Intracellular water in *Artemia* cysts (brine shrimp)

Investigations by deuterium and oxygen-17 nuclear magnetic resonance

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ABSTRACT The dormant cysts of *Artemia* undergo cycles of hydration-dehydration without losing viability. Therefore, *Artemia* cysts serve as an excellent intact cellular system for studying the dynamics of water-protein interactions as a function of hydration. Deuterium spin-lattice (T_1) and spin–spin (T_2) relaxation times of water in cysts hydrated with D_2O have been measured for hydrations between 1.5 and 0.1 g of D_2O per gram of dry solids. When the relaxation rates ($1/T_1$, $1/T_2$) of ^2H and ^{17}O are plotted as a function of the reciprocal of hydration (1/H), an abrupt change in slope is observed near 0.6 g of D_2O (or $H_2{}^{17}O$)/gram of dry solids, the hydration at which conventional metabolism is activated in this system. The results have been discussed in terms of the two-site and multisite exchange models for the water-protein interaction as well as protein dynamics models. The ^2H and ^{17}O relaxation rates as a function of hydration show striking similarities to those observed for anisotropic motion of water molecules in protein crystals.

It is suggested here that although the simple two-site exchange model or *n*-site exchange model could be used to explain our data at high hydration levels, such models are not adequate at low hydration levels (<0.6 g H_2O/g) where several complex interactions between water and proteins play a predominant role in the relaxation of water nuclei. We further suggest that the abrupt change in the slope of $1/T_1$ as a function of hydration in the vicinity of 0.6 g H_2O/g is due to a change in water-protein interactions resulting from a variation in the dynamics of protein motion.

INTRODUCTION

Water is the major constituent of organisms, yet little is known of its exact structure in cells. A few insights concerning water's functional role in cells are, however beginning to emerge. Our current understanding of the physicochemical state of cellular water and its consequences for cellular function have been reviewed by several investigators (1–10).

Nuclear magnetic resonance (NMR) techniques have been widely used for the elucidation of the physicochemical state of cellular water in the living cell (1–5, 8–10). It has been observed that the relaxation times (T_1, T_2) of cellular water and the self-diffusion coefficients of cellular water protons are reduced compared to those of bulk water (3–5, 8–10). Water-macromolecular interactions have been considered to be primarily responsible for these reduced parameters. Physical models that describe such water-macromolecular interactions are the two-site–bound and free-exchange models (2, 3, 11, 12), the polarized multilayer hypothesis of Ling (6, 7), and, recently, two similar protein dynamics models (13, 14).

One biological system that we have found useful for testing water-macromolecular interactions is the cyst of the brine shrimp *Artemia,* because they can undergo repeated cycles of hydration-dehydration without any significant loss of viability. Earlier using *Artemia,* we investigated the hydration dependence of the relaxation times as well as the self-diffusion coefficient of cellular water protons (5, 15–18). A detailed study of the diffusive motion of water by neutron scattering techniques revealed a reduction in both the translation and rotational diffusion coefficient (16). This reduction in the diffusive motion, however, could not account for the observed reductions in the relaxation times in the brine shrimp (16, 17). In fact, finding a satisfactory explanation for the reudction (i.e., relative to bulk water) of the spin-lattice (T_1) and the spin–spin (T_2) relaxation times of water protons and their dependence on hydration has proven difficult. A large part of the problem arises from the fact it is difficult to separate the intra- and intermolecular contributions to relaxation in water protons. To obtain a more precise interpretation of the relaxation rates for cellular water, we have extended our earlier work by studying deuterium and ^{17}O relaxation times as a function of hydration in *Artemia* cysts. In both deuterium (^2H) and ^{17}O NMR spectroscopy, the relaxation mechanisms are dominated by nuclear electric quadripolar relaxation, and hence the relaxation is due primarily to intramolecular motions. These relaxation rates are therefore better probes of the changes in the anisotropic reorientational motion of water molecules.Our results on the water-protein interactions in *Artemia* cysts show striking similarities with those observed in protein single crystals (19).

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MATERIALS AND METHODS

Description of *Artemia* cysts

The cysts of *Artemia*, a primitive crustacean known as the brine shrimp, can undergo cycles of hydration-dehydration without any significant loss of viability (20–24). Each cyst is ~200 μm in diameter and is composed of an embryonic mass of ~4,000 morphologically similar, closely packed cells surrounded by an acellular shell of ~8 μm in thickness. It is important to understand that there is virtually no extracellular space due to very tight cell packing; therefore, results may be interpreted in terms of intracellular events (22, 24). When dried over desiccants, the cells retain only residual water (<1% by weight) and are ametabolic (they do not engage in detectable metabolism) but viable if rehydrated.

