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Sodium-23 NMR Spin–Lattice Relaxation Rate Studies of Mono- and Bis-Intercalation in DNA

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ABSTRACT: ^{23}Na spin–lattice relaxation rate ($1/T_1 = R_1$) measurements have been used to study the intercalation of a series of 9-aminoacridine derivatives in DNA. The ^{23}Na relaxation rate is strongly dependent upon the amount of intercalator added to a sodium DNA solution. The results are analyzed by a combined use of the ion condensation theory and the quadrupolar relaxation theory of polyelectrolyte solutions. This interpretation shows that the major effect in lowering the relaxation rate by intercalation is not due to the release of sodium ions but is caused by a substantial decrease in the relaxation rate R_b for the remaining bound sodium ions. Likewise, titration of NaDNA solutions with $MgCl_2$ shows that condensation of Mg^{2+} on the DNA double helix reduces R_b. A good agreement between experiment and theory is found if the average lengthening following intercalation of a 9-aminoacridine moiety is assumed to be approximately 2.7 Å. The distinction between mono- and bis-intercalation is clearly indicated by the results. The two bis-intercalating drugs examined are found to bis-intercalate only up to $r \leq 0.02$. For $r > 0.02$ the drugs apparently mono-intercalate.

The overall conformation and physical properties of DNA in solution are strongly influenced by association with counterions, e.g., Na^+. Sodium-23 NMR spectroscopy has been applied in several studies of DNA. Association of sodium and other simple cations to DNA in solution has been examined by using ^{23}Na line width measurements (Andersen et al., 1978; Bleam et al., 1980, 1983; Mariam & Wilson, 1983; Nordenskjold et al., 1984; Braunlin et al., 1986; Delville et al., 1986). Recently, T_1 spin relaxation measurements have been introduced in the investigations (Nordenskjold et al., 1984; Delville et al., 1986).

Intercalation is one of several modes whereby drugs interact with DNA. A planar part of the drug is inserted in between adjacent stacked base pairs of a double-stranded DNA. The intercalation process results in a helix extension. This increases the average phosphate to phosphate distance and decreases thereby the DNA charge density. Positive charges on the intercalator neutralize some of the anionic charge of DNA, which also reduces the charge density. Counterions, e.g., Na^+, are associated (called bound) to the DNA to stabilize the helix structure. The intercalation process will reduce the relaxation rate of the bound Na^+ due to the charge density reduction and hindrance of DNA internal motion. Furthermore, the charge density reduction results in a release of bound Na^+. These effects strongly influence the spin relaxation rates of ^{23}Na measured by NMR spectroscopy.

Mariam and Wilson (1983) have studied the intercalation of ethidium bromide in DNA by using ^{23}Na NMR line width measurements. A dramatic decrease of the line width upon intercalation was found. Several problems arise when ^{23}Na line widths are used as the relaxation parameter. Non-Lorentzian line shapes may appear due to the spin dynamical behavior of spin $I = {}^3/_2$ nuclei. Furthermore, line broadening caused by intermediate fast exchange between free and bound sodium ions cannot be excluded together with line shape effects created by the presence of medium-range order in the DNA solution (H. Eggert, J. Dinesen, and J. P. Jacobsen, unpublished results). Although spin dynamics also complicate spin–lattice relaxation (T_1) behavior, an average T_1 spin relaxation time can be obtained independently of the exchange phenomena. The field dependency of ^{23}Na T_1 in DNA solution indicates that the bound sodium ions are in intermediate motional narrowing range (Eggert et al., unpublished results), and relaxation can therefore be treated as single exponential (Bull, 1972). These facts have prompted us to use T_1 relaxation measurements instead of line width measurements to follow intercalation processes.

