The effective correlation time τ in jaw cysts determined from 400 MHz NMR T_1 and T_2 measurements

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Abstract. Macromolecular crowding is a general phenomenon of biological systems, and the evaluation of a correlation time for a specific molecule is difficult when the molecular crowding exists. The determination of an effective correlation time may therefore give useful insights into molecular dynamics of such systems. In this work, the relaxation rates in the mixture of D₂O (80%) and cystic fluid (20%) were measured with a NMR operating at 400 MHz for three types of cysts (non-infected radicular, infected radicular and hemorrhagic). The effective correlation times (τ values) were then determined by using a formula derived from the observed relaxation rates. The τ value of the infected cyst was found to be longer than those of the others for the studied cases. The present data suggest that an effective correlation time for fluids with macromolecular crowding can be determined from NMR relaxation measurements.

Keywords: Correlation time, relaxation time, cysts and NMR

1. Introduction

NMR relaxation studies on the molecular dynamics of macromolecules in biological fluids have been frequently investigated due to the extreme efficiency and specificity of functions of biological macromolecules [1–3,6,9,14,15,19,21,24,31,32]. Diffusion of such molecules have also been studied by NMR and MRI for the same reasons [10,11,18,20,22,23,27,29,30]. The correlation time of the motion in solutions containing one biological macromolecule (τ), which modulates relaxation mechanism, is fairly estimated [3,9,14,15,19,24,32]. However, macromolecular crowding is a general phenomenon of biological systems, and the determination of the correlation time for a specific molecule is difficult when molecular crowding exists [10,20,27,29,30]. A determination of the effective correlation time may therefore give useful insights into the molecular dynamics of such systems. Furthermore, the concept of average or effective correlation time becomes important to explain the source of the relaxation in protein solutions [3,14]. The estimation of effective τ values of jaw cysts may also be useful for such studies.

On the other hand, jaw cysts can be classified as radicular and hemorrhagic cysts on the basis of clinical evaluation, fluid aspiration, radiological and histopathological findings [13]. Then radicular cysts can be divided into two subcategories that is called non-infected and infected radicular cysts. Non-infected

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radicular cysts are characterized by a clear or yellowish fluid which contains several proteins, cholesterol, lipids and ions as well as inflammatory cells, whereas infected radicular cysts were characterized by a dense and purulent fluid containing materials in non-infected radicular cysts and intensive inflammatory cells [13]. Hemorrhagic cysts contains blood cells in addition to those of non-radicular cysts. It is clear that jaw cysts are a good example for macromolecular crowding in biological systems.

The observed NMR spin–lattice ($1/T_1$) and spin–spin ($1/T_2$) relaxation rates in biological fluids are caused by the dipole–dipole interaction of water protons modulated by the motions of the hydrated solids [32]. Under the assumption of isotropic and uncorrelated motions, the motions of the solids can be represented by an effective correlation time which is the sum of the inverse of several correlation times [4,7]. Then the effective correlation time in a heterogeneous solution can be determined from observed T_1 and T_2 measurements by using the relaxation ratio [4,7,8]. NMR relaxation studies in biological fluids have been made mostly over MRI frequency range. Using much higher frequencies such as 400 MHz for such studies may therefore be interesting.

In this work, the observed relaxation rates ($1/T_1$ and $1/T_2$) in the mixture of D_2O (80%) and the cystic fluid (20%) were measured. The relaxation rates ($1/T_{1f}$ and $1/T_{2f}$) in the mixture of D_2O (80%) and bidistilled water (20%) were also determined. The τ values were then determined by using a formula derived from the $(1/T_1 - 1/T_{1f})/2(1/T_2 - 1/T_{2f})$ ratio.

2. Materials and methods

2.1. Preparation of samples

Three typical fluids of jaw cysts; a serum like fluid (non-infected cyst), a purulent and dense fluid (infected cyst) and a fluent bloody fluid (hemorrhagic cyst) were selected for the determination of the effective correlation time representing molecular crowding. The samples were obtained from the Department of Oral and Maxillofacial Surgery. The clinical evaluation for diagnosis of cysts was confirmed by radiological and histological studies. Since high signal intensity did not allow to measure relaxation times, a mixture of 0.1 ml of each cyst fluid with 0.4 ml of D_2O were prepared for the T_1 and T_2 measurements in cysts, and also a mixture of 0.1 ml of bidistilled water with 0.4 ml of D_2O were prepared to determine T_{1f} and T_{2f} in water. Then the samples were transferred into NMR tubes with 5 mm of radius.