In these cysts, metabolic activity exhibits an interesting dependence on hydration levels (20). In particular, there are two hydration levels of metabolic importance. The hydration levels are measured as grams of H_2O (or D_2O or $H_2{}^{17}O$) per gram of dry solids, hereafter referred to as g H_2O (or g D_2O or g $H_2{}^{17}O$)/g. Below a hydration level of 0.3 g H_2O/g, the cysts are virtually ametabolic. Around 0.3 g H_2O/g, restricted metabolic pathways are initiated, but the cysts do not resume embryonic development. Conventional metabolism occurs above 0.6 g H_2O/g; however, the fully hydrated cyst contains 1.3 g H_2O/g at which the *cells* contain ~1.6 g H_2O/g (21). The *Artemia* system is well studied; details of the ultrastructure, development, and biochemical-biophysical properties of the system are available (20–25).

Procedures for hydration of cysts

We used two different procedures for the hydration of cysts. For hydrations >0.4 g D_2O/g, cysts were immersed in D_2O overnight at 4°C and then air dried or desiccated to attain different hydration levels. Hydrations <0.4 g D_2O/g were achieved by soaking the cysts overnight in D_2O solutions containing different concentrations of NaCl. (Because the shell of the cyst is impermeable to NaCl, the amount of water absorbed is determined by the water activity of the NaCl solution.) The cysts were then rinsed free of salts and surface dried quickly. Hydration levels were determined gravimetrically by determining the dry weight after heating at 103–105°C for 24 h. The same procedures for hydrating the cysts were followed for hydration studies in $H_2{}^{17}O$ (50% enriched).

The effect of hydration in D_2O on the viability and metabolism of the cysts has been investigated by Clegg (24). He found that the percentage of viable larvae produced in D_2O was smaller (66%) than those produced in water (88%). While the rate of development in D_2O was reduced by a factor of 2–3, the larvae appeared perfectly normal in their morphology and swimming motions, although their mobility was sluggish compared with that of controls (24).

NMR measurements

For both ^2H and ^{17}O NMR measurements, the cysts were placed in 10 mm NMR tubes. An SXP spectrometer (Bruker Instruments, Inc., Billerica, MA) equipped with a superconducting magnet was operated at a magnetic field of 42.84 kgauss, corresponding to frequencies of 28 MHz for deuterium and 24.73 MHz for ^{17}O. Data were recorded using a model 1074 digital signal averager (Nicolet Instrument Corp., Madison, WI), operated by an Interdata 7/16 computer. The 90° pulse width for deuterium nuclei was 50 μs, whereas that for ^{17}O was 60 μs. The ^2H and ^{17}O spin-lattice relaxation times (T_1) were determined using the standard inversion recovery method (26). For hydrations >0.2 g D_2O/g, the spin–spin relaxation times (T_2) were measured using the standard Carr-Purcell method with Meiboom-Gill modification (CPMG sequence) (27). T_2 at low hydrations (<0.2 g D_2O/g) could not be determined by the CPMG technique due to very fast signal decay, and hence no T_2 values were reported for hydration below 0.2 g D_2O/g. The error estimated on the basis of reproducibility of the measurement is shown in the figures. All measurements were made at room temperature (21–23°C).

Data analysis

The deuterium T_1 data could be fitted to a single exponential as shown in Fig. 1. Thus the spin-lattice relaxation times and the relaxation rates are represented by a weighted average because single exponential relaxation decay was observed. The T_2 relaxation behavior, on the other hand, could be decomposed into two components, with the slower relaxing component constituting 75–85% of the total signal at the hydration of 1.45 g D_2O/g (Fig. 1 *b*). The relative percentage of the two components depends on hydration. This behavior was observed even at very low hydrations (<0.4 g D_2O/g). In the case of deuterium spin-spin relaxation, the major component decaying with a T_2 ~25 ms (at full hydration) corresponds to more mobile cellular water, whereas the fast decaying component (T_2 ~1.5–2 ms) is likely due to a fraction of much less mobile (or immobilized) water molecules and/or exchangeable protons on the macromolecules. The results reported in this paper for deuterium spin–spin relaxation behavior (T_2) as a function of hydration represent relaxation times of the more mobile cellular water that constitutes the major component of the relaxation decay. The relative percentages of the two components as well as the T_2 of the fast-decaying component are sensitive to the fit.

In the case of ^{17}O NMR, only the spin-lattice relaxation time (T_1) could be determined due to S/N ratio considerations except at the full hydration value for which the spin–spin relaxation time (T_2) could also be determined using CPMG sequence. The ^{17}O relaxation decay (T_1) could be fitted to a single exponential as in the case of ^2H NMR. A typical relaxation decay curve for ^{17}O is shown in Fig. 2.

