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Sodium-23 and lithium-7 NMR spin–lattice relaxation measurements in the study of intercalation in DNA

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Abstract

Sodium-23 spin–lattice relaxation rate (the reciprocal relaxation time) measurements have been used to study the intercalation of 9-aminoacridine in calf thymus DNA. The results are analyzed by a two state model based on the counterion condensation theory and a theory for the quadrupolar relaxation of counterions in polyelectrolyte solutions. It is shown that change of the solvent from H_2O to D_2O has a negligible effect on the intercalation process. Furthermore, an attempt is made to analyze the dependence of the ^7Li spin–lattice relation rate on intercalation of 9-aminoacridine in LiDNA. It is shown that both quadrupolar and dipolar mechanisms contribute to the bound ^7Li relation rate, and that both these contributions are reduced upon intercalation of 9-aminoacridine.

Keywords: Intercalation; 9-Aminoacridine; DNA; ^{23}Na; ^7Li, NMR spin–lattice relaxation

1. Introduction

A large variety of organic molecules containing a planar part bind strongly to DNA by inserting the planar part in between adjacent stacked base pairs in the double helix structure of DNA. This process is called intercalation, and was first described by Lerman [1].

The classic intercalation process results in an extension of the helix, whereby the average phosphate to phosphate distance is increased. This results in an overall decrease of the DNA charge density. Furthermore, positive charges on the intercalator neutralize some of the anionic charge of DNA which also reduces the charge density.

In solutions of DNA and a simple counterion (e.g. ^{23}Na and ^7Li) some of the counterions are associated (called bound) to DNA, whereby the helix structure is stabilized. It has been shown that the NMR relaxation rates of $^{23}Na^+$ counterions bound to DNA are significantly enhanced compared to the relaxation rates of $^{23}Na^+$ in solutions without DNA [2,3]. This is due to effects produced by the electric field of the DNA polyion. These effects include motional effects and effects on the local field gradients around the quadrupolar relaxing $^{23}Na^+$. Intercalation reduces the bound

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relaxation rate due to the reduction of the charge density, which reduces the average field produced by the DNA polyion. Furthermore, the charge density reduction results in a release of bound Na^+ to the bulk region of the solution characterized by a smaller ^{23}Na relaxation rate. Consequently, the intercalation process strongly reduces the average relaxation rate of $^{23}Na^+$ in DNA solutions.

This effect was first observed by Mariam et al. [4]. They observed a dramatic decrease of the ^{23}Na line width upon addition of ethidium bromide to NaDNA. Recently, we introduced ^{23}Na spin–lattice relaxation rate measurements in the study of intercalation in NaDNA [5]. It was shown that the ^{23}Na spin–lattice relaxation rate (R_1) is a sensitive parameter for the investigation of intercalation. The intercalation of a series of derivatives of 9-aminoacridine was studied by such measurements [5,6]. The observed decrease of R_1 upon intercalation was analyzed by a simple model [5] based on a combined use of the counterion condensation theory [7] and the theory for the quadrupolar relaxation of counterions in polyelectrolyte solutions, introduced by Van der Klink et al. [8].

In this study we have investigated the intercalation of 9-aminoacridine in solutions of calf thymus NaDNA in both H_2O and D_2O by ^{23}Na-R_1 measurements in order to obtain information on the effect of solvent change on the intercalation process. For the analysis of the ^{23}Na-R_1 studies we have introduced a revised model.

The applicability of ^{23}Na-R_1 measurements as a method to study the binding of intercalators to DNA has urged us to measure the spin–lattice relaxation rates of other types of monovalent counterions. In this study we report the effect of intercalation of 9-aminoacridine in LiDNA on the ^7Li spin–lattice relaxation rate. The relaxation of $^7Li^+$ is caused by both quadrupolar and dipolar interactions [9,10]. The separation of the ^1H-^7Li dipolar contribution from other relaxation contributions is performed by measuring the relaxation rate in both H_2O and D_2O as the dipolar contribution from the ^1H-^7Li interaction is replaced by the much less effective ^2D-^7Li dipolar interaction in D_2O. Consequently, all ^7Li-R_1 measurements in this study have been performed in both H_2O and D_2O.

2. Experimental

Four types of 10^{-3} *M* PIPES-buffers were used in this study: a NaPIPES-buffer in both H_2O and D_2O (abbreviated NaPIPES(H_2O) and NaPIPES (D_2O)) and LiPIPES buffers in the same solvents denoted likewise. The buffers contained 10^{-4} *M* EDTA and were adjusted to neutral by addition of either NaOH/NaOD or LiOH/LiOD. The concentration of Li^+ and Na^+ in the buffers has been measured to be approximately 2 m*M*.

Calf thymus NaDNA (Sigma Type 1) was first dissolved in NaPIPES(H_2O) (3.5 mg/ml) by magnetic stirring at 0–4°C for 48 h, and then filtered through 0.45 μm Sartorius cellulose acetate membranes. Portions of 10 ml solution were sonicated for 10×10 s at 0–4°C. The average molecular weight of the sonicated DNA has been estimated to $(1\text{-}2) \times 10^6$ Da by gelelectrophoresis. The DNA was dialyzed several times against NaPIPES(H_2O) at 4°C followed by precipitation with ethanol at −10°C. The DNA was then washed in ethanol and air-dried overnight. The dry DNA samples were then redissolved for 48 h at 4°C in either NaPIPES(H_2O) or NaPIPES(D_2O) followed by filtration through 0.45 μm Sartorius cellulose acetate membranes to obtain both the normal NaDNA and NaDNA samples in the deuterated solvent from the same preparation.

