Sodium-23 Nuclear Magnetic Resonance Studies of Sodium Aminocarboxylic Acid Complexes

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Abstract: Nuclear magnetic resonance (nmr) relaxation time measurements of ^{23}Na have been used to study sodium ion complexes with ethylenedinitrilotetraacetic acid (EDTA), N′-(2-hydroxyethyl)ethylenediamine-N,N,N′-triacetic acid (HEEDTA), and nitrilotriacetic acid (NTA) in aqueous solution. The pH dependence of the ^{23}Na spin-lattice relaxation time (T_1) demonstrates a chelate effect and identifies the important chelating groups. Analysis of ^{23}Na spin-lattice relaxation times as a function of total sodium ion and total ligand concentrations permits measurement of the complex formation constant (K_f) and the relaxation time of the bound sodium (T_{1B}). The results are (at 25°, ionic strength variable between 0.7 and 1.0): for HEEDTA, $K_f = 8.7 \pm 0.9\ M^{-1}$, $T_{1B}^{-1} = 610 \pm 30\ sec^{-1}$; for NTA, $K_f = 9.6 \pm 1.5\ M^{-1}$, $T_{1B}^{-1} = 525 \pm 40\ sec^{-1}$ (95% confidence limits given). In addition it is shown that histidine is ineffective in complexing sodium, contrary to what has been previously reported.

The chemistry of the sodium ion in aqueous solutions is of wide importance, especially in biological systems.[2-3] In particular, sodium ion complexes appear to be important in promoting the permeability of natural and artificial membranes to sodium and other group I cations.[2] Nuclear magnetic resonance (nmr) is useful for the study of the formation of reversible complexes of ^{23}Na ions in solution; the change in the ^{23}Na spin-lattice relaxation time (T_1) when complexes are formed can be related quantitatively to the equilibria involved. This effect is due to the large nuclear quadrupole moment of the ^{23}Na nucleus which couples the nuclear spin to its surroundings. These couplings, which dominate the spin-lattice relaxation, are very sensitive to changes in the electronic environment of the quadrupolar nucleus.

Previous studies have been reported detailing the effect of electrolyte concentration on the relaxation rate or line width of ^{23}Na in concentrated electrolyte solutions.[4-7] The work of Jardetzky and Wertz[4] was the only one to deal with organic chelating ligands to any extent, but their line-width measurements appear to have been dominated by field inhomogeneity as pointed out by Eisenstadt and Friedman.[6]

In this paper we report the application of ^{23}Na spin-lattice relaxation time measurements to the investigation of possible Na^+ complexes of ethylenedinitrilotetraacetic acid (EDTA), N′-(2-hydroxyethyl)ethylenediamine-N,N,N′-triacetic acid (HEEDTA), nitrilotriacetic acid (NTA), and histidine. Our choice of these aminocarboxylic acids for this initial work was motivated by their similarity to many amino acids and the extensive study previously afforded this group as chelating agents. Using a different approach from that used in the papers[4-7] cited above, we are able to detect a chelate effect, identify the important chelating groups, and determine the conditional formation constant and the relaxation rate of complexed Na^+.

Theoretical Section

Magnetic relaxation of nuclei with total spin quantum number $I > 1/2$ is ordinarily dominated by nuclear quadrupole relaxation. Quadrupole relaxation has been extensively treated elsewhere.[8,9] For a nucleus such as ^{23}Na with spin $I = 3/2$ the spin-lattice relaxation time T_1 is given in the limit of extreme narrowing by[8]

$$\frac{1}{T_1} = \frac{2\pi^2}{5}\left(\frac{e^2qQ}{h}\right)^2 \tau_c \qquad (1)$$

where the symbols have their usual meaning.[10] Of particular interest is the electric field gradient eq (actually the principal component of the field gradient tensor assumed to be axially symmetric). If the quadrupolar species is bound by a covalent bond or bonds in a site of noncubic symmetry, eq will be nonzero and quadrupole relaxation will occur.[11] For nuclei such as ^{23}Na with a large nuclear quadrupole moment (eQ), even moderately covalent bonding will make nuclear quadrupole relaxation the predominant mechanism for spin-lattice relaxation. On the other hand, quadrupolar species, such as Na^+ or Cl^- ions, in a symmetrically solvated environment will have relatively long spin-lattice relaxation times.[5,12] If we consider a quadrupolar ion, M, in rapid equilibrium between a "free" (*i.e.*, solvated) state and a state in which it is bound to a coordinating ligand, (L)

