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SODIUM-23 NMR STUDIES OF CATION-DNA INTERACTIONS

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Sodium-23 NMR has been used to study the extent to which monovalent cations associate with double stranded DNA in aqueous solution (28°C, pH = 7.5). On the basis of the two site model for rapid exchange the ^{23}Na linewidth can be related to the fraction of sodium ions associated with DNA. To test the applicability to this system of the condensation model for the association of small counterions with polyelectrolytes, the concentration dependence of the sodium linewidth has been determined by making additions of NaCl to solutions of tetraethyl- or tetrabutylammonium DNA. ([P], the DNA phosphate concentration was about 0.02 M.) The resulting titration curves extend over a wide range of the ratio [Na]/[P] (0.3–30). When [Na]/[P] $\gtrsim$ 3 only sodium is associated, and the extent to which it compensates the charges on DNA does not vary with the addition of salt, at least until [Na]/[P] $\approx$ 30, the highest concentration examined. When [Na]/[P] $\lesssim$ 3 the tetraalkylammonium species is also associated with DNA; an equation has been derived to account for the effect on the ^{23}Na linewidth of the competition between sodium and another monovalent cation. Based on the assumption that the fraction of uncompensated charge remaining on DNA after the condensation of both species is constant, this equation fits *all* the linewidth data if the charge fraction is in the range 0.25 ± 0.10. The value required by the condensation model for DNA in the presence of monovalent counterions is $\xi^{-1} = 0.24$. The reasonable agreement between experimental and theoretical values of the charge fraction and its invariance with respect to large variations in the concentration of added salt indicate that even in moderately concentrated solutions of DNA, the association of sodium can usefully be described in terms of the condensation model. If the theoretical value of the charge fraction is assumed, it follows from fitting the titration curves that the approximate relative affinities for DNA of Na^+, Et_4N^+, and Bu_4N^+ are in the ratio 20:5:1, and the transverse relaxation rate of condensed sodium is 180 ± 10 s^{-1}.

1. Introduction

Various simple strong electrolytes (NaCl, $MgCl_2$, etc.), found at relatively high concentrations in many biological systems, can affect the structure, stability, and reactions of biological macromolecules in aqueous solution. In particular, the nonspecific electrostatic association of alkali metal ions with highly charged polyanions is known to influence their conformation, melting characteristics, and binding equilibria [1–3]. An accurate quantitative description of this association is especially necessary for the analysis of kinetic and thermodynamic data. When a charged ligand (such as Mg^{+2} or a protein) binds to a nucleic acid in a solution containing excess salt (NaCl), some sodium ions must be displaced. The release of these small ions makes a significant contribution to the change in the electrostatic free energy for the reaction. For example, the equilibrium dialysis experiments of Latt and Sober [4] on the interaction of oligolysines with polyribonucleotides have recently been reinterpreted by Record et al. [5]. They successfully accounted for the salt-dependence of the binding equilibrium by relating the extent of sodium ion association to a single parameter, the mean linear charge density along the axis of the polyanion. This approach is founded on the model of counterion "condensation", introduced by Oosawa [6], and developed by Manning [7,8].

The concept of condensation may be illustrated by considering the simplest hypothetical system: an ex-

tremely dilute aqueous solution of a weak polyacid. Suppose that this macromolecule has become fully charged as the result of titration with a strong base $M(OH)_z$, where M is a small cation of valence z. (Additional strong electrolyte(s) of the type MX_z may also be present.) The structure of the fully charged polyanion may be such that the average axial spacing between negatively charged groups is too small for the macromolecule to be thermodynamically stable in solution. To prevent this instability some of the cations associate with the polyanion ("condense"), thereby lowering the inherent structural value of ξ, a reduced linear charge density parameter, to a critical value, ξ_{net}. Prior to condensation, ξ is given by the ratio of the length β_L, 7.1 Å for water at 25°C *, to b, the mean distance between charges projected onto the cylindrical axis of the polyanion. Since a single type of counterion is present, ξ_{net} is the reciprocal of the absolute value of its charge [9]. Thus, after the requisite number of cations condenses, the uncompensated "charge fraction" remaining on each monomeric unit is given by the ratio $\xi_{net}/\xi = b/z\beta_L$. If b is so large that this ratio exceeds unity, then no counterions will condense. For many polyelectrolytes, including both double and single stranded DNA, $\xi_{net}/\xi < 1$.

Proceeding from this model of counterion condensation, Manning has demonstrated the derivation of thermodynamic and transport variables for dilute polyelectrolyte solutions containing excess simple electrolytes with a common counterion [9]. Recently the applicability of the model has been extended. Assuming that Na^+ (or K^+) undergoes condensation, Record et al. have obtained quantitative thermodynamic interpretations of the salt-dependence of nucleic acid helix-coil transitions [10,11] and the interactions, both specific [12] and nonspecific [13,14], between proteins and nucleic acids. Record [10,13] and Manning [15,16] have treated condensation in poly-electrolyte solutions containing monovalent and (alkaline earth) divalent counterions. The utility of the concept of monovalent counterion condensation, even in relatively concentrated solutions and in situations involving complex binding equilibria, has been borne out by the results of a steadily growing number of experimental studies, which in part form the subject of recent reviews [17]. However, there have been few studies providing a direct measure of the extent of counterion condensation. A clear indication of the applicability of the model may be obtained by verifying that in solutions containing only one cationic species the charge fraction on a polyanion remains constant as the concentration of the counterion is increased. But quantitative verification of the value of the charge fraction postulated by the condensation model, ξ_{net}/ξ, is especially desirable.

Magnetic resonance has frequently afforded a direct and sensitive probe of the interactions of small ligands with macromolecules [18]. But of the metallic cations common in biological systems only ^{23}Na can yield sufficiently accurate data on moderately concentrated samples (0.01–0.1 M). It has been found that the presence of polyanions such as polyacrylate [19], polyphosphate [20], polystyrenesulfonate [21], and DNA [22] in aqueous solutions of NaCl causes a considerable increase in the observed relaxation rate of the sodium nucleus. In general when a nucleus is associated with a macromolecule, its relaxation rate, R_B, is much faster than R_F, its relaxation rate when free in solution *. Provided that the rate at which sodium exchanges between these environments is much greater than either of their characteristic relaxation rates, the observed relaxation rate, R, is proportional to the population-weighted average of R_B and R_F. Then the concentration dependence of ^{23}Na linewidths in polyelectrolyte solutions can be expressed in terms of these relaxation rates and the parameter(s) characterizing the extent of sodium association. However, if the condensation model correctly describes this association, the data cannot supply separate values for ξ_{net}/ξ and R_B unless another type of monovalent counterion also associates with DNA in competition with sodium.

In the present investigation measurements of the ^{23}Na linewidth, $\Delta\nu_{1/2}$, were used to follow the sodium

* The parameter $\beta_L = e^2/\epsilon kT$, where e is the electronic charge; ϵ, the dielectric constant of the solvent; k, Boltzmann's constant; and T, the absolute temperature. The product (ϵT) is only slightly temperature-dependent in aqueous solution.