The intercalation mode of binding was first proposed by Lerman (1961) for binding of aminoacridines to DNA. Since then, intercalation of this class of compounds has been widely studied by several methods (Le Pecq et al., 1975; Wakelin et al., 1978; Wright et al., 1980; Assa-Munt et al., 1985; Wirth et al., 1988). Compounds containing two aminoacridine functional groups joined by a molecular linker chain are po-

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tential bis-intercalating agents. Assa-Munt et al. (1985) have shown that bis(acridine) derivatives with linker chain shorter than 9 Å mono-intercalate under conditions used in their ^1H NMR studies, whereas those bis(acridines) with chains of 9.8 Å or longer bis-intercalate. Previously, these properties of the compounds were established from the measured values of the binding constants since a 10-fold increase of the values was observed on going from mono-intercalation to bis-intercalation (Wakelin et al., 1979).

The aim of the present work has been to test the applicability of ^{23}Na T_1 measurements as a method to study the binding of intercalators to DNA. It is shown that ^{23}Na T_1 is an extremely sensitive and capable parameter in studies of intercalation in DNA solutions.

Relaxation Theory

^{23}Na is a quadrupolar nucleus with spin quantum number $I = {}^3/_2$. The spin–lattice relaxation process of sodium ions in aqueous solution is consequently mainly caused by the quadrupolar interaction. In general, the quadrupole relaxation can be described by a weighted sum of two exponentials (Hubbard, 1970). For $\omega_o\tau_c < 1.5$ the relaxation is essentially single exponential from an experimental point of view (Forsen & Lindman, 1981; Bull, 1972; Lindman & Forsen, 1981).

The sodium ions of a DNA solution can be in a perturbed region (denoted bound sodium ions, Na_B^+) and a bulk region (denoted free sodium ions, Na_F^+) characterized by different intrinsic relaxation rates. The exchange rate between the two states has been examined (Eggert et al., unpublished results). Assuming a two-state model, intermediates fast exchange was inferred at 5.9 T for sonicated calf thymus DNA at 27 °C. For fast and intermediate fast exchange rates the longitudinal relaxation rate of the observed ^{23}Na signal is given by

$$R_1 = 1/T_1 = p_b R_b + p_f R_f \quad (1)$$

where p_b and p_f are average fractions of bound and free sodium ions, respectively.

Equation 1 can be rewritten in a more comprehensive way (Bleam et al., 1983):

$$R_1 = R_f + (\Delta R)([P]/[Na])a \quad (2)$$

where $\Delta R = R_b - R_f$ and a is the number of moles of sodium ions bound per mole of DNA phosphate:

$$a = p_b([Na]/[P]) = [Na]_b/[P] \quad (3)$$

where [Na] and [P] denote the concentrations of sodium ions and DNA phosphate, respectively. Subscript b refers to the concentrations of the bound state.

The intercalation process results in a release of sodium ions given by $[Na]_b^0 - [Na]_b$, where $[Na]_b^0$ is the concentration of bound sodium ions before addition of intercalator. The number of moles sodium ions released per mol of bound intercalator is denoted by n:

$$n = ([Na]_b^0 - [Na]_b)/[I]_b = \frac{a_0[P] - a[P]}{[I]_b} \quad (4)$$

a_0 is the value of a before addition of intercalator and [I] the concentration of the intercalator. Equations 2 and 4 then yield

$$R_1 = R_f + a_0(\Delta R)[P]/[Na] - np_I(\Delta R)([P]/[Na])r \quad (5)$$

where $p_I = [I]_b/[I]$ is the fraction of bound intercalator and $r = [I]/[P]$.

Equation 5 shows that R decreases linearly as a function of added amount of intercalator, r, provided that n and p_I are independent of r and that ΔR either is unaffected by the intercalation or is a linear function of r. A change of ΔR as a function of r can be described by the expansion:

$$\Delta R = (\Delta R)_0 - (\Delta R)_0\alpha r + (\Delta R)_0\beta r^2 + \ldots \quad (6)$$

When terms to higher than second order are neglected, eq 6 can be introduced in equation 5 to give the result:

$$(R_1 - R_f)([Na]/[P]) = a_0(\Delta R)_0 - [a_0(\Delta R)_0\alpha + np_I(\Delta R)_0]r + [a_0(\Delta R)_0\beta + np_I(\Delta R)_0\alpha]r^2 \quad (7)$$

From this equation it can be deduced that a plot of measured values of R versus r should give a straight line for small values of r. The intersection of this line with $R_1 = R_f$ occurs at a value of $r = r_s$ given by

$$r_s = a_0/(a_0\alpha + np_I) \quad (8)$$

α can be determined by use of eq 8 if a_0, n, and p_I are known. The value of p_I is often assumed to be 1.00 due to the large binding constants observed for most intercalators. a_0 has been determined to be 0.76 (Manning, 1978).

