Interactions of Spermidine and Methylspermidine with DNA Studied by Nuclear Magnetic Resonance Self-Diffusion Measurements

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ABSTRACT The NMR pulsed field gradient self-diffusion method has been used to study the self-diffusion of the polyamine spermidine and the polyamine analog methylspermidine (completely *N*-methylated spermidine). The self-diffusion coefficient, *D*, was measured in solutions of calf thymus DNA prepared from nucleosome core particles (with an average length of 120 base pairs) as a function of the concentration ratio of polyamine to DNA phosphate. A study of the self-diffusion quotient, D/D_0 (where D_0 is the diffusion coefficient for free polyamine, not associated with DNA), in additions of spermidine and methylspermidine to solutions of NaDNA/NaCl, gave almost identical results with complete association of polyamine to DNA in the initial part of the titrations, indicating similar affinities for DNA. A large influence on the measured self-diffusion coefficients was detected for methylspermidine in NaDNA solutions with different concentrations of NaCl, which shows a considerable salt effect on the polyamine-DNA association. No notable differences in D/D_0 for methylspermidine were observed in competitive titrations of solutions of Li- and NaDNA, indicating that sodium and lithium ions behave similarly in their interactions with DNA. In titration experiments of methylspermidine into MgDNA solution, the results showed that the polyamine association is less effective than in the case of NaDNA, because of competition from magnesium binding to DNA. Comparisons with calculations based on the electrostatic Poisson-Boltzmann cell model were performed. It is suggested that the interaction is primarily of electrostatic nature, with no binding to specific sites on the DNA molecule.

INTRODUCTION

Double-helical DNA in solution is a highly negatively charged polyelectrolyte, and it is clear that long-range electrostatic interactions with any positively charged species present will profoundly affect (in fact, to a large extent often dominate) the nonideal behavior in the system. Over the past 20 years considerable attention has been devoted to experimental studies of the interaction between DNA and counter-ions of different types and as a function of varying solution conditions (Record et al., 1981; Braunlin, 1995). Furthermore, much work has been directed toward the development and application of polyelectrolyte theories for describing electrostatic interactions and their physical consequences in DNA systems (Record et al., 1981; Lamm et al., 1984). In these theoretical studies the interest has to a large extent been focused on the interactions and distribution of monovalent counter-ions around the DNA polyion. Recently, the interaction and biological significance of multivalent cations interacting with nucleic acids have received much attention in experimental work (Braunlin, 1995). Among those multivalent cations that are of significant interest are the polyamines. Polyamines interact with polyanionic nucleic acids and with membranes in vivo (Cohen, 1978; Tabor and Tabor, 1984). To clarify the biological significance of these interactions, their molecular and thermodynamic consequences have been extensively investigated in vitro. Perhaps the most interesting recent observation in this context is the polyamine-induced collapse and condensation of nucleic acids to compact forms (Gosule and Schellman, 1978; Chattoraj et al., 1978), which indicates a biological role for polyamines in DNA packing processes. On the basis of crystal studies, it has been assumed (Saenger, 1984) that polyamines bind in a highly specific manner to well-defined sites on DNA. X-ray studies (Liquori et al., 1967; Quigley et al., 1978; Drew and Dickerson, 1981; Kopka et al., 1983; Jain et al., 1989) have shown several types of polyamine binding sites on nucleic acids. In contrast to the X-ray results, DNA-polyamine interactions in solutions studied by nuclear magnetic resonance (NMR) are most simply interpreted as a nonspecific and mainly electrostatic association (Burton et al., 1981; Wemmer et al., 1985; Braunlin et al., 1986; Padmanabhan et al., 1988; Besley et al., 1990; Padmanabhan et al., 1991). It should be noted that in general the development and application of polyelectrolyte theories to the interaction of multivalent cations with DNA have not been very frequent, and this is particularly evident in the case of polyamines.

Different NMR methods have recently been extensively used in studies of DNA-counter-ion interactions, among them some polyamine-DNA systems (Padmanabhan et al., 1991; Gibbs and Johnson, 1991; Andreasson et al., 1993). Gibbs et al. (Gibbs and Johnson, 1991) studied the ^{1}H NMR self-diffusion and spin-lattice relaxation times of one divalent and one trivalent polyamine analog as a function of NaCl concentration. A large salt effect on the polyamine self-diffusion coefficient was detected, and the results were interpreted within a two-state model describing the polyamine as either associated with DNA or free in the aqueous bulk phase. In a previous report from this laboratory (Andreasson et al., 1993), we used the ^{1}H-NMR self-diffusion method to study the interaction of the completely *N*-meth-

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ylated polyamine analog methylspermidine (Me_8Spd) with DNA of different lengths. The results were very well reproduced by a two-state diffusion model. This model describes the polyamine diffusion as a fraction weighted average due to DNA-associated Me_8Spd ("bound" ions), with the diffusion coefficient determined by that of the DNA molecule itself, and that of free Me_8Spd characterized as unaffected by DNA (as in a DNA-free solution). The DNA-associated polyamines were interpreted as being electrostatically trapped by the polyion field, but with considerable translational mobility along DNA. Sodium was the counter-ion of choice in the NMR studies mentioned above. Lithium as counter-ion to DNA has been studied by NMR in only a few cases (Nilsson et al., 1985; Stilbs, 1987; Einarsson et al., 1990; Einarsson et al., 1991; Schultz et al., 1992; Andreasson et al., 1994). Comparative studies of lithium and sodium ion binding to double-helical DNA (Ross and Scruggs, 1964a; Ross and Scruggs, 1964b; Bleam et al., 1980) have shown that lithium binds with a somewhat larger affinity to DNA than does sodium.

Other methods have also yielded important information on the nature of DNA-polyamine interactions. Plum et al. (Plum and Bloomfield, 1990) measured the thermal melting profile for poly[d(AT)] · poly[d(TA)] as a function of concentration of the three trivalent cations spermidine, methylspermidine, and hexaammine cobalt(III). They found that the three cations bind to double-helical DNA with similar affinities, but spermidine binds somewhat more tightly. Braunlin et al. used equilibrium dialysis to determine binding constants for the interaction of the polyamines putrescine^{2+}, spermidine^{3+}, and spermine^{4+} with double-helical DNA, as obtained from analysis of the data within the McGhee-von Hippel theory for binding of ligands to an infinite one-dimensional lattice (Braunlin et al., 1982). These authors studied the dependence of the binding constants on NaCl salt concentration. The results of this study were concluded to be consistent with a predominantly electrostatic interaction, although the nature (site-localized or diffusive and delocalized) of the polyamine-DNA association on the DNA surface could not be elucidated on the basis of the data.