Theoretical background

In the case of a deuterium nucleus ($I = 1$) and oxygen-17 nucleus ($I = 5/2$) the relaxation is caused by nuclear quadripolar interactions modulated by molecular motions. In contrast, the relaxation of the hydrogen nucleus of water is caused by the dipolar interactions between the protons within and between water molecules as well as cross-relaxation between the water protons and protein protons, and proton chemical exchange. Quadripolar relaxation of duterium and oxygen-17 nuclei in cells could be considered as being entirely caused by intramolecular motion such as rotational motion (19, 28–30). The relaxation behavior is more complex in ^{17}O ($I = 5/2$) than for ^2H because the relaxation rate has contributions from three components (30–33, 35, 44). In the case of ^2H relaxation, chemical exchange between the solvent deuterons with the exchangeable sites in the protein should also be considered. Oxygen-17 relaxation is not affected by cross-relaxation or chemical exchange. However, exchange of whole water molecules should be considered even in the case of ^{17}O relaxation.

The motion of water molecules in the cell is rather complex due to the interaction of water with the proteins and other macromolecules as well as cellular organelles leading to a distribution of correlation times characterizing the motion of water molecules in the cell (2, 19, 37–40). The motion of water molecules interacting with the cellular components is considered to be anisotropic in protein crystals (19) and other heterogeneous systems such as aqueous solutions of macromolecules, whole cells, and tissues (36, 43–45, 49, 50).

Various physical models have been proposed to explain the reduced relaxation times for water nuclei (^1H, ^2H, and ^{17}O) and their hydration