* The subscripts B and F, denoting "bound" and "free", are standard but may have misleading connotations in the present context. "Bound" does not here imply "immobilized at some definite site on the polyelectrolyte". "Free" should not be taken to mean that the relaxation rate of unassociated cations is completely unaffected by the presence of polyelectrolyte.

chloride titrations of two quaternary ammonium salts of double stranded DNA in aqueous solution. The cations chosen were tetraethylammonium (Et_4N^+) and tetrabutylammonium (Bu_4N^+). The ratio of sodium to DNA phosphate, [Na]/[P], was varied from 0.3 to 30. At sufficiently high concentrations of added salt the functional form of the titration curves should be determined solely by the extent to which sodium associates with DNA. If $\Delta\nu_{1/2}$ is a linear function of [P]/[Na], this extent of association must be constant and hence the charge fraction on DNA must be invariant with respect to the addition of sodium, but it cannot be evaluated separately from the relaxation parameter R_B. If over the entire range of salt concentrations examined the condensation model applies, then the charge fraction should have the constant *non-zero* value, $1/\xi$. (For monovalent counterions $\xi_{net} = 1$.) But a possible alternative explanation of the linear functional form of the high salt data is supplied by a mass action treatment. Provided that the apparent binding constant for the sodium-DNA interaction is sufficiently large, virtually all of the phosphate charges could be compensated by bound sodium. In this interpretation as [Na]/[P] exceeds one, the binding density of sodium would rapidly approach unity, so that the charge fraction attains the constant value *zero*. Only by analyzing linewidth measurements made at low concentrations of added salt is it possible to evaluate the charge fraction on DNA and thus test the applicability of the condensation model to these moderately concentrated polyelectolyte solutions over a wide range of ionic strengths.

The data also permit the evaluation of the mean relaxation rate characteristic of sodium associated with DNA. Once R_B is known, ^{23}Na NMR measurements should provide useful quantitative information about the binding of charged ligands such as proteins which, as they interact with the DNA polyanion, must expel sodium ions. In an analogous way $^{35}Cl^-$ NMR has been employed as an indirect probe of the oxygen equilibrium in human hemoglobin [23,24]. Similarly, the extent of association of various other alkali metal or alkaline earth ions with DNA could be studied by determining how known concentrations of these ions diminish the observed relaxation rate of ^{23}Na. Within these series of monovalent and divalent cations the relative affinities for DNA are still not well established [15].

Aside from its evident intrinsic interest, double stranded DNA has certain properties which recommend it as a model system for investigating the association of counterions by ^{23}Na magnetic resonance. It is generally accepted that in aqueous solution the double helix is in the B form, and thus has a well-characterized geometry not significantly modified by solvation or the ionic composition of the medium (if the ionic strength does not exceed unity) [25,26]. The distance between phosphate charges, measured along a single strand, is 6.6 Å; but the parameter b, the mean spacing between charges projected onto the helical axis, is only 1.7 Å. Thus the structural linear charge density, ξ, the characteristic parameter for the condensation model, has the value 4.2. Recent theoretical calculations indicate that this relatively large value should have the favorable consequence of extending the applicability of the condensation model to solutions containing DNA in the concentration range of interest here [16]. The virtually unique structural rigidity of the double helix has led to the frequent use of DNA to test theoretical models of rod-like polyelectrolytes [3]. Particularly at the relatively low molecular weights ($\approx 5 \times 10^5$) used in this study, the macromolecule will be sufficiently inflexible that the electric field which it produces at the nuclei of associated counterions should be relatively uniform. According to the theoretical treatment by van der Klink et al. [19] of ^{23}Na quadrupolar relaxation in polyelectrolyte solutions, R_B should be insensitive to changes in solution composition if the equipotential surfaces surrounding the polyelectrolyte are cylindrical. Since the diameter of the B form of helical DNA is 19.4 Å [27], the surface charge density on this polyanion is exceptionally low. It is therefore likely that the association of sodium with DNA produces relatively little mutual dehydration [15]. The absence of any such specific effects capable of producing short-range stabilizing forces tends to favor the stability of the charge fraction with respect to increasing ionic strength [16]. Furthermore, when a comparatively large surface area is available for associated counterions, specific steric or electrostatic interactions among them should be infrequent. Consequently, R_B should not vary significantly as the relative abundance of different types of associated ions is changed by titration (when [Na]/[P] $\lesssim 3$).

2. Materials and methods

2.1. Preparation of samples and titration procedure

Buffers and salts were of the highest purity available. Doubly distilled deionized water was used in all solutions. High molecular weight calf thymus DNA was obtained from the Worthington Co. Stock solutions approximately 15 ml in volume were made by dissolving DNA overnight at 4°C to a final concentration of approximately 8 mg/ml in a solution containing 0.1 M NaCl and 0.01 M tris (hydroxymethyl) aminomethane buffer at pH = 7.8. ("*Tris*" buffer was obtained from the Sigma Corp.) According to the basic procedure given by Cohen and Eisenberg [28], these solutions were sonicated to produce DNA molecules of approximate molecular weight 5×10^5. Aliquots of these sonicated solutions were then placed in dialysis tubing which had previously been washed in hot EDTA solution and subjected to the following series of dialyses at 4°C. First, to remove divalent metal ion contaminants, the solutions were dialyzed against 0.01 M EDTA (pH ≈ 7) for several hours and then against two changes of a solution containing 0.01 M *tris* buffer for an equal time period. Finally, exhaustive dialysis (36–48 hours) was carried out against many changes of a quaternary ammonium bromide solution approximately 0.002 M in *tris* buffer. The resulting fairly concentrated solutions (≈0.02 M in DNA monomer) contained the desired tetraalkylammonium salt of DNA (Et_4N^+ or Bu_4N^+) and a minimum of simple salt. From a Donnan effect calculation the ratio of excess salt to DNA monomers was estimated to be no greater than 1:2.

It has been observed in this laboratory that the cationic form of the *tris* molecule interacts with double stranded DNA to some degree [29]. If the extent and nature of this interaction are similar to the association of Et_4N^+ and Bu_4N^+ with DNA, and especially if the concentration of *tris* is kept at a minimum, then its tendency to compete with sodium ions should be a negligible effect. Support for this assumption was gained by performing titrations of Bu_4NDNA at two different *tris* concentrations. Anionic buffers, such as phosphate, were avoided because at low salt their tendency to affect the relaxation rate of "free" sodium could become important [30].

Concentrations of DNA phosphate, [P], were determined by measuring the absorbances of diluted aliquots at 260 nm with a Gilford spectrophotometer, Model 2400-S. Immediately after sonication and again before initiating any NMR measurements the native state of the DNA was verified by melting a sample and observing the expected hyperchromicity (≈30%) at 260 nm. The ratio of absorbances at 260 nm and 280 nm, 1.9, indicated minimal protein contamination. During the course of dialysis a small amount of precipitation, possibly caused by the presence of protein, was noted. After all dialyses were completed each sample was centrifuged before the DNA content was determined. Total loss of material during dialysis never exceeded 10%. One sample was treated by hot phenol extraction [31] to remove all traces of protein before dialysis. The NMR measurements performed on this solution were in close agreement with those obtained from samples which had not been extracted with phen

For the purpose of linewidth measurements, samples 4.5 ml in volume and 10% in D_2O were placed in 10 mm NMR tubes. Prior to beginning each titration the absence of sodium from each sample was ascertained by determining that after several hours of accumulation no ^{23}Na signal could be detected. Additions of sodium were made by pipetting microliter volumes of concentrated NaCl stock solutions directly into the sample tube, which had been removed from the insert. At the end of each titration the total volume increase in the sample was never more than 10%. Since the sodium in these solutions was, at least in the early stages of titration, very dilute by the standards of the NMR measurement, long time periods were required to obtain linewidth data of the desired accuracy. Thus the complete titration of a sample extended over a period of several weeks. When each titration was concluded the native state of the DNA was verified spectrophotometrically. More concentrated solutions of DNA were found to deteriorate over the time scale required for the acquisition of a complete set of titration data, of the necessary accuracy.

