Plant Physiol. (1988) 87, 813–817
0032-0889/88/87/0813/05$01.00/0

^{23}Na-NMR Studies of the Intracellular Sodium Ion Concentration in the Halotolerant Alga *Dunaliella salina*[1]

Received for publication September 28, 1987 and in revised form March 18, 1988

MICHAL BENTAL, HADASSA DEGANI, AND MORDHAY AVRON*
Departments of Isotope Research (M.B., H.D.) *and Biochemistry* (M.A.), *The Weizmann Institute of Science, Rehovot 76100, Israel*

ABSTRACT

The Intracellular Na^+ concentration in the halotolerant alga *Dunaliella salina* was measured in intact cells by ^{23}Na-NMR spectroscopy, utilizing the dysprosium tripolyphosphate complex as a sodium shift reagent, and was found to be 88 ± 28 millimolar. Intracellular sodium ion content and intracellular volume were the same, within the experimental error, in cells adapted to grow in media containing between 0.1 and 4.0 molar NaCl. These values assume extracellular and intracellular NMR visibilities of the ^{23}Na nuclei of 100 and 40%, respectively. The relaxation rate of intracellular sodium was enhanced with increasing salinity of the growth medium, in parallel to the intracellular osmosity due to the presence of glycerol, indicating that Na^+ ions and glycerol are codistributed within the cell volume.

Members of the genus *Dunaliella* (division Chlorophycophyta, order Volvocales, family Polyblepharidaceae) have the capacity to tolerate and adapt to a very wide range of salt concentrations (0.1–5.5 M NaCl). This is achieved through the ability of the algae to survive the initial osmotic stress and then adjust their intracellular solute content to the new level required for the maintenance of a similar cell volume. Glycerol has been established to be the major osmoregulator and compatible solute in *Dunaliella* (4, 7). However, there is much controversy concerning the role of other ions, primarily Na^+, in osmoregulation (1).

It is generally accepted that the intracellular Na^+ level in *Dunaliella* is lower than that of the surrounding medium. This is also supported by the sensitivity of several of its enzymes to NaCl (16, 27). However, different values have been reported for the magnitude of the transcellular sodium gradient, ranging from an intracellular to extracellular concentration ratio of 0.5 (17, 29) to less than 0.1 (13, 22, 25). There is also a disagreement as to whether the internal Na^+ content is a function of the external NaCl concentration (2, 18) or not (13, 22, 25). We believe that the origin of the discrepancy lies in the technical difficulties of separating the relatively small volume of low-salt-containing cells from the large volume of extracellular medium containing massive amounts of salt. Although *Dunaliella* is very flexible osmotically, it is very fragile mechanically, making the physical separation of undamaged cells (with unknown ionic content) from the medium extremely difficult. Even small contamination of the cells with NaCl from the outside will produce misleading results. Methods involving extensive washings may cause cell damage or induce ion exchange, while different extracellular markers have yielded contradictory information about the extracellular space due to adhesion to the cell surface, interference of the surface coat, or uptake by the algal cells. This also accounts for the variety of values for the intracellular volume reported by different investigators.

Recently, Hajibagheri *et al.* (21) have used x-ray microanalysis to determine the intracellular Na^+ content in *Dunaliella*. Although in this technique only the intracellular space is examined, it involves fixation steps which may induce changes in the composition of the different cellular compartments.

It is clear from the data obtained so far that if one could use a noninvasive technique for determination of intracellular Na^+ that does not require separation of the cells from the external medium or their disruption, many of the methodological problems mentioned above will be avoided. We decided, therefore, to use NMR spectroscopy for determining noninvasively the intracellular Na^+ content in intact *Dunaliella salina* adapted to different NaCl concentrations. In recent years, ^{23}Na-NMR spectroscopy coupled with the use of Na^+ shift reagents (10, 20) has permitted measurements of Na^+ content and fluxes in living cells and tissues (8, 9, 11, 15, 20, 23). A drawback in employing ^{23}Na-NMR techniques for *in vivo* studies is the uncertainty regarding the NMR-visibility of the intracellular ^{23}Na nuclei (3, 12). However, since the visibility should not vary for the same species of cells under different environmental conditions, comparative studies of intracellular content are valid. Verification of the cells' viability and well being under the conditions of the NMR experiment (*i.e.* aerated in the dark at 4°C) can be achieved through ^{31}P NMR studies of the phosphate metabolites and microscopic observation (5). We believe that the results reported in this communication provide a definitive answer to what has been a controversial issue.