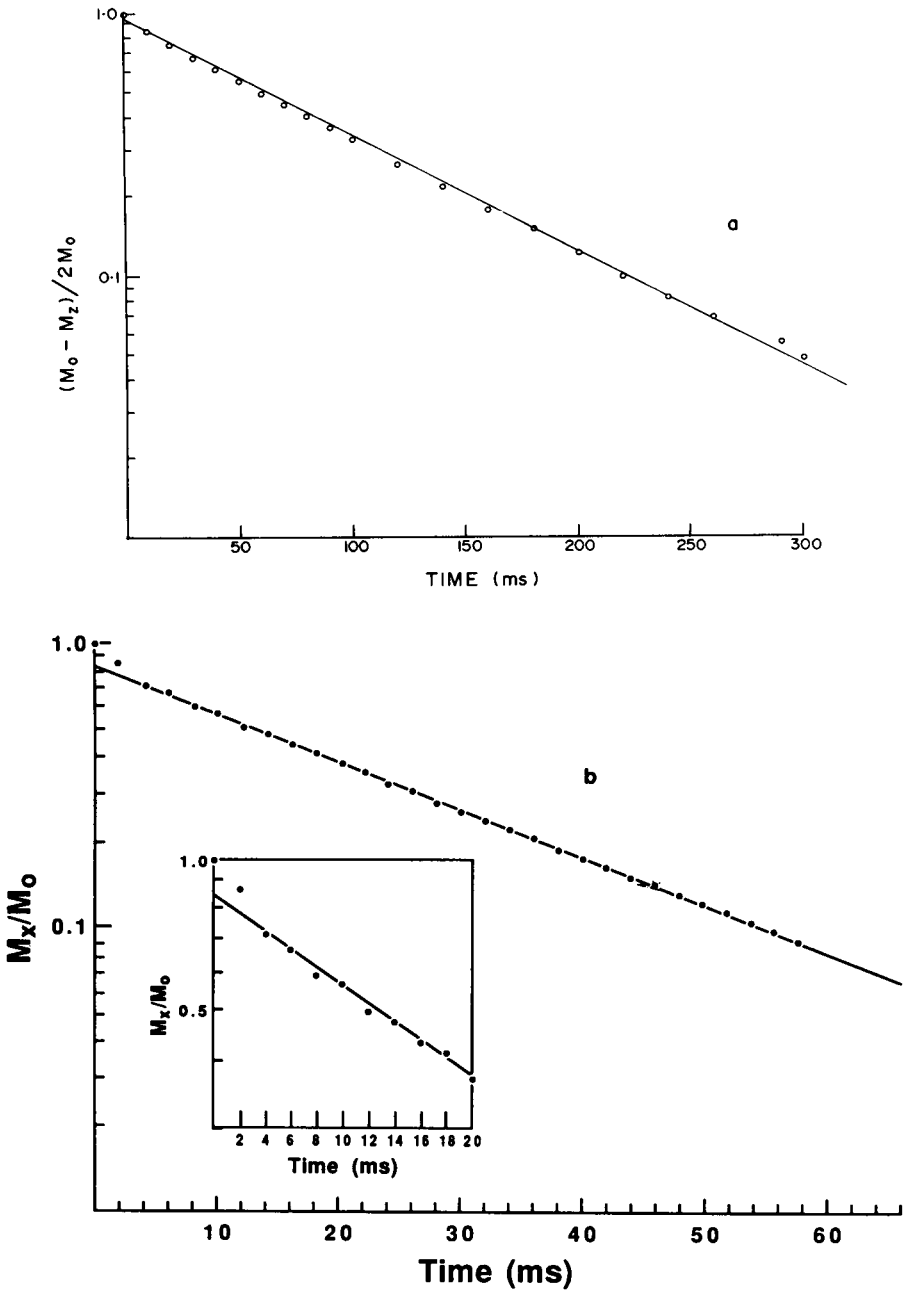

FIGURE 1 (*a*) Deuterium spin-lattice relaxation (T_1) of water in fully hydrated *Artemia* cysts (1.45 g D_2O/g). Upper data points (*open circles*) represent the ratio $(M_o - M_z)/2M_o$, in which M_o is the equilibrium magnetization and M_z is the magnetization as a function of time after the 180° pulse in inversion recovery sequence. (*b*) The data points represent the ratio M_x/M_o as a function of time after the 90° pulse in CPMG sequence, in which M_x is the echo height. The solid line represents the relaxation behavior of the major component in the multiexponential fit. The decay of M_x/M_o during the first 20 ms is given in the inset, demonstrating the multiexponential behavior of T_2.

dependence. These models range conceptually from the simple two-fraction fast exchange model (TFFE) of Zimmerman and Brittin (11), the bimodal log power distribution of the correlation times for molecular motions (37, 38), the polarized multilayer theory of Ling (6), the anistropic dual-motion model with fast exchange of water between bound and free states (48), and other models (12, 41–43, 45–47). Of these, the phenomenological two-state model is used most frequently. Recently, however, two similar protein dynamics models (13, 14) have been proposed based on an entirely different mechanism to explain the observed frequency and hydration dependence of relaxation rates of water protons or deuterons. In the model of Rorschach and Hazlewood (13) the relaxation mechanism is the modulation of the dipolar interaction of the protons that are closely associated with the proteins, either as side-chains or as water of hydration ("bound protons"). These

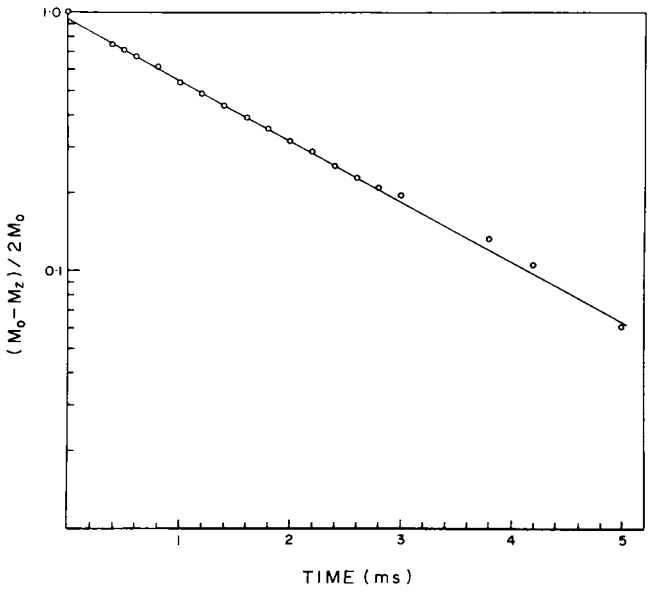

FIGURE 2 The spin-lattice relaxation of ^{17}O in *Artemia* cysts hydrated with $H_2{}^{17}O$ (50% enriched). The hydration of the cysts is 1.20 $H_2{}^{17}O$/g of dry cysts.

protons participate in the motion of the protein segments, which can be thought of as similar to the motion of a string vibrating in a viscous medium. If the string is overdamped, then the motion will be Brownian-like, and the different modes of vibration of the string will have correlation times that depend on the wavelengths of the permitted modes. The various possible modes for a string of fixed length will thus produce a spectrum of correlation times which depends on the physical properties of the string and the viscous medium. Each mode will produce its own contribution to the relaxation process, and the total relaxation rate of the "bound" fraction, R_{1b}, can be determined by an integration over the mode distribution. The bulk water will be relaxed by exchange or cross-relaxation with the "bound" phase.

The TFFE model is based on the hypotheses that the protons (or deuterons) of water molecules exchange between two environments and the exchange rate is fast compared to the NMR relaxation rates. In other words, a fraction of the water molecules are bound to the cellular macromolecules and these water protons (or deuterons) are in fast exchange with the bulk of the water protons (deuterons) in the cell. According to this model, the quadripolar relaxation rate is:

$$R_1 = R_s + \frac{x}{H}(R_{1b} - R_s) \qquad (1)$$

where R_s is the relaxation rate ($1/T_1$) of water nuclei in the bulk phase, X is the amount of bound water per gram of dry substance (proteins and other macromolecules), H is the hydration (grams of H_2O or D_2O/gram of dry substance), and R_{1b} is the relaxation rate ($1/T_{1b}$) of water molecules bound to the macromolecules. Such a model then predicts a linear relationship between the observed relaxation rate ($1/T_1$) and $1/H$ with the intercept on the $1/T_1$ axis corresponding to the relaxation rate ($R_s = 1/T_1$) for free liquid D_2O (or $H_2{}^{17}O$). A linear dependence between $1/T_1$ and $1/H$ would also be expected for a multisite exchange model.

