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Analysis of sodium-23 nuclear magnetic resonance spin–lattice relaxation for the study of the intracellular sodium state

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Abstract

Sodium-23 Nuclear Magnetic Resonance relaxation spectroscopy has been used to investigate the state of intracellular Na^+ in control and CCl_4-treated rat livers. The analysis of spin–lattice relaxation rates at 1.88 and 7.07 Tesla based on a two-site exchange model led to estimates of pertinent modulation times. Also it has been found that a relatively high quantity of Na^+ ($P_B = 1.59 \times 10^{-2}$) is bound to charged sites of intracellular macromolecules or membranes. The degree of binding strongly decreases in CCl_4 treated rat livers.

Keywords: Sodium-23 NMR; Spin–lattice relaxation; Intracellular sodium state

1. Introduction

It is well known that Na^+ ions play a fundamental role in a variety of vital cell functions. Among the methods employed to gather information on this matter, the ^{23}Na Nuclear Magnetic Resonance technique is gaining increasing attention due to the use of the chemical shift reagent dysprosium(III) tripolyphosphate $Dy(PPP_i)_2^{7-}$, which separates the intracellular and the extracellular ^{23}Na signals [1–6].

Recently, particular attention has been addressed to the investigation of “free” and “bound” sodium ions inside a biological cell and the ^{23}Na NMR relaxation spectroscopy has been used successfully to this aim [6–8]. However, both the complexity of the biological systems and the theory of the relaxation spectroscopy of 3/2 spin nuclei has strongly limited the information obtained from the analysis of the spin–lattice T_1 and spin–spin T_2 relaxation times [6,8]. In particular, T_2 data give the greatest difficulties of analysis since it was found in a number of cellular systems that the spin–spin relaxation mechanism is generally described by complex exponential decay; on the other hand the spin–lattice relaxation can be described in terms of a single exponential decay. The interpretation is made even more difficult when the chemical exchange among a number of binding sites is introduced [9–11].

Following these considerations and in the attempt to overcome partly these overall complexities, we have collected the longitudinal relaxation times T_1 of the intracellular ^{23}Na of different tissue samples at two magnetic field strengths. Two kinds of cellular systems were investigated, i.e. normal rat livers and CCl_4-intoxicated rat livers. The cellular components have different organizations in the two systems and therefore these samples are useful probes for testing the potentiality of the NMR technique to obtain information on the sodium ions inside the cell.

2. Materials and methods

2.1 Chemicals

N-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid (HEPES) was purchased from Sigma. Dysprosium trichloride ($DyCl_3$) and pentasodium tripolyphosphate (PPP_i) were obtained from Aldrich. Deuterated water (D_2O, 99.75%) was purchased from Carlo Erba. Other chemicals were from Merck.

2.2 Buffers

The following isotonic buffer medium was used for the rat livers: 60 m*M* NaCl, 5 m*M* HEPES, 2 m*M* $MgSO_4 \cdot 7H_2O$, 1 m*M* $CaCl_2 \cdot 2H_2O$, 1 m*M* Na_2HPO_2, 5 m*M* D(+)-glucose with an osmolarity of 295 mos*M*, pH 7.4.

2.3 Rat livers

Male Sprague–Dawley rats (Charles River) weighing 200–250 g, fed on a standard diet (Ditta Piccioni, Brescia, Italy) and water *ad libitum*, were killed by decapitation. Organs were quickly removed and the portions (300 mg) immersed in an isotonic buffer were used for NMR determinations without further manipulation.

2.4 NMR measurements

Sodium-23 spectra of rat livers were recorded on a Varian FT-80-A spectrometer and on a Varian VXR-300 spectrometer at the field strengths of 1.88 and 7.07 Tesla respectively. All measurements were carried out with a broad-band probe at 25 ° C using 10-mm sample tubes.

The spin–lattice relaxation times were obtained by the inversion recovery method and calculated by a non-linear three-parameter regression with percentage standard errors never greater than 5%.

2.5 Shift reagent

The shift reagent was obtained from 0.1 *M* $DyCl_3$ and 0.1 pentasodium tripolyphopshate (PPP_i) in D_2O solution, with a ratio of $Dy/PPP_i = 0.5$. The complex $Dy(PPP_i)_2^{7-}$ was added to the medium to give a final concentration of 9 m*M*.

2.6 Calculations

The simulation and plotting were done with the mathematical package MATHEMATICA (Mathematica Wolfram Research Inc.).

3. Results and discussion

For non-exchanging systems the spin–lattice relaxation mechanism of a $\frac{3}{2}$ spin nucleus occurs through the quadrupolar interaction of its quadrupole moment and the electric field gradient at its site.