MATERIALS AND METHODS

Growth of Cells and Their Preparation for the NMR Measurements. *Dunaliella salina* was grown in batch cultures (5). Algae at the logarithmic phase (1–2.5 × 10^6 cells/ml) were concentrated by centrifugation at 4°C (2000*g* for 10 min). All subsequent operations were performed at 0 to 4°C. The cells were washed twice with an isoosmotic NaCl medium or with a medium containing 0.3 M Na^+ made isotonic with glycerol. The medium also contained 25 mM HCO_3^-, 5 mM KNO_3, 0.2 mM KH_2PO_4, 5 mM $MgSO_4$, 0.3 mM $CaCl_2$, and shift reagent at the indicated concentration at pH = 8.2. In all experiments, the final cell suspension (1–2 ml of 2 × 10^9 cells/ml) was transferred to a 10 mm NMR tube and immediately aerated by passing humidified air through silicon tubing immersed in the cell suspension.

Shift Reagent. The shift reagent, dysprosium tripolyphosphate ($Dy[PPP]_2^{-7}$), was prepared by mixing 0.35 M $DyCl_3 \cdot 6H_2O$ (Alfa Chemicals) in 0.1 N HCl with a 0.35 M solution of $Na_5P_3O_{10} \cdot 6H_2O$ (Sigma) to give a 2.5:1 molar ratio.

[1] Supported by a grant from the United States/Israel Binational Science Foundation.

NMR Measurements. NMR experiments were performed at 4 ± 1°C (unless indicated otherwise), using a Bruker CXP-300 FT NMR spectrometer. ^{23}Na-NMR spectra were recorded at 79.39 MHz, using 90° pulses and 0.2 s repetition time. An external reference contained 8.75 mM $DyCl_3$ and 26.25 mM $Na_5P_3O_{10}$. T_1 measurements were performed by using the inversion recovery method, applying 180°-τ-90° pulses. Each measurement included 19 variable τ values and was completed within approximately 30 min. The data were fitted to a uniexponential decay using a nonlinear best fit analysis (26).

Intracellular Volume Determination. The extracellular space was determined according to Gupta and Gupta (20), by comparing the normalized integrated intensity of the signal due to extracellular Na^+ ions (A_{out}) with that of Na^+ ions in a control containing the resuspension buffer (with the shift reagent) and no cells, in an identical sample geometry (A_{buf}). The integrated intensities of the signals were normalized according to the signal of the external reference. The fraction of the total volume occupied by the cells, S_{in}, was obtained from

$$S_{in} = 1 - A_{out}/A_{buf}$$

and the ratio of the extracellular to intracellular volume was calculated as

$$V_{out}/V_{in} = A_{out}/(A_{buf} - A_{out}).$$

Intracellular Na^+ Concentration. Intracellular Na^+ concentration was calculated from the ratio of the integrated intensities of the resonances due to the intracellular (A_{in}) and extracellular (A_{out}) Na^+ ions, the ratio of the extracellular to intracellular volume and the extracellular Na^+ concentration. The intracellular Na^+ concentration is given by:

$$[Na^+_{in}] = (A_{in}/A_{out}) \times (V_{out}/V_{in}) \times [Na^+_{out}]$$
$$= (A_{in}) \times [Na^+_{out}]/(A_{buf} - A_{out})$$

This calculation assumes that both the intracellular and extracellular Na^+ ions are 100% visible; otherwise, appropriate correction factors must be introduced.