To obtain more insight into the nature of polyamine-DNA interactions and the role and importance of the electrostatics in this counter-ion association phenomenon, it is important to obtain experimental information at varying solution conditions, particularly with respect to effects of added salt and to the type, amount, and valency of the supporting electrolyte in the system. This originates from the sensitivity of the electrostatic interactions to changes in these environmental conditions and the consequent practice of thus being able to discuss and test different polyelectrolyte theories and models in terms of their ability to predict such effects. It is also important that experimental data are obtained for such physical quantities that enable both qualitative and quantitative comparisons with theoretical predictions from polyelectrolyte theories. Currently it is not feasible to experimentally determine the counter-ion distribution in polyelectrolyte solutions directly, and experimental measurements of quantities that are related to this distribution function are therefore of great interest. The counter-ion diffusion coefficient is a well-defined physical property, the direct measurement of which does not involve any model dependence. This is in contrast to, e.g., counter-ion spin relaxation measurements, where a model of the relevant dynamic processes is necessary to obtain dynamic information on diffusional properties. The counter-ion diffusion coefficient may also be theoretically calculated from a knowledge of the mean electrostatic potential outside the polyion, or it can be obtained directly from Brownian dynamics or molecular dynamics simulations.

In the present work we have applied the pulsed gradient spin-echo method to measure the polyamine self-diffusion coefficient of Me_8Spd in DNA solutions, under different solution conditions. We have performed four separate studies, namely:

1. Measurements of the self-diffusion coefficient of the trivalent polyamine spermidine (Spd), $N^+(H)_3$-$(CH_2)_3$-$N^+(H)_2$-$(CH_2)_4$-$N^+(H)_3$, and the trivalent completely *N*-methylated polyamine analog $N^+(CH_3)_3$-$(CH_2)_3$-$N^+(CH_3)_2$-$(CH_2)_4$-$N^+(CH_3)_3$, methylspermidine (Me_8Spd), in solutions of NaDNA/NaCl.
2. Measurements of the self-diffusion coefficient of Me_8Spd in solutions of NaDNA/NaCl at two different sodium chloride concentrations.
3. Measurements of the self-diffusion coefficient of Me_8Spd in solutions of LiDNA/LiCl. Here we also measured the self-diffusion coefficients of the lithium ions.
4. Measurements of the self-diffusion coefficient of Me_8Spd in solutions of MgDNA/$MgCl_2$.

The purpose of the experiments was to obtain polyamine diffusion data under different solution conditions to obtain information on the nature and the importance of the electrostatic interactions for the polyamine-DNA association. In the present paper we have analyzed the experimental data within the framework of the relatively simple Poisson-Boltzmann cylindrical cell model. This model, as we have previously (Andreasson et al., 1993) applied it to the polyamine-DNA system, is based on treating all mobile ions as point charges. Clearly, this is quite a crude approximation, given the discrete nature of the polyamine charge distribution in, e.g., methylated spermidine. However, this model should be a natural starting point for the interpretation of the experimental data in the present study, and for more refined theoretical modeling using the Monte Carlo simulation method, which is presently in progress in our laboratory.

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 or LiCl solutions. The average length of the DNA was 120 base pairs, as determined by polyacrylamide gel electrophoresis. On the basis of densitometric determinations of the gels, 90% of the sample

was in the range of 110–150 base pairs. The DNA phosphate and the total salt concentrations in the four experiments were: 1) 17.7 mM DNA-P; 21.9 mM Na^+ (titration with Me_8Spd and Spd). 2) 14.0 mM DNA-P; 28.7 mM Na^+ (titration with Me_8Spd). 3) 23.4 mM DNA-P; 26.5 mM Li^+ and 1.4 mM Na^+ (titration with Me_8Spd). 4) 20.7 mM DNA-P; 10.4 mM Mg^{2+} and 2.1 mM Na^+ (titration with Me_8Spd). The DNA-P concentrations were determined from the UV absorbance at 260 nm by using an extinction coefficient of 6600 M^{-1} cm^{-1} (Nordenskiöld et al., 1984). The total salt concentrations were determined by atomic absorption. All DNA samples were lyophilized and redissolved in D_2O. Spermidine · 3HCl was purchased from Sigma. High purity was confirmed by ^1H NMR. Methylated spermidine was synthesized according to the method of Sommer et al. (1971) and analyzed by elemental analysis and ^{13}C NMR.

^1H and ^7Li NMR pulsed gradient spin-echo self-diffusion measurements (Stejskal and Tanner, 1965) were performed with a Bruker MSL-200 spectrometer. The magnetic field gradient pulses were generated with a home-built gradient driver (Stilbs, 1987). In all measurements the temperature was maintained constant at 19 ± 0.3°C. To avoid temperature gradients across the sample, the temperature was controlled with air precooled by a thermostated water bath. Measurements were made in 10-mm NMR tubes with a total DNA/D_2O solution volume of 2 ml. In this type of experiment the amplitude follows the relation

$$A \approx \exp(-\gamma^2 G^2 \delta^2 D(\Delta - \delta/3)), \quad (1)$$

where γ is the magnetogyric ratio of the observed nucleus, G is the gradient strength (applied parallel to the static magnetic field), δ is the gradient duration, D is the self-diffusion coefficient, and Δ is the common *rf* 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 150 or 200 ms in the experiments with Me_8Spd, whereas in the experiments with Spd, Δ = 185 ms was the optimal delay to avoid J-modulations. The magnitude of G was 5.6 ± 0.2 or 10.9 ± 0.2 G cm^{-1}, depending on the self-diffusion coefficient to be measured in the given experiment. G was calibrated by measuring the self-diffusion of water. The self-diffusion coefficient of Spd was obtained from the overlapping signals of the eight methylene protons adjacent to the amine groups, at the shift 3.1 ppm. Evaluation from the less intense signals of the remaining methylene protons at 1.7 and 2.1 ppm gave identical diffusion coefficients, within experimental uncertainty. The self-diffusion coefficient of Me_8Spd was obtained from the signal of the 24 methyl protons at the shift 3.2 ppm. The "free" self-diffusion coefficients, D_0, of the species Me_8Spd and Li were obtained from the figures by extrapolation from the titration of Me_8Spd, corresponding to the quotient [Me_8Spd]/[DNA-P] going to infinity. D_0 of Spd was measured for a 0.14 M solution, because for this system aggregation precludes a determination of D_0 by extrapolation. The D_0 values obtained were

