^{1}H-NMR RELAXATION INVESTIGATION OF WATER BOUND TO BOVINE ROD OUTER SEGMENT DISK MEMBRANES

ELENA GAGGELLI, NERI NICCOLAI, AND GIANNI VALENSIN
Institute of General Chemistry, Universtiy of Siena, 53100 Siena, Italy

ABSTRACT Spin-lattice relaxation times T_1 in deuterated aqueous dispersions of lecithin and rod outer segment disk membranes were measured at various concentrations and temperatures. Fast chemical exchange between two loosely defined phases of water molecules was shown to fit the data, allowing the dynamic features of "bound" water and the hydration of the biological membrane to be evaluated. The state of the water was shown to be also involved in vision physiology.

INTRODUCTION

In the field of vision research, an important task is to understand the structure and function of photoreceptor membranes. In fact, the action of light on rhodopsin, which is the major protein component of the rod outer segment (ROS) disk membrane, is the first event in dim light vision (1), and may be directly coupled to permeability changes in the disk membrane (2, 3).

In the present communication, we report the NMR relaxation parameters of water protons in the ROS disk membrane based on the following:

(*a*) water is the main membrane component and it is involved in stabilizing the lipid bilayer structure (4) as well as the cell physiology (5);

(*b*) the structural and motional order of water in biological samples has often been investigated in terms of nuclear relaxation rates (6);

(*c*) the water spin-lattice relaxation rate ($1/T_1$) has been shown to reflect the dynamic and conformational features of macromolecular solutes (7–9).

MATERIALS AND METHODS

Egg yolk lecithin and deuterium oxide (D_2O) were obtained from Merck & Co., Inc. (Rahway, N.J.). The dispersions were made by dissolving the lipid in $CHCl_3$, evaporating the solvent under nitrogen and adding D_2O 95%. Bovine ROS were obtained using the method of Chabre et al. (10), from which purified and lyophilized membranes were obtained. They were then buffered with Tris-HCl 2 mM in D_2O 95% at pH = 8 and nitrogen saturated to minimize oxidative damage to the polyunsaturated fatty acids. The samples were prepared under a weak red light to avoid denaturation of rhodopsin.

The spin-lattice relaxation times were measured using a Bruker WH 90 FT-NMR spectrometer operating at 90 MHz (Bruker Instruments, Inc., Manning Park, Billerica, Mass.) equipped with a Nicolet BNC-12 computer and using the $180 - \tau - 90$ pulse sequence (Nicolet Instrument Corp., Madison, Wis.). The pulse duration was carcfully selected for every experiment. The temperature was measured by a Bruker temperature control unit with an accuracy of ± 1°C. T_1 was evaluated by computer fitting of the recovery curve of longitudinal magnetization; the T_1 values were averaged over five experiments and are accurate at ±4%. In every case a single exponential decay was detected.

RESULTS AND DISCUSSION

The experimental water proton relaxation rates in biological samples have been alternately interpreted in terms of models for water mobilities and for relaxation mechanisms. It has been recently shown (9) that cross-relaxation between water protons and macromolecular protons provides the most effective relaxation pathway for the water molecules in the immediate neighborhood of the macromolecular surface, and that this process is usually smeared out at high isotopic dilution. For this reason, two loosely defined phases of water molecules (free and bound) exchanging rapidly have been usually successful in fitting the observed relaxation parameters, for erythrocyte ghosts (11) and red cell membranes (12).

Fig. 1 shows the water proton $1/T_1$ against the solids fraction (x = percent by weight of solids, $1 - x$ = percent by weight of water) for both lecithin and ROS membrane vesicles. The linear dependence suggests that, whichever the relaxation mechanism, the macromolecules are relaxing solvent protons directly and independently. If a formal two-phase model is assumed, the observed relaxation rate can be expressed (12, 13):

$$1/T_1 = c(1/T_{1b} - 1/T_{1f})\, x/(1 - x) + 1/T_{1f}, \quad (1)$$

where c equals grams bound water per grams of solids and $1/T_{1f}$ and $1/T_{1b}$ are the relaxation rates in the free and bound phase, respectively. From the intercept of the two

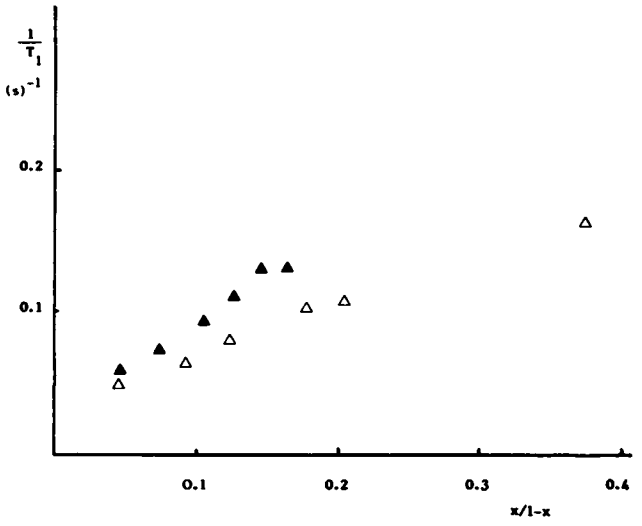

FIGURE 1 Water proton spin-lattice relaxation rate vs. the lecithin and ROS membrane solids fraction (x = percent by weight of solids, 1 − x = percent by weight of water). ▲, ROS; △, lecithin.