RESULTS AND DISCUSSION

^{23}Na-NMR Measurements of the Intracellular Na^+ Ion Content. ^{23}Na-NMR was used to monitor noninvasively the intracellular sodium ions of *Dunaliella*. The signal due to the external ^{23}Na was separated from the intracellular one by addition of the paramagnetic shift reagent $Dy(PPP)_2^{-7}$ to the medium in which the cells were suspended. A typical ^{23}Na-NMR spectrum (Fig. 1) consisted of three well-resolved resonances, intracellular, extracellular, and that of the external reference.

Interference of the shift reagent with the metabolism of *Dunaliella* was ruled out by the observation that the growth rate of cells in the logarithmic phase of growth was not affected by the shift reagent at the concentrations used in the NMR experiments. Examination of ^{23}Na-NMR spectra of aerated cells during prolonged (12 h) incubation at 4°C or at room temperature revealed no change in the extracellular sodium chemical shift, indicating that the shift reagent remained intact. In several experiments with cells adapted to low salinities (≤0.5 M NaCl) and resuspended in a medium containing glycerol and only 0.15 M NaCl, leakage of Na^+ from the cells was observed. However, this never involved more than 20% of the observed intracellular signal and ceased about 2 h past the time of harvesting.

A computer SAS nonlinear fitting procedure (NLIN) (26) was employed for lineshape analysis of the ^{23}Na-NMR spectra. Using single lorentzian lines for each resonance provided the best fit for the three peaks. An example of such a fit is presented in Figure 2. The simulated spectrum (B) almost exactly overlaps the experimental one (A), and therefore they are shown next to each other. The integrated intensities of the signals were derived from this analysis and used for the determination of the intracellular volume, Na^+ visibility, and intracellular content.

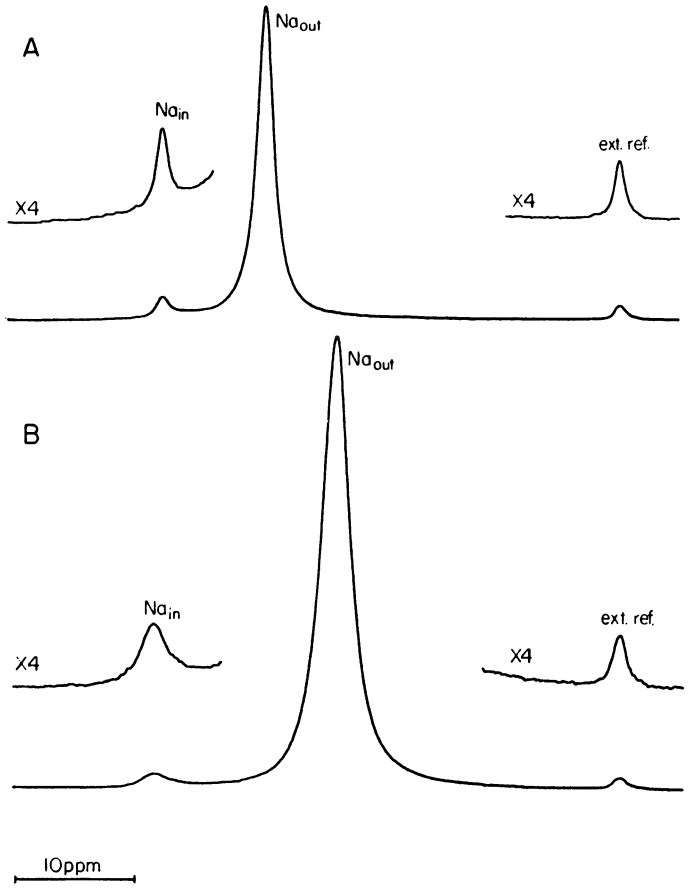

FIG. 1. ^{23}Na-NMR spectra of *D. salina* adapted to different salinities. Experimental conditions were as described under "Materials and Methods." Each trace was obtained by accumulating 400 scans (90° pulses, 0.2 s delay) and was processed using line broadening of 5 Hz. A, Cells grown and measured in media containing 0.1 M NaCl. The measuring medium contained 4 mM shift reagent. B, Cells grown in 3.0 M NaCl, and measured in a medium containing 0.3 M Na^+, 3.65 M glycerol, and 9 mM shift reagent. Na_{in}, signal due to intracellular Na^+; Na_{out}, signal due to extracellular Na^+. The same external reference (ext. ref.) was used in both measurements.