$D_0(Me_8Spd) = 0.44 \pm 0.02 \times 10^{-9}$ m^2 s^{-1}; [Na]/[DNA-P] = 1.24

$D_0(Spd) = 0.48 \pm 0.02 \times 10^{-9}$ m^2 s^{-1}; [Na]/[DNA-P] = 1.24

$D_0(Me_8Spd) = 0.44 \pm 0.02 \times 10^{-9}$ m^2 s^{-1}; [Na]/[DNA-P] = 2.05

$D_0(Me_8Spd) = 0.48 \pm 0.02 \times 10^{-9}$ m^2 s^{-1}; [(Li + Na)]/[DNA-P] = 1.19

$D_0(Li) = 0.96 \pm 0.05 \times 10^{-9}$ m^2 s^{-1}; [(Li + Na)]/[DNA-P] = 1.19

$D_0(Me_8Spd) = 0.41 \pm 0.02 \times 10^{-9}$ m^2 s^{-1}; [Mg]/[DNA-P] = 0.5

For comparison it can be noted that D_0 measured for a 0.14 M Me_8Spd solution gives a value of 0.42 ± 0.02 × 10^{-9} m^2 s^{-1}. The small difference in obtained D_0 values of Me_8Spd in the NaDNA and LiDNA solutions was probably caused by different D_2O content in the two samples. To check this we calibrated the Li^+ diffusion coefficient for 20 mM LiCl solutions in different H_2O/D_2O mixtures. On the basis of these results, the D_2O content was estimated to be about 80% in the DNA/LiCl solution and about 99% in the DNA/NaCl solution. The measurements in DNA solutions were performed as titration experiments, in which microliter amounts of a stock solution of Me_8Spd or Spd (49.6 mM and 49.5 mM, respectively) were added to the DNA sample. The error in the self-diffusion measurements is estimated at ±7% (very small values of [Me_8Spd]/[DNA-P]) and at ±5% ([Me_8Spd]/[DNA-P] > 0.1), based on reproducibility. The fraction of bound ions, P_B, was obtained by solving the Poisson-Boltzmann (PB) equation within the cylindrical cell model (Nilsson et al., 1985; Braunlin et al., 1987).

RESULTS

The experimental Me_8Spd self-diffusion coefficients obtained for titration of core-length NaDNA/NaCl solution ([Na]/[DNA-P] = 1.24) are given in Table 1. At very low polyamine concentrations, the retardation of the self-diffusion of Me_8Spd due to interaction with DNA is considerable. Thus, $D = 0.024 \times 10^{-9}$ m^2 s^{-1} for the first point in the titration, compared to $D_0 = 0.44 \times 10^{-9}$ m^2 s^{-1}. In the initial region of the titration the self-diffusion coefficient is almost constant for Me_8Spd in the DNA solution. The self-diffusion coefficient then increases with increasing Me_8Spd concentration and gradually approaches D_0.

TABLE 1 Values of self-diffusion coefficients for methylspermidine in core-length DNA solutions at various contents of methylspermidine

[DNA-P] (mM)	[Me_8Spd] (mM)	D ($\times 10^9$ m^2 s^{-1})
17.64	0.089	0.024
17.58	0.266	0.025
17.5	0.527	0.028
17.3	1.05	0.029
17.1	1.72	0.035
16.7	2.69	0.053
16.3	3.78	0.092
16.1	4.38	0.12
15.7	5.39	0.17
15.4	6.36	0.21
14.9	7.64	0.25
14.5	9.01	0.27
13.9	10.6	0.31
13.4	12.1	0.33
12.6	14.1	0.34
11.8	16.5	0.36
11.1	18.5	0.38
10.4	20.4	0.39
8.6	25.7	0.40
7.3	29.2	0.43
6.4	31.8	0.42

[Na^+]/[DNA-P] = 1.24.

In Table 2 we present the experimental Spd self-diffusion coefficients obtained for the titration of core-length NaDNA/NaCl ([Na]/[DNA-P] = 1.24). Because of the low signal-to-noise ratio in the NMR experiment, we did not succeed in measuring this coefficient at very low Spd concentrations. The values for Spd in Table 2 are very similar to those obtained for Me_8Spd up to the concentration where the DNA-spermidine solution forms a precipitated aggregate.

In Table 3 we present the experimental Me_8Spd self-diffusion coefficients obtained for titration of core-length NaDNA/NaCl ([Na]/[DNA-P] = 2.05, [Na] = 29 mM). At low [Me_8Spd] the coefficients are slightly higher than those obtained for Me_8Spd in NaDNA/NaCl with lower salt concentration ([Na]/[DNA-P] = 1.24, [Na] = 22 mM), presented in Table 1. However, for larger [Me_8Spd] the self-diffusion coefficients of Me_8Spd begin to increase considerably more rapidly than for the lower salt case presented in Table 1.

The experimental Me_8Spd and Li self-diffusion coefficients obtained by titration of core-length LiDNA/LiCl ([Li] + [Na] = 28 mM) solution are given in Table 4. The self-diffusion coefficients of Me_8Spd are very similar to those obtained in the former titration listed in Table 3. The measured self-diffusion coefficients of Li increase rapidly when Me_8Spd is added to the solution.

Finally, in Table 5 the diffusion data were obtained from a sample of MgDNA/$MgCl_2$ titrated with Me_8Spd. A qualitative difference as compared to the data for the monovalent counter-ions can immediately be discerned. Here there is no constancy in the value of the Me_8Spd diffusion coefficient at low additions of polyamine.

DISCUSSION

As a basis for the discussion of the results given above in Tables 1–5, we will present figures showing the polyamine diffusion quotient D/D_0, as a function of the concentration ratio [Polyamine]/[DNA-P], and for some of the figures together with theoretically calculated curves obtained from the two-state PB model. To emphasize the interesting initial part of the titration, only the data corresponding to the titration up to a value of [Polyamine]/[DNA-P] = 1 will be shown.

