Nuclear Magnetic Relaxation Studies of the Interaction Between ^{7}Li Ion and Nucleotides in Aqueous Solution

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Abstract

^{7}Li and ^{13}C nuclear magnetic relaxations were studied in order to obtain information on the complexing of ^{7}Li ion with nucleotides in an aqueous solution. It is concluded from the observed ratios of the spin-lattice relaxation times of the complexes in the H_2O and D_2O solutions that both the electric quadrupolar and the magnetic dipolar mechanisms contribute almost equally to the ^{7}Li relaxation in 5′ CMP-LiCl system. Anions also affect the relaxation time of ^{7}Li nucleus in the complexes of the nucleotides in an aqueous solution. Spin-lattice relaxation time measurements of Li nucleus in LiCl/nucleotide systems suggest that the ^{7}Li ion interacts mainly with the phosphate group. No drastic change in the T_1 was observed for the Li nucleus in the complexes, and this result seems to indicate that the Li ion is still hydrated even in the case of the complex with the nucleotides. Some experiments concerning the pH effect have been done for the understanding of the change in the ionization of the nucleotides.

1. Introduction

The alkali metal ions in biological systems play an important role in the highly orderd structure of the nucleic acids[1,2] as well as the membranes[3,4]. In order to obtain detailed information on the interactions between the metal ions and the biomolecules in a solution, both ^{13}C and ^{1}H NMR resonance studies[5-7] have been proved to be very useful. Moreover, NMR resonances of ions, for example alkaline[8], alkaline earth cation[9,10] and/or halogen anions[11], will give information on the environments, which are related with the complexing. In a previous paper[7], the interaction between the Li ion and the nucleoside was reported, but no detailed investigation had been carried out. Then, we carried out a ^{7}Li NMR study for obtaining more detailed information on the Li complexes of the nucleotides (side). And the results of the relaxation studies of the ^{7}Li ions in the presence of nucleotides will be presented and we will discuss the two kinds of contributions, namely the electric quadrupolar contribution and the magnetic dipolar contribution to the ^{7}Li spin-lattice relaxation in the LiCl-nucleotides

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solution. This discussion will be helpful to build up a picture of Li complexing of the nucleotides.

2. Experimental

LiCl and LiBr of the special grade were purchased from Wako Co., and Cytidine, 5′ cytidine monophosphate (5′ CMP), 5′ cytididine diphosphate (5′ CDP) and 5′ cytidine triphosphate (5′ CTP) were obtained from Sigma or Kojin Co. Divalent metallic ions were removed by shaking the aqueous solution of nucleotides with Chelex-100. All the solutions were degassed by a repetition of the freeze and pump-thaw method until dissolved gasses were sufficiently removed. The solution was then re-frozen in a sample and the tube sealed. The spin-lattice relaxation time, T_1, was measured by a Bruker SXP 4–100 Pulse and FT spectrometer at a resonance frequency 34.98 MHz for ^{7}Li and 22.63 MHz for ^{13}C, respectively. The pulse sequence used was 180°-t-90° and the logarithm of the differences between the equilibrium magnetization and the magnetization after each 90° pulses were plotted as a function of time, and good linearity was obtained and T_1 was determined from the gradient. The pH and pD were adjusted with NaOH and HCl for H_2O solution and NaOD and DCl for D_2O solution. The pH was measured with a HITACHI-HORIBA pH meter.

