

Effects of Different Cations on the Hydrodynamic Radius of DNA

Bryant S. Fujimoto, Julia M. Miller, N. Susan Ribeiro, and J. Michael Schurr

Department of Chemistry, University of Washington, Seattle, Washington 98195 USA

ABSTRACT The effects of different cations on the hydrodynamic radius (R_H) of a 48-bp synthetic DNA are measured by time-resolved fluorescence polarization anisotropy of intercalated ethidium. Relative statistical errors in R_H are only ~1%. With increasing cation concentration, Na^+ causes a small decrease in R_H , Cs^+ causes a somewhat larger decrease by up to 0.5 Å at 100 mM, and $(\text{CH}_3\text{CH}_2)_4\text{N}^+$ causes an increase in R_H by ~0.5 Å at 100 mM. The qualitatively different effects of these monovalent cations indicates that the changes in R_H with cation concentration do not arise primarily from electrolyte friction. Divalent cations cause much larger increases in R_H with increasing cation concentration. Mg^{2+} causes an increase in R_H by up to 1.0 Å at 24.4 mM, and Mn^{2+} causes an increase in R_H by up to 1.6 Å at 24.4 mM. These effects are independent of DNA concentration. There is some positive correlation between the order of effects of the different cations on R_H and the order of their effects on interhelical hydration forces. It is suggested that these different ions affect R_H either by altering the hydration layer or possibly by some effect on DNA structure, such as stabilizing bends.

INTRODUCTION

In principle, counterions could affect the rotational dynamics of DNA in any of three ways. They might significantly enhance the rotational friction factor via fluctuating interionic forces in a manner analogous to electrolyte friction (Schurr, 1980; Schurr and Schmitz, 1986; Schmitz, 1993); they might affect the hydrodynamic radius (R_H) directly by altering the hydration layer of the DNA; or they might alter the structure of the DNA in some way that affects its R_H , for example, by stabilizing bends in the molecule. Electrolyte friction would be expected to depend on the valence, but not the kind of counterion, whereas alterations to either the hydration layer or DNA structure would be expected to be quite sensitive to cation type.

Evidence for substantial “hydration” forces of significant range between DNA molecules comes from osmotic compression studies in which the interhelical spacing of hexagonal arrays were measured as a function of osmotic pressure (Rau and Parsegian, 1984, 1992; Podgornik et al., 1989; Leikin et al., 1991). The results indicate remarkably large variations in the force versus distance curves with changes in counterion type. There are significant differences in the force versus distance curves not only between monovalent and divalent cations, but also between different divalent cations and between different monovalent cations. Among monovalent ions, the hydration forces at close distances increase in the order $F(\text{Na}^+) < F(\text{Cs}^+) < F((\text{CH}_3)_4\text{N}^+) \leq F((\text{CH}_3\text{CH}_2)_4\text{N}^+)$. For the divalent ions, $F(\text{Mn}^{2+}) > F(\text{Mg}^{2+})$ at short distances below the condensation transition induced by Mn^{2+} , and $F(\text{Mn}^{2+}) < F(\text{Mg}^{2+})$ at long distances beyond that transition. The question arises whether there is any correlation between the effects of these cations on the strength

of interhelix hydration forces at short distances and the effects of these same ions on the R_H of DNA in solution.

Raman spectra of concentrated calf-thymus DNA samples in the presence of 100-mM concentrations of different divalent cations showed significant variations with cation type (Duguid et al., 1993). Such information suggests that divalent cations do actually perturb the DNA structure, although the extent of the perturbation is difficult to assess quantitatively.

Until now there have appeared no reports regarding the effects of different cations on the R_H of DNA. We describe herein measurements of the R_H of a 48-bp synthetic DNA by fluorescence polarization anisotropy (FPA) of intercalated ethidium. For a DNA of such high axial ratio this technique is sensitive primarily to azimuthal rotation around the helix-axis.

