Polyammonium Cation Diffusion in Aqueous Solutions of DNA As Studied by Pulsed Field Gradient NMR

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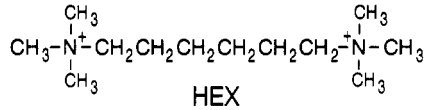
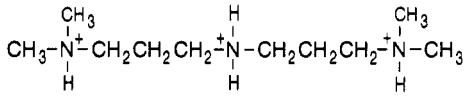
Polyammonium cations (PACs) such as the protonated forms of putrescine, cadaverine, spermidine, and spermine are thought to play an essential role in a number of different biological cell functions involving nucleic acids and membranes.[1-4] Of particular interest are the strong electrostatic interactions between PACs and the biological polyanion DNA.[2-5] Polyammonium cations are thought to be important in influencing DNA structure as well as in mediating interactions of other ligands with DNA.[4,6] In addition, the PAC–DNA system provides an interesting model for testing theories of counterion–polyelectrolyte interactions in solution.[7-9]

Our focus here concerns the effect of these interactions on the macroscopic diffusive mobility of PACs in aqueous DNA solutions. Previous NMR line width, relaxation, and NOE studies indicate that PACs maintain a high degree of rotational mobility even when strongly associated with DNA under conditions of low solution ionic strength.[3,10,11] These observations are consistent with nonspecific, delocalized interactions between a PAC and the polyanion. It has recently been postulated that PACs also maintain a high degree of axial mobility along the DNA chain.[10] We address this issue by reporting measurements of tracer diffusion coefficients of HEX and IBDMPA cations in aqueous solutions of calf thymus DNA as functions of NaCl concentration. Briefly, we find that



HEX



IBDMPA

the tracer diffusion coefficients of PACs associated with DNA can be more than 2 orders of magnitude lower than when not associated.

Materials and Methods

Calf thymus NaDNA (type I, "highly polymerized") was obtained from Sigma. The DNA was dissolved in H_2O and dialyzed against two changes of 1 M NaCl and 10 mM EDTA followed by five changes of deionized H_2O. The resulting DNA solution was gradually adjusted to pH 6.6 with NaOH. This solution was then lyophilized, redissolved in D_2O (Aldrich, 99.9 atom % D), and lyophilized again before it was dissolved in D_2O to make a stock solution with a DNA concentration of 5 g/L. Absorbance measurements at 260 nm indicated that the DNA phosphate concentration was approximately 18 mM.

Hexamethylenebis(trimethylammonium) bromide monohydrate (HEX) ($(CH_3)_3N(CH_2)_6N(CH_3)_3Br_2 \cdot H_2O$) and 3,3′-iminobis(*N*,*N*-dimethylpropylamine) (IBDMPA) ($HN[(CH_2)_3N(CH_3)_2]_2$) were obtained from Aldrich. A 100 mM stock solution of HEX was prepared by dissolving the monohydrate in D_2O, lyophilizing, and redissolving in D_2O. A 100 mM solution of the trivalent cation of IBDMPA was prepared by titrating a 100 mM aqueous solution of the free base with HCl to pH 6.6, lyophilizing, and dissolving in D_2O. At pH less than 7.0, IBDMPA is expected to be completely protonated and thus trivalent.

Solutions of DNA and PAC were then prepared by slowly adding the PAC to the DNA stock solution while slowly stirring the solution for a final PAC concentration of 1 mM. For the IBDMPA solution, a small amount of DNA precipitated upon addition of the PAC and then gradually redissolved. The two solutions thus prepared were gently stirred with a magnetic stir bar overnight at room temperature. Aliquots of 3.5 mL of these solutions were then transferred to 10-mm-o.d. NMR tubes containing a known mass of NaCl. The samples were mixed by periodically vortexing at room temperature over the course of 24 h.

^1H PFG NMR experiments were performed on a Brüker AC-250 with a custom-built probe and a computer-controlled gradient driver.[12] The Longitudinal Encode Decode (LED) version of the PFG experiment,[13] which avoids errors caused by transient field disturbances following a gradient pulse, was used for all of the diffusion measurements. The LED experiment is essentially a stimulated echo (STE) experiment[14] in which the echo is stored with an rf $\pi/2$ pulse and then, after a "settling period", is recalled with another rf $\pi/2$ pulse for detection. When large gradient pulses (>200 G/cm) are used with unshielded gradient coils, a settling period of up to 100 ms may be required. We find that the LED sequence permits the measurement of diffusion coefficients with magnitudes less than 10^{-10} cm^2 s^{-1}. The diffusion experiments were performed by varying either the gradient pulse duration or the amplitude while maintaining constant echo time and diffusion time Δ. Data were analyzed by nonlinear least-squares regression of the equation[15]

