion is also valid for the effect of the mean field approximation within the PB model (Nilsson et al., 1987). A more serious approximation is that of modeling DNA as a continuously charged cylindrical surface. Since real DNA does have a potential that varies along the cylinder axis, $D_{\parallel}$ is not identical with D_0. This assumption should fail completely if the polyamine molecules are translationally immobilized by site-specific binding. For Li^+ ions in Li–DNA solution, we have previously found that the PBS model gave a very reasonable description of the LiCl dependence of the diffusion of this monovalent ion (Nilsson et al., 1985). However, the diffusion of the divalent Ca^{2+} ion in DNA solution was not correctly described at low concentration ratios of calcium(II) to DNA phosphate (Nilsson et al., 1985). Finally, the PBS model considers the DNA to be of infinite length. Although this assumption is of minor consequence for the calculation of the electrostatic potential, it may lead to serious error when applied to calculating diffusion coefficients (see below).

The two-state model is motivated by the following considerations. The experimental diffusion coefficient of Me_8Spd approaches a limit of 0.03×10^{-9} m^2 s^{-1} at low Me_8Spd/P ratios. The simplest explanation of this observation is that polyamine molecules near the DNA surface suffer considerable restrictions of their macroscopic translational motion. If such restrictions are particularly severe, then as a lower limit the diffusion coefficient of Me_8Spd should approach that of the DNA molecule itself. The diffusion coefficients that we have measured appear to be at or near this limit. Nicolai and Mandel (1989) studied the diffusion of core length DNA ($\sim$150 base pairs, obtained from chicken erythrocytes) by dynamic light scattering (DLS) and sedimentation coefficient measurements. They obtained an infinite dilution limiting value of the translational self-diffusion coefficient of core length DNA that was 0.03×10^{-9} m^2 s^{-1}. This value is, within experimental uncertainty, identical to the limiting value that we have obtained at low Me_8Spd/P ratios for the diffusion coefficient of Me_8Spd. We note that a recent forced Rayleigh scattering (FRS) study of dye-labeled core length DNA (Wang et al., 1991) gave values of the self-diffusion coefficients for DNA at finite salt and DNA concentrations that are considerably smaller than those obtained by Nicolai and Mandel. However, since the FRS method measures the diffusion of a rather large probe molecule attached to DNA, we believe that it is more appropriate to use the DLS results (Nicolai & Mandel, 1989) in the interpretation of our data. The results of Nicolai and Mandel are obtained from an extrapolation to infinite dilution and should thus provide an upper bound for the DNA diffusion coefficient under the finite concentration conditions of our experiments. It should also be noted that any DNA concentration dependence of the DNA diffusion coefficient should not influence the shape of the early part of our titration curve since the DNA concentration is constant in this interesting regime.

For fast exchange between the sites, the two-state model applied to counterion self-diffusion measurements is given by (Stilbs & Lindman, 1982)

$$D = P_B D_B + P_F D_F \quad (3)$$

Thus, the counterion diffusion coefficient is a population-weighted average of the diffusion of the ions associated with DNA and those free in the bulk. We next assume that D_B is given by the diffusion coefficient of the DNA molecule itself and that D_F is given by D_0. The resulting expression for the diffusion quotient is

$$D/D_0 = P_B(D_{DNA}/D_0) + (1 - P_B) \quad (4)$$

P_B is calculated from the cylindrical Poisson–Boltzmann equation by integrating the radial distribution function for the trivalent ion within a cylindrical shell of 5 Å (Braunlin et al., 1987). The values of P_B as a function of concentrations of Me_8Spd and DNA are given in Table I. For reasonable choices of this cutoff distance near the chosen value, the effect of varying this distance is of minor importance (Bleam et al., 1983). D_{DNA} has been given the value obtained by Nicolai and Mandel (1989), as discussed above. It can be seen that the curve calculated from the two-state model is nearly superimposable on the experimental curve. The present data thus clearly support the notion that the binding of the polyamine methylspermidine to DNA is strongly localized, so that its diffusion coefficient is determined by that of the DNA.

Diffusion as a Function of DNA Molecular Weight. Additional support for the above picture of polyamine–DNA interaction is obtained from a comparison of the curves of the diffusion quotients of Me_8Spd in solutions of duplex DNA of variable length. In Figure 2 we present the diffusion quotients in solutions of the 8-mer $d(GC)_4$ and in solutions of sonicated high molecular weight calf thymus DNA of an average base pair length of 7500. For comparison, the data from Figure 1 for core length DNA are also presented. It should be noted that the scale on the horizontal axis is logarithmic, spanning two decades in the value of D/D_0. The difference in the limiting behavior at low ratios of Me_8Spd/P clearly demonstrates that the diffusion coefficient of methylspermidine is highly dependent on the DNA molecular weight. The limiting value in $d(GC)_4$ solution is nearly identical to the value obtained for the diffusion of this oligonucleotide itself, from dynamic light scattering, by Eimer and Pecora (1991) (D_{DNA} = 0.15