2.2. Instrumentation and the accuracy of the measurement

The instrument employed was a Bruker HX90E high resolution NMR spectrometer, supplemented so that a variety of frequencies can be derived from a PRD 7828 synthesizer, amplified by broadbanded cir-

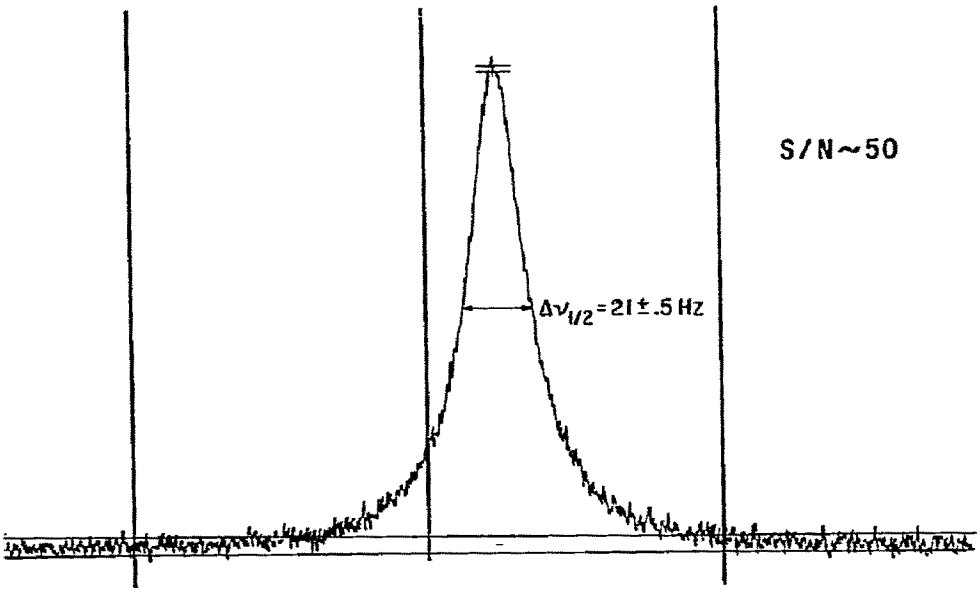

Fig. 1. Reproduction of a typical ^{23}Na Lorentzian spectrum, for a sample containing 0.0175 M Bu_4NDNA and 0.05 M NaCl.

cuitry, and transmitted to an appropriately tuned probe insert. For the present study a 10 mm ^{13}C insert, tuned to receive at 23.81 MHz for sodium, was used together with a ^{13}C pre-amplifier. Measurements were made in the pulsed mode with a single coil configuration. Free induction decays (FID's) were accumulated into the memory of a Nicolet 1080 minicomputer. Fourier transformation and phasing were accomplished with the computer, and the resulting spectrum was plotted to obtain the linewidth of the peak. Because relaxation times of ^{23}Na are comparatively short (<0.1 s), a 25 μs pulse ($\approx 90°$) was selected. Pulse delays were dictated by the time of accumulation into 8 K memory locations and the accuracy with which the peak could be represented with discrete memory channels. Total accumulation times varied from 20 h for samples containing 0.005 M Na^+ (36000 FID's with 2 s delays) to 15 min for samples containing more than 0.2 M Na^+ (100 FID's with 8 s delays). It was determined that the improvement in signal-to-noise obtained by the chosen accumulation times was not offset by any systematic broadening resulting from long-term drifts in field homogeneity.

Field-frequency stability was maintained by locking onto an internal deuterium reference (D_2O). To ensure the maximum, reproducible field homogeneity, the deuterium signal was optimized by means of the magnet shim controls before each series of accumulations. The success of this procedure was checked by measuring the linewidth of ^{23}Na in a standard sample (0.15 M NaCl). The shim settings were adjusted until this linewidth was no greater than 6 Hz †. The observed linewidths were not subjected to empirical corrections for inhomogeneous broadening, as the uncertainty of this procedure is probably greater than the actual error. Long-term homogeneity was reasonably well maintained by a Bruker "auto-shim" accessory on the spectrometer. This device senses the decline in reference signal intensity resulting from changes in homogeneity and automatically feeds back compensating corrections to the sensitive y-field gradient control. This cumulative adjustment, as well as the intensity of the reference signal itself, was monitored during the course of long term accumulations. If substantial deviations occurred, the FID's were discarded and a new series of accumulations was begun. The temperature of the probe (and sample) were stable at 28 ± 0.5°C.

In fig. 1 a typical ^{23}Na frequency domain spectrum

† In 20% D_2O, the ^{23}Na linewidth may be computed from published NMR data (Eisenstadt, Friedman [32]) and the viscosity data for D_2O/H_2O mixtures (Baker and La Mer [33]).

is reproduced. (The sample contained 0.05 M NaCl and 0.0175 M Bu_4NDNA.) For samples which were relatively dilute in sodium (< 0.1 M), the accumulated FID's were damped by an exponential multiplier in the computer memory prior to Fourier transformation in order to filter out random noise from that portion of the FID where the signal has essentially decayed to zero. The signal-to-noise ratio in the resulting Lorentzian spectrum was thereby much improved, while care was taken to ensure that the damping was not strong enough to cause discernible broadening of the peak or shifts in the base line. In all spectra from which linewidths were obtained, the S/N was at least 25:1; in many cases (when $[Na^+] > 0.02$ M) it exceeded 50:1. Visual averages of the noise were performed to establish the base line and maximum of the peak. The reproducibility of this method of measuring linewidths was found to be satisfactory. Experimental error appeared to be chiefly due to minor fluctuations in field stability; variability introduced by the necessity of periodically repositioning the sample tube and/or probe insert was minimized by the reshimming procedure detailed above. The error bars appearing in figs. 2–4 were determined by multiple measurements on representative samples. The estimated relative error in the linewidth data varied from about 5%, for samples in which $[Na^+] < 0.02$ M, to 3%, when $[Na^+] > 0.1$ M.

3. Theoretical interpretation and results

3.1. Using the two site model to interpret ^{23}Na linewidths

Since the relaxation rate of ^{23}Na in polyelectrolyte solutions is at least several times faster than in solutions of unassociated simple electrolytes at comparable concentrations [19–22], this enhancement must be due, at least in part, to the association of sodium with the macromolecule. To analyze the effect of association on the observed ^{23}Na relaxation rate the "two site" model is commonly assumed. (See for example ref. [22].) But it has not been demonstrated that sodium binds to specific sites on a polyelectrolyte like DNA. In the current model of the condensation layer [16], associated ions remain fully hydrated and mobile on the surface of the macromolecule. It is therefore important to know that if this description is correct, the continuum of "sites" traversed by diffusing sodium ions in a polyelectrolyte solution can still be divided into two classes whose characteristic relaxation rates, R_B and R_F, do not vary with the addition of sodium chloride. (Refer to the comments on these symbols in the Introduction.) From the work of Eisenstadt and Friedman on ^{23}Na relaxation in simple electrolyte solutions [30,32] and the experimental results of van der Klink et al. [19] on sodium polyacrylate solutions, it appears that the dynamic features of counterion condensation are consistent with the two site model. Detailed justification of this model requires a rather lengthy consideration of the ^{23}Na quadrupolar relaxation mechanism and other aspects of the NMR measurement which will be presented elsewhere [34]. If the observed relaxation rate, R, can be analyzed in terms of only two classes of magnetic environments, it should be possible to quantify accurately the equilibrium distribution of the ions between them. The total *amount* of sodium present is designated N. Then, in the two site model:

$$N = N_B + N_F, \qquad NR = N_B R_B + N_F R_F. \qquad (1a, b)$$

For ^{23}Na, both R_B and R_F are large enough that the effect of broadening caused by field inhomogeneity on the width of the signal is negligible. Thus, the transverse relaxation rate is accurately related to the measured linewidth: $R = 1/T_2 = \pi\Delta\nu_{1/2}$.