LiDNA was prepared from sonicated NaDNA by dialysing against LiPIPES(H_2O). After the dialysis, the LiDNA was ethanol precipitated, washed and redissolved in either LiPIPES(H_2O) or LiPIPES(D_2O) by a procedure similar to the one used for NaDNA.

DNA concentrations, [P], expressed in terms of nucleotide equivalents per liter were determined spectrophotometrically ($\epsilon_{260} = 6600\ M^{-1}\ cm^{-1}$) on a Shimadzu UV-160 spectrophotometer. All the DNA samples displayed a A_{260}/A_{280} ratio between 1.87 and 1.90. Phenol extraction followed by ether extraction and ethanol precipitation performed on a NaDNA sample to remove traces of protein before dialysis did not change this ratio.

Neither had it any effect on the results of the types of titrations performed. The hyperchromicity of all the DNA-samples was measured by alkaline denaturation and shown to be in an acceptable range (27–30%).

The sodium concentration, $[Na^+]$, was measured from the NMR spectrum by comparing with the signal from a calibrated capillary reference containing the ion and a shift reagent (240 mM NaCl and 60 mM $DyCl_3$). Prior to measurements of the signal intensity, the DNA samples were diluted 2–3 times with 100 mM $MgSO_4$ to exclude Na^+ from the bound state. The signal/noise (S/N) ratio of the signal from the DNA sample was thereby improved considerably. Careful calibration of the intensity measurements showed that the uncertainties on the $[Na^+]$ determinations were less than 5%. A similar method has been used to determine $[Li^+]$ in LiDNA solutions by use of ^7Li NMR spectra. The uncertainties of the $[Li^+]$ determinations are generally higher than the uncertainty on the $[Na^+]$ determinations, but are less than 8%.

9-Aminoacridine hydrochloride (Aldrich) was used without further purification. Stock solutions were prepared by dissolving 9-aminoacridine in either H_2O or D_2O. The concentrations were determined by UV spectroscopy ($\epsilon_{401} = 10100\ cm^{-1}\ M^{-1}$).

All DNA samples for NMR were made 4 mM by diluting with either distilled H_2O (DNA in H_2O-buffer) or D_2O (DNA in D_2O-buffer). The intercalator titrations were performed by adding successive aliquots of the 9-aminoacridine stock solutions in either H_2O or D_2O directly to the DNA sample in the NMR tube. The concentration of the 9-aminoacridine solution was adjusted so that the sample volume increased by less than 10% during the titrations. It has been shown that increasing the sample volume by 20% with water does not change the spin–lattice relaxation time T_1 significantly [5]. Titrations with the counterions (in the form of NaCl or LiCl solutions) were performed by a similar procedure.

All ^7Li and ^{23}Na NMR measurements were performed in 10 mm tubes on a Bruker AC-250 spectrometer. The temperature was 300 K unless otherwise stated. The inversion recovery (180°-τ-90°-aqc) pulse sequence was used for the T_1 measurements with 15 different values of τ for each experiment. Each ^{23}Na spectrum was the result of the accumulation of 320 scans, while ^7Li spectra were obtained from only 1 scan. The use of only 1 scan for ^7Li spectra in the inversion recovery experiments was necessary due to the slow relaxation of ^7Li and proper consideration of the sample stability. The T_1 values were obtained by a three-parameter linear least-squares fitting procedure. Each T_1 value is the average of at least 2 measurements.

3. Theoretical background

In this work we have used Manning's counterion condensation theory [7] in order to interpret the experimental results. Although approximative, this theory has shown to be quite successful in rationalizing the distribution of monovalent counterions in DNA solutions [2,11,12]. The counterion distribution is described by a two site model in which counterions are assumed to be either kept close to the polyion by a delocalized binding (denoted bound state), or in a free state unperturbed by the polyion. The relaxation of the counterions in each state is assumed to be described by a single relaxation rate. If the exchange rate between the sites is sufficiently fast, the observed relaxation rate is given by:

$$R_1 = R_f + (R_b - R_f)([P]/[M^+])a \qquad (1)$$

where R_b and R_f represents the relaxation rate in the bound and free state. Manning's [7] degree of condensation given as bound counterion charge per polyion charge is denoted by a, while [P] and $[M^+]$ are the concentrations of DNA and counterions respectively.

An important result of the condensation theory is that the degree of condensation of the counterions is independent of the concentration of the ion at concentrations lower than approximately 1 M, but a function of the charge density, λ, of the polyion. For a monovalent counterion a is given by:

$$a = 1 - 1/\lambda \qquad (2)$$

where λ is given by:

$$\lambda = (\alpha e^2)/(\epsilon bkT) \quad (3)$$

ϵ is the dielectricity constant of the solvent, k the Boltzmann constant, T the absolute temperature, e the elementary charge, α the degree of ionization, and b is the average intercharge distance in DNA.

The classic intercalation process is assumed to reduce the charge density by two effects. Firstly, intercalation results in an extension of the helix with an amount x. Secondly, the positive charges on the intercalator will reduce the overall anionic charge of the polyion. These two effects combined produce a significant decrease of the charge density of the polyion upon intercalation. If we assume a linear decrease of the anionic charge of the polyion and a linear increase of the helix extension upon addition of intercalator, the intercalation will reduce λ according to the expression:

$$\lambda = \frac{e^2(\alpha_0 - rqp_i)}{\epsilon kT(b_0 + rxp_i)} \quad (4)$$

q is the number of neutralized anionic charges on DNA per bound intercalator, α_0 and b_0 are the degree of ionization and the average intercharge distance in DNA before addition of intercalator, respectively, $p_i = [I]_b/[I]$ is the fraction of bound intercalator, [I] is the total concentration of intercalator and $[I]_b$ the concentration of intercalated intercalators and $r = [I]/[P]$.