3. Results

3.1 ^{7}Li relaxation

In Table 1 the ^{7}Li spin lattice relaxation times for the LiCl aqueous solution with and without the nucleotides are given. The observed T_1 for ^{7}Li ion is shorter in the complexes with the nucleotides than without the nucleotide. Furthermore, the results in Table 1 show that the relaxation time for ^{7}Li ion complex decreases by the complexing of the larger molecules. The data in Table 1 also indicate that the relaxation time for ^{7}Li of the molecules containing the phosphate group decreases with the number of phosphate group in the molecule. Our results for aqueous solution containing LiCl and nucleotides are in good correspondence with similar studies reported by R. G. Bryant[9]. In Table 2 we present the ^{7}Li relaxation time T_1 in the 5′ CMP-LiCl aqueous solution for the solvents, which contains different fractions of D_2O. The ratio of the spin-lattice relaxation time for D_2O solution to those for normal water solution was found to be 2.08. Analysis of the data will be discussed in the next section. The relaxation times of ^{7}Li ion are plotted in Fig. 1 as a function of pH for LiCl-5′ CTP, LiCl-5′ CDP and LiCl-5′ GTP system, respectively. The three systems exhibit similar behavior in the pH dependence of T_1, that is, the relaxation time increases with the lower pH value, but the levelling off of T_1 is observed at the pH around 6. Our results for pH dependence of T_1 are in good agreement with the data obtained by J. A. Magnusson et al.[10] through pH dependence of the line width.

In a previous paper[6], the changes in the chemical shifts induced by an

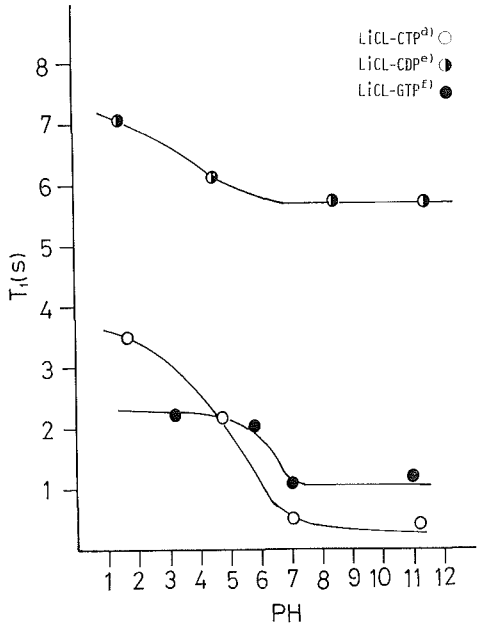

Fig. 1 The pH dependence of T_1 of the ^{7}Li nuclei of LiCl-CTP, LiCl-CDP and LiCl-GTP systems
d) 0.5 mnl/*l* LiCl; 0.1 mol/*l* 5′ CTP
e) 0.5 mol/*l* LiCl; 0.1 mol/*l* 5′ CDP
f) 0.5 mol/*l* LiCl; 0.1 mol/*l* 5′ GTP

Table 1 ^{7}Li spin lattice relaxation times (T_1) (sec.) of several nucleotide (side) complexes[a] in aqueous solution

Complexes	^{7}Li T_1 (s)
None	16.5
Cytidine	10.4
5′ CMP	6.5
5′ CDP	5.3
5′ GTP	0.5

a) 0.5 mol/*l* LiCl : 0.1 mol/*l* nucleoside and nucleotides.

Table 2 ^{7}Li-T_1 in 5′ CMP-LiCl[b] system (isotope effect)

5′ CMP cnmplexing	^{7}Li T_1 (s)
H_2O only	1.3
$H_2O/D_2O=1$	2.1
D_2O only	2.7

b) 1.0 mnl/*l* LiCl : 0.5 mol/*l* 5′ CMP at pH=7.2.

Table 3 ^{7}Li-spin lattice relaxation time (T_1) (sec.) in aqueous solution (anion effect)

Cnmplexes	T_1 (s)
LiCl[c]	13.6
LiBr[c]	15.1
LiCl-5′ GDP[d]	4.3
LiBr-5′ GDP[e]	3.2

c) 4 mol/*l* LiCl; 4 mol/*l* LiBr
d) 4 mol/*l* LiCl; 12.5 mg/0.5 cc 5′ GDP
e) 4 mol/*l* LiBr; 12.5 mg/0.5 cc 5′ GDP

addition of the metal salts to the dimethyl sulfoxide (Me_2SO) solution of nucleoside can not be explained as a single effect of either the metal ion or the anion. In this report, we checked the anion effect for the ^{7}Li relaxation time of the LiCl-5′ GDP and LiBr-5′ GDP systems in aqueous solutions. The results of the T_1s are given in Table 3. The relaxation time of LiCl-5′ GDP is apparently longer than that of LiBr-5′ GDP, although T_1 of Li was found to be shorter in LiCl than in LiBr in the nucleotides.