THEORY

Model of DNA motion

A DNA composed of $N + 1$ bp is regarded as a linear array of $N + 1$ disks each with height $h = 3.4$ Å and an R_H value. Each disk is connected to its neighbor(s) on either side by Hookean torsion and bending springs. The bending spring constant is chosen to yield our current best guess for the dynamic persistence length, namely $P = 1500$ Å (Allison et al., 1989; Song and Schurr, 1990; Fujimoto and Schurr, 1990; Hustedt et al., 1993; Schurr et al., 1992). The torsion spring constant between base pairs is taken to be $\alpha = 5.4 \times 10^{-12}$ dyne cm, which is intermediate between the values (determined by assuming $P = 1500$ Å) appropriate for ordinary linear DNAs ($\alpha = 4.7 \times 10^{-12}$ dyne cm) and for linearized pUC8 DNA ($\alpha = 5.8 \times 10^{-12}$ dyne cm) in 0.1 M NaCl (Fujimoto and Schurr, 1990; Schurr et al., 1992). For the present 48-bp DNA, the extracted value of the R_H is only weakly dependent on the assumed values of P and α , provided they are not absurdly high or low.

Received for publication 11 February 1994 and in final form 25 April 1994.

Address reprint requests to Dr. J. Michael Schurr, Department of Chemistry BG-10, University of Washington, Seattle, WA 98195. Tel.: 206-543-6681; Fax: 206-685-8665; E-mail: schurr@chem.washington.edu.

© 1994 by the Biophysical Society

0006-3495/94/07/304/05 \$2.00

The diffusion coefficients for the rigid-body rotations around the symmetry and transverse axes, (D_{\parallel} and D_{\perp} , respectively), are calculated using the results of Tirado and Garcia de la Torre (1980) as summarized by Eimer et al. (1990). In particular,

$$D_{\perp} = 3k_B T(\ln p + \delta_{\perp})/\pi\eta L^3 \quad (1)$$

$$D_{\parallel} = k_B T/[(3.814)\pi\eta LR_H^2(1 + \delta_{\parallel})] \quad (2)$$

where $k_B T$ is thermal energy, η is viscosity, L is the contour length, $p = L/(2R_H)$ is the axial ratio, and δ_{\perp} and δ_{\parallel} are end-plate corrections that are given in terms of p by

$$\delta_{\perp} = -0.662 + (0.917/p) - (0.050/p^2) \quad (3)$$

$$\delta_{\parallel} = 1.119 \times 10^{-4} + (0.6884/p) - (0.2019/p^2) \quad (4)$$

The intercalation of an ethidium dye molecule is assumed to increase the contour length by 2.7 Å (Hogan et al., 1979) and to increase the number of "base-pair" subunits and twisting and bending springs by 1. The additional springs are taken to exhibit the same torsion and bending constants as those between base pairs (Wu et al., 1991; Schurr et al., 1992; Smith et al., 1992).

FPA

The results obtained from FPA experiments on such a short DNA depend only very weakly on dynamical details of either the rapid local motions of the dye or the twisting and bending normal modes. However, the amplitudes of those rapid motions have a significant effect. The ethidium probe dye undergoes a rapid libration of small amplitude in its binding site (Magde et al., 1983; Shibata et al., 1985; Schurr et al., 1992). Our most recent measurements on a much longer DNA indicate a relaxation time, $\tau_{\text{int}} \leq 60$ ps (unpublished results). If libration of the transition dipole is assumed to take place in an isotropic harmonic deflection potential around two orthogonal axes, then its root-mean-square (rms) amplitude around each axis is estimated to be $\sim 7^\circ$. After this rapid isotropic dye-wobble has relaxed, the emission anisotropy can be written as (Schurr and Fujimoto, 1988; Schurr et al., 1992)

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} = r_0 \sum_{n=0}^2 I_n C_n(t) F_n(t) \quad (5)$$

where $I_{\parallel}(t)$ and $I_{\perp}(t)$ are the fluorescence intensities with polarizations parallel and perpendicular, respectively, to that of an infinitesimally short exciting pulse. The initial anisotropy, $r_0 = (2/5)A_F$, contains the amplitude reduction factor $A_F = \langle P_2(\cos \beta) \rangle^2$, where β is the angle between the transition dipole and its minimum-energy orientation. The amplitude reduction factor is the fraction of the correlation function that is not relaxed by the local rotational motion of the dye molecule. For small rms amplitudes of libration in an isotropic deflection potential $A_F = 1 - 6\sigma^2$, where σ^2 is the mean squared amplitude of local rotation of the transition dipole

around any single transverse axis. The A_F factor inferred from the best-fit initial anisotropy in this work is similar to the A_F factors observed for much longer DNAs, so the amplitude of dye-wobble is evidently comparable. The quantities I_n , $C_n(t)$ and $F_n(t)$ are described below.