TABLE 2 Values of self-diffusion coefficients for spermidine in core-length DNA solutions at various contents of spermidine

[DNA-P] (mM)	[Spd] (mM)	D ($\times 10^9$ m^2 s^{-1})
17.3	1.05	0.027
17.1	1.72	0.033
16.8	2.54	0.043
16.5	3.33	0.073
16.3	3.79	0.098

TABLE 3 Values of self-diffusion coefficients for methylspermidine in core-length DNA solutions at various contents of methylspermidine

[DNA-P] (mM)	[Me_8Spd] (mM)	D ($\times 10^9$ m^2 s^{-1})
13.94	0.207	0.032
13.88	0.414	0.033
13.8	0.820	0.045
13.6	1.35	0.061
13.4	2.13	0.10
13.2	3.00	0.17
13.0	3.49	0.21
12.8	4.31	0.26
12.6	5.11	0.29
11.9	7.33	0.30
11.2	9.96	0.34
10.1	13.9	0.39
9.1	17.5	0.40
7.6	22.7	0.42
6.6	26.2	0.43
5.8	29.0	0.43

[Na^+]/[DNA-P] = 2.05.

Comparison of Me_8Spd and Spd

Methylation of the amino groups of spermidine eliminates their ability to form hydrogen bonds. Because the charge is unchanged, other electrostatic interactions are expected to be largely unaffected. If the binding is determined by electrostatic interactions, then alteration of the ligand by methylation, which results in a change in volume but not charge, should not greatly alter the affinity of the ligand for DNA. However, relative affinities of the alkali metal ions for DNA indicate that increasing hydrated ion size (decreasing charge density) is correlated with decreasing affinity for DNA (Bleam et al., 1980). Although K^+ and NH_4^+ are generally considered to be of comparable size in aqueous solution, the

TABLE 4 Values of self-diffusion coefficients for methylspermidine and lithium in core-length DNA solutions at various contents of methylspermidine

[DNA-P] (mM)	[Me_8Spd] (mM)	D (Me_8Spd) ($\times 10^9$ m^2 s^{-1})	D (Li^+) ($\times 10^9$ m^2 s^{-1})
23.4	0		0.52
23.3	0.117	0.023	0.53
23.2	0.352	0.028	0.56
23.1	0.698	0.033	0.60
22.7	1.38	0.039	0.63
22.3	2.26	0.057	0.69
21.7	3.52	0.11	0.74
21.1	4.90	0.21	0.75
20.7	5.64	0.23	0.76
20.1	6.92	0.29	0.79
19.6	8.10	0.30	0.80
18.8	9.68	0.34	0.81
18.1	11.3	0.38	0.86
17.2	13.2	0.40	0.89
16.4	14.9	0.42	0.88
14.3	19.5	0.42	0.91
12.8	22.8	0.46	0.90
11.2	26.3	0.44	0.93
9.7	29.2	0.45	

TABLE 5 Values of self-diffusion coefficients for methylspermidine in core-length Mg-DNA solutions at various contents of methylspermidine

[DNA-P] (mM)	[Me_8Spd] (mM)	D ($\times 10^9$ m^2 s^{-1})
20.6	0.205	0.069
20.3	0.814	0.094
20.2	1.21	0.11
20.0	1.60	0.13
19.8	2.18	0.16
19.6	2.55	0.17
19.0	4.00	0.22
18.6	5.02	0.24
17.9	6.62	0.27
17.1	8.56	0.29
16.0	11.2	0.33
15.1	13.6	0.34
13.8	16.6	0.34
12.4	19.9	0.36
11.3	22.6	0.36
10.2	25.4	0.36
9.7	26.6	0.38

$[Mg^{2+}]/[DNA\text{-}P] = 0.5$.

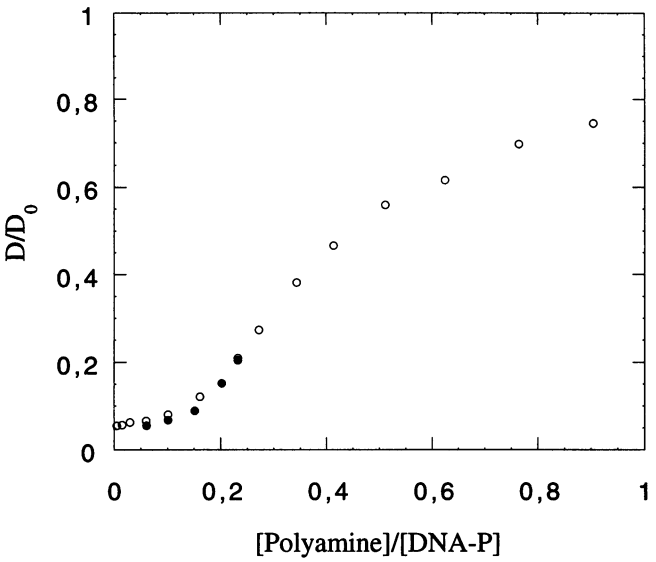

FIGURE 1 Experimental D/D_0 for methylspermidine (○) and spermidine (●) in solutions of NaDNA/NaCl, as a function of the [Polyamine]/[DNA-P] concentration ratio.

relative affinity of the latter for DNA is significantly greater, possibly as a result of hydrogen-bonding interactions. Various kinds of NMR measurements (Wemmer et al., 1985; Besley et al., 1990) give no indication of any hydrogen-bonding interactions between polyamines and sites on nucleic acids in solution. In a study of how the affinity for DNA is affected by methylation of the terminal ammonium groups on divalent polyamines, Padmanabhan et al. (1991) found that polyamines have a higher affinity for DNA (relative to Na^+) than the methonium ions. They interpreted the interactions between polyamines and methonium ions with DNA as purely electrostatic, and explained the obtained difference in affinity by the difference in size of the ions, which affects the distance of closest approach of the counter-ion to DNA.