3.2 ^{13}C relaxation

To evaluate the correlation time for 5′ CMP in the aqueous solution containing 5′ CMP and LiCl, the ^{13}C relaxation times were measured. The T_1 values observed for ^{13}C at the various sites of the 5′ CMP are listed in Table 4. Assignment of each peak of the ^{13}C spectrum in 5′ CMP was taken from the literature[12]. The carbons may be classified into three groups from the view point of the number of the protons attached to the carbon; a) two protons. The carbon at position 5′ having two protons show shorter T_1 of 0.17 (s). b) one proton. The carbons C-1′, C-2′, C-3′, C-4′, C-5, C-6 having one proton show nearly the same

T_1 value 0.31–0.38 (s), within an experimental error ±15%. c) no proton. The carbons at position 4 and 2 in 5′ CMP which have no proton show T_1 values 0.24 (s) and 0.30 (s), respectively.

It is worthy to mention that the relaxation time of C_5' having two protons in nearly one half of those carbons having one proton in the second group. In a previous paper[7], the carbons having no proton have longer T_1 values than that of the carbons with either one or two protons in DMSO system, but the T_1 values obtained in the aqueous solution show some values between two protons and one proton.

Table 4 C-13 spin-lattice relaxation times (T_1) (sec.) for 5′ CMP-LiCl complex in aqueous solution

Carbon atom	T_1 (s)
C-1′	0.38
C-2′	0.33
C-3′	0.31
C-4′	0.33
C-5′	0.17
C-4	0.24
C-2	0.30
C-5	0.37
C-6	0.33

4. Discussion

The nature of the hydrated Li^+ ion in the aqueous solution have been treated extensively by several investigators, and it is proposed from the relaxation measurements that the ^7Li ion in the aqueous solution exists in a tetrahedron consisting of four water molecules[13–16].

4.1 ^7Li relaxation

It is known that the ^7Li nucleus in aqueous LiCl solution relaxes partly by the magnetic dipole-dipole interaction and partly by the quadrupole interaction[13–16]. From our results obtained by the isotope effects (see Table 2) we can say that in the case of aqueous solution contaning LiCl as well as the biomolecules both the dipole-dipole and the quadrupole interaction contribute to the relaxation of ^7Li nucleus. This conclusion is derived from the following considerations. Since the gyromagnetic ratio of the deuteron is about one-seventh of that of the proton, the magnetic dipole-dipole interaction between the ^7Li nucleous and a deuteron is negligible compared to that between ^7Li and a proton. Then, one can reasonably assume that the ^7Li relaxation time in D_2O is determined mainly by the electric quadrupolar mechanism. Thus, the T_1 observed for the D_2O solution may be regarded as a pure quadrupolar contribution. This way of thinking is compatible with that reported by H. S. Keilman et al.[17]. The T_1 observed for the D_2O : H_2O solvent of the 1 : 1 ratio is increased by nearly 1.6. This result indicates the reduction of the Li-relaxation due to the decreased dipolar coupling and proves the contribution of the proton dipolar mechanism to the Li-relaxation in the aqueous solution. Thus, the relaxation rate of ^7Li ion in 5′ CMP-LiCl water system is determined with the two processes; the dipolar mechanism and the quadrupolar one, and the observed T_1 is represented as follows.

$$\left(\frac{1}{T}\right)^{Li}_{H_2O} = \left(\frac{1}{T_1}\right)_{d\text{-}d} + \left(\frac{1}{T_1}\right)_{quad} \tag{1}$$

where $\left(\frac{1}{T_1}\right)_{d\text{-}d}$ is the relaxation rate for the magnetic dipolar contribution, and