$$(M) + (L) \underset{}{\overset{K_f}{\rightleftharpoons}} (ML) \qquad (2)$$

the observed relaxation rate will be given by

$$\frac{1}{T_1} = \frac{X_F}{T_{1F}} + \frac{X_B}{T_{1B}} \qquad (3)$$

where X_F and X_B are the respective mole fractions of "free" and bound ion and T_{1F} and T_{1B} are the spin-lattice relaxation times of the "free" and bound ion. Equation 3 is valid in the "rapid exchange" limit which

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can be operationally defined as a situation where the nuclei in two sites behave as if only one species were present; experimentally the criteria are (a) spin-lattice relaxation is a single exponential, (b) the line shape is Lorentzian, and (c) the measured relaxation time varies continuously as ligand L is added. Criterion (a) requires $T_{1B}^{-1} \ll k_{exch}$ and (b) requires $\delta\omega$ (chemical shift) and $T_{2B}^{-1} \ll k_{exch}$; all these criteria appear to have been met by systems studied and described in this paper. A theoretical discussion of the meaning of "rapid exchange" has been given elsewhere.[13]

Defining relaxation rates $R = T_1^{-1}$, $R_F = T_{1F}^{-1}$, and $R_B = T_{1B}^{-1}$ and C_M and C_L as the analytical concentrations of M and L, respectively, and the formation constant for eq 2, $K_f = [ML]/[M][L]$, one can solve for X_B

$$X_B = K_f C_L/[1 + K_f C_L + K_f C_M - K_f C_M X_B] \quad (4)$$

and rewrite eq 3 as

$$R = R_F + (R_B - R_F)X_B \quad (5)$$

Equation 4 is a quadratic equation which we generally solve iteratively.

The relaxation rate R_F can be easily measured in the absence of L. However, for weak complexes it is not usually possible to measure R_B directly. Therefore, eq 4 and 5 must be considered to contain two undetermined parameters, K_f and R_B, with two independent variables C_L and C_M. Measurements of R as a function of C_L and C_M are fit to the model (eq 4 and 5) using a nonlinear least-squares computer program furnished by the University of Wisconsin Computing Center (GAUSHAUS). This program utilizes a method by Marquardt[14] which combines the Gauss (Taylor series) method and the method of steepest descent. Limits of errors of the parameters K_f and R_B quoted in this paper are rough 95% confidence limits estimated assuming a linear model.

If it becomes necessary to take into account equilibria involving either (M), (L), or (ML) other than the one expressed in eq 2, the equations for R as a function of C_L and C_M become much more complicated. Even if the equations can be solved numerically by a computer, the addition of more parameters would make a least-squares fit very difficult. Given present limitations on the accuracy of measuring R and the range of concentrations which can be used, the application of nmr to study multiple equilibria without additional information may not be feasible.

Experimental Section

Reagents used were of the highest available commercial purity and, with the exception of HEEDTA which was recrystallized from water, were used without further purification. Solutions were prepared by weighing the requisite amounts of the reagents; a few were checked by titration. Sodium chloride was used as a source for Na^+ since NaCl concentration has little effect on the ^{23}Na T_1 in the range of concentrations used.[5,7] Solutions were stored in polyethylene bottles and were adjusted to the desired pH using concentrated hydrochloric acid or tetramethylammonium hydroxide pentahydrate. Tetramethylammonium (TMA) ion was chosen because of its inability to form complexes with the ligands[15,16] and its negligible effect on the Na^+ relaxation times as determined in blank runs. Solution pH measurements were made at 25° using a Leeds and Northrup Model 7405 line-operated pH meter and a wide-range glass electrode. Conventional NBS buffers were used for standardization of the pH meter. The nature of our experiments required the use of solutions with a large and slightly variable ionic strength (0.7–1.0).