The protein dynamics model for the observed relaxation rate ($1/T_1$) can be reduced to the TFFE model under certain conditions (13). According to this model,

$$R_1 = \tfrac{1}{2}(R_{1p} + MR_T + R_s + R_T) - \tfrac{1}{2}[(R_{1p} + MR_T - R_s - R_T)^2 + 4MR_T^2]^{1/2}, \qquad (2)$$

where R_s is the relaxation rate of the solvent protons (or deuterons), R_{1p} is the relaxation rate of the protons or deuterons that are a part of the macromolecule (includes bound water molecules), R_T is the cross-relaxation and/or exchange rate for the transfer of magnetization between solvent and macromolecular protons (or deuterons), and M is the ratio of the number of solvent-protons (or deuterons) to the number of protein protons (or deuterons that exchange with exchangeable protons). In the high-frequency range and for higher hydrations, where we can take $MR_T \gg |R_s - R_{1p}|$ (13), the fast exchange conditions holds and Eq. 2 will reduce to:

$$R_1 = R_s + \frac{1}{(1+M)}(R_{1p} - R_s). \qquad (3)$$

Eqs. 1 and 3 are similar but some of the parameters have different physical meaning. R_{1p} in Eq. 2 for example, is dependent on the correlation time (τ). The complete details of the model are given in reference 13.

RESULTS

The deuterium relaxation times measured as a function of hydration for cysts hydrated in D_2O are shown in Fig. 3.

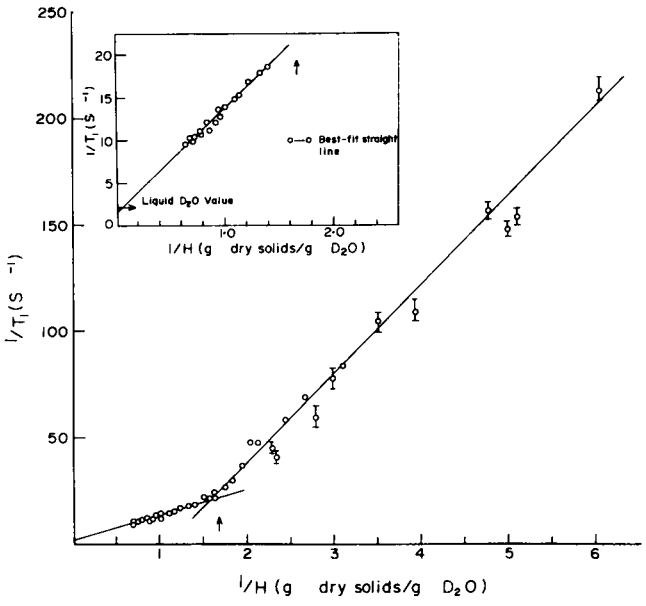

FIGURE 3 Deuterium spin-lattice relaxation rate ($1/T_1$) as a function of $1/H$. The solid lines are best fit straight lines in these regions. Error bars represent typical experimental error in different hydration regions. Arrows in the main figure and in the inset represent the hydration level of 0.6 g D_2O/g. Inset shows spin-lattice relaxation rate data vs. $1/H$ in the higher hydration region (>0.6 g D_2O/g). Solid line represents the best fit straight line to a linear form.

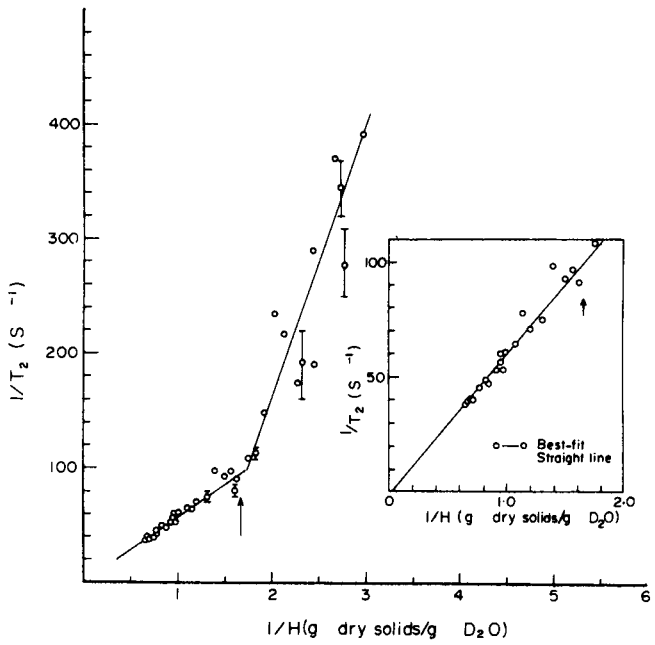

FIGURE 4 Deuterium spin–spin relaxation rates ($1/T_2$) as a function of $1/H$. Solid lines are the best fit straight lines in these hydration regions. Error bars represent the typical experimental error in $1/T_2$ in the different hydration regions. Arrow indicates the hydration level of 0.6 g D_2O/g. Inset shows the data in the higher hydration range (>0.6 g D_2O/g). Straight line shows the best fit of the data to a linear form of $1/T_2$ vs. $1/H$.

