^{23}Na NUCLEAR MAGNETIC RESONANCE RELAXATION STUDIES OF SODIUM ION INTERACTION WITH SOLUBLE RNA*

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Abstract.—Interactions between $^{23}Na^+$ and soluble RNA in aqueous solution are studied with the use of ^{23}Na nuclear magnetic resonance. At low concentrations of NaCl, the interactions obey a simple equilibrium model with a formation constant $\log (K_f)_s = 2.8 \pm 0.3$. The relaxation rate of the bound sodium is found to be $T_{1B}^{-1} = 222 \pm 19$ sec^{-1} compared to that of free sodium $T_{1F}^{-1} = 17.5$ sec^{-1}. At high NaCl concentrations, the system deviates from the model, possibly owing to aggregation of the soluble RNA.

Recently, there has been extensive interest in the application of nuclear magnetic resonance (NMR) relaxation measurements to the study of systems of biological interest. Electric quadrupole relaxation of ^{35}Cl ion has been used as an indirect probe of the interactions of mercury and zinc with biomolecules.[1-4] In addition, NMR studies of ^{23}Na permitted the conclusion that the sodium ion is partially bound in some tissues.[5,6] Efforts in this laboratory have been directed toward further development of NMR, especially electric quadrupole relaxation techniques, for the study of weak biomolecular interactions. Of special interest are the interactions of sodium ions in aqueous solution.

Theoretical.—The relaxation of quadrupolar nuclei ($I > 1/2$) has been discussed elsewhere.[7] For a nucleus such as ^{23}Na with nuclear spin $I = 3/2$, in the limit of extreme narrowing, the spin-lattice relaxation time T_1 is given by

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

where eQ is the nuclear quadrupole moment, eq is the electric field gradient at the nucleus (assumed here to be cylindrically symmetric), and h is Planck's constant. The rate of rotational diffusion of the electric field gradient at the nucleus is expressed by the correlation time (τ_c) for the orientation of the field gradient with respect to the applied magnetic field. If the angle between the electric field gradient and the applied magnetic field at time t is θ, and at time $t + \tau$ is θ', the usual assumption[7]

$$\frac{\langle P_2(\cos\theta)P_2(\cos\theta')\rangle}{\langle P_2(\cos\theta)^2\rangle} = \exp\left(\frac{-|\tau|}{\tau_c}\right) \tag{2}$$

provides a definition of τ_c. In equation 2, $P_2(\cos\theta)$ is the Legendre Polynomial, and the indicated averages are ensemble averages. In general, the larger the molecule, or the more restricted the motion, the larger τ_c will be; and the more covalent the bond between the quadrupolar nucleus and the molecule, the larger eq may be expected to be.

In cases where a nucleus undergoes rapid exchange between sites having different relaxation times, the observed average relaxation time is given by

$$T_1^{-1} = \sum_n X_n T_{1n}^{-1}, \tag{3}$$

where X_n is the average fractional population of site n, and T_{1n} is the relaxation time of the nucleus at that site.

In the case of an ion (M) in rapid equilibrium between a solvated ("free") state and a state in which the ion is bound to a site (S) on a macromolecule (all sites assumed to be identical), the relaxation rate $R = 1/T_1$ may be expressed as

$$R = R_F X_F + R_B X_B \tag{4}$$

where R_F and R_B are the respective relaxation rates of the "free" and bound ions, and X_F and X_B are the respective mole fractions of the "free" and bound ions. With the definitions of $[M]$ and $[S]$ as the respective molar concentrations of unbound ion and unbound site, $[MS]$ as the molar concentration of bound ion or site, C_m and C_s as the total concentrations of ions and sites, and the intrinsic formation constant as $(K_f)_s = [MS]/[M][S]$, equation 4 can be rearranged to give

$$(R - R_F) = (K_f)_s C_s (R_B - R_F)/[1 + (K_f)_s (C_m + C_s - [MS])]. \tag{5}$$

In the limiting case $C_s \ll C_m$, equation 5 reduces to

$$(R - R_F)^{-1} = [(K_f)_s C_s (R_B - R_F)]^{-1} + C_m [C_s (R_B - R_F)]^{-1}. \tag{6}$$

For weak complexes R_B cannot be measured directly, so that equation 6 must be considered to contain two unknowns. Measurements of R at constant C_s and varying C_m, with $C_s \ll C_m$, will thus permit measurement of the formation constant. From the linear equation 6, $(K_f)_s$ = Slope/Intercept, which is independent of any assumption concerning the concentration of sites other than $C_s \ll C_m$. If C_s can be estimated, R_B can be calculated from the slope. This is a practical method, since sensitivity places a lower limit on C_m, and solubility or availability of the macromolecule may place an upper limit on C_s; thus the case $C_s \ll C_m$ will often be unavoidable.