The ion condensation theory of Manning (1978) can give an estimate of the value of n in eq 8. According to this theory, the fraction a of counterions condensed on an infinite polyion per structural polyion charge is given by (Manning, 1978)

$$a = 1 - 1/\lambda \quad (9)$$

where λ is the charge density parameter expressed as

$$\lambda = 7.1/b \quad (10)$$

at 27 °C. b is the intercharge distance in the polyion (DNA). For native B form DNA, $b = 1.7$ Å (two phosphate groups for every 3.4 Å along the helix axis). If the intercalation results in an increase of the distance between the phosphate pairs with an amount x at the intercalation site, the simple ion condensation theory yields the following expression for the number of released sodium ions:

$$n = xm/7.1 + q \quad (11)$$

in which $m = 1$ for mono-intercalation and $m = 2$ for bis-intercalation. q is the charge of the intercalator. In eq 11 we assume that all the bound intercalator actually does intercalate properly in between adjacent base pairs.

The value of α in eq 8 can be estimated by use of the relaxation theory introduced by van der Klink (1974) applied to the case of DNA, for which the neutralization degree is equal to 1. The ion condensation concept (Manning, 1978) leads to an expression for the spin–lattice relaxation rate of the bound sodium nuclei of the type:

$$R_b = C\lambda(\lambda - 1) \quad (12)$$

where C contains all the parameters independent of the intercalation. If we assume a linear decrease of the charge on the DNA strain and a linear increase of the phosphate to phosphate distance upon addition of intercalator, the intercalation will reduce λ according to the expression

$$\lambda = \frac{7.1(2 - 2rq)}{3.4 + 2rxm} \quad (13)$$

in which we have assumed that all the added amount of the intercalator actually intercalates properly ($p_I = 1$). Full intercalation corresponds to $r = [I]/[P] = 1/4$ due to the neighboring exclusion principle.

Expansion of eq 13 gives

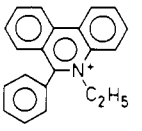
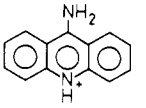
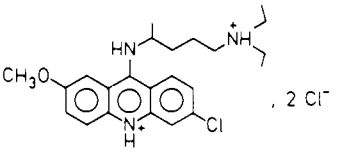
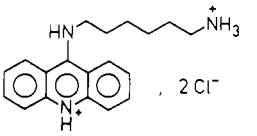
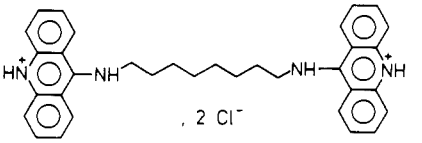
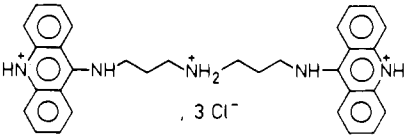
$$\lambda(\lambda - 1) = 13.4[1 - r(2.3q + 1.4xm)] + (\text{higher order in } r) \quad (14)$$