The smaller value of λ will reduce the observed relaxation rate R_1 as a consequence of two effects. Firstly, the degree of condensation, a, is reduced according to eq. (2), followed by a release of counter ions from the bound to the free state. Secondly, the relaxation rate, R_b, for the remaining bound counterions will decrease due to the smaller average field on the DNA strain after intercalation. Both of these effects will reduce the the relaxation rate, R_1, but the latter has been shown to be the most important one [5].

To describe the dependence of the bound relaxation rate upon intercalation is a formidable task, and no satisfactory theory exist at the moment. Correspondingly we use as an approximation the only theory with the necessary simplicity to be of practical use. This is the relaxation theory for quadrupolar relaxed counterions in polyelectrolyte solutions introduced by van der Klink et al. [8]. Although approximative [13–15], it will be shown that adopting results from this theory parameters describing the intercalation can be obtained. A discussion of the interpretation of these parameters and the limitation in the use of the whole model will be given below.

Van der Klink et al.'s theory [8] yields the following expression for the bound relaxation rate as a function of the charge density of the polyion:

$$R_b = C\lambda^2 \quad (5)$$

where C is a constant independent of the intercalation, and where λ is given by eq. (4). Combination of eqs. (1), (2) and (5) yields the following approximative expression for the observed quadrupolar relaxation rate of the bound counterions in DNA solutions

$$R_1 = R_f + (C\lambda^2 - R_f)([P]/[M^+])(1 - 1/\lambda) \quad (6)$$

If the relaxation in the bound state is not caused exclusively by the quadrupolar interactions like in the case of ^{7}Li, the following expression can be used to calculate the relaxation rate of the bound state:

$$R_b = R_f + (R_1 - R_f)([M^+]/[P])/(1 - 1/\lambda) \quad (7)$$

In this equation it has been assumed that the counterion distribution can be described by the condensation theory.

4. Results and discussion

4.1 Exchange rate and motional conditions in NaDNA solutions

The conditions used in this work to study the relaxation behaviour of $^{23}Na^+$ in NaDNA solutions yield ^{23}Na NMR signals in both NaPIPES (H_2O) and NaPIPES(D_2O) that deviates from Lorentzian line shapes, but only to a minor extent. This indicates a deviation from the extreme narrowing conditions [16]. The relaxation in the free

state is assumed to be identical to the relaxation of $^{23}Na^+$ in a solution of a simple electrolyte, in which extreme narrowing conditions are fulfilled. Deviation from Lorentzian line shapes in DNA is therefore caused by non extreme narrowing conditions in the bound state. The spin–lattice relaxation measurements do not exhibit any deviation from exponential relaxations, as the inversion recovery data can be fitted nicely to single exponential decays. These observations imply that the relaxation measurements on $^{23}Na^+$ in this work must have been performed under conditions outside, but close to the extreme narrowing limit probably in the intermediate narrowing range [17–19].

In order to examine the exchange rate, we have measured R_1 for $^{23}Na^+$ as a function of the temperature in the range 280–310 K in both NaDNA in NaPIPES(H_2O) and NaPIPES(D_2O). Plots of $\ln(R_1)$ as a function of the inverse temperature $1/T$ (Arrhenius plots) are linear with positive slopes (data not shown) in agreement with fast exchange between the two sites [20].

4.2 Counterion concentration dependency of R_1

Prior to intercalation experiments on NaDNA we have measured the observed relaxation rate of $^{23}Na^+$ in solutions of NaDNA in both NaPIPES (H_2O) and NaPIPES(D_2O) as a function of the Na^+ concentration, $[Na^+]$. The result is represented in Fig. 1, where the observed spin–lattice relaxation rate is shown as a function of $[P]/[Na^+]$. A linear dependency between R_1 and $[P]/[Na^+]$ is expected on the basis of Manning's condensation theory [7]. This follows from the assumptions that R_b, R_f and the degree of condensation, a, are independent of $[Na^+]$ (eq. 2). Anderson et al. [2] have verified these assumptions at low ionic strengths.

In Fig. 1 approximate linear trends call be observed for both types of solutions over the entire range of $[P]/[Na^+]$ ratios examined. A slight deviation from linearity may be inferred in both cases. Such deviations are often observed [11,12] and are probably caused by small deviations from extreme narrowing conditions in both solutions [12] or a slight protein contamination of the DNA

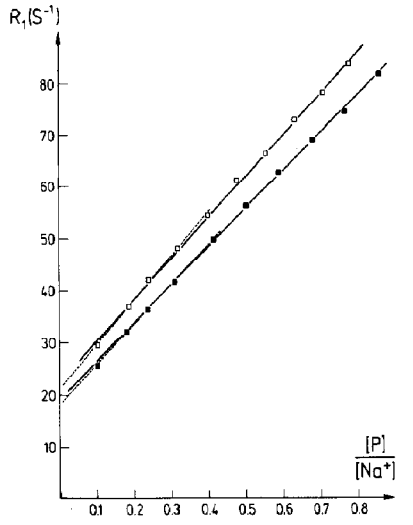

Fig. 1. The observed spin–lattice relaxation rate of $^{23}Na^+$ at 300 K in solutions of NaDNA in NaPIPES(H_2O) (■, [P] = 4.0 mM and $[Na^+]^0/[P] = 1.2$) and NaPIPES(D_2O) (□, [P] = 4.0 mM and $[Na^+]^0/[P] = 1.3$) as a function of the $[P]/[Na^+]$ ratio. The dotted lines represent the best linear lines through the experimental points below $[P]/[Na^+] = 0.45$.

[11]. The uncertainty on $[P]/[Na^+]$ is considerable at the high values. This makes it even more unjustified to conclude that the small deviations are due to breakdown of the applicability of the condensation theory.