$\left(\frac{1}{T_1}\right)_{quad}$ is for the electric quadrupolar contribution. The dipole-dipole term in Eq. (1) is divided in four terms.

$$\left(\frac{1}{T_1}\right)_{dd}=\left(\frac{1}{T_1}\right)_{Li\text{-}Hfs}+\left(\frac{1}{T_1}\right)_{Li\text{-}H\ other}+\left(\frac{1}{T_1}\right)_{Li\text{-}Li}+\left(\frac{1}{T_1}\right)_{Li\text{-}anion} \tag{2}$$

The first term expresses the contribution from the interactions with the protons in the first hydration sphere, the second term does that with the protons other than in the first sphere, and the third term from the interaction between the Li ions and the last term from the anions. The contribution of the third term in Eq. (2) is apparently negligibly small compared to the other contributions because of the smaller dipole moment of Li. Hertz et al.[14] and Woessner et al.[13] reported that the ratio of $\left(\frac{1}{T_1}\right)_{Li\text{-}H\ other}\Big/\left(\frac{1}{T_1}\right)_{Li\text{-}H\ other}$ is about three in the aqueous solution of the LiCl. Based on these results the contribution of the second term is nearly one third of the first term and is not so important as the first order approximation even in such a simple system as an aqueous solution of the alkaline salts. The observed changes in T_1 (see Table 1), which are more than the one third of the aqueous solution of the alkaline salts, are clearly caused by the addition of the biomolecules. This fact demonstrates that the effect from the nucleotides (side) is larger than from the second term in Eq. (2). Thus it would be sufficient in the following discussion, if one takes into account only the first term in the Eq. (2) as the interaction with water molecule and neglects the second term. One might consider that anion effects could not be neglected because the observed T_1 was changed depending on the species of the anion. However, the electric field might be disturbed by the anion species and the quadrupolar mechanism could be affected by the anion. Since the magnetic dipolar moments of the anion are smaller than the proton, the dipolar mechanism could not be a major path for the relaxation through the anion. Thus the last term in Eq. (2) may be neglected. Now, we introduce an unknown parameter, A, in the following equation.

$$\left(\frac{1}{T_1}\right)_{dd}=A\left(\frac{1}{T_1}\right)_{Li\text{-}Hfs} \tag{3}$$

In the case of simple alkaline salts solution, the constant, A, is about four thirds because the contribution from the second term is one third of the first term, and in the case of aqueous solution containing LiCl and nucleotides, generally the parameter A could be a small value between one and four-thirds, which corresponds to the complete neglect of the second term and the assumption of the unchanged environment outside of the first sphere, respectively. Thus, one may take A as the parameter to reflect the nature of the complex. Difference induced by replacement with D_2O are affected by the two facts; one is the different magnetic moment between deuteron and proton, and the other is the difference in viscosity between H_2O and D_2O[13-14]. The contribution of the dipole-dipole part in H_2O solution can be estimated from the observed relaxation rates both H_2O and D_2O.

$$\left(\frac{1}{T_1}\right)^{H_2O}_{d\text{-}d}=\frac{\left(\frac{1}{T_1}\right)^{Li}_{H_2O}-\left(\frac{1}{T_1}\right)_{D_2O}\zeta^{-1}}{1-\varepsilon} \tag{4}$$

where ζ^{-1} is the ratio of the effective fluidities around the Li^+ ion in D_2O and H_2O, respectively. ε is the ratio of the squares of the magnetic moments of the deuteron and the proton. $\varepsilon=0.063$. If the ^7Li ions are tightly bound to water molecules, the Li-complex may be regarded as the center of a rigid tetrahedron. In such a rigid system no intramolecular motion between the Li and the water molecules is assumed and the bulk motion of the complex is described by a single rotational correlation time. The spin-lattice relaxation rate of the ^7Li nucleus due to the dipolar mechanism is given by[18]

$$\left(\frac{1}{T_1}\right)_{\text{Li-Hfs}}=2\gamma_p^2\gamma_{Li}^2\hbar^2 n r^{-6}\tau_c \tag{5}$$