For such a nucleus the spin–lattice relaxation rate is given by [12,13]:

$$(R_1)_q = 0.2r_1 + 0.8r_2 \qquad (1)$$

where the fast r_1 and slow r_2 components are given by:

$$r_1 = 0.1(e^2qQ/\hbar)^2(1+\eta^2/3)\tau_c/(1+\omega^2\tau_c^2) \quad (2)$$

$$r_2 = 0.1(e^2qQ/\hbar)^2(1+\eta^2/3)\tau_c/(1+4\omega^2\tau_c^2) \quad (3)$$

In these equations eQ is the quadrupole moment and eq is the principal component of the field gradient tensor which depends on the electronic environment at the nucleus; $e^2qQ/\hbar$ is the quadrupole constant which gives the magnitude of the quadrupolar interaction; $\hbar$ is Planck's constant; η measures the electric field gradient deviation from the axial symmetry, here assumed null; τ_c is the correlation time modulating the quadrupolar interaction and ω is the angular Larmor frequency.

In the extreme narrowing limit ($\omega^2\tau_c^2 \ll 1$), eqs. (2) and (3) converge to the same form:

$$(R_1)_q = 0.1(e^2qQ/\hbar)^2\tau_c \quad (4)$$

In systems where the spins exchange between two different magnetic sites, one of which, say A, is in the extreme narrowing limit, the appropriate equations can be written as [9]:

$$R_1 = R_{1A} + (P_B/P_A)\left[0.2/\left(r_{1B}^{-1} + \tau_B\right) + 0.8/\left(r_{2B}^{-1} + \tau_B\right)\right] \quad (5)$$

where

$$R_{1A} = 0.2r_{1A} + 0.8r_{2A}$$

and where

$$\mathrm{r}_{1A(B)} = 0.4\pi^2\chi_{A(B)}^2\tau_{c_{A(B)}}/\left(1+\omega^2\tau_{c_{A(B)}}^2\right)$$

$$\mathrm{r}_{2A(B)} = 0.4\pi^2\chi_{A(B)}^2\tau_{c_{A(B)}}/\left(1+4\omega^2\tau_{c_{A(B)}}^2\right)$$

$\tau_{cA(B)}$ is the reorientational correlation time at site A(B), and P_B and τ_B are the fractional population and the lifetime of the species at site B respectively.

The spin–lattice relaxation rates of intracellular Na^+ ions in control and CCl_4-treated rat livers have been measured at two magnetic fields and the values are summarized in Table 1. It was found that the R_1 relaxation rates of both normal and CCl_4 treated rat livers are frequency dependent.

Table 1

Sodium-23 relaxation rates R_1 ($s^{-1} \pm$S.D.) for control and CCl_4-treated rat livers and relaxation parameters calculated from the two-site exchange model

Parameter	NaCl aqueous solution	Rat liver	
		Control	CCl_4
R_1 (1.88 Tesla)	16.7 ± 0.2	96.1 ± 3.9 (6) [a]	50.8 ± 1.7 (4)
R_1 (7.07 Tesla)	16.7 ± 0.1	64.5 ± 2.1 (5)	37.7 ± 0.7 (4)
P_B		1.59×10^{-2}	0.69×10^{-2}
τ_B (s)		1.5×10^{-4}	
τ_{cA} (s)		1.0×10^{-11}	
τ_{cB} (s)		2.0×10^{-9}	

[a] Number of cases analysed are indicated in parenteses.

From Table 1 it is seen that in both systems the quadrupole relaxation rates are much larger than that of free Na^+ in aqueous solution. Therefore from this observation we infer that binding of Na^+ to the charged sites of the macromolecules occurs, and accordingly, the two site model was adopted in the following analysis. It is to be pointed out that because of the high structural heterogeneity of these systems the estimates of the relaxation parameters are to be considered as statistical averages.

In general the use of two or more magnetic fields and a fitting procedure between experimental findings and calculated quantities can be of much help in rationalizing relaxation mechanisms. From eq. (5) it is clear that the six parameters determining the observed relaxation rates (i.e. τ_{cA}, χ_A, $\tau_{cB}\chi_B$, P_B and τ_B) would make the fitting process quite hard unless a sufficient number of experimental data were available or/and a way to reduce the degrees of freedom of the investigated system were possible by making reasonable assumptions or/and exploiting the existence of constraints.

In our case, while the first approach was not feasible, see experimental section, it was still possible making reliable assumptions on the value of some parameters.