The I_n are geometric factors, $I_0 = 0.25(3 \cos^2 \epsilon - 1)^2$, $I_1 = 3 \sin^2 \epsilon \cos^2 \epsilon$, $I_2 = 0.75 \sin^4 \epsilon$, where ϵ is the polar angle between the local symmetry axis and the equilibrium orientation of the transition dipole (Schurr, 1984; Schurr and Fujimoto, 1988; Schurr et al., 1992).

The twisting correlation functions are given by (Allison and Schurr, 1979; Schurr, 1984)

$$C_n(t) = (N+1)^{-1} \sum_{m=1}^{N+1} \exp \left[-n^2 \sum_{\ell=2}^{N+1} d_{\ell}^2 Q_{m\ell}^2 (1 - \exp(-t/\tau_{\ell})) \right] \cdot \exp[-n^2 D_{\parallel} t] \quad (6)$$

where

$$\tau_{\ell} = \gamma / (4\alpha \sin^2[(\ell-1)\pi/(2(N+1))]) \quad (7)$$

is the relaxation time of the ℓ th torsion normal mode,

$$d_{\ell}^2 = k_B T / (4\alpha \sin^2[(1-\ell)\pi/(2(N+1))]) \quad (8)$$

is its mean-squared amplitude,

$$Q_{m\ell} = (2/(N+1))^{1/2} \cos[(m-1/2)(\ell-1)\pi/(N+1)] \quad (9)$$

$\ell \neq 1$

is the projection of the ℓ th normal mode onto the m th subunit, and

$$\gamma = k_B T / (D_{\parallel}(N+1)) \quad (10)$$

is the friction factor/bp for rotation around the symmetry axis.

The tumbling correlation functions are given by the following modification of the result of Barkley and Zimm (1979):

$$F_n(t) = \exp \left[-(6-n^2)D_{\perp}t - (6-n^2)A_n \sum_{k=1}^{k_{\max}} (1 - \exp(-t/T_k)) / (2k+1)^2 \right] \quad (11)$$

where

$$A_n = -\ln(D_n(\infty)) / \exp \left[(6-n^2) \sum_{k=1}^{k_{\max}} 1 / (2k+1)^2 \right] \quad (12)$$

The bending relaxation times are given by

$$1/T_k = \frac{k_B T P \kappa_k^4}{4\pi\eta} \left[K_0(\kappa_k R_H) + \left(\frac{\kappa_k R_H}{2} \right) K_1(\kappa_k R_H) \right], \quad (13)$$

$k = 1, 2, \dots$

where $\kappa_k = (2k+1)\pi/2L$, P is the (dynamic) persistence

length, $K_0(X)$ and $K_1(X)$ are modified Bessel functions, and $D_n(\infty)$ is defined in Eq. 15 below. These equations and the reasons for the modifications have been described previously (Schurr et al., 1993; Nuutero et al., 1994). For this work, we choose $k_{\max} = 10$. The $k = 10$ mode only contributes $\sim 1\%$ of the total bending amplitude, so our results should not be affected by excluding modes of larger k from the sum. The particular modification of the BZ result in Eq. 11 is simply designed to enforce a smooth relaxation (with the approximately valid BZ relaxation times and relative amplitudes of the longer modes) from 1.0 at zero time to the known correct result at long times (Wu et al., 1987; Schurr et al., 1992)

$$F_n(t) = D_n(\infty) \exp[-(6 - n^2)D_{\perp} t], \quad (14)$$

where

$$D_n(\infty) = (Z_n)^{1/2} \exp(Z_n/3) (\pi^{1/2}/2) \operatorname{erf}(Z_n^{1/2}) \quad (15)$$

and $Z_n = (6 - n^2)L/4P$. The important feature of $F_n(t)$ is not the dynamics of the bending deformations, which are treated only very approximately, but their amplitude reduction after the bending modes have all relaxed, which is given accurately by $D_n(\infty)$ in Eq. 15. For $P = 1500 \text{ \AA}$, $R_H = 10.7 \text{ \AA}$, $L = (48)(3.4) + 2.7 = 165.9 \text{ \AA}$, $T = 293 \text{ K}$, and $\eta = 0.01002$ poise, one finds $T_1 = 1.8 \text{ ns}$, and $D_0(\infty) = 0.90$. Hence, the bending deformations relax so quickly that their dynamics have little influence on the best fit R_H . Because of the large assumed dynamic persistence length, bending deformations contribute only a small reduction of the total amplitude.