If P is the total *amount* of DNA phosphate in solution and sodium is the only counterion, then the fraction of polyanionic charge compensated by association will be:

$$r = N_B/P. \qquad (2)$$

Combining (1) and (2) and expressing the independent variable as a ratio of concentrations,

$$R = R_F + r(R_B - R_F)([P]/[Na]). \qquad (3)$$

Since in the two site approximation R_B and R_F do not depend upon the concentration of added salt, the observed linewidth can be a linear function of $[P]/[Na]$ if and only if r is constant. If the association of sodium with DNA conforms to the condensation model, r will be a constant determined solely by the reduced linear charge density parameter appropriate for the condensation of monovalent counterions. Since for DNA $b = 1.7$ Å, $1/\xi = 0.24$, and:

$$r = 1 - 1/\xi = 0.76. \quad (4)$$

On the other hand, r should be variable if a mass action model is appropriate, because the binding density of sodium should continue to change with the addition of salt until the binding capacity of DNA has been saturated ($r = 1$).

3.2. The high salt range of the titrations ([Na]/[P] > 2)

In fig. 2, ^{23}Na linewidth measurements for a solution of Et_4NDNA (0.021 M) and a solution of Bu_4NDNA (0.0175 M) are compared with the concentration dependence of $\Delta\nu_{1/2}$ for a solution in which sodium was the only counterion present (0.0053 M NaDNA). All three sets of data exhibit a linear functional form when the variable [P]/[Na] is lower than 0.2. Collectively these observations imply that when a large enough excess of salt has been added, virtually all of the bulky cations have been excluded from association with the DNA, so that their presence in solution has no discernible effect on the variation of the observed linewidths. Evidently Et_4N^+ is more difficult to displace than Bu_4N^+, since for the former salt curvature is apparent when [P]/[Na] > 0.2, whereas for the latter linearity extends to [P]/[Na] = 0.5. From the high salt region of the data it is clear that

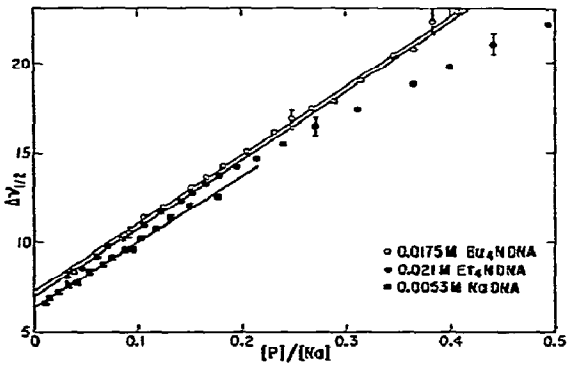

Fig. 2. Linewidth measurements (Hz) as a function of [P]/[Na] for solutions containing (0.021 M) Et_4NDNA, (0.0175 M) Bu_4NDNA, and (0.0053 M) NaDNA. The lines represent linear least squares fits (section 3.2) to the three sets of data; the slopes and intercepts are given in table 1. (The ordinate has been displaced.)

the continued addition of sodium to the solution does not alter the extent of its association with DNA. The linear dependence of $\Delta\nu_{1/2}$ on [P]/[Na] establishes the constancy of r.

The straight lines appearing in fig. 2 were derived from linear least squares fittings of eq. (3) to the three sets of linewidth measurements (15 points for the NaDNA solution, 13 points for the Et_4NDNA solution, and 18 points for the Bu_4NDNA solution.) For the solutions containing tetraalkylammonium, data were included in the fitting for all values of [P]/[Na] below the onset of detectable curvature. At each value of [P]/[Na], a predicted value of $\Delta\nu_{1/2}$ was computed using the slope and intercept obtained from a linear least squares fitting to all measurements performed at lower values of [P]/[Na]. The first two successive points whose predicted linewidths differed from their observed values by more than their experimental uncertainty (in the same direction) were taken to define the onset of detectable curvature. These points and all data for higher values of [P]/[Na] were therefore excluded from the linear fitting procedure. The success of this approach depended upon the relatively small scatter in the linewidths measured at the high salt concentrations.

The slopes, $r(R_B - R_F)$, and intercepts, R_F, determined from the linear least squares fitting to each set of data in fig. 2 are presented in table 1. Slight differences among the three values for the slope lie well within the limits of uncertainty. It is understandable that the intercept obtained for the sample containing the smallest amount of DNA should be the lowest. The ^{23}Na linewidth is still lower (approximately 6 Hz) in aqueous solutions of pure NaCl, up to 1 M [32]. The presence of a polyelectrolyte in solution is expected to exert a long range influence on the relaxation rate of nuclei not associated with the macromolecule. This effect will be discussed in section 4.2.

The simple linear relation between R and [P]/[Na] proves that r is constant, but does not permit the separation of this quantity from the relaxation rate R_B. Although the high salt data are consistent with the result expected from the condensation model that DNA should maintain a constant *non-zero* charge fraction as the sodium concentration is increased, they cannot confirm its theoretical value. If sodium is in sufficient excess (regardless of whether another type of cation is present) a mass action treatment, with a large ap-

Table 1
Linear least squares parameters for the high salt region of the titration curves

Sample	Slope [a]	Intercept [a]
	$r(R_B - R_F)$	R_F
0.0175 M Bu_4NDNA	38.1 (119.7)	7.2 (22.6)
0.021 M Et_4NDNA	38.3 (120.3)	6.9 (21.7)
0.0053 M NaDNA	38.5 (121)	6.3 (19.8)

[a] In Hz, values in parentheses are in s^{-1}.

parent binding constant, demonstrates that the "binding density" of sodium can approach the constant value $r = 1$. Thus, the observed linearity of $\Delta\nu_{1/2}$ at high salt might mean that the binding capacity of the DNA phosphates has been saturated, so that further addition of sodium merely increases the pool of free ions, N_F [see eqs. (1a, b)]. To determine the value of r it is necessary to analyze the non-linear, low salt region of the titration curves, where sodium is not the only ion associated with the DNA.

3.3. Modelling the competitive condensation of two types of monovalent counterion

Within the framework of the two site model for ^{23}Na relaxation, it should be possible to derive an expression to fit the non-linear, low salt, regions of the titration curves by taking proper account of the competition between sodium and the tetraalkylammonium cations which associate with DNA. The approach taken here is similar to Manning's recent treatment of condensation in polyelectrolyte solutions containing a mixture of monovalent and divalent counterions [16]. In addition to the two site model two basic assumptions are required. First, the charge fraction per DNA phosphate, $1/\xi$, must not depend upon the distribution of sodium or quaternary ammonium either in the condensation layer or free in solution. Hence, r, the extent of condensation by *both* types of counterion, will be constant, and the composition of the condensation layer will be governed by a linear relation. If N_B and Q_B are the amounts of condensed sodium and quaternary ammonium:

$$N_B + Q_B = rP, \tag{5}$$

[compare eq. (4)]. Furthermore, it is assumed that the relative abundance of the two cationic species in the condensation layer is proportional to their relative abundance in free solution:

$$N_B/Q_B = D \cdot N_F/Q_F. \tag{6}$$

Justifications for these assumptions will be considered in the next section. It should be noted that D need *not* be unity; it should be fairly independent of ionic strength. (Minor variations in D with the activity coefficients of the small ions are likely to lie well within the scatter of the low sodium data.) Finally, since R_F and $r(R_B - R_F)$ can be accurately evaluated from the linear least squares fitting to the high salt data (see table 1), it is convenient to define a dimensionless linewidth variable, x, proportional to the "condensed fraction" of sodium [compare eq. (3)].