Intracellular Volume Determination. The intracellular volume of *D. salina* cells adapted to different salinities (0.1–4.0 M NaCl) was determined using ^{23}Na-NMR, as described under "Materials and Methods." The fractional volume of 2×10^9 cells/ml was 0.19 ± 0.04 (n = 8). This corresponds to a volume of 95 ± 20 femtoliters per cell, which agrees with the value of 90 femtoliters per cell determined by Katz and Avron (22). There was no significant variation of the intracellular volume with the salinity of the growth medium, within the error of the measurements (± 12–33%). Therefore the value of 95 femtoliters per cell was used for determining Na^+ concentration at all salinities.

Sodium Visibility. The extracellular sodium nuclei were shown to be 100% visible by the addition of aliquots of a concentrated NaCl solution to a cell suspension containing 10 mM of the shift reagent $Dy(PPP)_2^{-7}$. The area of the external sodium signal was determined relative to the area of the signal due to 0.1 M NaCl solution inside a concentric tube.

The visibility of the intracellular sodium was determined by following the leakage of Na^+ from cells into the extracellular medium. As previously noted, *Dunaliella* cells adapted to 0.5 M

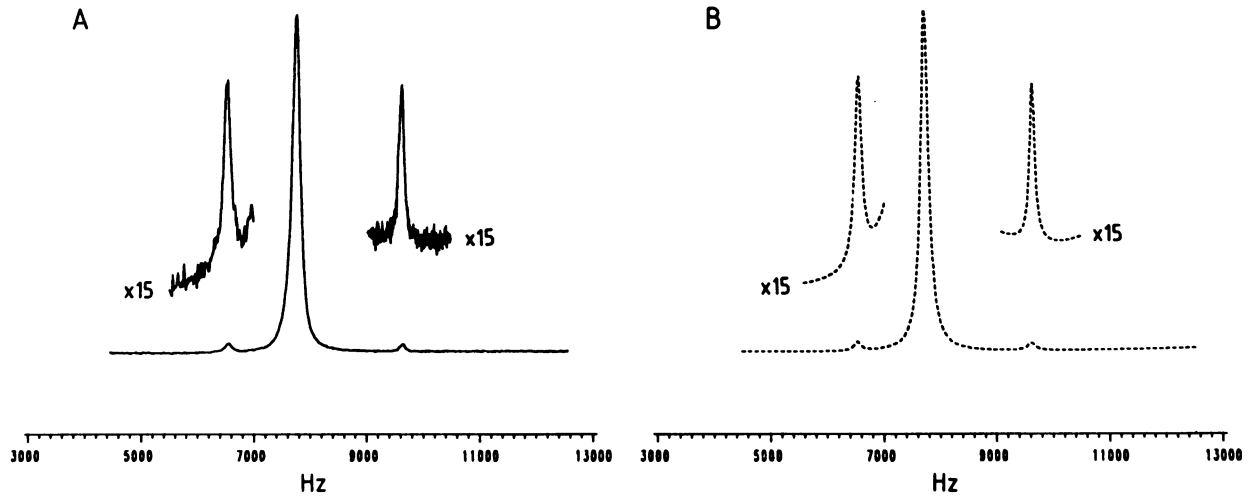

FIG. 2. Best-fit lineshape analysis of ^{23}Na-NMR spectrum of *D. salina* adapted to 1.0 M NaCl. Experimental conditions were as described in Figure 1. The medium contained 0.3 M NaCl, 1.19 M glycerol, and 9 mM shift reagent. A, Experimental spectrum; B, calculated spectrum. The integrated intensities of the three observed signals were $A_{out} = 1845 \pm 1.4\%$, $A_{in} = 36.1 \pm 11\%$, and $A_{ref} = 26.1 \pm 9.2\%$.