From the ordinate intercepts in Fig. 1 R_f can be obtained. Likewise, estimates for R_b can be calculated from the slopes by use of the R_f values and Manning's estimate for the degree of condensation ($a = 0.76$) (eq. 1). These estimates have been calculated for both solutions by performing linear least squares fitting of the data points below $[P]/[Na^+] = 0.45$. The results are shown in Table 1 together with the ratio of R_b and R_f.

Table 1

Sodium-23 ion relaxation rates measured at 300 K

Buffer	R_b [a] (s^{-1})	R_f [a] (s^{-1})	R_b/R_f [a]	R_f [b] (s^{-1})
NaPIPES(H_2O)	118	18.0	6.6	16.8
NaPIPES(D_2O)	131	21.3	6.2	20.5

[a] Obtained from the Na^+ titrations of NaDNA represented in Fig. 1.

[b] Measured on Na^+ in buffers without DNA.

The relaxation rate of $^{23}Na^+$ at 300 K is seen to increase 6–7 times from the free to the bound state (Table 1). Also included in Table 1 are the spin–lattice relaxation rates of $^{23}Na^+$ in the buffers without DNA. The R_f values obtained from the ordinate intercepts in Fig. 1 are slightly larger than the relaxation rates measured on the buffers. Minor differences are often [2,11,12], but not always [21] observed, when R_f in DNA solutions is estimated from R_1–[P]/[Na] plots. We have chosen to use the R_f values measured in the buffers without DNA in our calculations, since this value is supposed to be the most accurate determined one. The results of the performed calculations have a negligible dependence of which R_f estimate is used.

4.3 Intercalation in NaDNA

The spin–lattice relaxation rate has been measured as a function of the amount of 9-aminoacridine added to a solution of NaDNA in NaPIPES(H_2O). The results are shown in Fig. 2. Precipitation occurs at intercalator/DNA nucleotide ratios (r) higher than approximately 0.25.

A non-linear decrease of R_1 as a function of r is observed and a least squares fit of the data to the eq. (6) with λ given by eq. (4) has been performed. The parameters C and x were allowed to vary, while all other parameters were kept constant in the iterations.

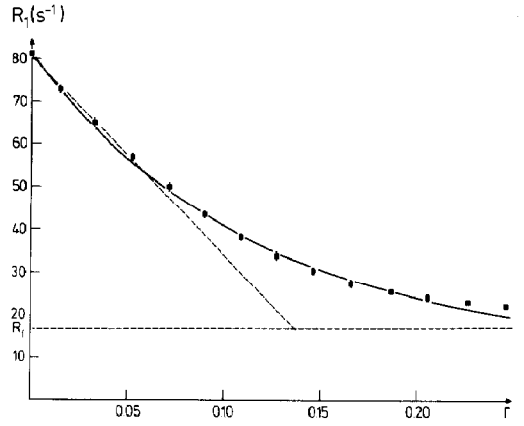

Fig. 2. The observed spin–lattice relaxation rate of $^{23}Na^+$ at 300 K in a solution of NaDNA in NaPIPES(H_2O) ([P] = 4.0 mM, [Na^+]/[P] = 1.2) as a function of r, the ratio of 9-aminoacridine to DNA nucleotide equivalents. The solid line represents the result of fitting the experimental points below 0.21 to eq. (6). The dotted line represents the free relaxation rate R_F (16.8 s^{-1}) measured on $^{23}Na^+$ in NaPIPES(H_2O) without DNA.

9-Aminoacridine is known to be a strong intercalator with a binding constant above 10^4 M^{-1} at low ionic strength [5,6]. By use of Scatchard's equation corrected for the neighbour exclusion principle [22] the ratio of bound to free intercalator molecules can be estimated. This has been done, and it was found that the ratio was greater than 50, even up to $r = 0.25$. Thus, we assumed that all the added intercalators actually do intercalate properly ($p_i = 1$). Since 9-aminoacridine is used in the form of the hydrochloride $q = 1$. The native B form of DNA is assumed to be dominant in aqueous solutions at low ionic strength. This implies that the average intercharge distance, b_0, is 1.7 Å. DNA is fully neutralized at pH = 7.0 and the degree of ionization α_0 is therefore equal to 1. Titrations were carried out at 300 K. At this temperature the relative permittivity of H_2O is 77.82. [P] and [Na^+] were determined as described above, giving a [P]/[Na^+] ratio of 0.83.

The fitting procedure yielded the estimates for C and x shown in Table 2. The last two points were omitted in the final fitting as this improved the fit considerably. The solid line in Fig. 2 representing the result of the fitting shows that the titration points in the range 0–0.21 can be fitted reasonably to eq. (6).

The reasonable fit should be interpreted with great care due to the weakness of the relaxation theory used. In particular it must be noted that the succes of eq. (6) in explaining the decrease in R_1 shows that eq. (5) when combined with other elements of the model seems to be a fairly good approximative description of the relationship between the bound relaxation rate and the charge density under these circumstances. This seems to be in accord with the results of Van Dijk et al. [23] in a study on sodium ion and solvent relaxation in aqueous solution of DNA.