$$x = \frac{N_B}{rN} = \frac{R - R_F}{r(R_B - R_F)}. \tag{7}$$

From (5), (6), and (7), it follows that:

$$[\mathrm{Na}]x^2 - (A[\mathrm{Na}] + B[\mathrm{P}])x + A[\mathrm{P}] = 0, \tag{8a}$$

where, if $D \neq 1$,

$$A = \frac{D}{r(D-1)}, \qquad B = 1 + \frac{[\mathrm{Q}]}{r[\mathrm{P}](D-1)}. \tag{8b}$$

To illustrate the applicability of (8a) to the fitting of sodium chloride titration curves it is useful to consider various limiting cases. First it should be noted that x does not depend upon the absolute concentration of any component in the solution; only ratios of concentrations, or amounts, appear in the quadratic coefficients. Since $[\mathrm{Q}]/[\mathrm{P}]$ does not vary during the course of a sodium chloride titration, x is (at low salt) a non-linear function of the single independent concentration variable, $[\mathrm{Na}]/[\mathrm{P}]$. In the extreme low salt limit, $[\mathrm{Na}]/[\mathrm{P}] \to 0$, x approaches the asymptote:

$$x \to \frac{A}{B} = \frac{D}{[\mathrm{Q}]/[\mathrm{P}] + (D-1)r}. \tag{9}$$

Since $r \leqslant 1$ and $[\mathrm{Q}]/[\mathrm{P}] \geqslant 1$, this asymptote increases monotonically with D; as $D \to \infty$, it attains its maximum value, $1/r$. From the high salt data it has already been inferred that the relative affinity of sodium for DNA, as judged by the persistence of linearity (fig. 2), is greater in the Bu_4NDNA solutions. From (9) it may be predicted that at low salt the ^{23}Na linewidths observed for the former solution will approach a higher

asymptote. In the extreme high salt limit, $[Na]/[P] \to \infty$, eq. (8a) reduces, as anticipated, to a form equivalent to (3): $x \to [P]/[Na]$.

When sodium is in sufficient excess, the presence of a second type of monovalent cation does not alter the linear relation between x and $[P]/[Na]$. What constitutes a "sufficient excess" is determined by the rather complicated dependence of the functional form of (8a) on the parameter D. The larger its value, the sooner x becomes linear with $[P]/[Na]$ as sodium is added to the solution. In the limit $D \to \infty$, eq. (8a) becomes:

$$(rx - 1)(x - [P]/[Na]) = 0. \tag{10}$$

The roots may be distinguished on physical grounds. When $[Na]/[P] \leqslant r$, $x = 1/r$, and the observed linewidth, R_B, does not depend upon the amount of sodium in solution because all of it is in the same magnetic environment. As more sodium is added, it all condenses and expels an equivalent number of quaternary ammonium cations, so that the charge fraction of DNA is maintained, in accordance with (5). When $[Na]/[P] > r$, $x = [P]/[Na]$ and the observed linewidths exhibit the linearity of (3) without an intermediate region of curvature. Eq. (10) indicates that if D is large enough, its effect on the titration curve will be minimal, and thus r may be determined with greater accuracy. In the opposite extreme, if the distribution of the two cations in the condensation layer is identical to their overall distribution in solution, then regardless of the relative amounts of the two cations it will be impossible to separate the quantities r and R_B. In this case $D = 1$ and over the entire range of $[Na]/[P]$, eq. (8a) reduces to:

$$x = \frac{[P]}{[Q] + [Na]} \tag{11}$$

[compare this expression with eq. (3)].

From the foregoing discussion it should be apparent that eq. (8a) can be used to fit the entire titration curve for a DNA solution containing ^{23}Na and any other type of monovalent cation which also undergoes condensation. (The silver ion, for example, would not be suitable; it is known to bind specifically to the bases of helical DNA [35].) In order to determine the extent of monovalent condensation from the NMR data, the composition of the condensation layer must be variable: more than one type of counterion must be condensed. The low salt region of the experimental titration curve is sensitive to this variability through two parameters, r and D. [See eqs. (5) and (6).] Because of the functional form of (8a) the variation in $\Delta\nu_{1/2}$ with added salt will depend almost exclusively on r, if D is large. Hence, as a second cation it was desirable to choose one whose relative affinity for DNA is much lower than that of sodium, but whose electrostatic interaction with DNA is sufficiently similar to that of sodium that eq. (5) should be upheld. The larger tetraalkylammonium cations appeared most likely to fulfill these requirements. However, it was found that even for these bulky species the parameter D is not very much greater than unity, so that the full eq. (8a) was needed to fit the non-linear portions of the linewidth data.

3.4. The low salt range of the titrations

The variation in ^{23}Na linewidth, $\Delta\nu_{1/2}$, as a function of the normalized amount of sodium present, $[Na]/[P]$, is shown in fig. 3 for two solutions of tetrabutylammonium DNA and in fig. 4 for a solution of tetraethylammonium DNA. The logarithmic form of the abscissa was chosen to provide a clearer representation of the low salt data ($[Na]/[P] < 2$) while covering most of the experimental range of $[Na]/[P]$ values. (For the 0.0175 M Bu_4NDNA and 0.021 Et_4NDNA solutions, some additional points corresponding to $[Na]/[P] > 10$ appear in fig. 2.) As anticipated on the basis of eq. (9) and previous qualitative conclusions about the relative affinities of the ions for DNA (in section 3.2), the asymptote approached by the sodium linewidths at very low added salt is higher for the Bu_4NDNA solutions than for the solution of Et_4NDNA. The solid lines in figs. 3 and 4 are theoretical curves calculated using eq. (8a) with r set equal to the value predicted by the condensation model for DNA: 0.76. The values obtained for the adjustable parameter D are 4.2 for the 0.021 M solution of Et_4NDNA and 20 for the 0.0175 M solution of Bu_4NDNA. In view of the approximate nature of the Donnan calculation (see the preceding section), 1.5 may be only an upper bound for $[Q]/[P]$. Lowering the value assigned to this ratio produces some variability in the corresponding value of D but does not significantly alter the sensitivity of the fit to the choice of r.

In determining the best fits to the nonlinear low

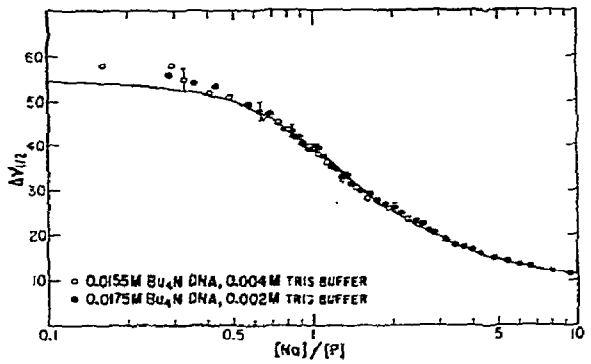

Fig. 3. Linewidth measurements (Hz) as a function of [Na]/[P] for a solution containing 0.0155 M Bu_4NDNA in the presence of 0.004 M *tris*, and a solution containing 0.0175 M Bu_4NDNA in the presence of 0.002 M *tris*. The line represents the best fit of eq. (8a) to both sets of data, with $r = 0.76$, as explained in section 3.4.