2.2. Relaxation measurements

The experiments were carried out on a BRUKER DPX-NMR spectrometer operating at 400 MHz. The signal of the cyst mixture is shown in Fig. 1. The signal belongs to water protons in the cyst mixture since the mixture of the bidistilled water and D_2O gives a similar signal at the same chemical shift.

The spin–lattice relaxation times (T_1) were measured by the inversion recovery method. Eleven delay times between 180° and 90° pulses were altered from 500 ms to 10 s. Pulse repetition time was set at 60 s to allow full recovery of the magnetization in the mixtures. T_2 measurements were carried out by CPMG method. In this case, echo delays were varied from 25 ms to 350 ms. Magnetization recovery curve for T_1 and magnetization decay curve for T_2 are given in Figs 2 and 3, respectively. These figures indicate that magnetization decay curve is a single exponential for the range of the delay and the echo times used. However, such samples may show a multi exponential decay when a wide range of the delay times were used.

Fig. 1. The water NMR Signal of the mixture of D₂O and jaw cysts recorded at 400 MHz.

The sample temperature was set at $22 \pm 0.1^\circ\text{C}$ by using a temperature controller unit. The experimental error, based on repeated measurements, was %3 for T_1 and %5 for T_2 .

2.3. Calculation of τ value

The following equation was used for calculation of τ by setting $(1/T_1 - 1/T_{1f})/2(1/T_2 - 1/T_{2f})$ equal to R

$$12R(\omega_0\tau)^4 + (37R - 8)(\omega_0\tau)^2 + (10R - 5) = 0, \quad (1)$$

where ω_0 is resonance frequency, being 400 MHz for the present case. This equation and its modifications have already been used for the calculation of τ values [4,7,8]. Since each cyst contains different types of molecules, the correlation time calculated from Eq. (1) should be identical to those of all molecules of each cyst fluid.

3. Results and discussion

The relaxation rates ($1/T_1$ and $1/T_2$) measured for the three types of cyst fluids are shown in Table 1. It is seen that the relaxation times of the cysts are different from each other and this is consistent with previous results reported for native cysts at 1T and also with other previous works [5,12,17,25,26]. The τ values calculated from Eq. (1) are also shown in Table 1. The τ value of the infected cyst is longest (about 3 ns), but the other τ values have similar values (about 2 ns). However, the observed relaxation

Fig. 2. Inversion recovery signals versus the delay times.

Table 1

The observed relaxation rates in the mixture of D₂O (80%) and the cyst fluids (20%) for three types of the jaw cysts. The calculated correlation times and viscosities measured by the Ostwald viscometer are also given. The $1/T_{1f}$ and $1/T_{2f}$ rates in the mixture of D₂O (80%) and bidistilled water (20%) were 0.07 and 1.07 s^{-1} , respectively

Parameters	Non-infected	Infected	Hemorrhagic
$1/T_1$ (1/s)	0.117	0.140	0.200
$1/T_2$ (1/s)	2.353	4.000	4.348
$\tau \cdot 10^{-9}$ (s)	2.3	2.9	2.2
Viscosity (cP)	1.11	1.14	1.22

rates in jaw cysts vary largely from one patient to other [5,26]. As a result, the values in Table 1 represent the studied cysts only.

Non-infected radicular cysts contain proteins, cholesterol, ions and chronic inflammatory cells [13]. The extra content of infected radicular cysts is intensive inflammatory cells, whereas extra content of hemorrhagic cysts is blood cells [13]. However, the mean concentration of each material in cyst fluid may be dependent on the cyst type. For example, cholesterol content in radicular cysts may be higher than that in hemorrhagic cysts. Also, the content of inflammatory cells in infected cysts is higher than

Fig. 3. Magnetization signals versus echo times in CPMG.

those in other cysts. Then the results in Table 1 are consistent with molecular content of the cyst fluids since the relaxation rates in cysts are dependent on their material content [5,12].

In biological fluids, some water molecules are bound to solids. The contribution of such solids to observed relaxation rates, $\Delta(1/T_i)$, can be written as

$$\Delta\left(\frac{1}{T_i}\right) = P_b\left(\frac{1}{T_{ib}}\right) \quad i = 1 \text{ and } 2, \quad (2)$$

where P_b and $1/T_{ib}$ represent the fraction of water bound to a solid and the relaxation rates of bound water, respectively [28]. The relaxation rates of the bound water are caused by proton dipole–proton dipole interaction modulated by molecular motions [32]. The possible molecular motions are rotational tumbling, water exchange between free and bound states and translational motion [3,14,32]. When all the motions are uncorrelated and under an assumption of isotropic motions, all the motions can be represented by an effective correlation time (τ). Such a correlation time may be written as

$$\frac{1}{\tau} = \frac{1}{\tau_r} + \frac{1}{\tau_m} + \frac{1}{\tau_t}, \quad (3)$$