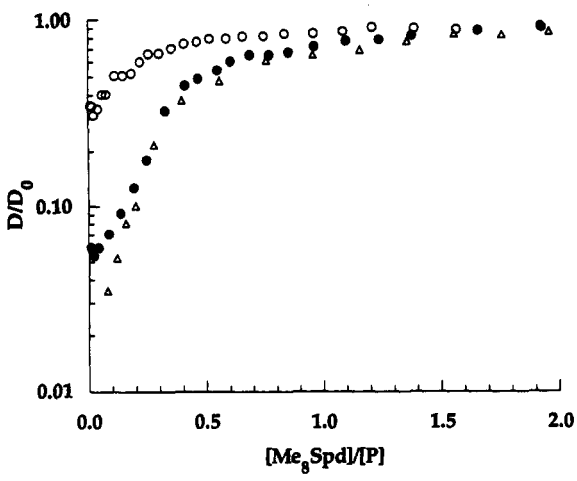

FIGURE 2: Experimental diffusion quotients for methylspermidine in solutions of $d(GC)_4$ (open circles), core length DNA (120 base pair) (filled circles), and sonicated high molecular weight calf thymus DNA (average 7500 base pair) (triangles), as a function of the ratio of methylspermidine to DNA phosphate concentrations, Me_8Spd/P, corresponding to data in Tables I, II, and III. Note the logarithmic scale on the vertical axis. The data for $d(GC)_4$ are given as a function of the ratio of concentration of methylspermidine to nucleotide concentration.

$\times 10^{-9}$ m^2 s^{-1}, corresponding to $D/D_0 = 0.33$). The value that we have obtained for the diffusion of $d(GC)_4$ by NMR is somewhat lower, corresponding to $D/D_0 = 0.28 \pm 0.05$. This difference is not large considering the experimental uncertainty and may reflect the expected decrease in the diffusion coefficient of $d(GC)_4$ at the finite oligonucleotide concentration of our measurements compared to the infinite dilution value obtained from DLS. The observation that the limiting value of the diffusion coefficient of methylspermidine is somewhat larger than that of $d(GC)_4$ may be then be due to incomplete binding of Me_8Spd to this short DNA fragment even at low concentration ratios. This idea is plausible in light of recent Monte Carlo simulations (Olmstedt et al., 1990). These calculations have shown that, for a small fragment the size of an 8-mer, end effects are considerable and the surface concentration of counterions is less than expected for an infinitely long polyelectrolyte. We note in this regard that we have measured the diffusion of $d(GC)_4$ first in a solution free of Me_8Spd and then during the titration and observed no systematic variation during this experiment (the uncertainty is rather large in these measurements; see Materials and Methods). This shows that for $d(GC)_4$, under the present conditions, neither DNA condensation, which would increase the diffusion coefficient, nor aggregation, which would decrease the diffusion coefficient, are influencing the results. However, since we cannot, for technical reasons, measure the core length DNA diffusion during titration with Me_8Spd, and since no independent measurements exist, we cannot rule out the possibility that alterations in this DNA conformation may occur as a function of polyamine addition. On the other hand, we do not believe that this possibility is very likely since the DNA concentration is too high for condensation and no visual signs of aggregation were observed.

The molecular weight dependence of the limiting diffusion behavior of Me_8Spd at low concentration ratios is also clear from the data for sonicated calf thymus DNA (average of 7500 base pairs). Due to experimental constraints, it was not possible to measure the diffusion at low concentration ratios where the diffusion is expected to level off. However, it is clear that in the beginning of the titration curve the diffusion of Me_8Spd is considerably slower than in the systems with DNA of lower molecular weight. Thus, at the lowest ratio that we could confidently measure, the value of D/D_0 was 0.03 for the sonicated DNA as compared to 0.06 for core length DNA. A dynamic light scattering measurement of the infinite dilution diffusion coefficient, for a monodisperse solution of 6500 base pair long DNA molecules, gave $D_{DNA} = 0.004 \times 10^{-9}$ m^2 s^{-1} (Voordouw et al., 1978). This result would indicate lower values of the diffusion quotients than we have observed. However, our sonicated DNA is highly polydisperse. For such a sample, analysis of the PGSE experiment may give an apparent diffusion coefficient which is larger than the true average value (Garver & Callaghan, 1991). Therefore, we have not attempted to apply the two-state model to this set of data. In the present context it should also be noted that Gibbs and Johnson (1991) measured the diffusion of the trivalent polyamine analogue 3,3′-diaminobis-(*N*,*N*-dimethylpropylamine) in solutions of unsonicated calf thymus DNA, the (moderately polydisperse) length of which is expected to be about 20 000 base pairs. At a polyamine to DNA phosphate concentration ratio of 0.056 and a low NaCl content, the diffusion quotient was found to be 0.005. The data of these workers thus support our observation of a strong molecular weight dependence of polyamine diffusion in DNA solution.