Spin-lattice relaxation times were measured at 25.0 ± 1.0° by the conventional 180°, τ, 90° two pulse sequence[17] using an NMR Specialties Model PS-60 pulsed nmr spectrometer at a frequency of 15.0 MHz. The maximum voltage of the free induction decay following the 90° pulse, which depends on the time τ between pulses, was measured using a Princeton Applied Research CW-1 boxcar integrator to improve the signal-to-noise ratio. Both the nmr spectrometer and the boxcar integrator were modified to allow a continuous sweep of τ, the spacing between the pulses, and continuous measurement and recording of $V(\tau)$, the maximum voltage of the free induction decay at time τ following the 180° pulse. This output is related to T_1 through

$$\ln [V(\tau \to \infty) - V(\tau)] = -\frac{\tau}{T_1} + (\text{constant}) \quad (6)$$

In addition, point-by-point averages were made in which the boxcar averaged the result of numerous 180°, τ, 90° sequences at a fixed value of τ; the average voltage was then read on a digital voltmeter. The pulse separations (τ) were measured with a Hewlett-Packard HP-5245L electronic counter and an HP-5262A time interval unit. This signal-averaging technique permitted useful measurements of T_1 at Na^+ concentrations as low as 0.01 *M*. At Na^+ concentrations of 0.1 *M*, least-squares fits of eq 6 gave standard deviations on the order of 1% or better. However, reproducibility between runs was generally on the order of 3–4%. Relaxation times reported in this paper can be considered to have an accuracy of about 3–4%.

Free induction decays from solutions containing saturated NaCl could be observed on an oscilloscope and were used to adjust the magnetic field to the resonance condition. No chemical shifts were observed between solutions containing saturated NaCl alone and 1 *M* Na–EDTA; we were generally able to ignore the possibility of a chemical shift in solutions where the free induction decay could not be directly observed. In all cases it appeared that chemical shifts were less than the effective line width of the sodium resonance. Absorption line shapes of ^{23}Na nmr in solutions of approximately equal concentrations of Na^+ and ligand were measured and found to be Lorentzian within experimental error for each of the various ligands studied; this confirmed our assumption of rapid exchange.

Results and Discussion

The nmr parameter of interest in this investigation, the ^{23}Na nmr relaxation rate, had a minimum value of 17.5 sec^{-1} for "free" Na^+. This value, taken to be R_F, was obtained with NaCl as the only solute at concentrations of 0.01 to 0.4 *M* and agrees with previously published results.[5,7]

The ^{23}Na relaxation rate is clearly pH dependent with Na^+ in the presence of a potential chelating agent as seen in Figures 1–3. The relaxation rate is definitely sensitive to the Na^+–aminocarboxylate interactions since the observed breaks in the relaxation rate *vs.* pH curves correspond to protonation at the various basic sites on the ligands.

The pH dependence of the ^{23}Na relaxation rate for a 1:1 solution of Na^+–EDTA is depicted in Figure 1. The small change in relaxation rate over the pH range 4.5–7 can be compared with the very large increase which occurs over the pH range 7.5–11 where the last proton is lost by a nitrogen of EDTA.[15] This illustrates the importance of the metal–nitrogen bonding. The ^{23}Na relaxation rate is, however, unaffected by the presence of equimolar trimethylamine at pH 11, which indicates that the sodium–nitrogen interaction in EDTA is largely due to a chelate effect. Unfortunately, this

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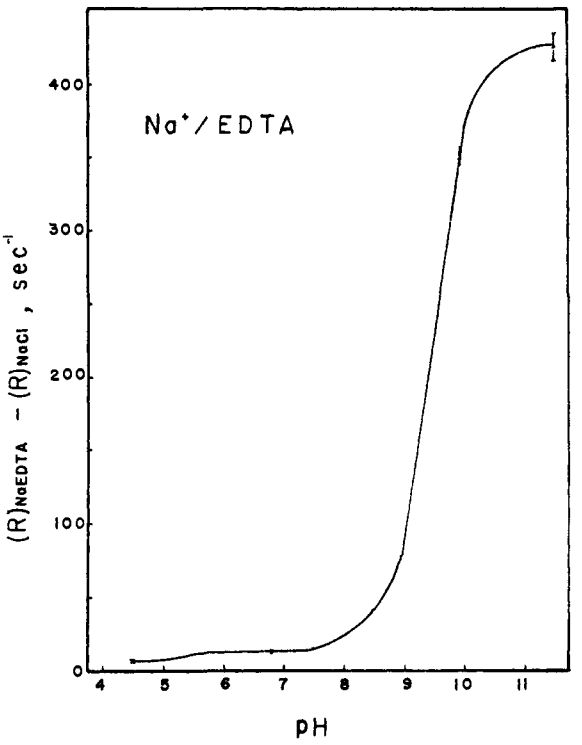


Figure 1. pH dependence of ^{23}Na T_1^{-1} for 0.100 M NaCl–0.100 M EDTA solution. The pH was adjusted using concentrated HCl and TMA hydroxide.