In Fig. 1, we give the self-diffusion quotients, D/D_0, for Me_8Spd and Spd, as functions of the concentration ratios [Me_8Spd]/[DNA-P] and [Spd]/[DNA-P], respectively. In the range $0 \leq [Me_8Spd]/[DNA\text{-}P] \leq 0.06$, the self-diffusion quotient, D/D_0, is almost constant upon addition of Me_8Spd to the DNA solution. For $[Me_8Spd]/[DNA\text{-}P] > 0.1$, D/D_0 increases rapidly at first, before approaching a plateau at its limiting value of 1. These results are identical to the ones obtained in our previous work (Andreasson et al., 1993) (in fact, the two titration curves are almost superimposable). Those previous results were obtained with a different DNA sample; the core-length DNA was prepared on a different occasion. Furthermore, the measurements were also made with a different NMR spectrometer system. This reproducibility is gratifying and indicative of the reliability of the NMR self-diffusion method in the present context. Data representing D/D_0 for Spd as a function of [Spd]/[DNA-P], presented in Fig. 1, are very similar to those obtained for Me_8Spd. The two curves are identical, within experimental error. The fact that D/D_0 is constant when adding Me_8Spd to a DNA solution with $[Me_8Spd]/[DNA\text{-}P] \leq 0.06$ shows that in this region all of the trivalent ions introduced by titration are associated with DNA (Andreasson et al., 1993). The consequence of this is that the fraction of bound polyamine, P_B, within the two-state model (see Eq. 2), is constant during that part of the titration. This observation is supported by calculations of the fraction of bound Me_8Spd, P_B, obtained by solving the PB equation within the cylindrical cell model. The values of P_B obtained from the calculation were negligibly different from unity (0.996–0.993 when [Me_8Spd]/[DNA-P] was in the range 0.005–0.06). This is in full agreement with previous studies (Braunlin et al., 1986; Padmanabhan et al., 1991), where the relaxation rate of sodium decreases linearly upon competitive titration with different polyamines to NaDNA solutions. Braunlin et al. (1986) found a linearity over the range $0 \leq [M^{3+}]/[DNA\text{-}P] \leq 0.1$ when adding hexaammine cobalt(III) or Spd to a NaDNA solution. Padmanabhan et al. (1991) found a linearity over the range $0 \leq [M^{2+}]/[DNA\text{-}P] \leq 0.2$ when adding divalent polyamines and their *N*-methylated analogs to NaDNA solutions. The obtained self-diffusion coefficients for Me_8Spd at these low [Me_8Spd]/[DNA-P] concentration ratios approach a limiting value which is close to that of the DNA molecule itself. This observation demonstrates that this polyamine analog is restricted severely in terms of radial motion away from the DNA surface. This could result from binding to a specific site on the DNA molecule, or from the polyamine analog being trapped within the confined domain of the finite-length DNA molecule. The curve representing D/D_0 for Spd as a function of [Spd]/[DNA-P], presented in Fig. 1, is very similar to the one representing D/D_0 for Me_8Spd. Unfortunately, the self-diffusion coefficient of Spd could not be measured at very low [Spd]/[DNA-P] concentration ratios, because of the low signal-to-

noise ratio in the NMR experiments. However, we find it probable that the self-diffusion behavior of Spd at very low [Spd]/[DNA-P] ratios will be similar to that of Me_8Spd, i.e., that it should be determined by the self-diffusion coefficient of the DNA. According to Fig. 1, it seems that in the studied range, $0.06 \leq [Polyamine^{3+}]/[DNA\text{-}P] \leq 0.23$, the two cations bind to core-length NaDNA with equal affinity. The simplest interpretation of this is that there are no effects of hydrogen-bonding interactions or any affinity effects due to ion size. Our results are in agreement with a DNA melting study by Plum et al. (Plum and Bloomfield, 1990), in which the affinities of Me_8Spd and Spd for double-helical poly-[d(AT)] · poly[d(TA)] were found to be roughly equal. In a sodium relaxation study, Burton et al. (1981) found that natural tri- and tetravalent polyamines (spermidine^{3+} and spermine^{4+}) interact more strongly with NaDNA than do polyamines not found in nature, of the same valency, independently of ion size. Of course, a comparison of the interactions of Me_8Spd and Spd with double-helical DNA would be much more interesting if condensation did not occur when Spd is added to the DNA solution. This can be achieved by using synthetically prepared DNA samples, like solutions of $d(GC)_4$ or $d(GC)_8$.

NaCl salt dependence

In Fig. 2, D/D_0 values obtained for Me_8Spd in a solution of core-length NaDNA, with $[Na^+]/[DNA\text{-}P] = 2.05$, are shown together with the corresponding curve of D/D_0 for $[Na^+]/[DNA\text{-}P] = 1.24$ (see Fig. 1). These two sets of experiments correspond to initial values of the total sodium concentrations of $[Na^+] = 29$ and 22 mM, respectively. When $[Me_8Spd]/[DNA\text{-}P] < 0.06$, the D/D_0 values obtained for Me_8Spd are somewhat higher in the DNA solution with higher salt concentration. Furthermore, when $[Me_8Spd]/[DNA\text{-}P] \geq 0.06$, the values of D/D_0 for Me_8Spd in the high-salt sample increase more rapidly. This results in consistently larger diffusion quotients than in the low-salt case for the same values of the ratio $[Me_8Spd]/[DNA\text{-}P]$. However, from the data of Tables 1 and 3, it is clear that the two curves do approach the same limiting value at high concentration ratios. Qualitatively similar salt dependence was also observed in the equilibrium dialysis study of spermidine-DNA binding (Braunlin et al., 1982). In that study it was found that the logarithm of the binding constant was linearly dependent on the logarithm of the sodium concentration. Furthermore, it is mainly the amount of total sodium, not the sodium-to-phosphate ratio, that determines the amount of polyamine binding. This fact is important and should be borne in mind in the discussion below. Quantitative comparison of the salt dependence observed in that study with the present results does not seem relevant, because of the considerable differences in solution conditions between the two studies. In the equilibrium dialysis study the $[Na^+]$ concentration was in the range of 50 to 160 mM, whereas the DNA phosphate concentration was between 0.3 and 7 mM, and the magnitude of the spermidine concentration was similar to that of the DNA.

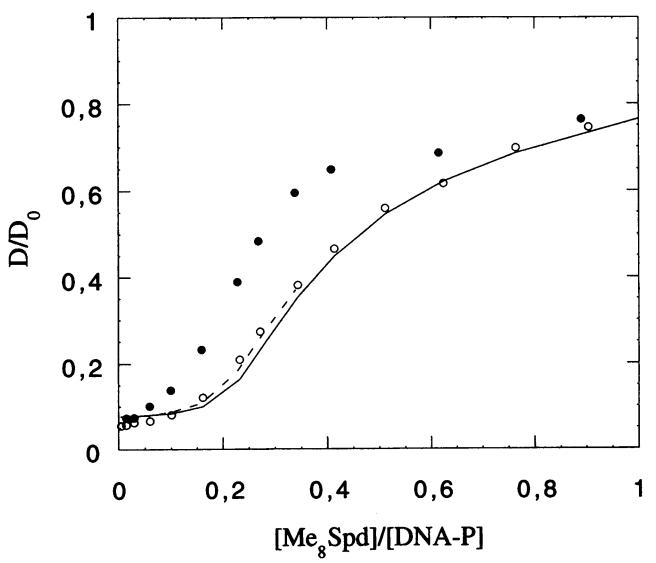

FIGURE 2 D/D_0 for methylspermidine as a function of the $[Me_8Spd]/[DNA\text{-}P]$ concentration ratio in solutions of NaDNA/NaCl with $[Na^+]/[DNA\text{-}P] = 1.24$ (○, experimental points; *solid line,* theoretical curve calculated from the Poisson-Boltzmann two-state model); NaDNA/NaCl with $[Na^+]/[DNA\text{-}P] = 2.05$ (●, experimental points; *dashed line,* theoretical curve calculated from the Poisson-Boltzmann two-state model).

