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THE DIFFUSIONAL WATER PERMEABILITY IN THE HALOTOLERANT ALGA *DUNALIELLA* AS MEASURED BY NUCLEAR MAGNETIC RESONANCE

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T_1 nuclear relaxation measurements of ^1H and ^{17}O of water have been applied to study the kinetics of the diffusional transport of water across the cytoplasmic cell membrane of *Dunaliella salina* and *Dunaliella bardawil*. The water permeability coefficients at 25°C were found to be $1.5 \cdot 10^{-3}$ cm/s and $1.8 \cdot 10^{-3}$ cm/s, respectively, with an activation energy of 3.7 kcal/mol. The results indicate that the cell membrane of *Dunaliella* exhibits high diffusional permeability to water, similar in magnitude to that found for other cells and model membranes, and a relatively low activation energy. This regularity is in contrast to the exceptionally low glycerol permeability of the membrane (Brown, F.F., Sussman, I., Avron, M. and Degani, H. (1982) Biochim. Biophys. Acta 690, 165–173).

Introduction

The halotolerant green algae *Dunaliella* can maintain high concentration gradients ($> 10^4$) of glycerol, between the intracellular fluid and the medium [1,2]. The most likely barrier to glycerol leakage is the cell membrane, which must therefore be almost impermeable to this small, usually highly permeable, substance.

Recently we have measured [3] the glycerol permeability of the *Dunaliella* cell membrane and found it to be extremely low: $P < 5 \cdot 10^{-11}$ cm/s at 17°C (i.e., $t_{1/2} > 400$ h). This finding raises the question whether the membrane is impermeable specifically to glycreol or does it slow down the permeation of other molecules too, particularly that of water. Permeation of water through the cell membrane is the first event in the osmoregulatory process and it is therefore important to characterize its kinetics.

In this study the exchange diffusional permeability of water was followed by NMR relaxation measurements [4]. The intrinsic difference in the chemical composition of the intracellular and extracellular media influences distinctively the corresponding water longitudinal relaxation rates and enabled us to perform the measurement without adding artificial relaxation reagents.

Materials and Methods

Sample preparation

Dunaliella salina was grown as previously described at 4 M NaCl in a growth chamber under continuous illumination [1]. *Dunaliella bardawil* was grown at 4 M NaCl in outdoor ponds with natural illumination [5]. The cells were harvested by centrifugation at 4°C for 10 min at $4000 \times g$. The pellet was resuspended in a minimal volume of a 4 M NaCl solution containing 40% ^2H_2O and

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the suspension was then concentrated by a similar centrifugation. For ^{17}O measurements the pellet was resuspended in $H_2^{17}O$ (6%) solution containing 4 M NaCl. Both, the supernatant and the closely packed cells were transferred to 5 mm or 10 mm NMR tubes for ^1H and ^{17}O studies, respectively. Cell numbers were determined in a Coulter Counter. Experiments were performed within 4 h after harvesting.

NMR measurements

Proton and ^{17}O relaxation studies were carried out at 90 MHz and 12.2 MHz, respectively, on a Bruker WH -90 pulse-FT spectrometer. In the proton studies the field was locked on internal ^2H_2O. In the ^{17}O measurements an external ^2H_2O field lock was used. The temperature was controlled to within 1.0 K with a variable temperature unit. Longitudinal relaxation rates were determined by the inversion recovery method.

Results and Discussion

The variation with temperature of the water ^1H and ^{17}O longitudinal relaxation rates, measured in suspensions of *Dunaliella salina* and *Dunaliella bardawil* and their corresponding supernatant solutions, are shown in Fig. 1. This figure includes also temperature dependence measurements of the ^1H T_1 relaxation after warming the algae suspension (10 min at 70°C) which causes complete leakage of the intracellular glycerol content [6] and other metabolites (Degani, H., Sussman, I. and Avron, M., unpublished data).

The ^{17}O relaxation rates in the algal suspension and the supernatant were found to be the same. This implies that the exchange of the water across the cell membrane is either much faster or slower than the intrinsic relaxation rate of the ^{17}O nuclei in the intra or extracellular media, respectively. Under such conditions the contribution of the exchange rate to the measured nuclear relaxation rate is negligible and can be ignored.

Generally the ^1H relaxation rate of water is slower than that of ^{17}O. Therefore the following may be expected: if the ^1H measurements in the cell suspension and the supernatant solution exhibited the same trend as the ^{17}O studies then water exchange is at the slow exchange limit and is

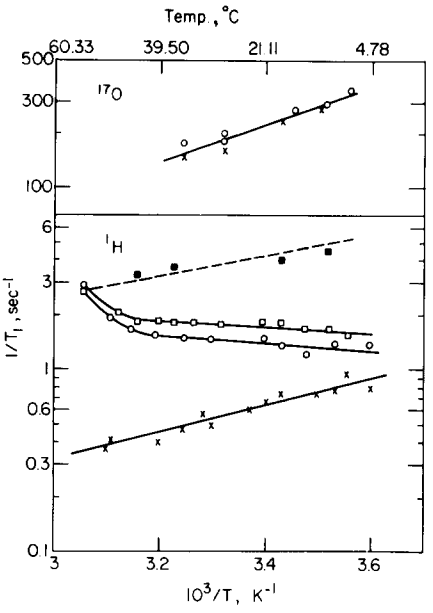

Fig. 1. Temperature dependence of ^1H and ^{17}O longitudinal relaxation rate of water in algal suspensions and their corresponding supernatants. The designation of the various relaxation rates is: ×——×, $1/T_1^{sup}$ in the supernatant; ○——○, $1/T_1$ in a suspension of *Dunaliella bardawil* ($2.9 \cdot 10^8$ cells/ml); (□——□) $1/T_1$ in a suspension of *Dunaliella salina* ($1.2 \cdot 10^9$ cells/ml); ■----■, $1/T_1$ in *D. salina* after warming the suspension to 70°C for 10 min.

