

How *Dunaliella* sp. can survive the high salt lake of Antarctica ?

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#### Summary

The genus *Dunaliella* do not have a rigid cell wall. The cell is enclosed by a thin elastic plasma membrane and therefore can respond rapidly to changes in osmotic stress by changing its cell volume. The response of the cell to changes in the extracellular osmotic stress occurs in two distinct steps. In the first step the cells rapidly shrink or swell under hypertonic or dilute stress. The second step of adaptation is slower than the first step and involves metabolic adjustment, by synthesis or elimination of glycerol in hypertonic or dilute stress. The Antarctic *Dunaliella* sp. had a large amount of glycerol content than *D. primolecta*. The alga is one of the ecological peculiarities of the Antarctic high salt lake. The *Dunaliella* sp. has many interesting characters in ecology and biochemistry as well because it was found to be halotolerant, cryotolerant and light-shield tolerant. We herein report the result obtained by the use of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR to characterize the intracellular glycerol metabolism in living *Dunaliella* sp. during adaptations to such stresses as salt, cryo and light-shielding.

In vivo  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR demonstrated that signal observed was assigned to be glycerol. The glycerol has been known as one of marker substance for osmoregulators in an adaptation experiment to salt stress.

In hypertonic salt stress shifting from 6% to 12% sodium chloride content, intracellular glycerol content increased, whereas in the hypotonic stress shifting from 3% to 1.5% sodium chloride intracellular glycerol decreased. In those stress experiments, in vivo  $^1\text{H}$ -NMR detected choline, alanine, lactate other than glycerol in *Dunaliella* sp..  $^{13}\text{C}$ -NMR demonstrated that glucose can be stored as glycogen. When *Dunaliella* sp. labeled with enriched  $^{13}\text{C}$ -labeled glucose was exposed to salt stress, changes such as degradation of glycogen were demonstrated. The results strongly suggest that biochemical change in cell membrane of *Dunaliella* sp. was occurred. Thus, in vivo NMR combined with stable isotope is extremely useful in a measurement for real time change in such metabolites as glycerol. And this approach is applicable not only to salt stress but also to cryostress or light-shielding stress.