Comparing the expansions in eq 6 and 14, we get

$$\alpha = [R_b^0/(\Delta R)_0](2.3q + 1.4xm) \quad (15)$$

According to this equation α can be calculated as a function

Table I

compound name	structure	compd no.
ethidium bromide		**1**
9-aminoacridine hydrochloride		**2**
quinacrine bis(hydrochloride)		**3**
9-[(6-amino-*n*-hexyl)amino]acridine bis(hydrochloride)		**4**
9,9′-(1,8-octanediyldiimino)bis[acridine] bis(hydrochloride)		**5**
9,9′-[iminobis(1,3-propanediylimino)]bis[acridine] tris(hydrochloride)		**6**

of x if the ratio $R_b^0/(\Delta R)_0$ is known. Values of R_b^0 can be obtained from eq 1 from a knowledge of a_0 (0.76), R_f (17.5 Hz), R_1^0 (the relaxation rate measured before addition of intercalator), and the [Na]/[P] ratio. At 27 °C the values are 91 Hz at 5.9 T and 130 Hz at 2.1 T, giving $R_b^0/(\Delta R)_0$ ratios of 1.21 and 1.16, respectively. In the calculation of α we have applied a value of 1.2, neglecting the small field dependence. The error introduced by this approximation is less than 5%. The calculated values of α are given in Table II.

Table II: Theoretical Values of α Calculated from Equation 15 for Different Values of x, m, and q

x	m	q	α^a	x	m	q	α^a
2.7	1	1	7.2	3.4	2	2	16.7
2.7	1	2	10.0	3.4	2	3	19.4
2.7	2	2	14.4	4.1	1	1	9.5
2.7	2	3	17.2	4.1	1	2	12.3
3.4	1	1	8.3	4.1	2	2	19.0
3.4	1	2	11.1	4.1	2	3	21.7

[a] A value of $R_b^0/(\Delta R)_0$ equal to 1.2 has been used (see text).

Materials and Methods

Calf thymus DNA was obtained from Sigma Chemical Co. (type I). DNA preparations and samples for NMR were made in a 10^{-3} M PIPES buffer adjusted to pH 7.0 with NaOH and containing 10^{-4} M EDTA. For sonication 4 mg/mL DNA was dissolved in buffer, stirred for 24 h at 4 °C, and filtered through 0.45–0.6-μm sartorius cellulose acetate membranes. Portions of 10-mL solution were sonicated for 10 × 10 s at high energy levels at temperatures from 0 to 4 °C. The average molecular weight of the sonicated DNA was estimated to (1–2) × 10^6 by gel electrophoresis. DNA concentrations were determined spectrophotometrically at 260 nm (ϵ_{260} = 6600 M^{-1} cm^{-1}) and are expressed in terms of nucleotide equivalents per liter. The sonicated DNA samples displayed an A_{260}/A_{280} ratio between 1.8 and 1.9. Extraction of sonicated DNA samples with chloroform did not change this ratio. Within experimental reproducibility (±1%) thermal denaturation produced a hyperchromic effect of sonicated DNA that was unchanged relative to an unsonicated sample. Total sodium concentrations were determined by atomic absorption spectroscopy.

Compounds **1–3** (see Table I) were commercial products. Compounds **4–6** were prepared as previously described (Hansen et al., 1983). The purity of the compounds was controlled by elemental analysis and UV–vis spectroscopy. The following extinction coefficients have been used: **1**, ϵ_{480} = 5860; **2**, ϵ_{401} = 10 100; **3**, ϵ_{424} = 9750; **4**, ϵ_{410} = 11 400; **5**, ϵ_{411} = 19 000; and **6**, ϵ_{410} = 19 600. Stock solutions of the intercalators were prepared by dissolving the compounds in distilled water to a concentration of 2–3 mM for **1–4** and 1–2 mM for **5** and **6**. All the compounds **1–6** were used as hydrochlorides.

All DNA samples for NMR were made 5 mM in DNA phosphate by diluting with either distilled water or buffer. This gave [Na^+]/[P] ratios of 1.2 and 1.5, respectively. The r_s values determined were found to be insensitive to this variation. The NMR intercalator titrations were performed by adding successive aliquots (corresponding to $r \sim 0.005$) of the intercalator stock solutions directly to the DNA solution in the NMR tube. For titrations up to $r \sim 0.05$ the volume increases by 10%. A control experiment, in which water alone was added up to 20% volume increase, showed that T_1 within measuring uncertainties was unchanged by such dilution. pH was measured to be 7.0 before and after the addition of intercalators.