lines $1/T_{1f} = 0.032\ s^{-1}$ is evaluated according to the isotopic dilution effect (14, 15). If $c = 0.43$ for lecithin membranes as suggested by water sorption isotherms (16), calorimetric measurements (17), and NMR studies (18, 19), $T_{1b} = 1.3$ s is calculated from the slope in Fig. 1.

For the bulk 95% D_2O at room temperature, the intramolecular dipole-dipole ^1H-^2H interaction, modulated by the fast rotational and translational diffusion ($\tau_c = 3 \times 10^{-12}$s)[20], is the main relaxation pathway for water protons. If, in the membrane dispersions, the relaxation mechanism does not change, $\tau_c = 2 \times 10^{-9}$ s can be evaluated from the T_{1b} value, showing a sizeable slowing down of the bound water. However, the relaxation mechanism of bound water is likely to be different from that of

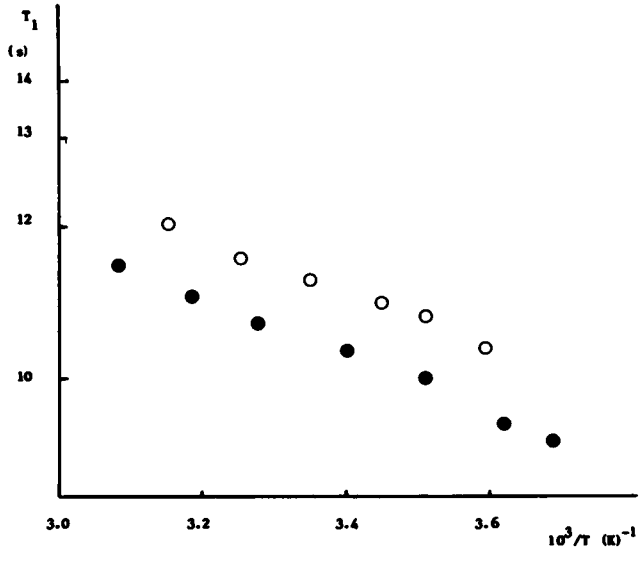

FIGURE 2 Temperature dependence of water proton T_1 for ROS and lecithin samples containing 10% solids by wt. ●, ROS; ○, lecithin.

TABLE I
SPIN-LATTICE WATER PROTON RELAXATION TIMES IN ROS DISK MEMBRANES AFTER A STRONG ILLUMINATING FLASH

Percent by weight of dry membrane	Time after flash (min)			
	0*	15	30	45
10	8.6	7.5	7.3	7.1
15	7.4	5.8	5.6	5.6

*Measured before the flash.

the free bulk water, as previously discussed. In this case the evaluated τ_c should be taken as an upper limit.

The concentration dependence of $1/T_1$ shows a larger slope for ROS membranes than for lecithin membranes. Since a similar $1/T_{1b}$ is expected in the two cases, the change in slope is attributable to a change in the amount of hydration water. Accordingly, c equals 0.79 gram of bound water per gram of ROS membrane is calculated from Eq. 1. This value agrees with the greater saturation hydration found for biological membranes than for globular proteins and phospholipids (21).

Fig. 2 shows the temperature-dependent T_1 data for samples containing 10% solids by weight. By taking an Arrhenius temperature dependence $\tau_c = \tau_c^\circ \exp(E_a/RT)$, an activation energy $E_a = 3$ kcal mol^{-1} can be calculated from the two curves. Since $E_a = 4.5$ kcal mol^{-1} is evaluated for the pure solvent, this finding could suggest that, in membranes, there is on average a smaller number of hydrogen bonds per water molecule than in the pure solvent. If the intermolecular interaction with macromolecular protons dominates the relaxation of bound water, the observed temperature dependence could be explained either by an enhanced rotational tumbling of macromolecular solute molecules or by an exchange contribution.

The concentration and temperature dependence point out a major structural role played by water in the organization of photoreceptor membranes. To check whether water is involved also in the physiological function, the NMR tube was illuminated by a white flash and the T_1 was measured thereafter. Since the T_1 measurement takes about 15 min, the early effects could not be detected. The NMR linewidth can be measured in few seconds but did not show any change up to 20 min after the flash. The T_1 data are summarized in Table I. The two-phase model indicates that a 20% increase in the bound water fraction occurred as a consequence of the action of light. Even if this is only a rough approximation (the change in protein conformation may well be contributing to the change in T_1), it does show that water is involved in the membrane function, and suggests future consideration.

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