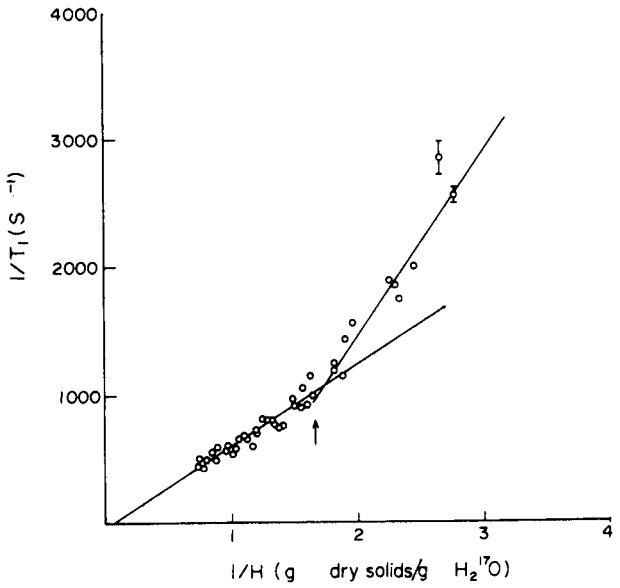

FIGURE 5 ^{17}O spin-lattice relaxation rate ($1/T_1$) of cyst water as a function of $1/H$. H is hydration defined as $H_2{}^{17}O$/g of dry cysts. Arrow represents the hydration level of 0.6 g $H_2{}^{17}O$/g. Error bars represent error determined in different hydration regions on the basis of reproducibility of measurements for the same hydration. Solid lines represent the best fit straight line in these hydration regions.

Two notable features of these data are apparent. First, with a reduction in hydration, the relaxation times decrease. This observation is similar to the hydration dependence of the proton relaxation times (T_1) observed in the case of several tissues (2, 3, 5) and cysts of *Artemia* (18), and of deuterium relaxation times in protein single crystals (19). Second, a change in the hydration dependence of relaxation times occurs around 0.6 g D_2O/g, the hydration level at which conventional metabolism resumes in the cysts (Fig. 3). Also noticeable is the approximate linear dependence of the deuterium relaxation rates ($1/T_1$) as a function of $1/H$ well above and below the hydration level of 0.6 g D_2O/g (indicated by an arrow in Fig. 3). The inset of Fig. 3 shows a best fit to a linear form of the relaxation rates ($1/T_1$) vs. $1/H$ in the higher hydration range (>0.6 g D_2O/g). The intercept of the best fit straight line is near the $1/T_1$ value of pure D_2O (2.22 s^{-1}).

Fig. 4 shows the deuterium spin–spin relaxation rates ($1/T_2$) of cyst water as a function of hydration down to a level of 0.3 g D_2O/g. Again the abrupt change in the slope if $1/T_2$ vs. $1/H$ plots occurs near the hydration level of approximately 0.6 g D_2O/g. The inset of Fig. 4 shows the linear fit of the data in the higher hydration range (>0.6 g D_2O/g).

Although the dominant contribution to deuterium relaxation times arises from intramolecular interaction due to rotation of water molecules, the exchange of deuterium in the macromolecules in the cysts could also be important. To circumvent this problem, the relaxation rate of the oxygen nucleus ($H_2{}^{17}O$) was determined as a function of *Artemia* hydration. Fig. 5 shows the ^{17}O spin-lattice relaxation rates ($1/T_1$) as a function of $1/H$. It seems apparent that the slope of the $1/T_1$ vs. $1/H$ plot changes abruptly near 0.6 g $H_2{}^{17}O$/g. Thus, the relaxation rate of ^{17}O varies with the hydration in a way qualitatively similar to that of deuterium.