These considerations are important for the interpretation of the obtained estimates for C and x. The obtained C value was used to calculate the value of the bound relaxation rate before addition

Table 2

Estimates for x, C and R_b^0 obtained from fitting of the results of the 9-aminoacridine titrations of NaDNA to eq.(6)

Buffer	r range	x (Å)	C	R_b^0 (s^{-1})
NaPIPES(H_2O)	0–0.25	4.4	6.7	118
NaPIPES(H_2O)	0–0.05	3.8	6.5	115
NaPIPES(D_2O)	0–0.05	3.9	7.2	128

of intercalator, R_b^0, from eq. (5) with λ given by eq. (3). The calculated value of R_b^0 is given in Table 2. Excellent agreement exists between the estimate for R_b^0 obtained from the 9-aminoacridine titration and the value for R_b (115 s^{-1}) obtained from the Na^+ titration of NaDNA in NaPIPES(H_2O) in Fig. 1. This shows that C has a negligible dependence on the intercalation as assumed (see theoretical background).

Although introduced in the model as a helix extension in the expression for the charge density the obtained estimate for x should be interpreted with great care. From the x value obtained, it is tempting to take x as the actual size of the extension induced by the intercalator as the obtained value of 4.4 Å seems reasonable, when compared to estimates obtained by other methods (2.0–5.0Å [24–27]). This is problematic, due to the lack of sound theory relating the effects of intercalation to the reduction of the bound relaxation rate discussed above. We shall therefore in this context use x only as an empirical parameter describing the effects of intercalation, and not particular relate it to the induced helix extension. In this framework the x-values can be used qualitatively to compare intercalation of different intercalators under the same experimental conditions or intercalation of the same intercalator under different experimental conditions.

4.4 Solvents effects on the ^{23}Na-NMR measurements on NaDNA

The intercalation of 9-aminoacridine in DNA in both NaPIPES(H_2O) and NaPIPES(D_2O) has been followed by ^{23}Na-R_1 measurements. The curves have been fitted to eq. (6) using the same values of the parameters as described above, but with the exceptions that ϵ_r was set to the value of the relative permittivity of D_2O at 300 K (77.53) and R_f to 20.5 s^{-1} (Table 1) for NaDNA in NaPIPES(D_2O).

The solid lines in Fig. 3 represent the result of these fittings, and the obtained values of x, C and R_b^0 are given in Table 2. As seen in Fig. 3, there is good agreement between the experimental data and the curves obtained by the fitting procedures for the titrations of NaDNA in both NaPIPES (H_2O) and NaPIPES(D_2O). The obtained estimates for x in the two buffers are close to each other and not significantly different (Table 2). Thus, we conclude that the intercalation process is unaffected by the change of solvent from H_2O to D_2O. Furthermore, by comparing these values with x-estimates obtained at 285 K, we have shown that the x-values are only slightly dependent of the temperature of the experiment. Thus the intercalation process does not seem to have significant temperature dependence in the range examined.

The estimate for R_b^0 in NaPIPES(H_2O) and NaPIPES(D_2O) obtained from the fittings (Table 2) is in excellent agreement with the values obtained from Table 1. The value for R_b^0 in NaPIPES(D_2O) is larger than the value in

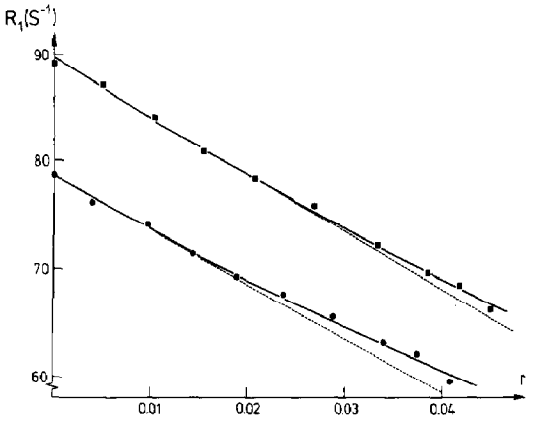

Fig. 3. The observed spin-lattice relaxation rate of $^{23}Na^+$ at 300 K in solutions of NaDNA in NaPIPES(H_2O) (●) and NaPIPES(D_2O) (■) ([P] = 4.0 mM [Na^+]/[P] = 1.2 in both solutions) as a function of r the ratio of 9-aminoacridine to DNA nucleotide equivalents. Solid lines represent the result of fitting the experimental points to eq. (6). The dotted lines represent the best linear lines through the first 4 points, and are included to stress the small deviations from linearity.

NaPIPES(H_2O). This is due to the higher viscosity of D_2O compared to H_2O.

4.5 Exchange rate and motional conditions in LiDNA solutions

The observed spin–lattice relaxation of $^7Li^+$ in LiDNA solutions in both LiPIPES(H_2O) and LiPIPES(D_2O) exhibits a single exponential decay. Consequently, the relaxation can be characterized by a single relaxation rate R_1. We have measured this relaxation rate as a function of the temperature in the range 280–310 K for LiDNA in both LiPIPES(H_2O) and LiPIPES(D_2O). The plots of $\ln(R_1)$ as a function of $1/T$ have both positive slopes (data not shown), and we therefore assume fast exchange between the two states in the solutions of LiDNA in both types of buffer.

Lithium-7 has a complicated relaxation behaviour with contributions from both quadrupolar and dipolar relaxation. The line shapes of the observed ^7Li signals from LiDNA in the two buffers deviate both slightly from exponential spin–spin relaxation behaviour. This is probably caused mainly by non-extreme narrowing conditions for quadrupolar relaxation in the bound state, since this relaxation mechanism is assumed to be dominant in both buffers.

4.6 Dependence of R_1 on counterion concentration in LiDNA solutions

The applicability of Manning's condensation theory in LiDNA solutions was examined by titrating solutions of LiDNA in both LiPIPES (H_2O) and LiPIPES(D_2O) with LiCl solutions. The results of these titrations are shown in Fig. 4, where the observed spin–lattice relaxation rate is plotted as a function of $[P]/[Li^+]$. An approximate linearity exists between $[P]/[Li^+]$ and R_1 in both solutions over the entire range of $[P]/[Li^+]$ ratios studied (the small curvatures in both plots are ascribed to the same effects as in the case of ^{23}Na). This is in agreement with the Manning's condensation theory.