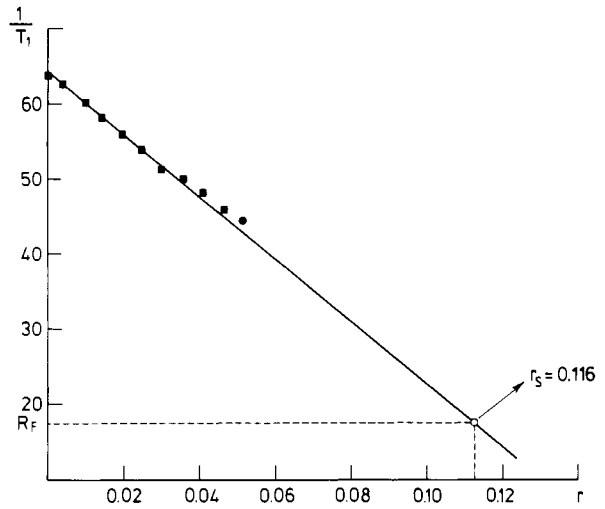


FIGURE 1: ^{23}Na spin–lattice relaxation rates, $1/T_1$, obtained at 5.9 T, versus the ratio of the intercalator 9-aminoacridine (**2**) to DNA phosphate. [P] = 5 mM, [Na]/[P] = 1.2. The temperature was 27 °C. Only the square symbols have been used in the linear regression analysis.

Table III: Experimental Value of r_s Obtained from Plots of $1/T_1$ versus r and Interpretation of the Results According to the Condensation Model[a]

	2.1 T			5.9 T		
intercalator	r_s	n^b	α^c	r_s	n^b	α^c
1	0.119	1.4	6.6	0.114	1.4	7.0
2	0.113	1.4	7.0	0.116	1.4	6.8
3	0.095	2.4	7.4	0.104	2.4	6.5
4	0.097	2.4	7.2	0.099	2.4	7.0
5	0.055[d]	2.8	14.5	0.080	2.8	8.9
6	0.054[d]	3.8	13.6	0.073	3.8	8.8

[a] The values have been obtained in the region $r < 0.05$ unless otherwise stated. [b] Calculated from eq 11. $x = 2.7$ Å has been chosen, but variation of x only affects n to a minor degree. [c] Calculated from r_s according to eq 8. $a_0 = 0.76$ and $p_1 = 1.00$ have been used. [d] Value obtained from the region $r < 0.02$.

The ^{23}Na NMR measurements at 2.1 T were performed on a JEOL FX90Q NMR spectrometer. External deuterium lock was used with D_2O in the outer tube of a double-walled tube system. The diameters of the inner and outer tubes were 8 and 10 mm, respectively. ^{23}Na NMR spectra at 5.9 T were recorded on a Bruker AC 250 and obtained without lock. The inversion–recovery (180°–τ–90°–acq) pulse sequence was used for the T_1 measurements with 15 different values of τ for each experiment. 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 two measurements. Unless otherwise stated, the temperature for the NMR measurements was 27 °C.

Results

The values of ^{23}Na spin–lattice relaxation rates ($1/T_1$) determined as a function of added amount of intercalator are shown in Figure 1 with 9-aminoacridine as the intercalator. A linear dependency is observed for small concentrations of the intercalator. The linear dependency can, according to eq 7 and 8, be extrapolated to yield the value of r_s at the intersection point with the line $1/T_1 = R_f$ ($R_f = 17.5$ s^{-1} at 27 °C). The value of r_s have been determined in this way for the intercalators **1**–**6** (Table I) and are given in Table III. At larger values of r ($r > 0.05$) the initial linear dependency does not seem to continue for any of the intercalators. The value of $r = 0.05$ corresponds to intercalation of one molecule per α-helix turn (10 base pairs). The measurements have been repeated several times at two different magnetic field strengths.