NaCl released Na^+ when resuspended in a medium containing glycerol and a very low (0.15 M) Na^+ concentration. Analysis of sequential spectra from such cells showed, in two independent experiments, that the decrease in the integrated intensity of the intracellular signal corresponded to 41 and 48% of the corresponding increase in the integrated intensity of the extracellular sodium signal. The changes, although small (approximately 20% of the total internal Na^+ content), were statistically significant. We therefore assume that the intracellular sodium nuclei in *Dunaliella* exhibit a 40% NMR visibility, in analogy to that previously determined in other cells such as *Escherichia coli* (8) and yeast (23) and in a variety of biological tissues (12). It should be noted, however, that it is very difficult to unequivocally determine the visibility of the intracellular ^{23}Na nuclei, and there exist contradicting reports by different investigators, even for the same system (*e.g.* 6, 19). The 40% NMR visibility of ^{23}Na could arise from rapid exchange between the bulk free internal Na^+ and a small fraction (<1%) of immobilized Na^+, causing two of the three permitted energy transitions to exhibit very short transverse relaxation times (T_2) due to quadrupolar interaction. Alternatively, the quadrupolar effect could be due to diffusion of the free sodium ions between domains of ordered polyelectrolytes (*e.g.* nucleic acids, polypeptides). The interaction between Na^+ and the polyelectrolytes is electrostatic and does not significantly limit the freedom of motion of the ions (12, 14). Both interpretations mean that virtually all of the intracellular Na^+ ions are free in solution. In addition, these considerations indicate that the visibility of such soluble Na^+ cannot be lower than 40%. Indeed, previous reports (3, 8, 12, 23) support this analysis.

Intracellular Na^+ Content. The Na^+ concentrations in *D. salina* adapted to different salinities were determined, assuming 40% visibility, (*i.e.* the relevant NMR signal intensities were multiplied by a correction factor of 2.5), and are summarized in Table I. The values in Table I provide an upper limit for the intracellular Na^+ concentration. If the visibility of the intracellular ^{23}Na nuclei was 100%, the values in Table I would have been 2.5-fold lower (the mean $[Na^+_{in}] = 35 \pm 11$ mM).

Cells adapted to 0.5 M and 1.0 M NaCl were measured both in an isoosmotic NaCl medium and in medium containing 0.3 M Na^+ made isoosmotic with glycerol. Similar values for the intracellular sodium content were obtained for cells resuspended in the two types of media (Table I), thus showing that the intracellular Na^+ level was not affected by partial replacement of extracellular NaCl by glycerol. For cells adapted to high salinities, an isoosmotic medium containing a combination of 0.3 M NaCl and glycerol was used. This was done to avoid high concentrations of the shift reagent, which caused substantial broadening of the sodium resonance. Although the presence of glycerol induced line broadening as well, the overall effect still allowed sufficient separation of the internal and external Na^+ signals even at high salinities (Fig. 1B). *Dunaliella* cells observed microscopically appear unaffected by this change in their medium. To further establish the cells' well-being under these conditions, we have recorded ^{31}P-NMR spectra of cells adapted to high salinities (2 and 4 M NaCl) and resuspended in isoosmotic NaCl or NaCl/glycerol medium. The spectra were similar in both media and exhibited a similar content of nucleotide triphosphates (primarily ATP). The possibility of very rapid efflux of Na^+ from the high-salinity-adapted cells upon transfer to the relatively low-Na^+ medium cannot be ruled out completely. However, the equivalent results obtained for cells grown with 1 M NaCl or lower and measured in the growth medium or in an isoosmolar medium containing 0.3 M NaCl and glycerol, the constancy of the values obtained with time, the conserved energy profile of the cells following the washes (reflected by the ^{31}P-NMR spectra), and the

Table I. *Intracellular Na^+ Concentration of D. salina Cells Adapted to Different Salinities*

Cells were grown and intracellular Na^+ concentration was calculated as described under "Materials and Methods." The intracellular volume was taken as 95 femtoliters per cell and 40% NMR visibility was assumed for the intracellular ^{23}Na nuclei. The asymptotic standard error in the estimation of the integrated intensities varied between 4 and 50% for the intracellular Na^+ and between 0.5 and 4% for the extracellular Na^+ signals.