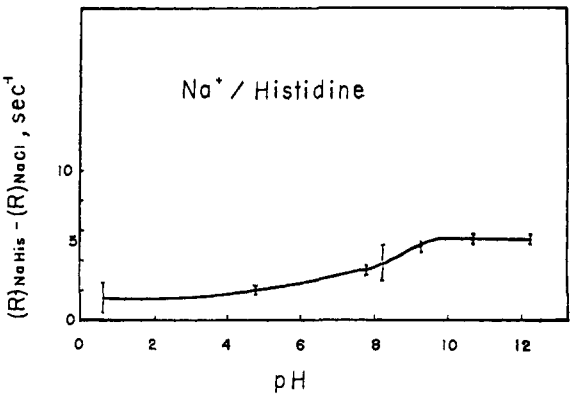
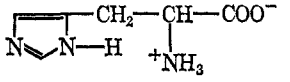


Figure 2. pH dependence of ^{23}Na T_1^{-1} for 0.185 M NaCl–0.185 M histidine solution. The pH was adjusted using concentrated HCl and TMA hydroxide.

most widely used chelating agent precipitates from solution below pH 4, making a complete pH study impossible.

In contrast to the large break noted in Figure 1 for EDTA, all the changes in relaxation rate for solutions of Na^+ with the naturally occurring amino acid histidine



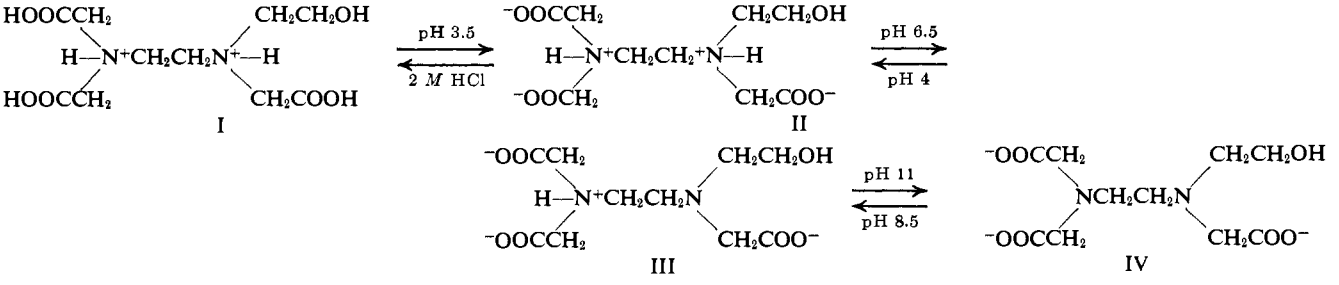
are small over the entire pH range. The results depicted in Figure 2 for histidine indicate only a small increase in Na^+ interaction as the protons on the carboxyl, imidazole, and amino groups are successively titrated. This emphasizes the importance of additional carboxylate groups for formation of a stronger chelate when compared with the EDTA results. Our conclusion of only a very weak Na^+–histidine interaction is not consistent with the conclusion of Jardetzky and Wertz[4] that histidine forms a relatively strong chelate with Na^+.

The aminopolycarboxylic acid HEEDTA, unlike EDTA, is soluble over the entire pH range employed from 2 M HCl to pH 12.7. This encompasses species ranging from one with all carboxylates and both nitrogens protonated (I) to the trianion (IV). The protonation scheme has been worked out by Reed[18] and a simplified version is presented (Scheme I).

The nonzero value of $R_{NaHEEDTA} - R_{NaCl}$ for pH <3.5, as shown in Figure 3, may be due to a weak interaction, perhaps ion pairing, of Na^+ with the carboxylate groups of HEEDTA or to nonspecific interactions of Na^+ with its aqueous environment due to slight changes in viscosity and volume effects. The increase in ^{23}Na relaxation rate from pH 3.5 to 6.5 may be attributed to the formation of a very weak complex of Na^+ with the monoprotonated HEEDTA species (III). The large increase in relaxation rate from pH 8.5 to 11.5 is a manifestation of the formation of a stronger chelate with the trianion (IV) utilizing sodium–nitrogen bonding.