Equation (1) and Manning's estimate for the degree of condensation in DNA solutions containing monovalent counterions, 0.76, can be used to obtain estimates for R_f and R_b from the titration curve in Fig. 4 by a procedure similar to the one used for sodium. The values of R_f and R_b are given in Table 3 and show that the bound relaxation rate in LiPIPES(H_2O) is significantly larger than the corresponding value of the relaxation rate in LiPIPES(D_2O). This indicates that at least the ^1H–^7Li dipolar interactions in LiPIPES(H_2O) contribute significantly to the relaxation rate in the bound state. A quantitative analysis of this effect is made below. The estimates for R_f obtained from the R_1–$[P]/[Li^+]$ plots are only slightly larger than the relaxation rates measured

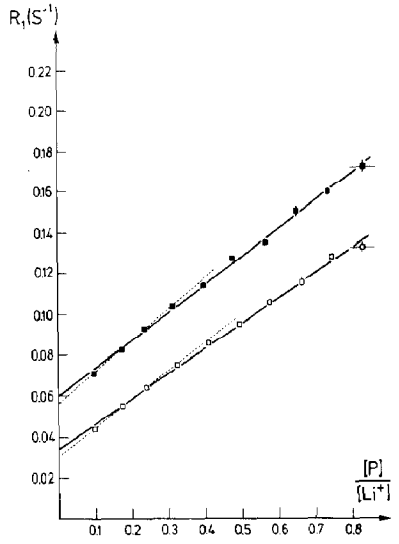

Fig. 4. The observed spin-lattice relaxation rate of $^7Li^+$ at 300 K in solutions of LiDNA in LiPIPES(H_2O) (■) and LiPIPES(D_2O) (□) ($[P] = 4.0$ mM and $[Li^+]^0/[P] = 1.2$ in both solutions) as a function of the $[P]/[Li^+]$. The dotted lines represent the best linear lines through the data points below $[P]/[Li^+] = 0.4$.

Table 3

Lithium-7 ion relaxation rates measured at 300 K

Buffer	R_b [a] (s^{-1})	R_f [a] (s^{-1})	R_f [b] (s^{-1})
LiPIPES(H_2O)	0.243	0.058	0.057
LiPIPES(D_2O)	0.202	0.032	0.029

[a] Obtained from Li^+ titrations of LiDNA represented in Fig. 4.

[b] Measured on Li^+ in buffers without DNA.

on the DNA free buffers as observed for sodium (Table 3).

4.7 Intercalation in LiDNA

The contribution from the different relaxation mechanisms to the observed spin–lattice relaxation rate of $^7Li^+$ in the free state may be separated by the following procedure: The relaxation rates of $^7Li^+$ at a concentration of 5 mM in LiPIPES(H_2O) and LiPIPES(D_2O) (model systems for the free state) at 300 K are 0.0575 s^{-1} and 0.029 s^{-1}, respectively (Table 3). These rates are in reasonable agreement with rates measured in LiCl solutions under similar conditions [9,10]. In these solutions extreme narrowing conditions apply. It is assumed that only quadrupolar and solvent-lithium dipolar interactions (^1H–^7Li in LiPIPES(H_2O) and ^2D–^7Li in LiPIPES(D_2O)) contribute to the observed relaxation rate (omitting terms describing the ^7Li–^7Li interactions due to the low concentration of Li^+). Then according to Hertz et al. [10] the following expressions apply for the observed relaxation rates of $^7Li^+$ in H_2O and D_2O:

$$R_1(H_2O) = R_{dd} + R_q \quad (8)$$

$$R_1(D_2O) = \zeta(R_{dd}\delta + R_q) \quad (9)$$

where R_{dd} represents the ^1H–^7Li dipolar and R_q the quadrupolar contribution to the observed relaxation rate in H_2O, ζ is the ratio of the effective viscosities around Li^+ in D_2O and H_2O and can be estimated to be 1.15 [10], and δ is the ratio of the squares of the magnetic moments of ^2D and ^1H.

Use of eqn. (8) and (9) yields: $R_q = 0.023$ s^{-1} and $R_{dd} = 0.034$ s^{-1}, which implies that the major contribution (60%) to the relaxation rate in the free state in LiPIPES(H_2O) is due to the ^1H–^7Li dipolar relaxation.

The separation of the different contributions to the relaxation rate in the bound state is somewhat more complex. Initially, we have calculated estimates for this rate by using the observed relaxation rate obtained during titrations of LiDNA in both LiPIPES(H_2O) and LiPIPES(D_2O) with 9-aminoacridine, the result of which is shown in Fig. 5.

These calculations were based on eq. (7) with λ given by eq. (4). $[Li^+]/[P]$ ratios of 1.2 were found in the solutions of LiDNA in both LiPIPES(H_2O) and LiPIPES(D_2O). The reduction of the charge density due to intercalation in LiDNA is assumed to be similar to the situation in NaDNA. Consequently, p_i and q have been set equal to 1.0 and x to 3.9 Å. The results of calculations of the bound relaxation based upon this assumption are shown in Fig. 6.