NaCl in Growth Medium	Intracellular Na^+
M	*mM*
0.1	58; 74
0.25	56; 58
0.5	98; 112; 78[a]; 145[a]
1.0	62; 68[a]
2.0	114[a]
3.0	117[a]
4.0	93[a]
Mean	88 ± 28 (n = 13)

[a] Data obtained from experiments in which the cells were resuspended in isoosmotic medium containing glycerol and 0.3 M NaCl.

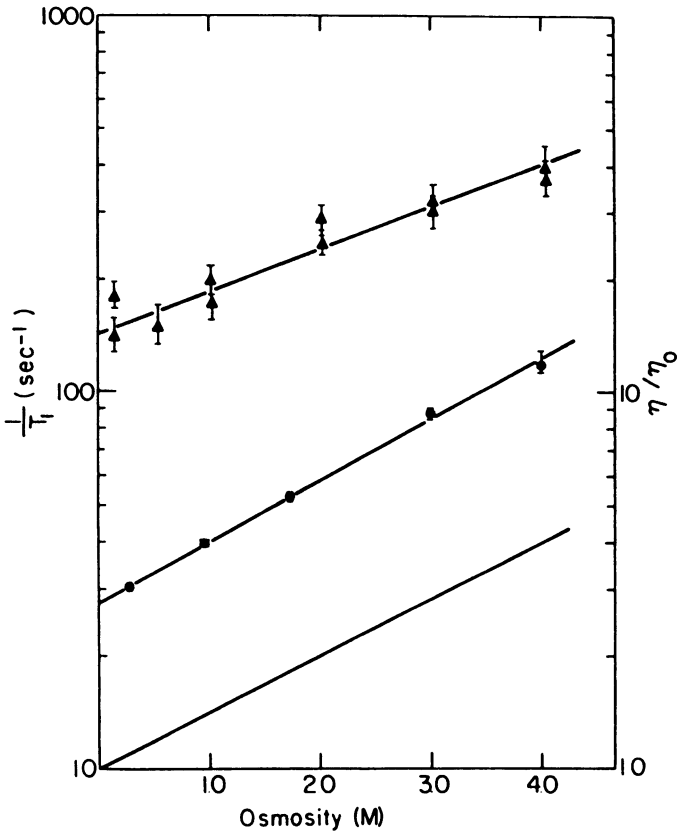

FIG. 3. Effect of the growth medium osmosity on the T_1 relaxation rates of the intracellular Na^+ in *D. salina*. The osmosity of a solution is defined as the molar concentration of an NaCl solution having the same osmotic pressure. Solid line: the change in the relative viscosity η/η_0, (the viscosity of a solution relative to the viscosity of pure water) of glycerol-water mixtures as a function of their osmosity (data from [28]). (●), The relaxation rate, $1/T_1$, of the ^{23}Na nuclei of 50 mM NaCl in water-glycerol mixtures as a function of their osmosity. (▲), The relaxation rate, $1/T_1$, of intracellular Na^+ ions in *D. salina* cells as a function of the medium salinities in which they were grown. Each point represents an independent experiment, the error bars delineate the 95% confidence interval obtained from the best-fit procedure as described under "Materials and Methods."

microscopic appearance of the cells make it unlikely that such leakage takes place.

From the results of Table I, it is apparent that the intracellular Na^+ concentration was invariant, within the experimental error, with the extracellular salinity, and was considerably lower than that of the growth medium: the extracellular/intracellular concentration ratio varied between 1.5 in cells grown in 0.1 M NaCl to as high as 40 for cells adapted to 4.0 M NaCl. These results are comparable to the values reported by Pick *et al.* (24). A similar value of approximately 90 mM, can be calculated from a weighted average of the the Na^+ concentrations determined by x-ray microanalysis for the cytoplasm and vacuoles of *Dunaliella parva* cells adapted to 0.4 and 1.5 M NaCl (21).