When the experimental FPA data are fitted using Eq. 5, there are only two adjustable parameters, namely r_0 and R_H . For each trial value of R_H used in the fit, D_{\perp} and D_{\parallel} can be calculated by Eqs. 1 and 2, respectively. These quantities together with the assumed $\alpha = 5.4 \times 10^{-12} \text{ dyne cm}$ and $P = 1500 \text{ \AA}$ allow the calculation of $C_n(t)$ and $F_n(t)$ at all times.

MATERIALS AND METHODS

DNA sample and buffers

A nonself-complementary 48-bp DNA with the sequence shown below was synthesized by standard solid phase phosphite triester techniques on an Applied Biosystems 380A DNA synthesizer: 5' GCCGTCGCGACGCTCGCGGCAGGCCAGCGGTCGCGCAGCGGCTCGGC. After deprotection, the two oligonucleotides were ethanol-precipitated twice and then chromatographed on a Sephadex G-50 column in deionized water. Selected fractions were run on a denaturing 20% polyacrylamide gel containing 7 M urea. Fractions with a minimum of failure sequences were pooled and lyophilized. Approximately equal amounts of the complementary sequences were combined and dialyzed against 400 mM NaCl, 50 mM Tris, 50 mM Na₂EDTA, pH 8.5. The sample was then annealed by placing it in an Eppendorf tube, which was floated on top of 2 liters of 60°C water in a beaker. The beaker was placed in an ice bucket, covered, and left to anneal for 8 to 12 h. The annealed double-strand molecules were separated from any remaining single-strand species by chromatography using a hydroxylapatite column (Bio-Rad Laboratories, Chicago, IL) and eluted with a sodium phosphate (pH 6.8) gradient from 10 mM to 500 mM. Selected fractions were run on a nondenaturing 12% polyacrylamide gel. Those fractions that showed only double-strand DNA were pooled, lyophilized, and then dialyzed against 100 mM NaCl, 10 mM Tris, 1 mM Na₂EDTA, pH 7.5. This solution was used as the stock solution for the experiments.

Unless otherwise specified, all solutions contained 450–500 $\mu\text{g/ml}$ of the DNA, 20 mM NaCl, 10 mM Tris, 0.2 mM Na₂EDTA, pH 7.5 at 20°C plus the indicated amount of added salt. The salt concentration was varied by adding a small amount of a 1 M solution to the fluorescence sample. The concentrations of the other buffer salts were kept constant to within 2.5% by adding appropriate amounts of concentrated solutions of the buffer salts. Magnesium chloride 6-hydrate, manganous chloride, sodium chloride, and the disodium salt of EDTA were purchased from J. T. Baker, Inc. (Phillipsburg, NJ). Cesium chloride was purchased from BRL. Tetraethylammonium acetate tetrahydrate was purchased from Aldrich Chemical Co. (Milwaukee, WI). Tris base was purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were used without further purification.

Fluorescence measurements

Time-resolved fluorescence measurements of intercalated ethidium were made using the single-photon-counting system described earlier (Schurr et al., 1992). The dye laser produced pulses with a full width at half-maximum of $< 20 \text{ ps}$. For samples containing 450–500 $\mu\text{g/ml}$ DNA, ethidium bromide was added to a concentration of 1 dye molecule/1000 bp. For samples containing 50 $\mu\text{g/ml}$ DNA, ethidium bromide was added to a concentration of 1 dye molecule/500 bp. The dye was excited at 575 nm and the fluorescence was detected at 630 nm.

The experimental sum ($s(t) = I_{\parallel}(t) + 2 I_{\perp}(t)$) and difference ($d(t) = I_{\parallel}(t) - I_{\perp}(t)$) curves are convolutions of the true sum and difference responses ($S(t)$ and $D(t)$, respectively), with the instrument response function $e(t)$:

$$s(t) = \int_0^t dt' e(t') S(t - t') \quad (16)$$

$$d(t) = \int_0^t dt' e(t') D(t - t') \quad (17)$$

The data were fitted using a convolute-and-compare approach based on the Marquardt algorithm to minimize the sum of squares of residual differences. Excitation data (light scattered from a dilute solution of polystyrene latex spheres) were collected both before and after the fluorescence data. The two sets of excitation data were added together and used as the instrument function.