The calculated values of the bound relaxation rate are given as a function of r. The relaxation rates of the bound state are seen to be significantly larger in LiPIPES(H_2O) than in LiPIPES (D_2O) over the entire range of r values examined. This indicates that ^1H–^7Li dipolar interactions contribute significantly to the relaxation of ^7Li in the bound state in solutions of LiDNA in LiPIPES(H_2O) over the entire range of r-values studied. Furthermore, the almost parallel linear dependencies in Fig. 6 show that the ^1H–^7Li

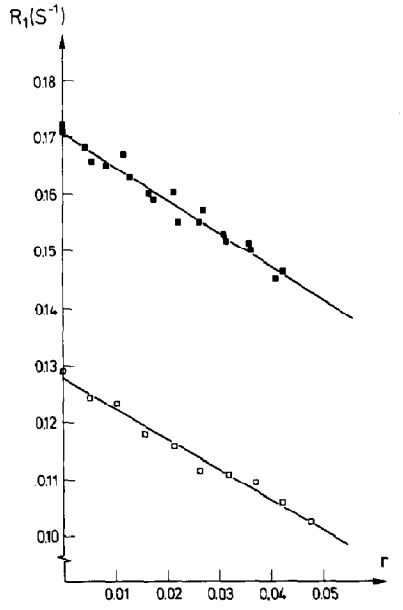

Fig. 5. The observed spin–lattice relaxation rate of $^7Li^+$ at 300 K in solutions of LiDNA in LiPIPES(H_2O) (■), data from two experiments and LiPIPES(D_2O) (□) ([P] = 4.0 mM and $[Li^+]/[P] = 1.2$ in both solutions) as a function of r. Solid lines represent the best linear lines through the experimental points.

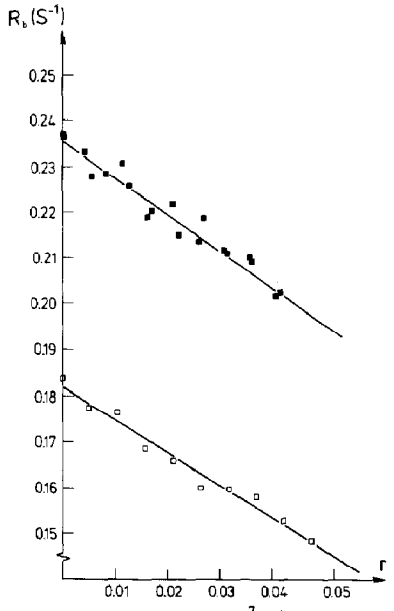

Fig. 6. The bound relaxation rates of $^7Li^+$ at 300 K in solutions of LiDNA in LiPIPES(H_2O) (■) and LiPIPES(D_2O) (□) calculated from the observed rates in Fig. 6 as described in the text as a function of r. Solid lines represent the best linear lines through the experimental points.

dipolar contribution is only slightly affected by the intercalation process.

A more quantitative analysis requires separation of the different contributions in a way similar to the one used for the free state. However, it is more complicated in the bound state since more than two relaxation mechanisms may contribute to the observed relaxation rate. This is due to the fact that the concentration of Li^+ in the volume of the bound state according to Manning's theory may be so high that ^7Li–^7Li and ^{31}P–^7Li interactions might be significant. Accordingly, the observed bound relaxation rate in LiPIPES(H_2O) is described by the following equation:

$$R_b(H_2O) = R^b_{dd} + R^b_q + R^b_e \qquad (10)$$

where R^b_e represents the contribution from all other mechanisms than ^1H–^7Li dipolar and quadrupolar interaction. The bound relaxation rate R_b in LiPIPES(D_2O) is assumed to be given by:

$$R_b(D_2O) = \zeta_b\left(R^b_{dd}\delta + R^b_q + R^b_e\right) \qquad (11)$$

where ζ_b has been taken equal to 1.11 based upon the viscosity correction factor obtained from the bound relaxation rates of $^{23}Na^+$ in H_2O and D_2O.

The observed values of the bound relaxation rates of $^7Li^+$ in LiDNA in LiPIPES(H_2O) and LiPIPES(D_2O) before addition of intercalator are 0.236 s^{-1} and 0.184 s^{-1}, respectively. Use of eqs. (10) and (11) then yields: $R^b_{dd} = 0.08\ s^{-1}$ (33%) and $(R^b_q + R^b_e) = 0.16\ s^{-1}$ (67%).

Comparing these rates to the ones obtained in the free state implies that the ^1H–^7Li dipolar rate is approximately doubled from the free to the bound state. This may be due to a more restrictive motional behavior of Li^+ in the bound state. If other dipolar interaction than ^1H–^7Li is neglected ($R^b_e = 0$), then the results show that the relaxation rate of the quadrupolar mechanism is increased 6.8 times from the free state to the bound. This is comparable to the enhancement obtained for the quadrupolar relaxation of $^{23}Na^+$ in NaDNA solutions in NaPIPES(H_2O) (Table 1).

Since further relaxation mechanisms may contribute ($R^b_e \neq 0$), the value of the enhancement must be taken as an upper limit for the increase of the quadrupolar relaxation rate of $^7Li^+$ from free to bound state.

A linear least square fit of the experimental data revealed the following relation:

$$R_b(H_2O) = (0.24 - 0.76r)s^{-1}$$

$$R_b(D_2O) = (0.18 - 0.68r)s^{-1}$$

Use of these equations combined with eqs. (10) and (11) and $\zeta_b = 1.11$ yield the following expressions:

$$R^b_{dd}(r) = (0.08 - 0.15r)s^{-1}$$

$$R^b_q(r) + R^b_e(r) = (0.16 - 0.61r)s^{-1}$$

According to these calculations the ^1H–^7Li dipolar contribution is seen to be slightly reduced on intercalation. The effect is small, as R^b_{dd} is reduced by less than 10% at $r = 0.05$, corresponding to one intercalated molecule per 10 base pair.