Two sources of the observed DNA molecular weight dependence of the Me_8Spd diffusion may be imagined. If the polyamine is completely immobilized on the DNA, then the diffusion coefficient will equal that of the DNA molecule. A less immediately obvious mechanism giving the same behavior would arise if the polyamine were trapped by the polyion field near the surface of a defined length DNA molecule. At low concentration ratios, almost all Me_8Spd molecules (around 99%) will be closely associated with DNA. It is quite conceivable that these molecules are constrained to undergo translational motion in close proximity to the DNA and cannot escape from the ends of the molecule on the time scale of the PGSE experiment. The PGSE diffusion experiment measures the mean square displacement, $\langle x^2 \rangle^{1/2} = 2Dt$, during a time t that is roughly given by Δ, the interval between the gradient pulses. For a finite DNA length, translational diffusion back and forth on the DNA during a finite time Δ should result in an extremely small value of the measured diffusion coefficient. In the case of "core length" DNA of 120 base pairs, the length of the DNA is about 40 nm, which then gives an upper bound on the mean square displacement for a molecule confined to the polyion surface. During an experiment with Δ = 140 ms, this corresponds to a diffusion coefficient of about 0.6×10^{-14} m^2 s^{-1}, which is considerably smaller than the value of 0.03×10^{-9} m^2 s^{-1} for the DNA molecule. Under these circumstances, the macroscopic mean square displacement of the polyamine should thus be dominated by the displacement of the DNA, and the effective diffusion coefficient of the polyamine should be determined by the diffusion coefficient of the DNA molecule. For the present experiments (Δ = 70 or 140 ms), this analysis holds equally well for the 8 and 7500 base pair DNA molecules.

CONCLUSIONS

This investigation has shown that the translational self-diffusion of the polyamine analogue methylspermidine in solutions of double-helical DNA of 8, ~120, and 7500 (average) base pairs is highly dependent on DNA molecular weight. The diffusion coefficient at low ratios of methylspermidine to DNA phosphate approaches a limiting value that is close to that of the DNA molecule itself. This

observation demonstrates that this polyamine interacts in a highly localized manner with DNA. Such localization could result from binding to a specific site on the DNA molecule. Alternatively, it could reflect the trapping of Me_8Spd molecules within the confined domain of the finite length DNA molecule. Irrespective of the value of the intrinsic surface diffusion constant, the resultant restriction in mean square displacement would result in a measured macroscopic diffusion constant equal to that of the DNA molecule. Nonetheless, even if this second model is correct, it does seem unlikely that such surface diffusion would be extremely rapid and occurring at an approximately diffusion-controlled rate, as suggested by Besley et al. (1990). Both experimental (Einarsson et al., 1990) and theoretical (Guldbrand & Nordenskiöld, 1987) work indicates that the surface diffusion of ions close to DNA is distinctly retarded when compared to free diffusion.

Regarding the distinction between these two quite different models, it is not possible to discriminate between them on the basis of the present data. Additional measurements with instrumentation capable of performing the PGSE experiments using large gradients would enable the use of short Δ values. In this situation, end effects could be neglected, and discrimination might be possible. At present, in light of the ^1H NMR NOE study by Wemmer et al. (1985), the interpretation that does not invoke site binding appears more plausible.

Our data for the diffusion quotients, D/D_0, as a function of the ratio of methylspermidine to DNA phosphate, Me_8-Spd/P, in core length solution were very well reproduced by a two-state diffusion model, where the measured diffusion coefficient is a population-weighted average of the diffusion coefficients for methylspermidine bound to DNA and free in the bulk. The theoretical curve was obtained using the value of D_{DNA} obtained from dynamic light scattering (Nicolai & Mandel, 1989) and calculating the fraction in the bound state, P_B, from the Poisson–Boltzmann cylindrical cell model. Although the Poisson–Boltzmann model applied to the chain-like and rather large methylspermidine molecule is a clear oversimplification, it probably gives a reasonable description of the amount of bound trivalent cation. The quantitative success of the two-state model, as demonstrated by the close correspondence between the calculated and experimental curves in Figure 1, depends heavily on the use of the PB model for calculating the fraction of bound ions. The PB model predicts that binding should be almost quantitative at low amounts of added Me_8Spd. This prediction is in good agreement with experimental binding studies on Me_8Spd, which also predict binding to be quantitative under these conditions (Plum & Bloomfield, 1990). Furthermore, the success of the two-state model does depend significantly on the value used for the diffusion coefficient of the bound polyamine. Here this value was assumed equal to the diffusion coefficient of DNA, as determined from DLS measurements. This diffusion coefficient is valid for DNA at infinite dilution and therefore provides an upper bound to the true value of D for the finite DNA concentrations of our experiments.

The present results have been obtained for the *N*-methylated analogue of the naturally occurring polyamine spermidine. We believe that this study is relevant for the natural unmethylated polyamines as well, since it seems unlikely that the natural polyamines would bind less tightly than the methylated analogues. Some hydrophobic interaction with the DNA molecule may well be introduced by the methylation. However, the distance of closest approach between the spermidine and DNA charges should be larger for the methylated analogue, which is also incapable of forming specific contacts via hydrogen bonding. In fact, a recent experimental study has shown that the DNA binding constant of spermidine is somewhat stronger than that of methylspermidine (Plum & Bloomfield, 1990).

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