Errors

Several different kinds of errors are encountered in the determination of R_H , not all of which are relevant to the conclusions of the present paper. 1) Statistical errors (σ_{RH}) that arise in fitting a single data set are obtained by standard linearized error-propagation rules. The relative single-data-set statistical errors (σ_{RH}/R_H) typically lie in the range 0.1 to 0.3%. Such statistical errors are much smaller than the variations in best-fit R_H from one data set to the next, and consequently are discounted as meaningless. 2) Several (four in this case) different data sets acquired in succession are fitted separately, whereupon the average value and standard deviation (σ_{RH}) of the resulting best-fit R_H values are calculated in the usual way. The value of σ_{RH} computed in this way is referred to as the reproducibility error. Typically, the relative reproducibility error (σ_{RH}/R_H) of these measurements is $\sim 1\%$. This is the most meaningful estimate of the statistical precision of the present measurements. 3) Another kind of reproducibility error is obtained by comparing best-fit R_H values from measurements on one day with those obtained from measurements on the same sample a week or two later. These time-lagged reproducibility errors are comparable to those in (2) above. This indicates that any systematic effects of temporal drift in instrument performance or in sample quality on R_H are practically negligible. 4) Yet another kind of reproducibility error is obtained by comparing best-fit R_H values for one DNA sample with those for a separately synthesized and purified DNA sample. All of the measurements described here pertain to the same DNA sample; consequently, such sample-to-sample reproducibility

errors do not affect the precision of the present results. However, observations on other samples indicate that systematic sample-to-sample variations might occur. Possible origins of such variations will be discussed in subsequent work.

The extremely high precision implied by the 1% reproducibility errors (for this single sample) arises in part because R_H varies as the square root of the pertinent rotational relaxation time that is directly monitored by these experiments. Relative errors in the latter quantity are actually $\sim 2\%$.

Systematic errors in R_H arise from the sample-to-sample variability noted above, and also from any errors in the assumed model and its input parameters, such as the rise/bp h , the torsion constant α , and dynamic persistence length P . Such systematic errors may well affect the absolute values of R_H , but do not affect the relative variations of R_H with concentration of different ions, which are the main objective of this work. In fact, the relative variation of R_H with Mg^{2+} concentration was measured three different times on two different DNA samples and was reproduced to within 1% each time. Thus, the observed 15% relative variation of R_H with Mn^{2+} far exceeds the relative reproducibility errors of these measurements.

RESULTS

The R_H of our DNA was measured in 100 mM NaCl, 10 mM Tris, 1 mM Na_2EDTA , and also in 20 mM NaCl, 10 mM Tris, 0.2 mM Na_2EDTA (pH 7.5 and 20° for both samples). The results were $R_H = 10.4 \text{ \AA}$ for the 100 mM NaCl solution and $R_H = 10.7 \text{ \AA}$ for the 20 mM NaCl solution. Before measuring the effect of a particular cation on the R_H of a given sample, a measurement was always made in 20 mM NaCl, 10 mM Tris, 0.2 mM Na_2EDTA pH 7.5 at 20°C . A total of five such determinations, each consisting of 4–12 individual measurements, were made on different DNA samples under these conditions. The values of R_H obtained were all between 10.6 and 10.8 \AA . Of the more than 100 individual fits of difference data that were performed during this study, fewer than 10% had reduced $\chi^2 > 1.10$, and none had reduced $\chi^2 > 1.20$.