The ^{23}Na relaxation rate as a function of the NaCl and $HEEDTA^{3-}$ concentrations at pH 11.5 is depicted in Figure 4. The concentration dependence of ^{23}Na T_1^{-1} is described by the two-parameter model outlined in the Theoretical Section; the best values for the parameters are $K_f = 8.7 \pm 0.9\ M^{-1}$ and $R_B = 610 \pm 30\ sec^{-1}$ (limits represent 95% confidence interval) for a Na^+–$HEEDTA^{3-}$ complex at 25.0 ± 1° and at a variable ionic strength of 0.7–1.0. The lines indicated on Figure 4 were calculated using these parameters. The deletion of the data obtained from the 0.050 or 0.392 M NaCl solutions with $HEEDTA^{3-}$ was found to have no significant effect on the determined values of K_f and R_B. The inclusion of data obtained at higher $HEEDTA^{3-}$ concentrations gave the same values for K_f and R_B within the experimental error. In addition, a

Scheme I



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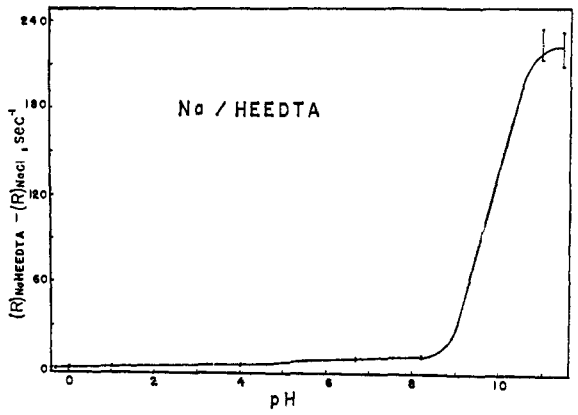

Figure 3. pH dependence of ^{23}Na T_1^{-1} for 0.100 M NaCl–0.095 M HEEDTA solution. The pH was adjusted using concentrated HCl and TMA hydroxide.

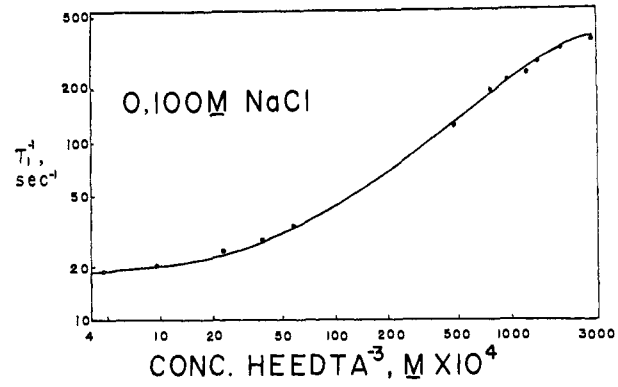

Figure 5. Log–log plot of ^{23}Na T_1^{-1} of 0.100 M NaCl as a function of HEEDTA^{3-} concentration at pH 11.5. The curve is calculated using the model presented in the Theoretical Section and the mean value of K_f and R_B. The concentration of TMA hydroxide is roughly 0.1–0.4 M and is varied from solution to solution to maintain constant pH.