DISCUSSION

The results on the T_1 and T_2 of ^2H and ^{17}O water in *Artemia* cysts show that these relaxation times differ significantly from those observed for cyst water protons (49). This difference arises because dipolar interactions modulated by intra- and intermolecular motions contribute to proton relaxation, whereas ^2H and ^{17}O relaxation is caused by modulation of the electric quadripolar interactions by intramolecular motions only. This difference in relaxation mechanism facilitates a more straightforward interpretation of the results for ^2H nd ^{17}O NMR than for protons. Also in the case of ^2H relaxation, the spin-lattice

relaxation was found to be single exponential while the transverse relaxation behavior was found to be multiexponential. This behavior was consistent with the behavior observed in the case of ^1H as well as ^2H by us in other systems as well as by others in biological systems (49, 50–53). The explanation for such multiexponential relaxation is not unique and can be explained in more than one way (50, 52, 53). The observation of single exponential behavior for T_1 can be explained qualitatively by assuming that the exchange among the populations of water in the cysts is rapid compared to T_1 resulting in a single average T_1 for the deuterons. On the other hand, the exchange of water deuterons among the different populations is slow compared to the T_2 resulting in multiexponential behavior.

As in the case of ^1H relaxation of water, the relaxation times of ^2H and ^{17}O are much shorter than those observed for bulk liquid. In cysts, T_1 for ^1H is 9% that of bulk H_2O; for ^2H 22% that of ^2H_2O; and for ^{17}O 29% that of $H_2{}^{17}O$. These results are consistent with data from other intact biological systems (2, 3, 5, 8). For review, these reductions in the relaxation times in tissues are attributed to reduced rotational motion of the water molecules, which are analogous to those observed in protein solutions (33–37, 40, 42, 44–46, 48, 49).

Detailed consideration of the ^2H and ^{17}O relaxation rate data for cyst water shown in Figs. 3–5, respectively, reveals an interesting feature. In all cases for ^2H and ^{17}O the relaxation rates vary in a linear manner with $1/H$ on either side of the hydration level of 0.6 g D_2O/g. The slopes of these lines, however, differ significantly. Our results are analogous to the hydration dependence of ^2H relaxation rates observed by Borah and Bryant (19) for water in protein single crystals. It is therefore appropriate to compare our results from an intact biological system to those obtained by these authors in protien single crystals of lysozyme (19). In the case of protein single crystals, the hydration level of 0.30–0.35 g D_2O/g, marked by a dramatic change in slope, has been interpreted as the value at which the protein has a full complement of water (19). For *Artemia* cysts, the hydration level of ~0.6 g D_2O/g, at which we observed a change in slope, is the hydration level above which conventional metabolism starts. Proteins constitute ~0.48 g/g of dried cysts (22), and are the major component for H_2O absorption (22). The 0.6 g D_2O/g level probably corresponds to a hydration above the full complement of hydration water required for most of the proteins present in the cysts. At present we choose to interpret the break in the slopes of the relaxation rate data of water nuclei at 0.6 g D_2O/g simply as a change in water-protein interactions due to differences in protein motions above and below this hydration level (see references 18, 19, 48 for comparison). We further suggest that these interactions may be related to the processes required for the onset of conventional metabolism (e.g., changes in protein conformation) (20, 21). It is also possible that at hydrations below 0.6 g D_2O/g, protein aggregation might be taking place resulting in changes in water-protein interactions. Although the contributions from intermolecular interactions and cross-relaxation mechanisms are significant in ^1H relaxation, the intramolecular motion is dominant in the relaxation of the deuterium and oxygen nuclei. Thus, we propose that the changes in the relaxation rates which are observed at hydrations <0.6 g/g reflect changes in the rotational motion of water molecules associated to the proteins; thus, the motion of the proteins is transferred to the water molecules. At hydrations >0.6 g/g, the realxation rates again vary monotonically with hydration; however, the relaxation process is now further complicated by exchange (13). This interpretation may be further evaluated by determining the frequency dispersion of the various nuclei as a function of hydration.

Table 1 compares the T_1/T_2 for ^1H, ^2H, and ^{17}O for water nuclei of fully hydrated cysts. Unlike muscle tissue (29), the three T_1/T_2 ratios differ significantly. An important implication of these findings is that different mechanisms contribute to the relaxation rate for the three nuclei of water and that the effect of the proteins on the relaxation behavior of ^1H, ^2H, and ^{17}O is different. For deuterium and ^{17}O, only quadripolar intramolecular motion contributes to the relaxation. The difference in the T_1/T_2 for ^2H and ^{17}O can be explained if one recognizes that there is a constant exchange (slow or fast) of water molecules among different environments. This could involve either an exchange of hydrogen (or deuterium) or of whole water molecules. Exchange of hydrogen (or deuterium) can occur between water molecules or between water and exchangeable nonwater protons or proteins in the cysts. Both types of exchange contribute to ^2H and ^1H relaxation, whereas only the exchange of the entire water molecule contributes to ^{17}O relaxation. Therefore, these results indicate the presence of both types of exchange occurring in the present system.