The results of our FPA measurements are summarized in Fig. 1. Among the monovalent cations examined, Na^+ has the smallest effect on R_H , causing only a slight decrease. Cs^+ causes a slightly larger decrease in R_H amounting to $\sim 0.5 \text{ \AA}$

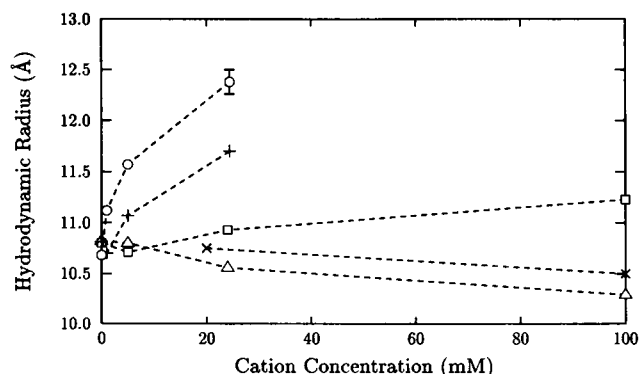


FIGURE 1 R_H as a function of salt concentration for five different salts. Δ , CsCl ; \times , NaCl ; \square , tetraethylammonium acetate; $+$, MgCl_2 ; \circ , MnCl_2 . With the exception of the NaCl sample, all samples contained 450–500 $\mu\text{g/ml}$ of the DNA, 20 mM NaCl, 10 mM Tris, 0.2 mM Na_2EDTA , pH 7.5 at 20°C in addition to the indicated amount of salt. The NaCl samples contained 10 mM Tris, 0.2 mM Na_2EDTA , pH 7.5 at 20°C plus either 20 mM or 100 mM NaCl. All the error bars are approximately the same size, so for clarity only one is drawn. The error range represents $\pm 1 \text{ SD}$.

at 100 mM concentration. Tetraethylammonium acetate causes an increase in R_H by $\sim 0.5 \text{ \AA}$ at 100 mM concentration. Inasmuch as the relative standard deviations in R_H measurements are $\sim 1\%$, these $\sim 5\%$ relative changes in R_H are statistically quite significant. In contrast, the two divalent cations both cause substantially larger increases in R_H , by $\sim 1.0 \text{ \AA}$ in the case of Mg^{2+} and by $\sim 1.6 \text{ \AA}$ in the case of Mn^{2+} .

The increase in apparent R_H caused by Mg^{2+} and Mn^{2+} does not appear to be caused by aggregation. This was tested by measuring the apparent R_H in solutions containing 25 mM of the chloride salt of either divalent cation with each of two different DNA concentrations, namely 500 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$. Despite the 10-fold change in DNA concentration, there is no significant change in the measured R_H for either MgCl_2 or MnCl_2 . Given that the extent of any aggregation and its consequent effect on R_H should be very sensitive to DNA concentration, we conclude that aggregation cannot be the cause of the substantial increases in R_H with Mg^{2+} or Mn^{2+} concentration.

The increase in R_H does not appear to be caused by any sort of damage to the DNA. In such a case, the R_H of a solution with a low Mg^{2+} or Mn^{2+} concentration would be expected to increase slowly over time and approach that of a solution with a higher concentration of the divalent cation at an earlier time. We prepared two samples containing 500 $\mu\text{g/ml}$ of DNA, one with 1 mM MgCl_2 and one with 1 mM MnCl_2 . Two sets of measurements were made on each sample 18 days apart. The samples were stored at 5°C between measurements. The initial values of R_H were $10.7 \pm 0.2 \text{ \AA}$ and $11.0 \pm 0.3 \text{ \AA}$ for 1 mM MgCl_2 and 1 mM MnCl_2 , respectively. After 18 days, the measured values of R_H were $10.8 \pm 0.1 \text{ \AA}$ and $11.1 \pm 0.1 \text{ \AA}$, respectively (error ranges are $\pm 2 \text{ SD}$). After these measurements were made, the original solutions with the highest concentrations (24.4 mM) of these two cations were remeasured. After 28 days the R_H value of the DNA sample with 24.4 mM MnCl_2 had changed from $12.4 \pm 0.2 \text{ \AA}$ to $12.0 \pm 0.5 \text{ \AA}$, and after 23 days the R_H value of the DNA sample with 24.4 mM MgCl_2 had changed from $11.7 \pm 0.3 \text{ \AA}$ to $11.7 \pm 0.5 \text{ \AA}$. We conclude that these samples are stable over periods of 18 to 28 days at either low or high concentrations of divalent cations. We conclude that the increases in R_H do not arise from damage induced in the DNA by either Mg^{2+} or Mn^{2+} .