Effect of Salinity on the Relaxation Rate of Intracellular ^{23}Na Nuclei. Using the inversion recovery technique, we determined the longitudinal relaxation rate ($1/T_1$) of the intracellular ^{23}Na nuclei in *D. salina* cells adapted to different salinities and the $1/T_1$ of 50 mM NaCl in different glycerol/water mixtures. The relaxation rate of the intracellular sodium increased with increasing salinity of the growth medium. A similar increase in $1/T_1$, which correlated with the increase in the relative viscosity (η/η_0) due to the presence of glycerol, was observed in the glycerol/water mixtures (Fig. 3). However, the absolute $1/T_1$ values in the cells were higher. The faster relaxation rates in the cells may be due to the dependence of the intracellular viscosity on other intracellular components. This is more pronounced at lower salinities, where the cells contain a lower glycerol concentration (4) than at high salinities, where the contribution of glycerol to the intracellular viscosity is higher, and can explain the more moderate slope of the dependence of $1/T_1$ in the cells. An almost parallel increase was observed in $1/NT_1$ of the glycerol carbons with increased salinity (5). These results show that Na^+ ions and glycerol are codistributed within the different compartments of the *Dunaliella* cell.

CONCLUSIONS

The results presented indicate that the intracellular Na^+ concentration in *Dunaliella salina* is around 90 mM (upper limit) and does not change, within the experimental error, with changes in the extracellular salinity. It is much lower than that of the extracellular environment and thus seems not to contribute significantly to osmotic control in this alga. Moreover, it seems that the intracellular sodium content is strictly regulated. The T_1 measurements suggest that glycerol, the major osmoregulator of *Dunaliella*, and Na^+ are located within the same cellular compartments. This makes unlikely the possibility that *Dunaliella* consists of two compartments, one containing a low Na^+ concentration and osmoregulated by glycerol and the other permeable to sodium ions (13, 18).

Acknowledgments—We are grateful to Nir Katzenellenbogen for his invaluable help with the computer analysis of the spectra. We would also like to thank Michal Kam for performing some of the early ^{23}Na-NMR experiments.

LITERATURE CITED

1. AVRON M 1986 The osmotic components of halotolerant algae. Trends Biochem Sci 11: 5–6, 360–361
2. BALNOKIN YV, YY MAZEL 1985 Permeability of the plasma membrane to sodium ions in halophilic algae of the genus *Dunaliella*. Sov Plant Physiol 32: 23–30
3. BELTON PS, RG RATCLIFFE 1985 NMR and compartmentation in biological tissues. Prog NMR Spectrosc 17: 241–279
4. BEN AMOTZ A, I SUSSMAN, M AVRON 1982 Glycerol production by *Dunaliella*. Experientia 38: 49–52
5. BENTAL M, M OREN-SHAMIR, M AVRON, H DEGANI 1988 ^{31}P and ^{13}C-NMR studies of the phosphorus and carbon metabolites in the halotolerant alga *Dunaliella salina*. Plant Physiol 87: 320–324
6. BOULANGER Y, P VINAY, A TEJEDOR, J NOEL 1986 ^{23}Na NMR investigations of isolated kidney tubules. Book of Abstracts, Society of Magnetic Resonance in Medicine Fifth Annual Meeting, Vol 3. Society of Magnetic Resonance in Medicine, Inc, pp 645–646
7. BROWN AD, LJ BOROWITZKA 1979 Halotolerance of *Dunaliella*. *In* M Levandosky, SH Hunter, eds, Biochemistry and Physiology of Protozoa. Academic Press, New York, pp 139–190
8. CASTLE AM, RM MACNAB, RG SHULMAN 1986 Measurement of sodium concentration and sodium transport in *Escherichia coli* by ^{23}Na NMR. J Biol Chem 261: 3288–3294
9. CASTLE AM, RM MACNAB, RG SHULMAN 1986 Coupling between the sodium and proton gradients in respiring *Escherichia coli* cells measured by ^{23}Na and ^{31}P NMR. J Biol Chem 261: 7797–7806
10. CHU SC, MM PIKE, ET FOSSEL, TW SMITH, JA BALSCHI, CS SPRINGER 1984 Aqueous shift reagents for high-resolution cationic NMR. III $Dy(TTHA)^{3-}$, $Tm(TTHA)^{3-}$, and $Tm(PPP)_2^{7-}$. J Magn Reson 6: 33–47
11. CIVAN MM, H DEGANI, Y MARGALIT, M SHPORER 1983 Observation of ^{23}Na in frog skin by NMR. Am J Physiol 245 (Cell Physiol 14): c213–c219
12. CIVAN MM, M SHPORER 1978 NMR of ^{23}Na and ^{39}K in biological systems. *In* LJ Berliner, J Reuben, eds, Biological Magnetic Resonance, Vol 1. Plenum Press, New York, pp 1–30
13. EHRENFELD J, JL COUSIN 1982 Ionic regulation of the unicellular green alga *Dunaliella tertiolecta*. J Membr Biol 70: 45–57
14. FORSEN S, B LINDMAN 1981 Ion binding in biological systems as studied by NMR spectroscopy. *In* D Glick, ed, Methods of Biochemical Analysis, Vol 27, John Wiley & Sons, New York, pp 289–486
15. GERASIMOWICZ WV, SI TU, PE PFEFFER 1986 Energy facilitated Na^+ uptake in excised corn roots via ^{31}P and ^{23}Na NMR. Plant Physiol 81: 925–928
16. GIMMLER H, R KAADEN, U KIRCHNER, A WEYAND 1984 The chloride sensitivity of *Dunaliella parva* enzymes. Z. Pflanzenphysiol 144: 131–150
17. GIMMLER H, R SCHIRLING 1978 Cation permeability of the plasmalemma of