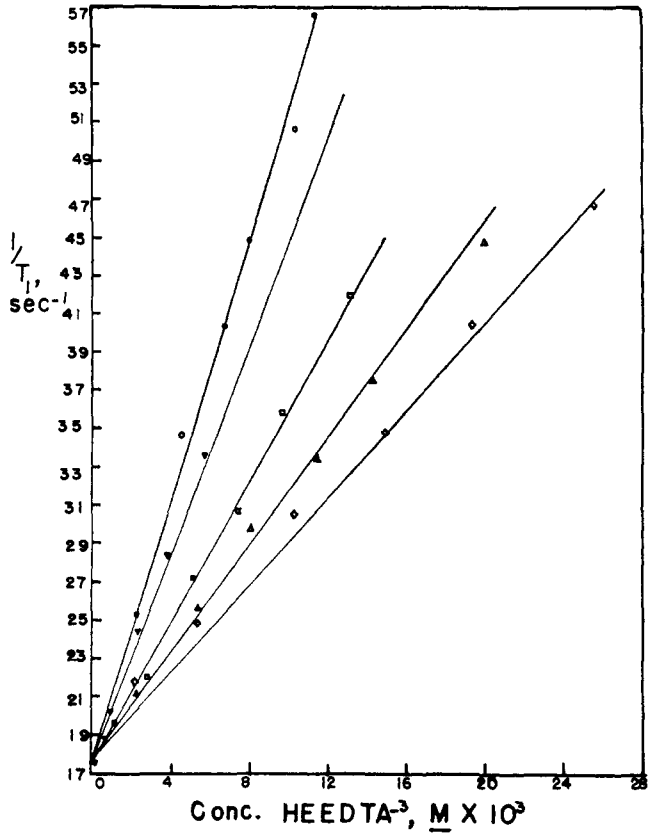

Figure 4. ^{23}Na T_1^{-1} as a function of NaCl and HEEDTA^{3-} concentration at pH 11.5. Curves are drawn using eq 4 and 6 with K_f = 8.7 and T_{1B}^{-1} = 610 sec^{-1} as calculated: ○, 0.050 M NaCl solution; ▽, 0.100 M NaCl solution; □, 0.200 M NaCl solution; △, 0.294 M solution; ◇, 0.392 M NaCl solution. The concentration of TMA hydroxide is roughly 0.1–0.4 M and is varied from solution to solution to maintain constant pH.

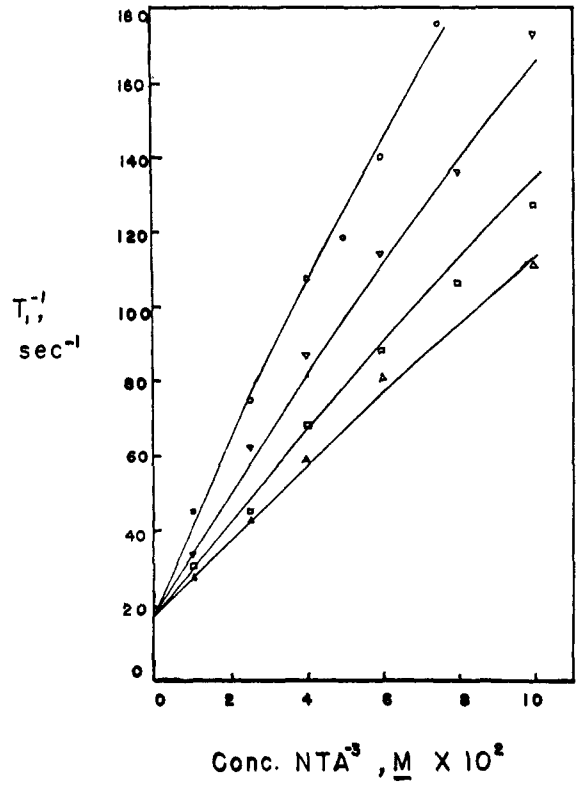

Figure 6. ^{23}Na T_1^{-1} as a function of NaCl and NTA^{3-} concentration at pH 11.5. Curves are drawn using eq 4 and 6 with K_f = 9.6 and T_{1B}^{-1} = 525 sec^{-1} as calculated: ○, 0.100 M NaCl solution; ▽, 0.200 M NaCl solution; □, 0.300 M NaCl solution; △, 0.400 M NaCl solution. The concentration of TMA hydroxide is roughly 0.1–0.4 M and is varied from solution to solution to maintain constant pH.

four-parameter model was developed to include the possibility of formation of a 2:1 complex. The "best fit" values for the data using the four parameter model were the same for the 1:1 complex as were obtained from the two-parameter model, and the formation constant for the 2:1 complex was virtually zero (0.7). This provides some justification for the applicability of our two-parameter model and, consequently, the assumed 1:1 stoichiometry. Figure 5 shows the dependence of ^{23}Na T_1^{-1} for 0.100 M NaCl over a wide range of concentrations of HEEDTA^{3-} at pH 11.5. The curve is calculated from the model using the parameters from the nonlinear least-squares fit given above. This also corroborates the two-parameter model presented in the Theoretical Section and, consequently, the assumed 1:1 stoichiometry of the complex.