It should be noted that the small difference in the DNA phosphate concentration ([DNA-P] = 17.7 mM and 14.0 mM for $[Na^+]/[DNA\text{-}P] = 1.24$ and $[Na^+]/[DNA\text{-}P] = 2.05$, respectively) may cause a small difference in the self-diffusion coefficients of DNA itself. We have studied the self-diffusion behavior of the oligomeric $d(GC)_8$ as a function of sodium concentration and [DNA-P] (Andreasson et al., 1996). The measured self-diffusion coefficients of $d(GC)_8$, D_{DNA}, were constant in the range of added sodium ($1.14 < [Na^+]/[DNA\text{-}P] < 20.0$), but a slight [DNA-P] dependence was detected (an increase in D_{DNA} of ~25% was seen when [DNA-P] decreased from 22.0 mM to 5.0 mM). It is reasonable to assume a similar [DNA-P] and $[Na^+]$ dependence on the self-diffusion coefficients of the studied core-length DNA. This precludes the possibility that the obtained salt-effect is a result of differences in the DNA diffusion coefficient (see Eq. 2 below) in the two systems.

A salt effect on the self-diffusion coefficient is expected from polyelectrolyte theory. The higher concentration of sodium ions near the DNA surface in the high-salt case reduces the electrostatic potential of DNA, which reduces the electrostatic interaction between Me_8Spd and the polyion. This should result in higher values of the self-diffusion coefficients of Me_8Spd in the DNA solution with higher sodium content. In our previous paper (Andreasson et al., 1993) we found excellent agreement between the experimental D/D_0 values of Me_8Spd in solutions of core-length NaDNA ($[Na^+]/[DNA\text{-}P] = 1.20$) and the calculated ones, obtained by solving the cylindrical PB equation combined

with a two-state self-diffusion model (Stilbs and Lindman, 1982):

$$D/D_0 = P_B(D_{DNA}/D_0) + (1 - P_B). \quad (2)$$

D is the self-diffusion coefficient of Me_8Spd. D_{DNA} is assumed to be equal to D_B, the self-diffusion coefficient of Me_8Spd in the bound state. D_0 is assumed to be equal to D_F, the self-diffusion coefficient of Me_8Spd in the free state, obtained as the limiting value of D at high values of [Me_8Spd]/[DNA-P]. P_B is the fraction of bound Me_8Spd, obtained from the cylindrical PB equation. D_{DNA} was taken from a work by Nicolai et al. (Nicolai and Mandel, 1989): $D_{DNA} = 0.03 \times 10^{-9}$ m^2 s^{-1}. The parameter that determines the salt dependence of the D/D_0 ratio in Eq. 2 is obviously P_B. In Fig. 2, the theoretically calculated titration curves for the two NaCl salt contents obtained from Eq. 2 and solution of the PB equation are also displayed. It can be noted that for the high-salt case the convergence in the solution of the PB equation is problematic at high amounts of added Me_8Spd, and therefore the calculated curve for these high salt points is not displayed. Furthermore, in Table 6, numerical values of P_B and D/D_0 values resulting from Eq. 2 are presented for some points of the titrations. According to the PB data in Fig. 2 and Table 6, the difference between the experimental D/D_0 values at the two salt contents is considerably larger than the difference between the theoretical ones. The predicted salt effect, although qualitatively correct, is in fact only marginal for the given change of total sodium concentration, whereas the experimental effect is considerable. At [Me_8Spd]/[DNA-P] = 0.06, the experimental D/D_0 for Me_8Spd is ~50% higher in the DNA solution with [Na^+]/[DNA-P] = 2.05 than the D/D_0 for Me_8Spd in the DNA solution with [Na^+]/[DNA-P] = 1.24. On the other hand, a difference of only ~3% is predicted by Eq. 2, and at [Me_8Spd]/[DNA-P] = 0.23 the corresponding differences are 144% (experimental) and 13% (theoretical), respectively. Clearly, the predicted salt effect from the PB model is too small, and considerably larger amounts of added sodium would be needed to obtain theoretical effects of the magnitude observed in the experiments. In the cylindrical cell 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 ions are treated as point charges. All of these approximations may, of course, contribute to the bad agreement with the experimental D/D_0 values when [Na^+]/[DNA-P] = 2.05. The most serious approximation, however, probably is the treatment of Me_8Spd as a trivalent point charge. This leads to an overestimation of P_B, and with that a lower value of D/D_0 than the true value. This effect, however, may be compensated to some extent by the mean field approximation within the PB model, which underestimates the accumulation of counter-ions close to the charged polyion due to neglect of ion-ion correlations, particularly in the presence of multivalent ions (Torrie and Valleau, 1982; Paulsen et al., 1988). The underestimation of the association of counter-ions within the PB model is generally larger in the high coupling limit, i.e., for high polyion charge density, high counter-ion valency, and low added salt concentration. Therefore, this underestimation of P_B within the PB model may be less serious at high salt concentrations, whereas the approximation of treating Me_8Spd as a three-valent point charge should still give an overestimation of P_B in the PB calculations at high salt concentrations. We therefore tentatively ascribe the experimentally observed salt dependence of the diffusion of Me_8Spd to an electrostatic effect that is not correctly captured within the PB model. To test this conjecture, it is necessary to perform calculations within a polylectrolyte model that includes a more refined description of the electrostatic interactions in the system, and particularly the polyamine charge distribution. Such calculations are currently in progress in our laboratory; these are made using the Monte Carlo simulation method. Preliminary results (Lyubartsev and Nordenskiöld, to be published) indicate that the salt dependence of the polyamine association to DNA is in fact better described by a more accurate description of the electrostatics in the system.