To illustrate this method, the binding of Na^+ to yeast soluble RNA in aqueous solution was investigated. For the calculation of R_B, it was assumed that binding of Na^+ took place on the phosphate and that the affinity of Na^+ to the various soluble RNA's in yeast soluble RNA and to the various phosphates was approximately the same.

Experimental.—Neutral solutions (pH = 7.0 ± 0.2) of yeast soluble RNA (Type III, Sigma Chemical Co.) were prepared with the requisite quantities of NaCl just prior to taking the NMR measurements. Measurements of the ^{23}Na T_1 were made at 24.5 ± 1.0°C, using an NMR Specialties Inc. Model PS-60 pulsed NMR spectrometer operating at 15.005 MHz. Details of the experimental method will be published separately.[8] CW recordings of some of the ^{23}Na NMR absorption line shapes were made; these were found to be Lorentzian in shape within experimental error, supporting our assumption of rapid exchange.

Results.—The results for the ^{23}Na relaxation rate as a function of NaCl concentration at four concentrations of soluble RNA are given in Figure 1. The initial portions of the curves for the three lower soluble RNA concentrations show the linear behavior predicted by equation 6. From the linear part of these curves, $(K_f)_s$ was calculated, and the results were reported in Table 1. The scatter in the results is due primarily to the difficulty in determining the intercept. In addition, a least squares best fit was obtained using the first two points at 10 mg/ml, the first 6 points at 5 mg/ml, and the first 5 points at 2.46 mg/ml, as-

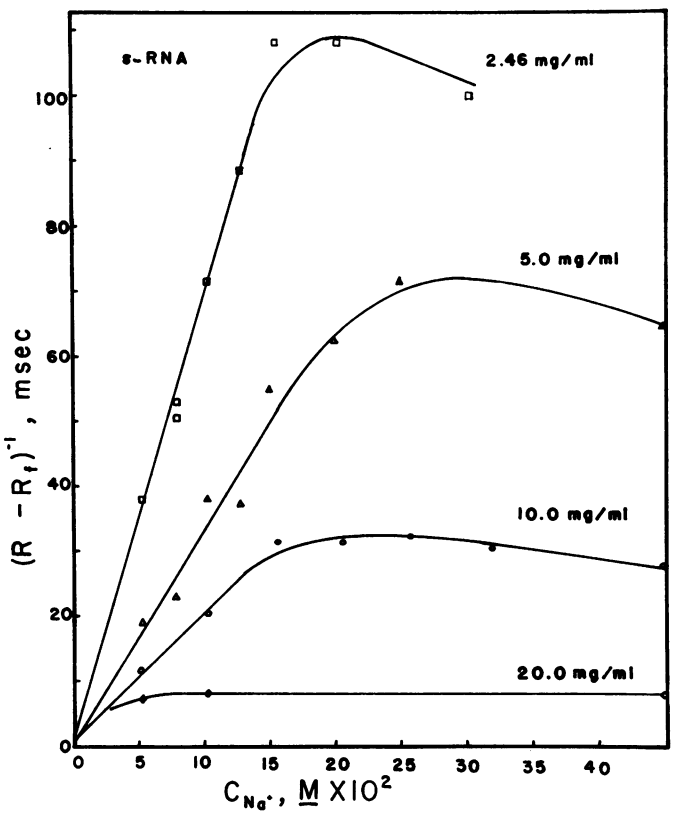

FIG. 1.—Relaxation rate of ^{23}Na ion in solutions with yeast soluble RNA. $R_F = 17.5\ sec^{-1}$ in all cases. The value of R_F is in agreement with previously published results.[17] All solutions were run at pH = 7.0 ± 0.2. Values of log $(K_f)_s$ based on lines shown are given in Table 1.

suming that C_s was proportional to the weight of soluble RNA used. This calculation gave a mean value of log $(K_f)_s = 2.8$ with limits for a single standard deviation $2.44 < \log (K_f)_s < 3.00$. Actually, the assumption $C_s \ll C_m$ may not be strictly valid for the first two points at 20.0 mg/ml and 10.0 mg/ml soluble RNA and the first point at 5.0 mg/ml. That these points are consistent with the limiting behavior is apparently owing to a cancellation of the last two terms in the numerator of equation 5, because $C_s \simeq [MS]$.