In this equation, n is the number of water molecules in the complex, r is the distance between ^7Li nuclei and protons in the complex, and τ_c is the rotational correlation time of the complex. On the other hand, the spin lattice relaxation by the quadrupolar interaction can be represented by the following equation in the extreme narrowing case[19],

$$\left(\frac{1}{T_1}\right)_{\text{quad}}=\frac{2}{5}\left(1+\frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{\hbar}\right)^2\tau_c \tag{6}$$

where e is the unit electric charge, q is the electric field gradient at the nucleous observed, Q is the nuclear quadrupole moment, τ_c is the correlation time for reorientation of the electric field gradient with respect to the direction of the applied magnetic field, and η is the asymmetry parameter. The field gradient for a Li ion in an aqueous solution is reasonably assumed to be small because of the symmetrical nature of the tetrahedron around the Li ion, and q in Eq. (6) is actually neglected. Thus, the resulting relaxation time through the quadrupolar mechanism must be long. When a Li ion coordinates with a molecule, the field gradient q is produced due to the loss of the higher symmetry and also the correlation time for the ^7Li ion increases because of the bulkiness of the complexing. Both effects may contribute to the decrease of the relaxation time for the Li complex. Actually, our results (see Table 1) can be explained by the above consideration.

4.2 Evaluation of the rotational correlation time

(1) Correlation time of Li. The correlation time τ_c is estimated from the value observed for the T_1 of the 5′ CMP-LiCl system on the following assumptions. The indirect coordination, which will be discussed in a later section, is assumed and the Li nucleus is surrounded by the four water molecules of the tetrahedron, which is assumed to maintain the same size as in the aqueous solution. From this assumption the distance between the Li nucleus and a proton in the water molecule is taken as 2.45 Å[13]. The value observed for the dipolar contribution is estimated as $\left(\frac{1}{T_1}\right)_{dd}^{H_2O}=0.35\,(s^{-1})$, inserting the value for $\left(\frac{1}{T_1}\right)_{H_2O}^{Li}=0.77\,(s^{-1})$ and $\left(\frac{1}{T_1}\right)_{D_2O}^{Li}=0.37\,(s^{-1})$ into Eq. (4). One can derived the contribution due to the first hydration sphere proton from Eq. (3) and $\left(\frac{1}{T_1}\right)_{\text{Li-Hfs}}=0.35/A$. Inserting this value and the distance $r=2.45$ Å into Eq. (5), respectively, the correlation times (τ_c)

can be estimated for the two extreme cases, $0.83\times10^{-10}(s)$ for $A=\frac{3}{4}$, $1.11\times10^{-10}(s)$ for $A=1$. The actual correlation time in the system which contains biomolecules as well as the alkaline salts might be taken between $0.83\times10^{-10}(s)$ and $1.11\times10^{-10}(s)$.

(2) Correlation time of ^{13}C. As mentined in the section of the results, the T_1 of the carbons having the two protons is found to be nearly one half of that having one proton. This fact supports the assumption that the relaxation mechanism is the dipolar coupling with the protons and can be described with a single correlation time. Assuming that the relaxation of ^{13}C is determined by a single correlation time τ_c, the longitudinal relaxation of carbon is given in the extreme narrowing conditions ($\omega\tau_c\ll1$) by the equation