It should also be pointed out that D_{DNA} in the present study, as obtained from the two-state model (Eq. 2) at very low [Me_8Spd]/[DNA-P] concentration ratios, seems to be lower than the value predicted by Nicolai et al. As mentioned before, D_{DNA} increases somewhat with increasing dilution (Andreasson et al., 1996). Because the value of Nicolai et al. (Nicolai and Mandel, 1989) is a measure of the self-diffusion coefficient at infinite dilution, we believe that the true D_{DNA} at the present [DNA-P] (~17.8 mM) should be lowered to at least 0.024×10^{-9} m^2 s^{-1}, as in Table 1.

Gibbs et al. (Gibbs and Johnson, 1991) studied the self-diffusion behavior of polyammonium cations in solutions of high-molecular-weight calf thymus DNA. The self-diffusion coefficients of the trivalent 3,3′-iminobis-(*N*,*N*-dimethylpropylamine) ($HN[(CH_2)_3\text{-}N(CH_3)_2]_2$) in solutions of

TABLE 6 Experimental and theoretical D/D_0 and P_B at some points in the titration for Me_8Spd in solutions of core-length NaDNA with different salt concentrations

	[Na^+]/[DNA-P] = 1.24			[Na^+]/[DNA-P] = 2.05		
[Me_8Spd]/[DNA-P]	P_B	D/D_0 (theo.)	D/D_0 (exp.)	P_B	D/D_0 (theo.)	D/D_0 (exp.)
0.06	0.993	0.075	0.066	0.991	0.077	0.10
0.23	0.902	0.16	0.21	0.880	0.18	0.39
0.27	0.828	0.23	0.27	0.805	0.25	0.48
0.34	0.697	0.35	0.39	0.680	0.37	0.59

DNA in that work are of interest, in comparison with the results from the present work. When [IBDMPA]/[DNA-P] = 0.056 ([DNA-P] = 18 mM), the obtained self-diffusion coefficients for IBDMPA increased with 60% upon increase of the total sodium concentration $[Na^+]$ from 23 mM to 28 mM. This result should be compared with the corresponding values in the present study at $[Me_8Spd]/[DNA\text{-}P] = 0.06$: $[Na^+]/[DNA\text{-}P] = 1.24$ ($[Na^+] = 22$ mM), $D = 2.9 \times 10^{-11}$ m^2 s^{-1} and $[Na^+]/[DNA\text{-}P] = 2.05$ ($[Na^+] = 29$ mM), $D = 4.5 \times 10^{-11}$ m^2 s^{-1}, which gives a difference in D between the two salt contents of about 55%. The similarity of the results of these two studies thus shows that the polyamine diffusion is directly sensitive to the total amount of sodium in the DNA system. It can also be noted that Magdalenat et al. (1974), in a study of the self-diffusion behavior of Sr^{2+} in solutions of a linear acidic polysaccharide, chondroitin sulfate, found a sodium concentration dependence in their data, which is similar to our results.

Titration of Me_8Spd in LiDNA solution

In Fig. 3 the obtained D/D_0 values for Me_8Spd and Li^+, in solutions of core-length LiDNA ($[Li^+]/[DNA\text{-}P] = 1.19$, $[Li^+] + [Na^+] = 28$ mM), are plotted as a function of $[Me_8Spd]/[DNA\text{-}P]$. In addition, the corresponding D/D_0 versus $[Me_8Spd]/[DNA\text{-}P]$ curves for $[Na^+]/[DNA\text{-}P] = 1.24$ ($[Na^+] = 22$ mM) and $[Na^+]/[DNA\text{-}P] = 2.05$ ($[Na^+] = 29$ mM) are given. It is clear that the Me_8Spd diffusion rates are almost identical for the LiDNA system and the NaDNA system, for which the total monovalent salt concentrations are also almost identical. The discrepancy for the two final points seems to be due to data scatter, because inspection of the preceding points (not displayed; see Tables 3 and 4) at the end of the titration reveals that the curves then continue to be very similar. This result clearly indicates that the polyamine-DNA interaction is very similar in the two systems. The interpretation of this is that the amount of polyamine association and its ability to displace monovalent counter-ions are independent of counter-ion type. However, the observation drawn from other studies, that in the absence of polyamine the Li^+ association to DNA is more effective than that of Na^+, is not addressed by the present results (see below). The curve representing D/D_0 for the diffusion of Li^+ is very steep at low values of $[Me_8Spd]/[DNA\text{-}P]$, because of an effective exchange of lithium ions from a "bound" state near the polyion, with low self-diffusion coefficient, to a "free" state in the bulk with higher self-diffusion coefficient, when Me_8Spd is titrated to the DNA solution.

Bleam et al. (1980), in a competitive titration of NaDNA, studied the affinity of various univalent ions for DNA, using ^{23}Na NMR. They found a correlation between increasing binding affinity and decreasing hydrated radius of the counter-ions, which was interpreted as resulting from purely electrostatic interactions between the polyion and its hydrated counter-ions. Although hydrated Li^+ is larger than hydrated Na^+, it was found to bind with higher affinity than Na^+ to DNA. They explained this fact by stating that the binding of lithium may have some covalent character. Ross et al. (Ross and Scruggs, 1964a, b) found, in studies of the electrophoretic mobility of calf thymus DNA, that Li^+ binds to DNA more strongly than does Na^+. Bartenev et al. (1983) have suggested that Li^+, which has a very small ionic radius (unhydrated) and a very stable water shell (hydrated), should have a different kind of interaction with B-DNA.

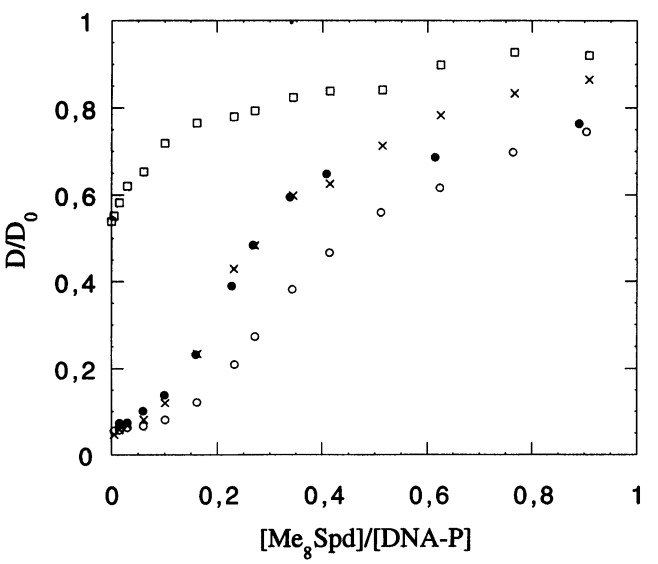

FIGURE 3 Experimental D/D_0 as a function of the $[Me_8Spd]/[DNA\text{-}P]$ concentration ratio for methylspermidine in solutions of NaDNA/NaCl with $[Na^+]/[DNA\text{-}P] = 1.24$ (○) and $[Na^+]/[DNA\text{-}P] = 2.05$ (●) and for methylspermidine (×) and lithium (□) in solutions of LiDNA/LiCl.