slower than the ^1H relaxation rate of the supernatant. Alternatively if the ^1H studies exhibited a distinct trend (namely ^1H relaxation rates in the suspension will be faster than in the supernatant) then conditions of fast exchange prevailed for the ^{17}O measurements and the exchange rate falls in the kinetic region for ^1H measurements. The data in Fig. 1 clearly indicate that the latter is actually occurring. Thus, the ^1H relaxation rate in the algal suspension is dominated by the exchange rate and exhibits a behaviour typical to a kinetic region.

The fraction of fast relaxing inner water protons, $(H_2O)_i$, in the algal suspension is small relative to the slow relaxing external water protons, $(H_2O)_o$. Therefore the following simplified equation for determining the exchange rate can be applied [7].

$$\frac{1}{\tau^i} = \frac{(H_2O)_o}{(H_2O)_i}\left(\frac{1}{T_1} - \frac{1}{T_1^{sup}}\right) \qquad (1)$$

where $1/\tau^i$ is the mean life time of water inside the cells, and $1/T_1$ and $1/T_1^{sup}$ are the measured relaxation rates in the algal suspension and supernatant, respectively. The temperature dependence of the exchange rates calculated according to Eqn. 1 is plotted in Fig. 2. The activation energy for the exchange process was found to be rather small 3.7 kcal/mol. At about 47°C there is a conspicious change in the Arrhenius plot. This change is attributed to the change in the cell membrane properties which are reflected in the leakiness of all the intracellular glycerol into the medium [6]. This temperature dependent leakiness behaves in a mode that resembles a phase transition event. The nature of this change is being currently investigated.

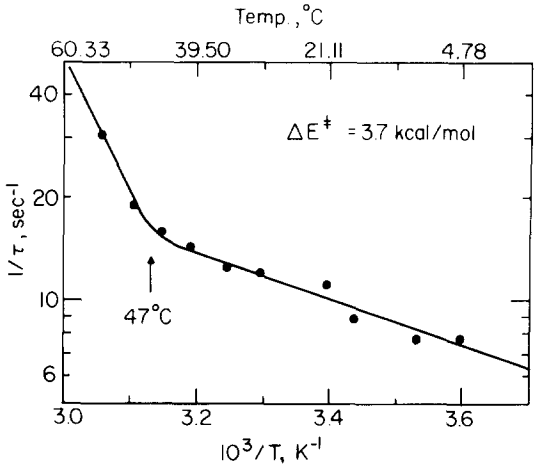

Fig. 2. Temperature dependence of the water diffusion exchange rates in *Dunaliella bardawil.*

The water diffusional permeability coefficient P_d was calculated using the relation

$$P_d = \frac{V}{A} \cdot \frac{1}{\tau^i} \qquad (2)$$

where V and A are the inner volume and surface area of the cells, respectively. The ratio V/A was calculated by assuming a spherical shape for the cells with an average radius, r, calculated from the measured volumes *V(D. salina)* 100 μm^3 and *V(D. bardawil)* = 400 μm^3 [8]. The cells are in fact ellipsoid in shape but the ratio of the long/short axis is close to 1. The results are summarized in Table I together with similar data obtained previously for other cells. A comparison between the various permeabilities indicates that the cell membrane of *Dunaliella*, as well as other cell membranes, exhibit high diffusional permeability to water, similar in magnitude to that found for pure lipid membranes.

The activation energy for water diffusion in *Dunaliella* is relatively low and similar in magnitude to that found for bovine and dog red cells. It was previously suggested that when water has an activation energy for water permeation much lower than for pure phospholipid membranes it may be

TABLE I

WATER PERMEABILITY COEFFICIENT AND ACTIVATION ENERGIES IN VARIOUS CELLS

System	Permeability at 25°C (cm/s)($\times 10^3$)	Energy of activation (kcal/mol)	Ref.
Dunaliella bardawil	1.8	3.7	This work
Dunaliella salina	1.5	3.7	This work
Halobacterium halobium	1.0	9	
Human erythrocytes	2.4	5.3–8.7	10, 11
Bovine erythrocytes	–	4.0	12
Dog erythrocytes	–	3.7	13
Chlorella vulgaris	2.1	–	14
Elechea leaf cells	3.0 (20°C)	–	15
Valnia etricularis	1.2	–	16
Nitella translucens	–	8.5	17
Phosphatidylcholine vesicles	2.9	10.5	7

crossing these membranes via an aqueous pore [13]. However, if the water concentration at the membrane-water interface is very high, the low activation energy may be a consequence of the fact that the rate limiting step for water permeation is the diffusion across the hydrocarbon region (the activation energy for water diffusion through saturated hydrocarbons is 2.6 to 3.4 kcal/mol [18]). Further studies with the isolated cell envelope may clarify this behaviour.

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