The ^{23}Na relaxation rate behavior of 0.100 M NaCl solutions of varying NTA^{3-} concentration was found to be similar to that of HEEDTA^{3-}. The dependence of the ^{23}Na relaxation rate on the concentration of NaCl

and NTA^{3-} is shown in Figure 6. The best values obtained from the nonlinear least-squares analysis for the formation of the Na^+–NTA^{3-} complex was calculated to be $K_f = 9.6 \pm 1.5\ M^{-1}$ and $R_B = 525 \pm 40\ sec^{-1}$. The lines in Figure 6 were calculated using these parameters. Deletion of the data obtained from the 0.400 *M* NaCl solutions of NTA^{3-} has no significant effect on the calculated values of K_f and R_B. The difference between the formation constant reported here, $10^{0.98}$, and that previously reported,[19] $10^{2.15}$, may be due to the vastly different solution conditions. The value of $10^{2.15}$ was determined at 20° by an extrapolation to zero concentration. The value of $10^{0.98}$ reported here was determined at 25° at a high and slightly variable ionic strength (0.7–1.0). The determined formation constants of such weak complexes are very dependent on ionic strength; this is evident from the reported formation constants for other weak complexes.[20] The method used here is not useful for determining thermodynamic formation constants; however, it is very useful for determining K_f under the *conditions of interest* since it is relatively free of interference effects.

A calculation of the values of K_f and R_B for EDTA was not attempted because it apparently forms both an $(Na\text{–}EDTA)^{3-}$ and an $(Na_2\text{–}EDTA)^{2-}$ complex.[21]

Conclusions

In this paper, a technique has been demonstrated for studying complex formation *via* measurements of the nmr spin-lattice relaxation rate of a quadrupolar nucleus with varying pH, metal ion concentration, and ligand concentration. A mathematical model was developed which describes the quadrupolar relaxation rate as a function of the analytical metal ion and ligand concentrations. Using this model and technique, the importance of a chelate effect was demonstrated, important coordinating groups were identified, and the conditional formation constant and nmr relaxation rate of complexed metal ion were determined.

The sensitivity of nmr relaxation time measurements for detecting complex formation is dependent on the relaxation rate of bound Na^+, R_B, which in turn is governed by the magnitude of the electric field gradient and the magnitude of the correlation time (τ_c). Formation of more covalent bonds in complexing would increase *eq* and complex formation with a macromolecule should increase τ_c. R_B for Na^+ bound to $HEEDTA^{3-}$ ($610\ sec^{-1}$) and to NTA^{3-} ($525\ sec^{-1}$) may be compared to R_B for Na^+ bound to s-RNA[22] ($222\ sec^{-1}$). Since $(\tau_c)_{s\text{-}RNA} > (\tau_c)_{HEEDTA}, (\tau_c)_{NTA}$, the differences in R_B must be ascribed to an increase in the field gradient due to sodium–nitrogen bonding in the aminopolycarboxylate complexes as compared to the more ionic bonding of sodium with the phosphate of s-RNA. Assuming the complexed species are free to rotate and τ_c is similar to the tumbling rate of H_2O molecules ($\sim 10^{-11}$ sec), the quadrupole coupling constant (e^2qQ/h) for the Na^+–aminopolycarboxylate complexes is about 4 MHz. This can be compared to values of 0.779, 0.842, and 0.334 MHz found for crystalline $NaClO_3$, $NaBrO_3$, and $NaNO_3$, respectively.[23,24] The bonding of Na^+ in the $HEEDTA^{3-}$ and NTA^{3-} complexes is still very ionic as shown by comparison with the values of the ^{35}Cl coupling constants ($Q_{Na} \sim Q_{Cl}$) in covalent carbon–chlorine bonds which range from 40 to 90 MHz.[25]

This and another paper[22] illustrate the utility of ^{23}Na nmr relaxation time measurements for studying Na^+ complexes with small molecules and macromolecules. This technique should be useful for biological studies since it is nondestructive and is useful under a wide variety of solution conditions. ^{23}Na quadrupole relaxation studies may be especially suited for study of the mechanism of Na^+ active transport across biological membranes. Extension of this technique to other group I cations is governed by their relative sensitivity in the order $^7Li \sim {}^{23}Na > {}^{87}Rb \sim {}^{133}Cs \gg {}^{39}K$, with ^{36}K nmr probably being useless for performing studies of this type because of signal-to-noise problems.

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