Titration of Me_8Spd in MgDNA solution

Fig. 4 shows the Me_8Spd diffusion curve in MgDNA solution. For this sample $[Mg^{2+}]/[DNA\text{-}P] = 0.50$, [DNA-P] = 20.7 mM, and the residual sodium concentration $[Na^+] = 2.1$ mM. For comparison with the total initial monovalent counter-ion concentrations in the other experiments, it is then more relevant to state the total initial counter-ion charge concentration, which equals 22.9 mM. Therefore, for comparison the Me_8Spd diffusion curve for the low-salt sodium, $[Na^+] = 22$ mM, is also shown in Fig. 4. The magnesium curve is very interesting, because it shows a qualitatively different behavior in the initial region of the titration than does the sodium case. Instead of an initially constant diffusion quotient, there is an immediate and steep rise. Within the two-state model (Eq. 2), this means that whereas in the sodium case practically all Me_8Spd added in the initial part of the titration is associated with DNA and is effectively displacing sodium, in the magnesium case on the other hand, the Mg^{2+} ions are able to compete with Me_8Spd for binding to DNA and not all Me_8Spd added associates with DNA. In the figure, the theoretically calculated PB

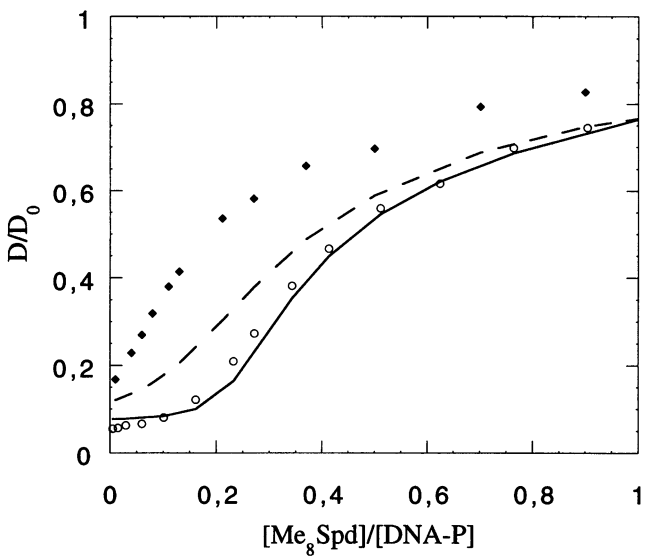

FIGURE 4 D/D_0 for methylspermidine as a function of the [Me_8Spd]/[DNA-P] concentration ratio in solutions of NaDNA/NaCl with [Na^+]/[DNA-P] = 1.24 (○, experimental points; *solid line,* theoretical curve calculated from the Poisson-Boltzmann two-state model); MgDNA/$MgCl_2$ with [Mg^{2+}]/[DNA-P] = 0.5 (◆, experimental points; *dashed line,* theoretical curve calculated from the Poisson-Boltzmann two-state model).

curve shows a behavior qualitatively similar to that of the experimental results. However, there is a clear quantitative discrepancy, with the PB curve being less steep in the beginning and generally predicting lower diffusion quotients. The reason for this is that the PB model predicts larger values of the fraction of bound polyamine than what seems to be at hand in the experiments. In light of the NaCl salt dependence results and the discussion of the PB model given in this connection above, such a discrepancy is expected. The treatment of Me_8Spd as a trivalent point charge is expected to exaggerate the electrostatic interaction between this polyamine and DNA and thus overestimate the amount of Me_8Spd in the close vicinity of the polyion. It should be noted, however, that within the two-state model the quantitative discrepancy between the PB model and the experiments in the prediction of the amount of DNA-associated Me_8Spd is not large. If Eq. 2 is used in combination with an experimentally determined diffusion quotient, P_B can be determined experimentally for a given point in the titration curve. Thus, for the two first points of the titration, values of $P_B = 0.90$ and 0.83 are obtained. The corresponding theoretically calculated numbers evaluated from the PB model are 0.95 and 0.93, respectively. Because of the form of Eq. 2, which is a weighted sum of two numbers differing by two orders of magnitude, these differences are then magnified when the experimental and theoretical diffusion quotients are compared.

CONCLUSIONS

This investigation has shown that in the studied range, 0.06 < [polyamine]/[DNA-P] < 0.23, there is no difference between the D/D_0 values obtained for Me_8Spd and Spd in solutions of core-length NaDNA. From these data, it seems that Me_8Spd and Spd associate with core-length DNA with equal affinity. Neither effects of hydrogen binding nor any effects of ion size could be detected. However, this study is not complete, because aggregation of the Spd-DNA solution occurred, which in itself is a symptom of a difference between Me_8Spd and Spd in the interaction with DNA. However, the similarity of the Me_8Spd and Spd interactions observed in solution in this and other studies indicates that this difference in aggregation is mainly due to a difference in the interaction of the two polyamines with DNA in the aggregated state. A qualitatively expected influence on the measured self-diffusion coefficients was found when the salt concentration in the core-length DNA solution was increased. This effect could not be quantitatively described by the PB equation together with the two-state self-diffusion model. No notable difference in the D/D_0 for Me_8Spd was observed between titrations into NaDNA and LiDNA, when experiments performed at similar total univalent salt concentrations were compared. It is interesting that the titration curve obtained for MgDNA is qualitatively different compared to the results for NaDNA, due to the competition of magnesium. In this context it is noteworthy that the prediction of the electrostatic PB model, although not quantitatively correct, has the ability to qualitatively predict the difference in Me_8Spd association to DNA between NaDNA and MgDNA at low polyamine-to-DNA ratios. This gives further evidence that the polyamine-DNA association is of mainly electrostatic origin. Further theoretical work to improve the description of the electrostatic interactions in this system therefore is highly motivated. Such calculations are currently in progress in our laboratory.

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