$$A/A_0 = \exp[-K^2(\Delta - \delta/3)D] \quad (1)$$

to the resulting echo amplitudes with A_0 and D as parameters. In eq 1, A is the echo amplitude, $K = \gamma g \delta$, γ is the magnetogyric ratio of the observed nucleus, δ is the duration of the field gradient pulses, g is the pulsed field gradient amplitude, Δ is the time interval between the leading edges of the field gradient pulses, and D is the tracer diffusion coefficient of the observed species. The echo intensity for a particular species was measured by integration over a well-resolved region of the Fourier transformed echo. In all cases the experimental data were well fit by the single-exponential function in eq 1.

Spin–lattice relaxation times (T_1) were measured by means of the inversion–recovery experiment for the same samples used in the PFG experiments. All experiments were performed at 25 °C, and no efforts were made to remove oxygen from the samples.

Results and Discussion

Spin–lattice relaxation times and tracer diffusion coefficients of the methyl group protons of the two PACs studied are shown in Figures 1 and 2, respectively, as functions of estimated total Na^+ concentration and in Table I as functions of the concentration of added NaCl only. For both of the PACs, T_1's appear to approach limiting values at low salt concentrations characteristic of the associated state. These values are approximately half of those for the unassociated PAC at high salt concentration. The small reductions in T_1's are in agreement with the modest increases in line width of a PAC upon association with DNA that were reported by Besley et al.[10] In contrast, the tracer diffusion coefficients of the PACs decrease apparently without bound with decreasing salt concentration. The tracer diffusion coefficient of the trivalent cation IBDMPA is more sensitive to salt concentration than that of the divalent cation HEX. Also, the tracer diffusion coefficient of HDO in the DNA solutions is insensitive to salt concentration.

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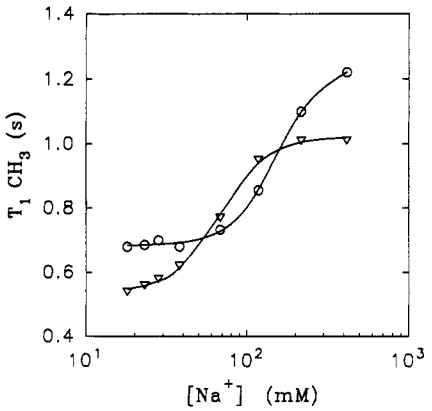

Figure 1. Proton spin-lattice relaxation times versus total Na ion concentration for IBDMPA (○) and HEX (▽). Lines only serve to guide the eye.

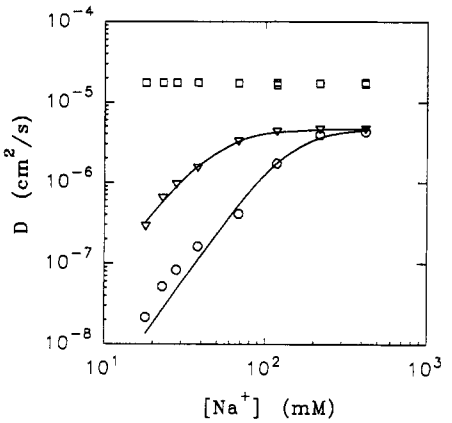

Figure 2. Tracer diffusion coefficients versus total Na ion concentration for HDO (□), IBDMPA (○), and HEX (▽). Lines represent least-squares fits of eq 3 to the data (see text).

Table I
PAC Tracer Diffusion Coefficients and Spin-Lattice Relaxation Times

[NaCl],[a] mM	HEX		IBDMPA	
	T_1, s	D, cm^2/s	T_1, s	D, cm^2/s
0.0	0.54	2.83×10^{-7}	0.68	2.16×10^{-8}
5.0	0.56	6.32×10^{-7}	0.68	5.20×10^{-8}
10.0	0.58	9.41×10^{-7}	0.70	8.31×10^{-8}
20.0	0.62	1.52×10^{-6}	0.68	1.63×10^{-7}
50.0	0.77	3.23×10^{-6}	0.73	4.08×10^{-7}
100.0	0.95	4.26×10^{-6}	0.86	1.73×10^{-6}
200.0	1.01	4.55×10^{-6}	1.10	3.92×10^{-6}
400.0	1.01	4.57×10^{-6}	1.22	4.23×10^{-6}

[a] Added NaCl only.