The deviation from the behavior predicted by equation 6 at higher NaCl con-

TABLE 1. *Intrinsic formation constant and relaxation rate of bound Na^+ for Na^+—soluble RNA complex as derived from Fig. 1. Values derived from least squares best fit are given in text.*

Concentration of soluble RNA, mg/ml	Log $(K_f)_s$	C_s(est.), M	R_B, sec^{-1}	T_{1B}, msec
10.0*	2.29	0.028	202.3	4.94
5.0	2.51	0.014	238.4	4.19
2.46	2.66	0.0069	227.3	4.40

* Note that model is not strictly valid at this concentration.

centrations could be due to an increase either in C_s or in R_B. Other experimental evidence[9] exists indicating that soluble RNA may form aggregates at higher salt concentrations. Such behavior would cause an increase in the rotational correlation time (τ_c) of the bound sodium and a corresponding increase in R_B (cf. equation 1). The alternative, that a change in configuration of soluble RNA is making more sites available, does not seem very likely.

If one assumes that C_s is equal to the concentration of phosphate groups, C_s can be estimated using the reported base composition of yeast soluble RNA.[10] This calculation is used to justify the assumption leading to equation 6 and to calculate the relaxation time (T_{1B}) of the bound Na^+ from the slopes of the linear portion of the curves (Fig. 1). The values of R_B and T_{1B} are reported in Table 1. Smaller values of C_s would give larger values for R_B. The mean value derived from the least squares best fit mentioned above is $R_B = 222 \pm 19\ sec^{-1}$.

Discussion.—Equation 1 permits the determination of the quadrupole coupling constant if the correlation time τ_c is known. If it is assumed that soluble RNA is a spherical molecule of radius r in a medium of viscosity η, the Debye-Stokes theory expresses the rotational correlation time as [7, 11]

$$\tau_r = 4\pi\eta r^3/3kT \tag{7}$$

and the diffusion coefficient as

$$D = kT/6\pi\eta r \tag{8}$$

where k is Boltzmann's constant, and T is the temperature. Diffusion coefficients have been measured for rabbit-liver soluble RNA[12] and *E. coli* soluble RNA,[13] giving 7.6×10^{-7} cm^2/sec and 7.8×10^{-7} cm^2/sec, respectively. Assuming D to be the same for yeast soluble RNA, τ_r was calculated to be 2×10^{-8} sec. Rabbit-liver soluble RNA has been reported to be a prolate spheroid of axial ratio 9:1.[12] However, this degree of eccentricity will not increase τ_r by more than a factor of 7.[14] Since the sodium-phosphate bond that causes eq could have more motional freedom than the soluble RNA itself, τ_r must be considered to be an upper limit to τ_c. If we assume that $\tau_c = \tau_r$, the quadrupole coupling constant (e^2qQ/h) is found to be 49 KHz. This can be compared to values of 779, 842, and 334 KHz found for crystalline $NaClO_3$, $NaBrO_3$, and $NaNO_3$, respectively.[15, 16] The small value of (e^2qQ/h) indicates a very ionic bond. The most likely errors in obtaining this estimate will tend to increase the value. However, a decrease of 2 orders of magnitude in either τ_c or the number of binding sites

available (C_s) will increase the quadrupole coupling constant by only a factor of 10.

The sensitivity of this method for detecting complex formation in dilute solutions of macromolecules will be greatly increased with larger values of R_B. Thus, the technique is favored by more covalent bonds. On the other hand, $(K_f)_s$ is most accurately measured when it is small, giving larger intercepts. Other methods of treating the data are available when C_s is larger, but the above conclusions still hold to a lesser extent. Rapid exchange is always desirable.

There are several possible areas of application for quadrupole relaxation studies of nuclei such as ^{23}Na. Important information can be obtained from the study of monomeric chelates using a somewhat different approach from that discussed above.[8] In addition to determining formation constants and relaxation rates for bound Na^+, information is obtained on the nature of the bonding, a chelate effect is observed, and the important chelating groups are identified.

The binding of metal ions to macromolecules is usually studied by equilibrium dialysis, conductimetric methods, and cation-sensitive glass electrode potentiometric methods. However, all three techniques have inherent limitations. Equilibrium dialysis requires a large excess of a second cation. The unknown and variable contribution of the partially complexed macromolecule to the conductivity limits this approach. Drift in the glass electrode potentials and changes in sensitivity with electrode aging must be recognized when using the cation-sensitive glass electrode. Another problem involves the possible interference of macromolecules with the electrode, resulting in a change of the value of the liquid junction potential.

The use of the NMR relaxation technique outlined in this paper overcomes the problems listed for the other methods and offers the additional advantage of providing information concerning the electronic environment and motional freedom of the metal ion bound to the macromolecule. The pH and other constituents in solution can be controlled. With NMR relaxation measurements, it may be feasible to do *in vivo* studies of small systems, because the method is nondestructive and requires no alteration or disruption of the system to detect ion-biomolecule binding. Quadrupole relaxation studies of ^{23}Na may be particularly useful for the study of Na^+-micelle interactions and the important problem of the mechanism of active transport of Na^+ across biological membranes.

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