TABLE 1 Spin-lattice ($1/T_1$) and spin–spin ($1/T_2$) relaxation rates for ^1H, ^2H, and ^{17}O of water in *Artemia* cysts in fully hydrated cysts (1.4–1.5 g/g)

Nucleus	Frequency MHz	$1/T_1$	$1/T_2$	T_1/T_2
^1H	30	3.66 ± 0.07	19.3 ± 0.5	5.3
^2H	28	10.4 ± 0.2	38 ± 2	3.7
^{17}O	24.7	439 ± 28	735 ± 81	1.7

Errors are assigned on the basis of the reproducibility of T_1 and T_2 measurements for same hydration on different samples.

Further evidence that the ^2H NMR relaxation rate has contributions different from those present in the ^{17}O NMR relaxation comes from the hydration dependence of the normalized relaxation rate $[(1/T_1)$ cyst$/(1/T_1)$ solvent] of deuterium and oxygen-17 shown in Fig. 6. It may be noticed that the normalized relaxation rates for deuterium are somewhat larger than those for oxygen-17 (Fig. 6) and this difference becomes progressively larger as the hydration is decreased. This observation is similar to that observed by Kakalis and Baianu (48) for deuterium and oxygen-17 transverse relaxation in lysozyme solutions but is at variance with that observed by Koenig et al. (35) who concluded that the ^1H, ^2H, and ^{17}O relaxation rates of lysozyme solutions are equal to each other after scaling to the free solvent nuclear spin-lattice relaxation rates. Therefore, our data cannot be analyzed simply in terms of TFFE model. We believe that a suitable model should include a multisite exchange with a distribution of correlation times for the motion of water molecules which includes anisotropic motion on the protein surface for a fraction of these water molecules.

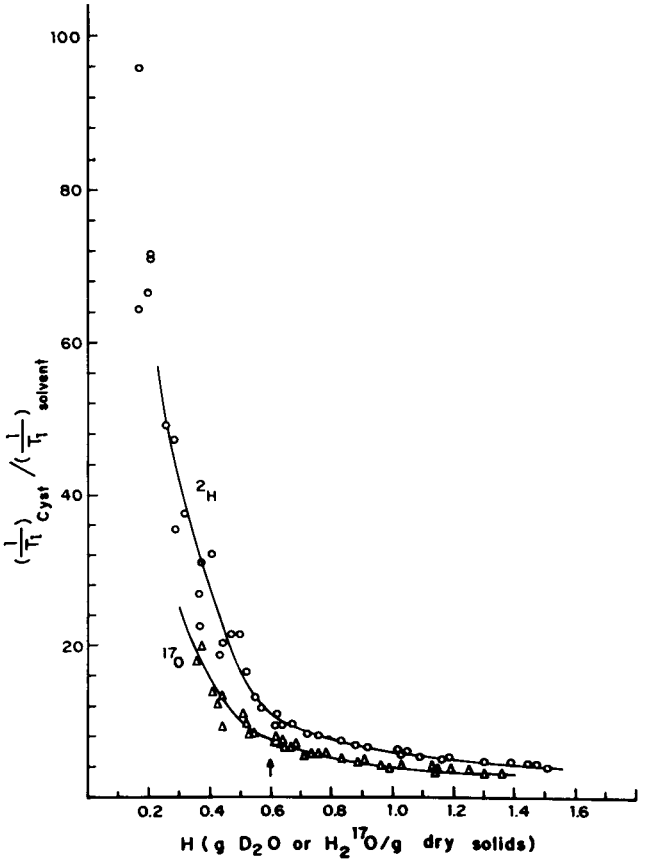

FIGURE 6 Hydration dependence of the normalized spin-lattice relaxation rate $[(1/T_1)$ cyst$/(1/T_1)$ solvent] for deuterium (O) and oxygen-17 (Δ) in the cysts. *H* is the hydration expressed as grams D_2O or $H_2{}^{17}O$ dry solids. Normalized spin-lattice relaxation rates have been calculated using $(T_1)^{-1}$ $(D_2O) = 2.22\ s^{-1}$ and $(T_1)^{-1}$ $(^{17}O) = 143.1\ s^{-1}$. The solid line is a hand-fit curve.

CONCLUSIONS

Our results on ^2H and ^{17}O relaxation behavior of *Artemia* cyst water show interesting differences from that of water protons in this same system. These findings demonstrate the advantage of using ^2H and ^{17}O NMR for an understanding of dynamical aspects of water in intact biological systems like *Artemia* cysts. In addition, the present study has demonstrated the sensitivity of ^2H and ^{17}O relaxation rates for detecting changes in water-protein interactions as a function of hydration, implying a possible correlation between such interactions and the hydration at which conventional metabolism occurs. Our results suggest that the behavior of water motion in this system is analogous to the anisotropic motion of water observed in protein single crystals. Finally, although two-site or multisite exchange models can be applied for qualitative description of the situation in the hydration region, our results over the entire hydration range indicate that these models represent oversimplified descriptions of water-protein interactions in intact biological systems.

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