The diffusion coefficients in Figure 2 and Table I were obtained with the diffusion time Δ = 503.5 ms. For IBDMPA diffusion times of 53.5 and 1003.5 ms were also used and no dependence of the observed diffusivity on diffusion time was found. Further, all PFG NMR results are well-fit single-exponential functions, and diffusivities were found to be unchanged after storage of samples for 9 days. These facts suggest rapid exchange of the PAC between accessible sites and averaging over any distribution of diffusional mobilities.

Record et al.[2,5,6] have shown that the association of polyammonium cations with DNA can be well described by a two-state model with an equilibrium distribution coefficient K_D given by

$$\log [K_D] = a \log [\mathrm{NaCl}] + b \qquad (2)$$

where a is proportional to the charge on the PAC with a proportionality constant of approximately 0.88. Here, K_D is the ratio of the concentrations of associated PAC to unassociated PAC. If exchange between associated and unassociated PAC is fast compared to the diffusion time Δ, then the diffusion coefficients of the PACs should be correlated by an equation of the form

$$D = \left[\frac{K_D}{1+K_D}\right]D_b + \left[\frac{1}{1+K_D}\right]D_f \qquad (3)$$

where D_b and D_f are the diffusion coefficients of the associated (bound) and unassociated (free) PACs, respectively. The lines in Figure 2 represent fits of eq 3 to the experimental data, with K_D given by eq 2, D_b set equal to zero, and a, b, and D_f as free parameters. Equation 3 describes the experimental diffusivities satisfactorily, but the resulting parameter estimates of a_{IBDMPA} = 2.8 and a_{HEX} = 2.6 are sensitive to errors in the Na ion concentration and the assumption that D_b = 0. These estimates are not, however, in agreement with the observation of Record et al.[3,6,7] that a should be proportional to the ligand charge. Interpretation of our results may be complicated by the fact that the charge on IBDMPA is pH dependent. Further, the ratio of PAC N^+ to DNA P^- was different for the two PACs since the total PAC concentration was 1 mM and the total DNA P^- was approximately 18 mM in both experiments.

More sophisticated treatments of counterion diffusion in polyelectrolyte solutions based on the cylindrical cell model are available.[7,8] The approximation of uniform axial charge density in the cylindrical cell model results in the conclusion that counterions should move freely along the polyion axis and thus that the minimum diffusion quotient D/D_0 should be 1/3.[7,16] The large reductions in macroscopic (long time) diffusion coefficients observed for the PACs in our studies do not support this view. The modest reductions in T_1's and NMR line widths observed with decreasing salt concentration support the notion that PACs maintain a high degree of rotational mobility upon association with DNA.

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References and Notes

(1) Chung, L.; Kaloyanides, G.; McDaniel, R.; McLaughlin, A.; McLaughlin, S. *Biochemistry* **1985**, *24*, 442.
(2) Braulin, W. H.; Strick, T. J.; Record, M. T., Jr. *Biopolymers* **1982**, *21*, 1301.
(3) Padmanabhan, S.; Richey, B.; Anderson, C. F.; Record, M. T., Jr. *Biochemistry* **1987**, *27*, 4367.
(4) Vertino, P. M.; Bergeron, R. J.; Cavanaugh, P. F., Jr.; Porter, C. W. *Biopolymers* **1987**, *26*, 691.
(5) Anderson, C. F.; Record, M. T., Jr. *Annu. Rev. Phys. Chem.* **1982**, *33*, 191.
(6) Record, M. T., Jr.; Mazur, S. J.; Melançon, P.; Roe, J.-H.; Shaner, S. L.; Unger, L. *Annu. Rev. Biochem.* **1981**, *50*, 997.
(7) Nilsson, L. G.; Nordenskiöld, L.; Stilbs, P.; Braunlin, W. H. *J. Phys. Chem.* **1985**, *89*, 3385.
(8) Yoshida, N. *J. Chem. Phys.* **1978**, *69*, 4867.
(9) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 934.
(10) Besley, S.; Cullis, P. M.; Partridge, R.; Symons, M. C. R.; Wheelhouse, R. T. *Chem. Phys. Lett.* **1990**, *165*, 120.
(11) Wemmer, D. E.; Srivenugopal, K. S.; Reid, B. R.; Morris, D. R. *J. Mol. Biol.* **1985**, *185*, 457.
(12) Saarinen, T. R.; Woodward, W. S. *Rev. Sci. Instrum.* **1989**, *59*, 761.
(13) Gibbs, S. J.; Johnson, C. S., Jr. *J. Magn. Reson.* **1991**, *93*, 395.
(14) Tanner, J. E. *J. Chem. Phys.* **1968**, *52*, 2523.
(15) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 282.
(16) Piculell, L.; Rymdén, R. *Macromolecules* **1989**, *22*, 2376.