the halotolerant alga *Dunaliella parva*. Z Pflanzenphysiol Bd 87: Suppl, 435–444

18. GINZBURG M, BZ GINZBURG 1985 Ion and glycerol concentrations in twelve isolates of *Dunaliella*. J Exp Bot 36: 1064–1074
19. GULLANS SR, MJ AVISON, T OGINO, G GIEBISCH, RG SHULMAN 1985 NMR Measurements of intracellular sodium in the rabbit proximal tubules. Am J Physiol 249: F160–F168
20. GUPTA RK, P GUPTA 1982 Direct observation of resolved resonances from intra- and extracellular ^{23}Na ions in NMR studies of intact cells and tissues using dysprosium (III) tripolyphosphate as paramagnetic shift reagent. J Magn Reson 47: 344–350
21. HAJIBAGHERI MA, DJ GILMOUR, JC COLLINS, TJ FLOWERS 1986 X-ray microanalysis and ultrastructural studies of cell compartments of *Dunaliella parva*. J Exp Bot 37: 1725–1732
22. KATZ A, M AVRON 1985 Determination of intracellular osmotic volume and sodium concentration in *Dunaliella*. Plant Physiol 78: 817–820
23. OGINO T, JA DEN HOLLANDER, RG SHULMAN 1983 ^{39}K, ^{23}Na and ^{31}P NMR studies of ion transport in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 80: 5185–5190
24. PICK U, A BEN-AMOTZ, L KARNI, CJ SEEBERGTS, M AVRON 1986 Partial characterization of K^+ and Ca^{2+} uptake systems in the halotolerant alga *Dunaliella salina*. Plant Physiol 81: 875–881
25. PICK U, L KARNI, M AVRON 1986 Determination of ion content and ion fluxes in the halotolerant alga *Dunaliella salina*. Plant Physiol 81: 92–96
26. SAS INSTITUTE INC. 1985 SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc, Cary, NC
27. SHEFFER M, M AVRON 1982 An unusual sensitivity to salt of ferredoxin-dependent photoreactions in *Dunaliella*. Plant Sci Lett 25: 241–246
28. WEAST RC ed 1984 CRC Handbook of Chemistry and Physics, Ed 64. CRC Press, Inc, Boca Raton, FL, pp D235–D236
29. ZMIRI A, BZ GINZBURG 1983 Extracellular space and cellular sodium content in pellets of *Dunaliella parva*. Plant Sci Lett 30: 211–218