If the lowest relaxation rate (i.e. the value observed in CCl_4 treated rats at 7.07 T) was taken as the upper limit for the free site relaxation rate and if a value of $\chi_A = 0.66$ MHz (i.e. the value estimated in NaCl solution [6,8] was used in eq. (4), an upper limit 2×10^{-11} s would be derived for τ_{cA}.

Further, according to eq. (5), the calculation of R_1 as a function of τ_{cB}, τ_B, and P_B (see caption to Fig. 1 for the investigated range) with $\chi_B = 1.70$ MHz —this value of the quadrupolar coupling constant has been used in similar cell systems [6,8]— τ_c being kept $\geqslant 2 \times 10^{-11}$ s, led to R_1 results not matchable with the experimental data, at the two field strengths for both samples. Therefore in the analysis that follows we have reasonably employed the value 10^{-11} s for τ_{cA} and the above reported values for χ_A and χ_B.

As for the remaining parameters, the study of the dependence of the ^{23}Na spin-lattice relaxation rate on τ_{cB}, P_B and τ_B, according to eq. (5), can help in clarifying the role of each parameter in the relaxation rate.

Figure 1 gives, as an example, the calculated relaxation rate for three typical exchange times. These plots show that for exchange times greater than 10^{-2} s (Fig. 1-I) the relaxation mechanism is determined by both the relaxation of the "free" site and the chemical exchange, whose weights depend on the fractional population of the "bound" site, and is frequency independent. This is the less favourable situation that can be met in

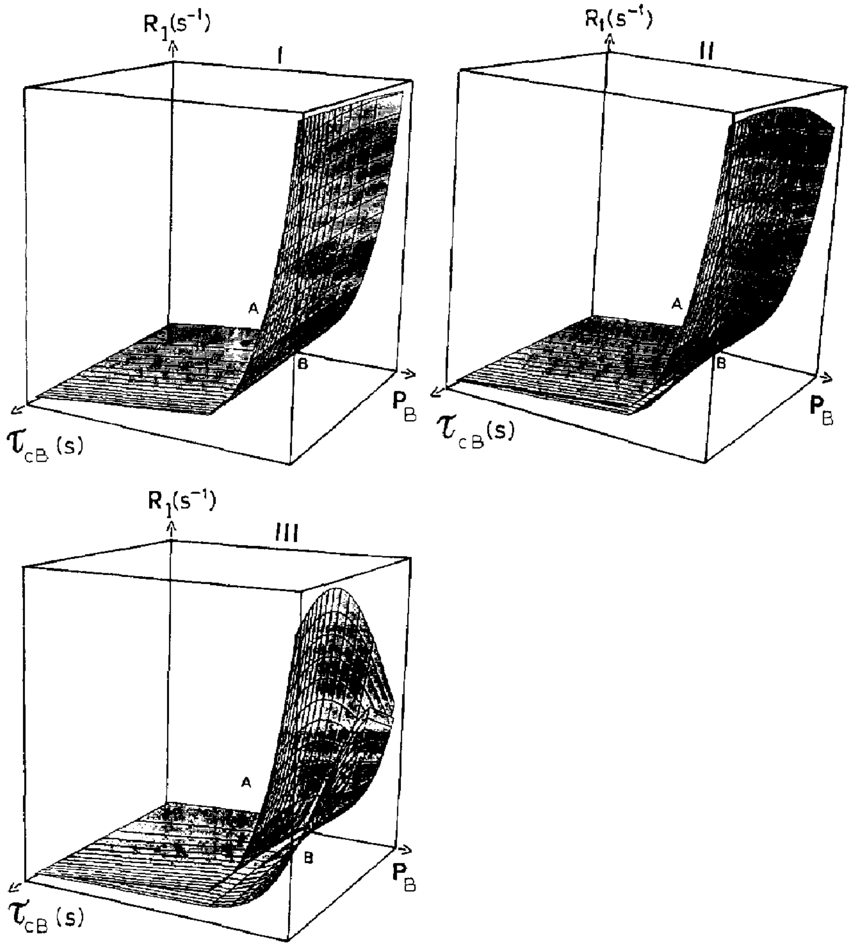

Fig. 1. Sodium-23 spin-lattice relaxation rate as a function of τ_{cB} and P_B at 1.88 Tesla (A) and 7.07 Tesla (B). The following parameters were used: $\tau_{cA} = 10^{-11}$ s; $10^{-10} \leqslant \tau_{cB} \leqslant 10^{-8}$ s; $10^{-5} \leqslant P_B \leqslant 10^{-2}$; (I) $\tau_B = 10^{-2}$ s; (II) $\tau_B = 10^{-3}$ s; and (III) $\tau_B = 10^{-4}$ s.