where τ_r , τ_m and τ_t represent the correlation time of rotational tumbling, lifetime of water in hydration sphere and translational motion, respectively [4,6]. The evaluation of each individual correlation time is difficult for systems involving molecular crowding. However, the effective τ value gives useful insights into the effective motion in such systems. In fact, it is seen that the motion in the infected cyst is slower

than those of others for the present case. This implies that the molecule related to effective motion in the infected cyst is larger than those in the others since larger molecules move slowly and has a longer correlation time. This is consistent with the presence of intensive inflammatory cells in the infected cysts. The similarity of the τ value of the non-infected cysts to that of the hemorrhagic cysts is reasonable since similar hydrated solids contribute to the relaxation rates of both cysts and since such water in hemorrhagic cysts exchange very rapidly across the membrane of blood cells [16]. Also, the concept of effective correlation time is important when the rotational correlation time of protein and the exchange time of water from protein surface are of nearly the same size [3]. In such case, the relaxation mechanism cannot be explained in terms of a specific correlation time since two uncorrelated motions contribute to the observed relaxation rate. Furthermore, Nuclear Magnetic Relaxation Dispersion (NMRD) experiments in protein solutions have provided better fits when weighted average correlation times are used [14]. Such studies implies that the effective correlation time has a physical meaning to analyze relaxation mechanism in protein solutions.

On the other hand, Eq. (3) implies that the effective correlation time may be dominated by one of individual correlation times. When $1/\tau_r$ dominates $1/\tau$, the effective radius of a rigid sphere may be calculated by using a modified Stokes–Einstein relation [9]. In this modification, the correction factor is $(\eta/\eta_s)^q$ where η and η_s are the viscosity of mixed and pure solvent respectively. The viscosity of the cysts, calculated by using an Ostwald viscometer, are shown in Table 1. Since the dilution of the cyst sample by D₂O approximates the η/η_s to unity, the present calculation of the effective τ makes possible the use of Stokes–Einstein relation without any modification whenever $1/\tau_r$ is known.

In conclusion, the present data suggest that an effective correlation time for fluids with macromolecular crowding can be determined from NMR relaxation measurements.

References

- [1] A.E. Garcia and G. Hummer, Water penetration and escape in proteins, *Proteins* **38** (2000), 261–272.
- [2] A.G. Krushelnitsky, V.D. Fedotov, J. Spevacek and J. Straka, Dynamic structure of proteins in solid state. ¹H and ¹³C NMR relaxation study, *J. Biomol. Struct. Dyn.* **14** (1996).
- [3] A. Van-Quynh, S. Willson and R.G. Bryant, Protein reorientation and bound water molecules measured by 1H magnetic spin–lattice relaxation, *Biophys. J.* **84** (2003), 558–563.
- [4] A. Yilmaz, M.Z. Köylü and H. Budak, Estimation of τ value in proton NMR relaxation times of dibenzo diaza 18-crown-6 ether derivative in solution, *C.P. Lett.* **427** (2006), 346–349.
- [5] B. Erol, U.N. Yilmaz, R. Tanrikulu and A. Yilmaz, Determinants of MR relaxation rates in jaw cysts: implications for diagnostic values of the relaxation times, *Dentomaxillofac. Rad.* **33** (2004), 183–187.
- [6] B. Halle, Protein hydration dynamics in solution: a critical survey, *Philos. T. Roy. Soc. B* **359** (2004), 1207–1224.
- [7] C. Kojima, A. Ono, M. Kainosho and T.L. James, DNA duplex dynamics: NMR relaxation studies of a decamer with uniformly ¹³C-labeled purine nucleotides, *J. Magn. Reson.* **135** (1998), 310–333.
- [8] D. Fushman, R. Weisemann, H. Thüning and H. Rüterjans, Backbone dynamics of ribonuclease T1 and its complex with 2/GMP studied by two-dimensional heteronuclear NMR spectroscopy, *J. Biomol. NMR* **4** (1994), 61–78.
- [9] D. Lavalette, C. Tétreau, M. Tourbez and Y. Blouquit, Microscopic viscosity and rotational diffusion of proteins in a macromolecular environment, *Biophys. J.* **76** (1999), 2744–2751.
- [10] D.S. Banks and C. Fradin, Anomalous diffusion of proteins due to molecular crowding, *Biophys. J.* **89** (2005), 2960–2981.
- [11] E.J. Chiu, D.C. Newitt, M.R. Segal, S.S. Hu, J.C. Lotz and S. Majumdar, Magnetic resonance imaging measurement of relaxation and water diffusion in the human lumbar intervertebral disc under compression in vitro, *Spine* **26** (2001), 37–44.
- [12] F.A. Timmer, M. Sluzewski, M. Treskes, W.J. van Rooij, J.L. Teepen and D. Wijnalda, Chemical analysis of an epidermoid cyst with unusual CT and MR characteristics, *Am. J. Neuroradiol.* **19** (1998), 1111–1112.
- [13] H. Harnish, *Clinical Aspects on Treatment of Cysts of the Jaws*, Buch-und Zeitschriften-Verlag, Berlin, 1974.
- [14] I. Bertini, M. Fragai, C. Luchinat and G. Parigi, 1H NMRD profiles of diamagnetic proteins: a model-free analysis, *Magn. Reson. Chem.* **38** (2000), 543–550.