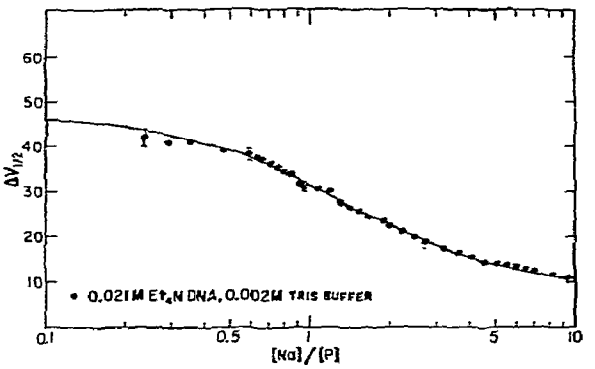

Fig. 4. Linewidth measurements (Hz) as a function of [Na]/[P] for a solution containing 0.021 M Et_4NDNA in the presence of 0.002 M *tris*. The line represents the best fit of eq. (8a) to the data, with $r = 0.76$, as explained in section 3.4.

sodium data for the Bu_4NDNA solutions, it was decided that closer conformity to the relatively large number of points in the vicinity of $[Na]/[P] = 1$ should outweigh the appearance of a possible systematic deviation at very low values of added salt. (See fig. 3.) Although the experimental uncertainty in these data is the largest, there is some reason to expect an upward trend in the linewidths, beyond that predicted by (8a), as $[Na]/[P] \to 0$. Manning [16] has estimated that the local concentration of monovalent counterions in the condensation layer surrounding DNA is approximately 1 M. Eisenstadt and Friedman [30] found that the relaxation rate of ^{23}Na is significantly enhanced in concentrated solutions containing sodium chloride together with other simple strong electrolytes. It was therefore important to estimate the magnitude of any additional concentration-dependent broadening caused by variable amounts of tetraalkylammonium in the condensation layer. For a blank solution, 0.25 M in NaCl, 0.75 M in Bu_4NBr and containing no DNA, the sodium linewidth was determined to be 9.5 Hz, about 3 Hz broader than for a blank solution containing only 1 M NaCl. (Note that the ratio $[Q]/[Na] \approx 3$ is the highest for which linewidth measurements were made on the Bu_4NDNA solutions.) Thus, it is likely that any broadening attributable to changes in the composition of the condensation layer when $[Na]/[P] < 0.5$ should have at most a slight effect on the titration curves obtained for the Bu_4NDNA solutions.

Because of the relatively large random scatter in the low sodium data and because D is not large enough for either tetraalkylammonium cation to warrant the use of eq. (10) to fit these data, it was not possible to determine the extent of counterion condensation with a high degree of precision. For the Et_4NDNA solution (fig. 4) the titration curve can be fit with a range of values for r: $0.6 < r < 1$. Since the Bu_4N^+ ion competes less effectively than Et_4N^+ with sodium, the range of values with which it is possible to fit the titration curves in fig. 3 is correspondingly narrower: $0.65 < r < 0.85$. For both Et_4NDNA and Bu_4NDNA the theoretical value 0.76 provides excellent fits of eq. (8a) to the low salt data ($[Na]/[P] < 2$). These curve fittings yield values of D which indicate that the relative affinities of Na^+, Et_4N^+, and Bu_4N^+ for DNA are approximately in the ratio 20:5:1. Moreover, if $r = 0.76$ it may be deduced from the more accurate high salt data for both Et_4NDNA and Bu_4NDNA that the intrinsic relaxation rate of condensed sodium is $180 \pm 10\,s^{-1}$.

4. Discussion

4.1. Implications of the constant charge fraction on DNA

The fundamental physical manifestation of the phenomenon of condensation is that in a polyelectrolyte solution containing counterions of a particular

valence type, the extent to which these ions compensate the charges on the polyelectrolyte through delocalized association does not depend on the overall concentration of the small ions. For some time prior to the theoretical development of the thermodynamic consequences of the condensation model, due chiefly to Manning [9], a small but convincing body of experimental evidence indicated the invariance of the charge fraction, even in moderately concentrated polyelectrolyte solutions (0.1 M and higher) and in the presence of appreciable excess concentrations of simple electrolytes. Some of the pertinent older literature has been surveyed by Felsenfeld and Miles [1]; the more recent supporting evidence has been critically reviewed by Record and Manning [17] (see also ref. [16]). The persistence of a non-zero charge fraction on a polyelectrolyte as the concentration of counterions capable of associating with it is increased appears to contradict the principle of mass action. However, a conventional mass action treatment is strictly applicable only if the small ions are bound reversibly to specific sites on the macromolecule. Mass action expressions have been used to describe the association of alkali metal and quaternary ammonium cations with helical DNA in a wide variety of investigations employing, for example, electrophoresis [36,37], equilibrium dialysis [38], and magnetic resonance [22,39]. But these experimental results can be reinterpreted in terms of the condensation model [15]. A particularly relevant example will be considered below (section 4.3), where it is shown that the ^{23}Na NMR measurements of Reuben et al. [22] are consistent with the conclusion that the charge fraction on DNA does not vary with the addition of sodium chloride. In the following discussion the consequences of describing the results presented in section 3 in terms of a mass action model are explored, and it is demonstrated that the description afforded by the condensation model is simpler and more plausible.

The linearity of the ^{23}Na titration curves in fig. 2 constitutes strong evidence that the number of sodium ions associated per phosphate charge, r, is constant, regardless of the concentration of added salt. The only possible alternative explanation of the linear functional form of the linewidth data is that the two site model must be failing (R_B varying) in a way which exactly compensates variations in the extent of sodium association. But this hypothesis appears extremely unlikely, because even if the total number of sodium ions associated per phosphate charge were changing, any resulting changes in the quadrupolar relaxation rate characteristic of associated sodium ions should be directly, rather than inversely, correlated. For example, from the experimental results of van der Klink et al. [19] it is clear that increasing the structural charge density on polyacrylate causes *both* r and R_B to increase (see also ref. [34]).

The simplest way to rationalize the linear high salt data using a mass action description of sodium association with DNA would be to assume that the apparent binding constant is so large that virtually all of the phosphate sites have been bound by sodium. Hence, r would equal one, and R_B, the parameter characterizing the relaxation rate of bound sodium, could be evaluated directly from the observed slope using eq.(3). However, the linewidths measured at low salt ($[Na]/[P] < 0.7$) for the Bu_4NDNA solutions (fig. 3) exceed the value of R_B deduced from the high salt data by setting $r = 1$. Since this value must constitute the absolute maximum for observed relaxation rates, the original assumption that the "binding capacity" of DNA has been saturated at high salt must be invalid. That the apparent magnitude of R_B could be significantly enhanced at low salt by the presence of associated Bu_4N^+ is improbable a priori and may be dismissed on the basis of the blank experiment described in section 3.4.

In view of the near equality of the slopes represented in fig. 2, it can be inferred that the presence of tetraalkylammonium cations has no influence on the parameters that characterize the functional form of the high salt data. Hence, if a mass action model can be used to account for the conclusion, based on the preceding arguments, that r has a constant value *less than one*, the appropriate expression must depend solely upon the concentrations of sodium and DNA phosphate. It can be shown that in order to simulate the linear concentration dependence of the linewidths using a mass action expression for sodium binding, the apparent dissociation constant, K_D, must increase as a linear function of the total sodium chloride concentration. While it can be expected from the known properties of simple electrolytes in solution that K_D should tend to increase with increasing ionic strength, a linear relation requiring a single parameter (the unknown constant "binding density", r) appears to have

no precedent, either empirical or theoretical. Of course, until the salt dependences of all the pertinent activity coefficients can be independently determined, it will not be possible rigorously to disprove the mass action model. Nevertheless, it seems reasonable to conclude that if the ionic strength dependence of K_D is fully as important in determining the extent to which sodium associates with DNA as are the concentrations appearing explicitly in the usual mass action expression, then the utility of this description is moot.