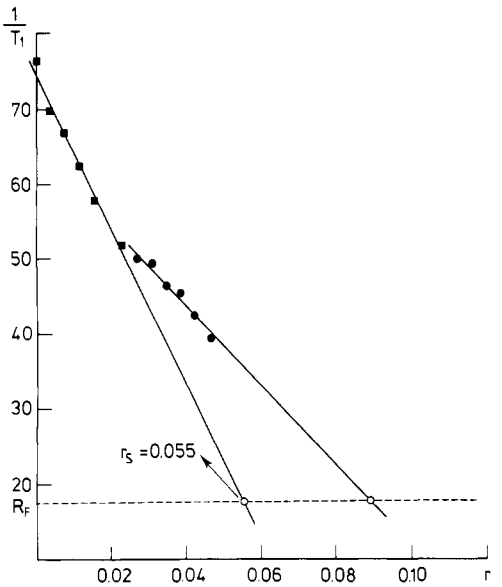


FIGURE 2: ^{23}Na spin–lattice relaxation rates, $1/T_1$, obtained at 2.1 T, versus r, the ratio of the intercalator **5** to DNA phosphate. [P] = 5 mM, [Na]/[P] = 1.5. The temperature was 27 °C. The two regression analyses have been performed on the square symbols and the circular symbols separately.

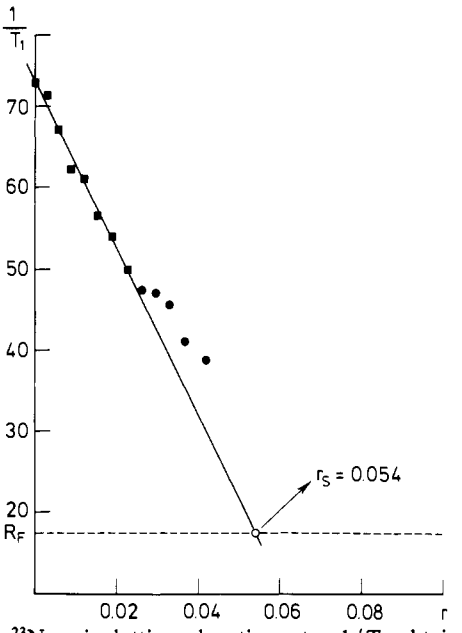


FIGURE 3: ^{23}Na spin–lattice relaxation rates, $1/T_1$, obtained at 2.1 T, versus r, the ratio of the intercalator **6** to DNA phosphate. [P] = 5 mM, [Na]/[P] = 1.5. The temperature was 27 °C. Only the square symbols have been used in the linear regression analysis.

The uncertainties of measurements are mainly due to sample preparation. Although ^{23}Na T_1 values can be obtained with high accuracy, the values obtained from identical DNA samples seem to fluctuate slightly and depend on the prehistory of the sample. This is probably caused by the dynamical behavior of the DNA strains and the possible existence of nonequilibrium ordering of the molecules in the samples.

Compounds **1**–**4** are expected to be mono-intercalating, whereas **5** and **6** are potentially bis-intercalating. This is reflected in different behavior of the plot of R_1 against r in the region $0 < r < 0.05$ (Figures 2 and 3). Contrary to compounds **1**–**4** the initial linear dependency of **5** and **6** is only fulfilled up to approximately $r = 0.02$ for the results obtained at 2.1 T.

In order to make direct comparison to a nonintercalating, but still sodium-releasing, situation, we have measured the

dependence of the sodium T_1 values in a NaDNA solution upon addition of $MgCl_2$. A linear dependency was observed up to a value of $r = 0.1$, and the value of r_s obtained was equal to 0.23.

Discussion

Bleam et al. (1983) have measured the ^{23}Na line width of a NaDNA solution as a function of addition of $MgCl_2$. They obtained values of r_s close to 0.27 at 20 °C. This is larger than the value of 0.23 found in this work from T_1 measurements. Use of eq 8 with $\alpha = 0$ and $p_1 = 1$ yields $n = 3.3$ from $r_s = 0.23$ if the theoretical value of the extent of counterion condensation ($a_0 = 0.76$), according to Manning's theory for double-stranded DNA, is used (Manning, 1978). However, the upper-limit value of n is equal to 2 in the case of magnesium ions. This implies that the assumption of R_b being constant ($\alpha = 0$) at increased Mg^{2+} concentrations is invalid. The condensation of Mg^{2+} on the DNA double helix changes the relaxation rates of the remaining bound sodium ions. With $n = 2$, a 17% decrease of R_b by addition of $MgCl_2$ up to $r = 0.1$ is able to account for the low value of r_s measured.