- [15] I. Bertini, Y.K. Gupta, C. Luchinat, G. Parigi, C. Schlörb and H. Schwalbe, NMR spectroscopic detection of protein protons and longitudinal relaxation rates between 0.01 and 50 MHz, *Angew. Chem.* **117** (2005), 2263–2265.
- [16] I.L. Cameron, V.A. Ord and G.D. Fullerton, Water of hydration in the intra- and extra-cellular environment of human erythrocytes, *Biochem. Cell. Biol.* **66** (1988), 1186–1199.
- [17] J. Asaumi, H. Konouchi, M. Hisatomi, H. Matsuzaki, H. Shigehara, Y. Honda and K. Kishi, MR features of aneurysmal bone cyst of the mandible and characteristics distinguishing it from other lesions, *Eur. J. Radiol.* **45** (2003), 108–182.
- [18] J.G. Li, G.J. Stanisiz and R.M. Henkelman, Integrated analysis of diffusion and relaxation of water in blood, *Magnet. Reson. Med.* **1** (1998), 79–88.
- [19] J. Gallier, P. Rivet and J. de Certaines, ^1H - and ^2H -NMR study of bovine serum albumin solutions, *Biochim. Biophys. Acta* **915** (1987), 1–18.
- [20] J. Han and J. Herzfeld, Macromolecular diffusion in crowded solutions, *Biophys. J.* **65** (1993), 1155–1161.
- [21] J.P. Korb and R.G. Bryant, Magnetic field dependence of proton spin–lattice relaxation times, *Magnet. Reson. Med.* **48** (2002), 21–26.
- [22] K.M. Donahue, R.M. Weisskoff and D. Burstein, Water diffusion and exchange as they influence contrast enhancement, *J. Magn. Reson. Imaging* **1** (1997), 102–110.
- [23] K. Nicolay, P.J. Braun, R.A. Graaf, R.M. Dijkhuizen and M.J. Kruiskamp, Diffusion NMR spectroscopy, *NMR Biomed.* **14** (2001), 94–111.
- [24] L. Grösch and F. Noack, NMR relaxation investigation of water mobility in aqueous bovine serum albumin solutions, *Biochim. Biophys. Acta* **453** (1976), 218–232.
- [25] M. Hisatomi, J. Asaumi, H. Konouchi, Y. Yanagi and K. Kishi, A case of glandular odontogenic cyst associated with ameloblastoma: correlation of diagnostic imaging with histopathological features, *Dentomaxillofac. Rad.* **29** (2000), 249–253.
- [26] M. Minami, T. Kaneda, K. Ozawa, H. Yamamoto, Y. Itai, M. Ozawa, K. Yoshikawa and Y. Sasaki, Cystic lesions of the maxillomandibular region: MR imaging distinction of odontogenic keratocysts and ameloblastomas from other cysts, *Am. J. Roentgenol.* **166** (1996), 943–949.
- [27] P. Bernado, J.G. Torre and M. Pons, Macromolecular crowding in biological systems: hydrodynamics and NMR methods, *J. Mol. Recognit.* **17** (2004), 397–407.
- [28] P. Mansfield and P.G. Morris, Water in biological systems, in: *NMR Imaging in Biomedicine*, J.S. Waugh, ed., Academic Press, New York, 1982, pp. 10–32.
- [29] R.J. Ellis, Macromolecular crowding: an important but neglected aspect of the intracellular environment, *Curr. Opin. Struct. Biol.* **11** (2001), 114–119.
- [30] R.J. Ellis, Macromolecular crowding: obvious but under appreciated, *Trends Biochem. Sci.* **26** (2001), 597–604.
- [31] R.J.P. Williams, Protein dynamics studied by NMR, *Eur. Biophys. J.* **21** (1993), 393–401.
- [32] S.H. Koenig, Classes of hydration sites at protein–water interfaces: The source of contrast in magnetic resonance imaging, *Biophys. J.* **69** (1995), 593–603.