$$(T_1)^{-1}=\hbar^2\gamma_c^2\gamma_{H_i}^2r_{CH_i}^{-6}\tau_cN \tag{7}$$

where γ_{H_i} and γ_c are the gyromagnetic ratios of ^1H_i and ^{13}C, and r_{CH_i} is the distance between ^{13}C and the i-proton. N is the number of protons attached directly to the carbon. Using Eq. 7, and the observed value of 0.35 s for the T_1 of a base carbon (the average T_1 values for the carbons at positions 5 and 6 in 5′ CMP) (see Table 4) one obtains $\tau_c=1.35\times10^{-10}(s)$ for the correlation time of 5′ CMP molecule, where 1.09 Å is used for the C-H distance. This value agrees well with the reported one for the τ_c of 5′ CMP[20] in aqueous solution. Hertz et al.[14] reported that the value for correlation time of ^{7}Li ion in the LiCl aqueous solution takes about $2\sim5\times10^{-11}(s)$. If the hydrated ^{7}Li ion binds to the nucleotide tightly, the correlation time of ^{7}Li would be equal to that for the ^{13}C in 5′ CMP. If Li ion is exchanged among the various binding environment, for example between the free ion and the tightly bounded site, the correlation time for the Li ion would be of some value falling between the free Li ion and that of ^{13}C. From the line of thought mentioned above one may say that the τ_c determined for the Li, which is a little smaller than that for the ^{13}C, is just reasonable. There is no detailed information on the environment of Li ion in the presence of the nucleotides the actual value for the parameter A is not determined, and therefore it is impossible to discuss the equilibrium constant of the Li complexing from this experiment.

(3) Indirect coordination of ^{7}Li ion to nucleotides. The reduction of T_1 by the complexing of Li with cytidine is merely 30%. If Li ion directly coordinates with the nucleoside molecule, the symmetry of the circumstance of the Li ion is quite destroyed. The field gradient q in such less symmetrical circumstances is different from zero which is expected for the Li ion in an aqueous solution. If so, the quadrupolar mechanism is now effective in the Li relaxation and a large decrease in T_1 can be expected for the direct coordination of the Li ion to the nucleoside molecule. However, the observed decrease in T_1 was not as large as expected, although the Li complex is evidently formed as stated above. This apparent contradiction might be circumvented by assuming that the tetrahedron of water molecules around a Li ion is not destroyed by addition of the nucleoside and the Li ion coordinates through the water molecule with the nucleoside molecule. In such an indirect coordination the field gradient, q, is not so different from that at a Li ion in an aqueous solution. Thus, to know the nature of the

ion complex, one must consider both the information of molecular motion of ion and that of the biomolecule. Regarding the anion effect in the alkaline salts solution Hertz et al.[14] found that the relaxation rates at a given concentration in the three soluble Li halides in H_2O are in the order of

$$\left(\frac{1}{T_1}\right)_{LiI} < \left(\frac{1}{T_1}\right)_{LiBr} < \left(\frac{1}{T_1}\right)_{LiCl}$$

M. Eisenstat et al.[21] reported that in NaCl and $NaClO_4$ solutions the behavior of ClO_4^- ion and Cl^- ion is strongly different; the ClO_4^- ion has a marked effect on the relaxation time, and the Cl^- ion shows little effect. The anion effects obtained in our experiments on the aqueous solution containing nucleotides are shown in Table 3. The relaxation rates of ^7Li nucleus was found larger in the bromide than in the chloride, that is

$$\left(\frac{1}{T_1}\right)_{LiCl-5'\ CMP} < \left(\frac{1}{T_1}\right)_{LiBr-5'\ CMP}$$

This result indicates that the anions affect the relaxation time of ^7Li nucleus. However, this inequality is just opposite to that for the systems having no nucleotides. From this result the nature of anions with and without the biomolecule seems to be different. More detailed aspects regarding the various anions in the biomolecule system will be reported in a future publication. Lastly, we describe the pH dependence of the spin-lattice relaxation time T_1. (see Fig. 1). If analogous treatment of the pH dependence of the line width reported by J. A. Magnusson et al.[10] may be applied to our case, the following expression can be given,

$$pK_f \approx \mathrm{pH} - pKa - p\mathrm{Li}^+ \qquad (8)$$

This equation is approximately valid when half of the potential ligand is in the form of Li complex. Where K_f is the formation constant of Li-nucleotide complex, Ka is the dissociation constant of secondary phosphate in nucleotide. Substituting the value of $pKa=6.5$ and with $\mathrm{pH}=6.0$ at the half conversion point, we obtain $K_f=6.3\ l/\mathrm{mol}$ in LiCl-GTP system.

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