The experimental ^{23}Na T_1 values of the DNA solutions show a clear dependence on the magnetic field strength. The values before addition of any intercalator are typically 13 ms at 2.1 T compared to 16 ms at 5.9 T (Eggert et al., unpublished results). This is caused by the slow motion outside the extreme motional narrowing limit of the bound sodium ions. Nevertheless, the values of r_s given in Table III are independent of the magnetic field strength within the limits of the uncertainties. r_s has equivalently been shown to be insensitive to temperature variations. This is in accordance with the theoretical expressions for r_s as given in eq 8 and 15. Even though R_b is both field and temperature dependent, α and thereby r_s depend on the ratio $R_b^0/(\Delta R)_0$. Within the range of magnetic fields and temperatures applied (27 and 34 °C) the variations in $R_b^0/(\Delta R)_0$ with field and temperature are expected to be experimentally insignificant.

The values of r_s obtained for the mono-intercalators **1**–**4** are substantially lower than the value of r_s obtained by addition of $MgCl_2$ to a NaDNA solution. This reflects that the effect of **1**–**4** is not a simple exchange of ions, but substantial conformational changes take place. The change of the T_1 values for **1**–**4** is, as noted earlier, linear up to $r = 0.05$ in accordance with eq 7. At this r value one molecule is in average intercalated per 10 base pairs, corresponding to one α-helix turn. In contrast, linearity is observed up to $r = 0.1$ for $MgCl_2$ titrations. Mariam and Wilson (1983) have studied the intercalations of **1** by ^{23}Na line width measurements. However, they reported only a few observations at $r < 0.05$. They interpreted their results in terms of a quadratic dependence of the bound sodium relaxation rate upon the DNA charge density and found qualitative agreement.

The values of r_s given in Table III can be interpreted according to the ion condensation model (eq 8, 11, and 13). A reliable value of n, the number of released sodium ions, given in Table III has been obtained by use of eq 11. The lengthening x of the DNA base-pair separation has been set to $x = 2.7$ Å in the calculations, but variations of x only affect n to a minor degree. α can be calculated from the experimental values of r_s by use of eq 8. The values of α obtained in this way are given in Table III and may be compared to the calculated values given in Table II.

The values of α calculated from the r_s values in the case of the intercalators **1** and **2** are closely in agreement with the theoretically predicted value of α in Table II ($m = q = 1$). The lengthening, x, of about 2.7 Å seems to be a reasonable estimate. The interactions **3** and **4** give smaller r_s values than **1** and **2**. Structurally, **3** and **4** differ by an extra positive charge on the side chain. Nevertheless, the values of α calculated in Table III are not in agreement with the theoretical values given in Table II for $m = 1$ and $q = 2$ no matter what value of α is used. A simple explanation for this divergency is that the charge parameter in eq 15 only reflects the charge localized on the intercalating part of the molecule, the acridine ring system. Since the charge on the aliphatic chain is outside the DNA base-pair region, it may not affect the charge density at the DNA strand to a substantial degree. On the other hand, this charge may substitute one of the bound sodium ions so $q = 2$ should be used in eq 11. A reasonable agreement between the theoretical values of α for $m = q = 1$ in Table II and the values of α calculated for **3** and **4** in Table III with $n = 2.4$ supports this interpretation. Furthermore, the results of **3** and **4** demonstrate that substitution of chloro and methoxy groups on the acridine ring does not affect the intercalation.