The dependence of the $R^b_q + R^b_e$ upon r represents approximately 80% of the totally observed reduction in R_b on intercalation. The reduction is

undoubtedly mainly due to the reduction in the quadrupolar relaxation rate (R_q^b). If it is assumed that $R_e^b = 0$, it can be shown that the reduction of the quadrupolar relaxation rate of $^7Li^+$ on intercalation is less effective than the corresponding reduction in the ^{23}Na quadrupolar relaxation rate. At $r = 0.05$ the $^7Li^+$ R_q^b rate is reduced to 80% of its initial value, whereas the ^{23}Na R_q^b rate is reduced to 70%. On the other hand if it is assumed, that the reduction of the quadrupolar relaxation rate of $^7Li^+$ is just as effective as the reduction of the rate for $^{23}Na^+$, it is necessary to conclude that $R_e^b \neq 0$. A final evaluation of the size of R_e^b cannot be performed from the present data. We will just point out that the existence of such a contribution could at least partly account for the slower relative decrease of the observed R^b for $^7Li^+$, if it was assumed that R_e^b is independent of the intercalation.

9-Aminoacridine titrations of LiDNA in the two buffers have been carried out at 285 K, and analysis identical to the one presented above has been performed. The general conclusions at 300 K also apply at 285 K. That is a 30% contribution from the dipolar relaxation rate to the bound rate before addition of intercalator, and a small decrease of this contribution upon intercalation. Likewise, the remaining contribution (70%) is less influenced by intercalation than the quadrupolar relaxation of $^{23}Na^+$ as observed at 300 K. Thus, the general relaxation behavior of Li^+ in LiDNA solutions seems little affected by temperature.

Few studies on the interaction of $^7Li^+$ with polyions have been published. In a study of Kielman et al. [28] the relaxation rate of $^7Li^+$ in solutions of polyphosphate at high concentration (0.3 M) was shown to be completely governed by quadrupolar interactions. Furthermore, it was found that the Li^+ polyion interaction was qualitatively different from the Na^+ polyion interaction. Particularly, it was found that the data indicated a small degree (15%) of site binding of Li^+ to the phosphate groups of the polyion, while the major part was free to move in the solution. We find no clear evidence for site binding of Li^+ in the present work. Nevertheless, such an effect would actually be able to explain the weaker dependence of the bound quadrupolar relaxation rate of Li^+ upon intercalation, since site bound Li^+ would tend to screen some of the phosphate anionic charges on the DNA polyion. The relative decrease in the charge density on intercalation would thereby be smaller in LiDNA than in NaDNA.

References

1 L.S. Lerman, J. Mol. Biol. 3 (1961) 18.
2 C.F. Anderson, M.T. Record Jr. and P.A. Hart, Biophys. Chem. 7 (1978) 301.
3 J. Reuben, M. Shporer and E.J. Gabbay, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 245.
4 Y.H. Mariam and W.D. Wilson, J. Am. Chem. Soc. 105 (1983) 627.
5 H. Eggert, J. Dinesen and J.P. Jacobsen, Biochemistry 28 (1989) 3332.
6 J. Dinesen, J.P. Jacobsen, F.P. Hansen, E.B. Pedersen and H. Eggert, J. Med. Chem. 32 (1989) 93.
7 G.S. Manning, Q. Rev. Biophys. 11 (1978) 179.
8 J.J. van der Klink, L.U. Zuiderweg and J.C. Leyte, J. Chem. Phys 60 (1974) 2391.
9 D.E. Woessner, B.S., Snowden and A.G. Ostroff, J. Chem. Physics 49 (1968) 371.
10 H.G. Hertz, R. Tutsch and H. Versmold, Ber. Bunsenges. Physik. Chem. 75 (1971) 1177.
11 M.L. Bleam, C.F. Anderson and M.T. Record Jr., Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 3085.
12 M.L. Bleam, C.F. Anderson and M.T. Record Jr., Biochemistry 22 (1983) 5418.
13 W.H. Braulin, T. Drakenberg and S. Forsen, Curr. Topics Bioenergetic 14 (1983) 97.
14 H. Gustavsson, B. Lindman and T. Bull, J. Am. Chem. Soc. 100 (1978) 4655.
15 B. Halle, H. Wennerström and L. Piculell, J. Phys. Chem. 88 (1984) 2482.
16 P.S. Hubbard, J. Chem. Phys. 53 (1970) 985.
17 M. Levij, J. de Bleijser and J.C. Leyte, Chem. Phys. Lett. 83 (1981) 183.
18 S. Forsen and B. Lindman, Methods Biochem. Anal. 27 (1981) 289.
19 T.E. Bull, J. Magn. Reson. 8 (1972) 344.
20 L. Nordenskiöld, D.K. Chang, C.F. Anderson and M.T. Record Jr., Biochemistry 23 (1984) 4309.
21 S. Padmanabhan, B. Richey, C.F. Anderson and M.T. Record Jr., Biochemistry 27 (1988) 4367.
22 W. Bauer and J. Vinograd, J. Mol. Biol. 47 (1970) 419.
23 L. van Dijk, M.L.H. Gruwel, W. Jesse, J. de Bleijser and J.C. Leyte, Biopolymers 26 (1987) 261.

24 L.S. Lerman, J. Cell. and Comp. Physiol. 64 (1964) 1.
25 W.D. Wilson and R.L. Jones, in: Intercalation chemistry, eds. M.S. Whittingham and A.J. Jacobsen (Academic Press, New York, NY, 1982), p. 445.
26 M. Wirth, O. Buchardt, T. Koch, P.E. Nielsen and B. Norden, J. Am. Chem. Soc. 110 (1988) 932.
27 M.E. Hogan and O. Jardetsky, Biochemistry 19 (1980) 2079.
28 H.S. Kielman, J.M.A.M. van der Howen and J.C. Leyte, Biophys. Chem. 4 (1976) 103.