To simulate the low salt data using mass action expressions for both sodium and tetraalkylammonium it would be necessary to introduce assumptions about the concentration dependences of *both* apparent binding constants, K_Q^D and K_N^D, which would be impossible to justify on the basis of the NMR measurements alone. Even if the simplest set of assumptions consistent with the high salt data were adopted, the resulting expression would contain at least as many parameters as does eq. (8a) and would be cubic in the measured linewidth, further amplifying the uncertainty in the fitted parameters owing to the random scatter in the low salt data. Figs. 3 and 4 show how closely these data can be simulated using eq. (8a), which was derived under the assumption that the charge fraction on DNA, and hence r, remains constant even when both tetraalkylammonium and sodium are present in the condensation layer. The conclusion that DNA has a charge fraction which is independent of the addition of sodium chloride (at least up to 0.6 M) is in accord with findings for a number of other polyelectrolytes deduced from a variety of experimental studies [15]. Very recently Manning has provided a theoretical explanation for the observation that the charge fraction on polyelectrolytes is invariant well beyond the limit of infinite dilution in the presence of simple electrolytes in considerable excess [16].

4.2. Assessing the quantitative results

From a consideration of the linewidth data at high salt concentrations (fig. 2) and the magnitude of the limiting value approached by the linewidths at low salt concentrations (fig. 3), it was deduced in the preceding section that the charge fraction on DNA is a non-zero constant. Then it was shown why this qualitative finding in itself constitutes a strong reason to prefer condensation over mass action, insofar as it affords a more practical way to describe the association of monovalent counterions with DNA. But to test the *quantitative* validity of this model using ^{23}Na NMR measurements, the parameter $r = 1 - \xi^{-1}$ must be evaluated. The approach to this problem taken in the present study was to vary the amount of sodium associated with DNA by titrating one of its tetraalkylammonium salts with sodium chloride. The resulting linewidth data conform to eq. (8a), which is a nonlinear function of [P]/[Na] at sufficiently high values of this ratio. Specifically, close simulations of all three experimental titration curves (in figs. 3 and 4) were obtained over the entire range of salt concentrations investigated by setting r equal to the theoretical value 0.76 and optimizing the fit by varying D. R_F is in effect independently given by the intercept of the linear high salt data; R_B is determined by their slope if r is fixed. This fitting procedure was found to be fairly sensitive to the choice of r, considerably less so to the choice of D.

In fact it is possible to fit eq. (8a) to the linewidth data in figs. 3 and 4 using a range of choices for r, centered on the value required by the condensation model for DNA. In section 3.4 this uncertainty was attributed to two sources: the relatively pronounced random scatter in linewidth measurements performed at the lowest salt concentrations; and the unexpected finding that D is not much greater than one for either tetraalkylammonium species. To sustain a favorable judgment concerning the quantitative accuracy of the condensation model on the basis of the success with which eq. (8a) has been used to fit the data, it is necessary at least to indicate how the various assumptions underlying the derivation outlined in section 3.3 can be justified. A convenient framework for this discussion is provided by considering the individual parameters, other than r, upon which the functional form of eq. (8a) depends.

The observed ^{23}Na transverse relaxation rate appears to share a deficiency common to many spectroscopic variables: it is an *averaged* quantity, not all of whose components can be independently determined by direct measurement or a theoretical calculation. It follows from the analysis presented in section 3.1 that knowledge of R_B suffices in order to deduce from the linewidth measurements the extent to which sodium is associated with DNA. The ^{23}Na NMR investigations conducted by Leyte and his colleagues [19–21] have

demonstrated that the quadrupolar relaxation rate of sodium associated with a polyelectrolyte is made up of two independent contributions. The dominant term, arising from the electric field gradient due to the cylindrical potential surrounding the polyelectrolyte, can be expressed as a function of known physical parameters and quantities for which reasonable estimates can be made [19]. However, the second contribution, arising from the field gradient due to the water dipoles adjacent to the associated ion, is nonnegligible and cannot be estimated a priori. The alternative of measuring R_B directly could have been accomplished (when [Na]/[P] < 1) had the affinity of sodium for DNA proved to be substantially greater than that of the tetraalkylammonium cations. The modest magnitude of the parameter D has the effect of introducing curvature into plots $\Delta\nu_{1/2}$ versus [P]/[Na] and thereby relegates R_B to the status of a parameter to be determined. Some justification for the assumption that this parameter has a constant value over the entire range of sodium chloride concentrations considered here has already been provided in section 4.1. More rigorous substantiation requires a detailed consideration of the validity of the two site model, presented elsewhere [34].

If r is set equal to the theoretical value 0.76, R_B can be evaluated from the linear least squares slope and intercept of the data presented in fig. 2 (see table 1). The resulting value, $R_B = 180 \pm 10\ s^{-1}$, is somewhat larger than the relaxation rate characteristic of sodium ions associated with polyacrylate [19] but only about half the value of R_B in polyphosphate solutions [20]. Although, for the reasons stated above, the constituent parameters in the theoretical expression for R_B cannot be uniquely assigned from its numerical value alone, definite qualitative inferences can be drawn bearing on the motional characteristics and hydration of the associated ions, and thus on the physical nature of the condensation layer. This topic will be addressed at greater length subsequently [34]. It should be noted that the evaluation of R_B may permit the effective use of ^{23}Na NMR as an indirect, but quantitative, means of studying the interactions of ligands such as proteins with DNA. Such an approach would be particularly valuable when independent probes of comparable sensitivity can be employed to gain as much information as possible about the relevant binding equilibria.

The values of R_F determined from the linear least squares fitting of the high salt data (22–23 s^{-1}) are significantly greater than the relaxation rate of ^{23}Na in moderately concentrated sodium chloride solutions (18–19 s^{-1}) (The highest salt concentration in the present investigation was 0.6 M.) An analysis using eq. (3) of the variation of ^{23}Na relaxation rates with the concentration of excess salt in solutions of sodium polyacrylate [19] and sodium polyphosphate [20] produces the same conclusion: R_F, the relaxation rate of sodium not directly associated with the polyelectrolyte, is greater than the relaxation rate of sodium in a blank solution containing the same amount of simple salt but no polyelectrolyte. Although this fact may appear to cast doubt on the interpretation of the high salt data outlined in sections 3.1 and 3.2, it can be explained by considering the results of an extensive study made by Eisenstadt and Friedman [30,32] of ^{23}Na NMR in solutions containing simple electrolytes. They found that even for nonassociating salts, at concentrations such that the direct encounters between sodium ions and oppositely charged species must be infrequent, the observed ^{23}Na relaxation rate does exhibit a definite concentration dependence. By examining a wide variety of systems they established a correlation between increases in the sodium relaxation rate and increases in the bulk viscosity and partial molar volume of the solvent brought about by varying the solute concentration. A particularly instructive example is afforded by their observation that in a dilute sodium chloride solution containing 0.2 M sucrose, the relaxation rate of sodium is enhanced by approximately 30%. Hence, even a *nonelectrolyte* molecule is capable of exerting a long-range effect on the quadrupolar relaxation of ^{23}Na. Although no rigorous theoretical treatment appears possible, the bulk viscosity of a sodium chloride solution is sufficiently increased by the presence of DNA at the concentrations in the present study ($\approx$0.02 M) that the values of R_F deduced from the intercepts of the high salt data (table 1) are completely reasonable. During the course of the sodium chloride titrations care was taken to avoid significant dilution of the samples, so that the concentration of DNA was kept nearly constant. Moreover, it is certainly permissible to neglect increases in the relaxation rate of "free" sodium owing to the steadily increasing concentration of sodium chloride in the samples [32]. On these grounds the assumption that R_F is a constant parameter, over the entire experimental range of salt concentrations is justifiable.