DISCUSSION

If the counterions contributed to R_H primarily via fluctuating interionic forces, one would expect R_H to depend strongly on counterion concentration over the range 0.01 to 0.1 M, and all counterions with the same charge should alter R_H in the same direction. In fact, there is very little change in R_H with NaCl concentration over this range, and the changes induced by Cs^+ and $(\text{CH}_3\text{CH}_2)_4\text{N}^+$ are of opposite signs. Thus, it seems most unlikely that electrolyte friction contributes significantly to the observed changes in R_H .

If these cations affect R_H by altering the hydration layer, then one might expect some correlation between the order of

their effects on R_H and the order of their effects on the measured hydration forces. In fact, the order of effects of the different monovalent cations on R_H , namely $R_H(\text{Cs}^+) < R_H(\text{Na}^+) < R_H((\text{CH}_3\text{CH}_2)_4\text{N}^+)$, does not exactly match the order of their effects on hydration forces ($F(\text{Na}^+) < F(\text{Cs}^+) < F((\text{CH}_3\text{CH}_2)_4\text{N}^+)$), but two of the three possible pairs are in the correct order, and the difference between $R_H(\text{Na}^+)$ and $R_H(\text{Cs}^+)$ is rather slight, so they are close to the correct order. The observation that $R_H(\text{Mn}^{2+}) > R_H(\text{Mg}^{2+})$ matches the order of their interhelix repulsions at very short distances. It thus appears that there might be some positive correlation between the effects of different ions on R_H and their effects on short-range interhelix repulsions. The minor discrepancy between the effects of Na^+ and Cs^+ on R_H and their effects on the hydration forces might be caused by the fact that measurements of R_H are performed on an isolated DNA whose hydration layers might differ somewhat from those of the DNAs in hexagonal arrays. The difference in behavior between the two divalent cations is probably caused by their different interactions with DNA. Mn^{2+} binds more strongly to the bases, whereas Mg^{2+} binds more strongly to the phosphates. If the different modes of binding affect the hydration layer of the DNA differently, this could perhaps account for the data.

The alternative possibility that the different cations affect R_H by variously altering the DNA structure cannot be ruled out. In such a case, the rather large changes in R_H induced by the divalent cations would very likely have to originate from induced bends, given that otherwise rather substantial alterations of the helix parameters would be required for which there is presently no direct crystallographic evidence.

Although these data indicate surprisingly large effects of certain ions on R_H , they do not establish whether the effect is caused by altered hydration or by the induction or stabilization of bends in the DNA, which would also increase both R_H and interhelix repulsions.

This work was supported in part by Grant P01-GM32681 from the National Institutes of Health.