The interpretation of the results in the way described above shows that the major effect in lowering the relaxation rate by intercalation is due to a sizable decrease in the relaxation rate of the bound sodium ions. From eq 6 and the values of α found for mono-intercalation R_b is found to be reduced by ~30% by intercalation of only one molecule per α-helix turn ($r = 0.05$). Intercalation reduces the DNA charge density by increasing the phosphate charge separation and by neutralizing anionic charge of the DNA. The field gradient at the bound sodium ions is thereby reduced, and a diminishing of the relaxation rate is a consequence. Furthermore, intercalation has been shown to stop or greatly hinder DNA internal motions at the binding site (Hogan & Jardetzky, 1980). This may also cause a reduction in R_b. Only a smaller reduction of the relaxation rate is caused by release of bound sodium ions.

The bis-intercalating molecules **5** and **6** exhibit a linear dependency of $1/T_1$ up to $r = 0.02$ at 2.1 T (Figures 2 and 3), only around half the region found for mono-intercalators. The r_s value obtained in this region is 0.054–0.055 (Table III). The values of n are estimated from eq 11 to be 2.8 and 3.8 for **5** and **6**, respectively. On the basis of these values α can be calculated to be 14.5 and 13.6 for the two compounds, which is close to the theoretical values of α for $m = q = 2$ in Table II. The lengthening of the base-pair separation seems still to be close to 2.7 Å. Molecules **5** and **6** are obviously bis-intercalating in the region $r < 0.02$. As for the mono-intercalators, the positive charge on the aliphatic chain of **6** does not contribute to a reduction of the charge density on the DNA strain and consequently does not affect the value of α.

The intercalator **5** yields clearly a further linear dependence of $1/T_1$ upon r in the region $0.02 < r < 0.05$ (see Figure 2). This observed second linear dependency is not appropriately described by the parameter r_s. However, from eq 7 and 11 and appropriate Table II values it appears that mono- and bis-intercalation can easily be differentiated in a more qualitative manner by considering the slope of the $1/T_1$ against r plots. For measurements performed under similar experimental conditions (same [Na]/[P] ratio, temperature, and magnetic field) the slope of the plot will differ by a factor of 2 for mono- versus bis-intercalation. The two slopes of the plot in Figure 2 actually differ by a factor of 2. This shows that the results of **5** can be interpreted as bis-intercalation in the region $r < 0.02$ and as a mono-intercalation for $0.02 < r < 0.05$.

The intercalator **6** shows the same trend as **5**. The initial linear dependency change is at $r \sim 0.02$. However, the experimental uncertainties on the data for $r > 0.02$ are too large

to permit definitive conclusions.

The measurements of the intercalation by **5** and **6** at 5.9 T do not give the same detailed information as the measurements at 2.1 T. The dynamic range available for T_1 at 5.9 T is too small to permit any reliable linear regression in the region $r < 0.02$. The whole region below $r < 0.05$ must be included to make any estimate of r_s. The results in Table III show that a mixture of mono- and bis-intercalation occurs in this region in agreement with the results at 2.1 T.

The theoretical expectation for the lengthening of the DNA base-pair separation following intercalation is 3.4 Å. In this study a good agreement between experiment and theory is found if the average lengthening is assumed to be approximate 2.7 Å. Viscosity enhancements and electric dichroism studies of intercalation in DNA (Hogan et al., 1979; Waring, 1981) showed that the increase in DNA length varies from drug to drug but is generally less than 3.4 Å. However, the experimental values are a measure of the *average* DNA lengthening. The actual base-pair separation may be different if other distortions of the DNA helix take place in addition. Several results (Hogan et al., 1979; Wilson et al., 1982; Warring, 1981) speak for the occurrence of more far-reaching perturbations of the DNA helix than implied by the simpled model originally proposed (Lerman, 1961).

Summary

The data presented above demonstrate that ^{23}Na NMR spin–lattice relaxation time measurements can yield substantial information about intercalation in DNA. Mono- or bis-intercalation is easily recognized, and detailed information about structural properties of the intercalation can be obtained.

Registry No. **1**, 69645-43-8; **2** (free base), 90-45-9; **3** (free base), 83-89-6; **4** (free base), 111557-08-5; **5** (free base), 57780-57-1; **6** (free base), 91790-15-7.

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