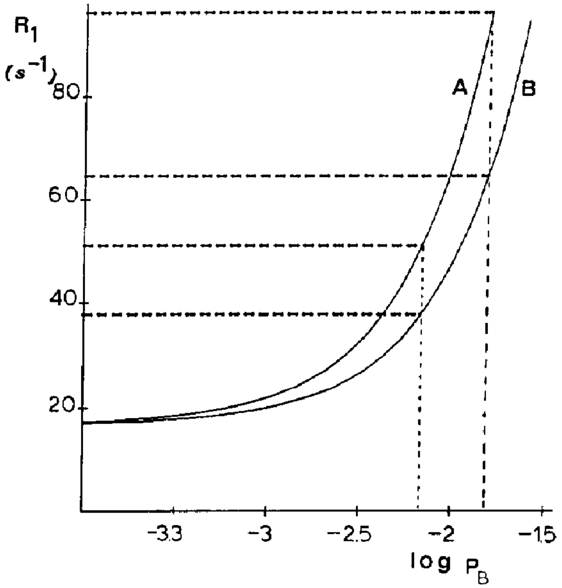

Fig. 2. Sodium-23 spin-lattice relaxation rate as a function of the fractional population P_B at 1.88 Tesla (A) and 7.07 Tesla (B). The following parameters were used: $\tau_{cA} = 10^{-11}$ s, $\tau_{cB} = 2 \times 10^{-9}$ s, $\tau_B = 1.5 \times 10^{-4}$ s. The upper pair of dashed lines refer to the experimental control values, the bottom pair show CCl_4-intoxicated values.

the relaxation. For values of τ_B smaller than 10^{-2} s (Figs. 1-II and 1-III), when the relaxation is frequency dependent, information can be obtained on all dynamical parameters and the fraction of bound Na^+ ions.

Following these results we have calculated R_1 as a function of P_B (see eq. 5) for values of τ_{cB} and τ_B in the ranges $(10^{-10}, 10^{-8})$ and $(10^{-5}, 10^{-2})$ respectively, (that is we have taken in Fig. 1 sections parallel to the plane R_1, P_B) and the estimates were compared to the experimental ones. It was found that matching R_1 pairs calculated at the two fields to those found experimentally could be achieved for the following set of data: $\tau_B = 1.5 \times 10^{-4}$ s, $\tau_{cB} = 2 \times 10^{-9}$. In particular we found that small deviations of τ_B from this value lead to inconsistent values of R_1 no matter what the other parameters were. The high quality of the agreement obtained is well indicated in Fig. 2 while the parameters obtained from the overall simulation are shown in Table 1.

From these data we infer that a significant quantity of Na^+ ($P_B = 1.59 \times 10^{-2}$) is bound to the charged sites of the cellular macromolecules or membrane components opposed to what has been found in other biological systems, as for example in red blood cells ($P_B = 2.5 \times 10^{-3}$) [6]. Furthermore, the degree of binding strongly decreases in CCl_4 intoxicated liver. This result possibly reflects the disruption of the membrane organization induced by the lipid peroxidation [3,4].

Thus we have shown the usefulness of an approach where use is made of a multi frequency relaxation study to investigate the binding of sodium ions in normal and intoxicated liver cells.

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References

1 R.K. Gupta and P. Gupta J. Magn. Reson. 47 (1982) 344.
2 M. Fossarello, N. Orzalesi, F.P. Corongiu, S. Biagini, M. Casu and A. Lai, FEBS Lett. 184 (1985) 245.
3 M. Casu, F.P. Corongiu, M.A. Dessì, M. Frau and A. Lai, Biochem. Biophys. Res. Commun. 134 (1986) 1079.
4 S. Banni, M. Casu, F.P. Corongiu, M.A. Dessì, A. Lai and C. Meloni, Chem.-Biol. Interactions 63 (1987) 207.
5 M. Casu, M. Fossarello, A. Lai, C. Meloni and A. Serra, Magn. Res. Med. Biol. 1 (1988) 197.
6 H. Shinar and G. Navon, Biophys. Chem. 20 (1984) 275.
7 H.J.C. Berendsen and H.T. Edzes, Ann. N.Y. Acad. Sci. 204 (1973) 459.
8 D.C. Chang and D.E. Woessner, J. Magn. Res. 30 (1978) 185.
9 T.E. Bull, J. Magn. Res. 8 (1972) 344.
10 U. Eliav, A. Baram and G. Navon, J. Chem. Phys. 89 (1988) 5584.
11 U. Eliav and G. Navon, J. Magn. Res. 88 (1990) 223.
12 P.S. Hubbard, J. Chem. Phys. 51 (1969) 1647.
13 P.S. Hubbard, J. Chem. Phys. 53 (1970) 985.