REFERENCES

- Allison, S. A., and J. M. Schurr. 1979. Torsion dynamics and depolarization of fluorescence of linear macromolecules. I. Theory and application to DNA. *Chem. Phys.* 41:35–59.
- Allison, S. A., R. H. Austin, and M. E. Hogan. 1989. Bending and twisting dynamics of short linear DNAs. Analysis of the triplet anisotropy decay of a 209 base pair fragment by Brownian simulation. *J. Chem. Phys.* 90:3843–3854.
- Barkley, M. D., and B. H. Zimm. 1979. Theory of twisting and bending of chain macromolecules: analysis of the fluorescence depolarization of DNA. *J. Chem. Phys.* 70:2991–3007.
- Duguid, J., V. A. Bloomfield, J. Benevides, and G. J. Thomas, Jr. 1993. Raman spectroscopy of DNA-metal complexes. I. Interactions and conformational effects of the divalent cations: Mg, Ca, Sr, Ba, Mn, Co, Ni, Cu, Pd, and Cd. *Biophys. J.* 65:1916–1928.
- Eimer, W., J. R. Williamson, S. G. Boxer, and R. Pecora. 1990. Characterization of overall and internal dynamics of short oligonucleotides by depolarized dynamic light scattering and NMR relaxation measurements. *Biochemistry* 29:799–811.
- Fujimoto, B. S., and J. M. Schurr. 1990. Dependence of the torsional rigidity of DNA on base composition. *Nature* 344:175–178.
- Hogan, M. E., N. Dattagupta, and D. M. Crothers. 1979. Transient electric dichroism studies of the structure of the DNA complex with intercalated drugs. *Biochemistry* 18:280–288.
- Hustedt, E. J., A. Spaltenstein, J. J. Kirchner, P. B. Hopkins, and B. H. Robinson. 1993. Motions of short DNA duplexes: an analysis of DNA dynamics using an EPR-active probe. *Biochemistry* 32:1774–1787.
- Leikin, S., D. C. Rau, and V. A. Parsegian. 1991. Measured entropy and enthalpy of hydration as a function of distance between DNA double helices. *Physiol. Rev. A.* 44:5272–5278.
- Magde, D., M. Zappala, W. H. Knox, and T. M. Nordlund. 1983. Picosecond fluorescence anisotropy decay in the ethidium/DNA complex. *J. Phys. Chem.* 87:3286–3288.
- Nuutero, S., B. S. Fujimoto, P. F. Flynn, B. R. Reid, N. S. Ribeiro, and J. M. Schurr. 1994. The amplitude of local angular motion of purines in DNA in solution. *Biopolymers* 34:463–480.
- Podgornik, R., D. C. Rau, and V. A. Parsegian. 1989. The action of interhelical forces on the organization of DNA double helices: fluctuation-enhanced decay of electrostatic double-layer and hydration forces. *Macromolecules* 22:1780–1786.
- Rau, D. C., and V. A. Parsegian. 1984. Measurement of the repulsive force between polyelectrolyte molecules in ionic solution: hydration forces between parallel DNA double helices. *Proc. Natl. Acad. Sci. USA.* 81:2621–2625.
- Rau, D. C., and V. A. Parsegian. 1992. Direct measurement of temperature-dependent solvation between DNA double helices. *Biophys. J.* 61:260–271.
- Schmitz, K. S. 1993. Macroions in Solution and Colloidal Suspension. VCH Publishers Inc., New York, 154–157.
- Schurr, J. M. 1980. A theory of electrolyte friction on translating polyelectrolytes. *Chem. Phys.* 45:119–132.
- Schurr, J. M. 1984. Rotational diffusion of deformable macromolecules with mean local cylindrical symmetry. *Chem. Phys.* 84:71–96.
- Schurr, J. M., and B. S. Fujimoto. 1988. The amplitude of local angular motions of intercalated dyes and bases in DNA. *Biopolymers* 27:1543–1569.
- Schurr, J. M., and K. S. Schmitz. 1986. Dynamic light scattering studies of biopolymers: effects of charge, shape and flexibility. *Annu. Rev. Phys. Chem.* 37:271–305.
- Schurr, J. M., B. S. Fujimoto, and S. Nuutero. 1994. Theory of relaxation of quadrupolar nuclei and deformable molecules in isotropic solutions. Application to DNA, *J. Magn. Reson. Ser. A.* 106:1–22.
- Schurr, J. M., B. S. Fujimoto, P. Wu, and L. Song. 1992. Fluorescence studies of nucleic acids: dynamics, rigidities and structures. In *Topics in Fluorescence Spectroscopy*, Vol. 3: Biochemical Applications. J. R. Lakowicz, editor. Plenum Press, New York. 137–229.
- Shibata, J. H., B. S. Fujimoto, and J. M. Schurr. 1985. Rotational dynamics of DNA from 10^{-10} to 10^{-5} seconds: comparison of theory with optical experiments. *Biopolymers* 24:1909–1930.
- Smith, S. B., L. Finzi, and D. Bustamante. 1992. Direct mechanical measurements of the elasticity of single DNA molecules by using magnetic beads. *Science* 258:1122–1126.
- Song, L., and J. M. Schurr. 1990. Dynamic bending rigidity of DNA. *Biopolymers* 30:229–237.
- Tirado, M. M., and J. Garcia de la Torre. 1980. Rotational dynamics of rigid, symmetric top macromolecules. Application to circular cylinders. *J. Chem. Phys.* 73:1986–1993.
- Wu, P.-G., B. S. Fujimoto, and J. M. Schurr. 1987. Time-resolved fluorescence polarization anisotropy of short restriction fragments: the friction factor for rotation of DNA about its symmetry axis. *Biopolymers* 26:1463–1488.
- Wu, P.-G., B. S. Fujimoto, L. Song, and J. M. Schurr. 1991. Effect of ethidium on the torsion constants of linear and supercoiled DNAs. *Biophys. Chem.* 41:547–565.