Since the affinities for DNA of Et_4N^+ and Bu_4N^+ where found to be at least several times smaller than that of sodium, varying the numerical values of D produces relatively insignificant variations in the simulated titration curves shown in figs. 3 and 4. Nevertheless, defining D according to eq. (6) in effect determines the possible functional forms of eq. (8a), so that D is a governing parameter, even though its precise magnitude is of little consequence for the purposes of the present study. Since eq. (6) happens to be identical to the ratio of two mass action expressions, it should be pointed out why this way of defining relative affinities is consistent with the prior assumption, embodied in eq. (5), that both sodium and the tetraalkylammonium cation undergo condensation. Since the phenomenon of condensation arises exclusively from the operation of *long range* electrostatic forces, the *extent* to which counterions condense on a polyelectrolyte (or the charge fraction remaining after condensation) depends only upon the charge spacing on the polyelectrolyte, the dielectric constant of the solvent, and the *charge type* of the counterion [9]. But when different types of counterions bearing the same charge are undergoing condensation, short-range specific forces dependent on the particular *nature* of the counterions become important in determining the *composition* of the condensation layer. Equations analogous to (5) and (6) have been derived by Manning [16] to model the competitive association of sodium and magnesium with polyelectrolytes. Essentially identical equations have been employed by Hen and Strauss [40] to interpret a large body of equilibrium dialysis data bearing on the competitive association of various monovalent ions with polyvinyl sulfonate. The internal consistency of their analysis strongly indicates that while the charge fraction on a polyelectrolyte does not depend on the chemical nature of the counterions (so long as they are of the same charge type), the relative abundance of these counterions in the condensation layer does. (Different pairs of counterions can exhibit substantially different values of the parameter D.) Hence, the compatibility of eqs. (5) and (6) can be upheld on empirical grounds. While it is possible that the inevitable increase in ionic strength accompanying the addition of sodium chloride should introduce some variability into D, the sensitivity of the titration curves obtained in the present study to the magnitude of this parameter is sufficiently small that no variability is apparent.

Although the values of D cited in section 3.4 must be regarded as highly approximate, they permit the conclusion that the affinity of Et_4N^+ for DNA is at least several times that of Bu_4N^+. It is reasonably certain that the larger tetraalkylammonium cations in aqueous solution have fully extented alkyl chains [41], so that the stronger affinity of Et_4N^+ may be attributed to its smaller size. However, an accurate quantitative determination of the relative affinities of these tetraalkylammonium species using ^{23}Na NMR was not the purpose of the present study and would entail varying the concentrations of these species as well as that of sodium. On the basis of the equilibrium dialysis measurements of Shapiro et al. [42] it can be concluded that the association of the larger tetraalkylammonium cation with DNA has no specific (nonelectrostatic) characteristics, at least at the concentration levels considered in the present study. Hence, despite the fact that the solvent structure immediately surrounding these cations differs from that surrounding sodium [43], if the condensation model is valid for sodium it should be valid for Et_4N^+ and Bu_4N^+ as well.

In the foregoing discussion the constancy of the parameters appearing in eq. (8a) has been demonstrated, and the assumptions entering into its derivation have been supported by drawing on the results of previous work, both theoretical and experimental. It follows that any uncertainty in the charge fraction obtained by an analysis of the linewidth measurements in terms of this equation should be attributed to experimental sources. The extent of this uncertainty ($\xi^{-1} = 0.25 \pm 0.10$) appears unavoidable in view of the limitations arising from the low sensitivity of the NMR measurement and from practical difficulties encountered at higher DNA concentrations (see section 2.1). Even in the absence of these problems, it is unlikely that the scatter in the low salt data could be significantly reduced unless the DNA concentration were increased to a point where both condensation and the two site model for relaxation begin to break down. Although higher magnetic fields would serve to increase the signal-to-noise ratio, it may be concluded from the work of van der Klink et al. [19] that at field strengths more than a factor of two greater than that employed here the condition of "extreme narrowing" is likely to be violated. In this situation the relaxation rate characteristic of associated sodium would not be a single exponential decay. Hence, linewidth measurements

would be essentially impossible to interpret, whereas experimental accuracy would be almost certainly insufficient to warrant a computer simulation of the non-Lorentzian lineshape. (Further discussion is given in ref. [34].) Thus, it appears that no experimental means are likely to be successful in significantly improving the precision with which the charge fraction on DNA can be determined by ^{23}Na NMR competition studies.

4.3. Comparisons with other work

The small number of previous studies specifically aimed at measuring directly the extent of association of monovalent cations with a polyelectrolyte in aqueous solution have almost exclusively utilized methods sensing changes in hydration, such as dilatometry. The conformity of the resulting body of experimental findings with the condensation model has been discussed by Manning [16]. Recently Reuben et al. [22] reported a number of ^{23}Na longitudinal relaxation time (T_1) measurements performed on solutions of NaDNA containing excess sodium chloride, whose concentration was varied. They interpret their data in terms of a mass action model for sodium binding to DNA phosphate by assuming that the apparent binding constant of sodium remains constant as the ionic strength of the solution is increased and that regardless of the concentration of DNA (which was varied threefold) the parameter R_F is equal to the ^{23}Na relaxation rate measured for a blank sodium chloride solution containing no DNA. Although the resulting nonlinear expression provides a reasonable two-parameter fit to the T_1 data, the first assumption is difficult to justify in view of uncertainty about the appropriate activity coefficients, and the second assumption is definitely at variance with the findings of previous studies on analogous systems. (See the discussion of R_F in section 4.2.) Owing to the presence of a second monovalent cation, the solution compositions examined in the present study (expressed as the ratio [Na]/[P]) were variable over a much broader range; however, to the extent that it is possible to compare the present linewidth data with the T_1 data of Reuben et al., the agreement is close. If R_F is regarded as a free parameter eq. (3) can be used to obtain a *linear* fit of the T_1 data, whose slope and intercept are equal, within experimental error, to those presented in table 1.

Our basic approach to achieving a measure of the extent to which monovalent counterions associate with DNA has been to monitor the competition between sodium and a tetraalkylammonium cation. Ideally, had D proved much greater than one, it would have been possible to eliminate this parameter and obtain a two parameter fit of the data using eq. (10). In view of the likelihood that there are no monovalent cations whose tendency to associate with DNA is substantially lower than that of tetrabutylammonium, it is appropriate at this point to consider possible alternative approaches using ^{23}Na NMR to determine the charge fraction on DNA. Since the condensation model posits a definite relation between the charge spacing on the polyelectrolyte and the extent to which counterions associate, if it were possible to vary in a known way the *structural* mean axial charge density on the polyelectrolyte, the parameter ξ^{-1} could be evaluated. Leyte and his colleagues have successfully adopted this approach: using ^{23}Na NMR measurements they demonstrated that sodium condenses to the extent predicted in solutions of polyacrylate [19], polyphosphate [20], and polystyrenesulfonate [21]. However, it does not appear feasible to alter the structural charge density on DNA in a known way using chemical means, such as varying the pH. Rather than competing sodium with a monovalent cation whose relative affinity for DNA is much lower, it seems reasonable to consider the opposite experiment: find a cationic species whose affinity for DNA is so much greater that when introduced in known amount, it would displace a known number of sodium ions. However, it seemed initially preferable to employ as a competing cation a monovalent species whose interaction with DNA was sure to be of no more specific character than is the association of sodium itself. Now that R_B has been determined with good accuracy in this way, ^{23}Na NMR may prove useful in gaining quantitative information about the more complicated binding characteristics of polyvalent cationic ligands with DNA.

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