Plant Physiol. (1980) 66, 1196–1197
0032-0889/80/66/1196/02/$00.50/0

Short Communication

Effect of Temperature on Glycerol Retention in the Halotolerant Algae *Dunaliella* and *Asteromonas*

Received for publication January 23, 1980 and in revised form July 8, 1980

KLAUS WEGMANN[1], AMI BEN-AMOTZ, AND MORDHAY AVRON
Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel

ABSTRACT

Algae of the genera *Dunaliella* and *Asteromonas* can maintain extremely high concentration gradients (>10^4) of glycerol between the intracellular space and the medium. This unique ability is highly temperature-dependent. Treating the algae for several minutes at temperatures exceeding 60 C causes complete release of all the internally held glycerol; 50% release occurs around 50 C, but essentially none is released below 40 C. A similar behavior was observed in several species of *Dunaliella*, and one of *Asteromonas* and is independent of the salt concentration of the medium. The underlying mechanism may involve a temperature-dependent conformational transition of a component of the cellular membrane which is essential for glycerol impermeability.

Cells of the wall-less halotolerant green algae *Dunaliella* and *Asteromonas* have been shown to accumulate glycerol as a major photosynthetic product (4, 6, 12). Glycerol was demonstrated to be the major osmoregulatory internal solute which enables these algae to grow in salt concentrations ranging from below 0.5 M to above 5 M (1, 4, 5, 7). The internal glycerol concentration has been shown to be proportional to the salt concentration in the growth medium and to reach values exceeding 4 M (1, 2, 4, 8). Nevertheless, *Dunaliella* can be grown in high salt containing media with little to no glycerol present in the growth medium (1). Thus, these algae possess a unique property, which enables them to maintain high concentration gradients (>10^4) of the rather membrane-permeable substance glycerol, between the intracellular fluid and the medium.

The study reported here illustrates that this property is highly temperature-dependent. All internal glycerol is released when the algae are exposed for a few minutes to temperatures which exceeds a critical value.

MATERIALS AND METHODS

Asteromonas gracilis (4), *Dunaliella bardawil* (3), *Dunaliella salina* (1), and *Dunaliella tertiolecta* (11) were grown as previously described. *A. gracilis*, *D. salina*, and *D. tertiolecta* were cultivated in a growth chamber under continuous illumination, and *D. bardawil* was cultivated in outdoor ponds with natural illumination.

Cell numbers were determined in a Thomas blood cell counting chamber or in a Coulter Counter. For temperature treatment, 2.5 ml culture medium containing 1 to 2 × 10^6 cells/ml were placed in a water bath maintained at the indicated temperature for the indicated time. The samples were centrifuged at room temperature and the glycerol content of the supernatant was determined as previously described (2).

RESULTS AND DISCUSSION

Figure 1 illustrates the glycerol release from cells of *D. bardawil* incubated for 10 min at the indicated temperatures. Less than 10% of the total glycerol was detected in the supernatant of cells treated at temperatures below 35 C. A dramatic increase in the leakage occurred between 45 to 55 C, and essentially all the glycerol leaked out from algae treated at temperatures above 60 C. The effect is similar in cells which were grown and heat-treated in salt concentrations ranging from 1 to 4 M NaCl. As previously noted (1, 2), the glycerol content of such cells and the amount released is a function of the salt concentration in the medium in which they were grown (Fig. 1, right).

The time course of glycerol release from *D. bardawil* at 60 C is illustrated in Figure 2. Clearly the release was rapidly effected. Between 3 to 6 min incubation, most of the glycerol had leaked out.

A similar release pattern has also been observed in *D. tertiolecta*, *D. salina*, and *A. gracilis* (Fig. 3), except for the percentage of glycerol present in the growth medium, which varied depending on the species and the salt concentration.

Viability tests conducted following 10 min incubation in various temperatures indicated that death of the cells paralleled the loss of glycerol as seen in Figures 1 to 3. Thus, essentially full viability was retained after treatment at 40 C but all cells were killed following treatment at 50 C.

Halotolerant algae of the genera *Dunaliella* and *Asteromonas* have the unique property of being able to maintain an extreme concentration gradient of glycerol across the cell, with little leak-

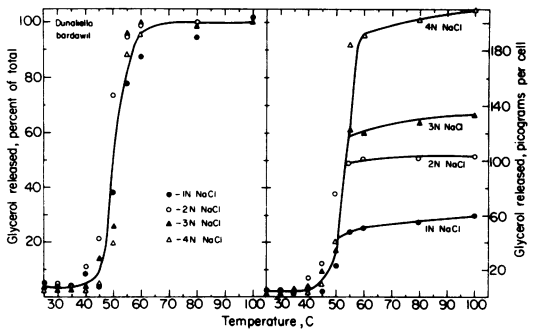

FIG. 1. Temperature dependence of glycerol release from *D. bardawil*. Incubation at the temperatures indicated was for 10 min.

[1] Present address: Institut fur Chemische Pflanzenphysiologie, Universitat Tubingen, D-7400 Tubingen, West Germany.

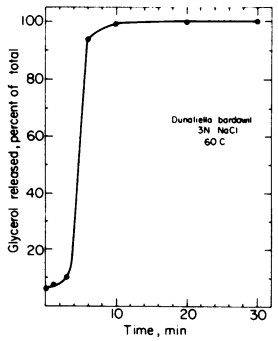

FIG. 2. Time course of glycerol release from *D. bardawil* incubated at 60 C. Algae were grown with 3 N salt and contained 130 pg glycerol/cell.

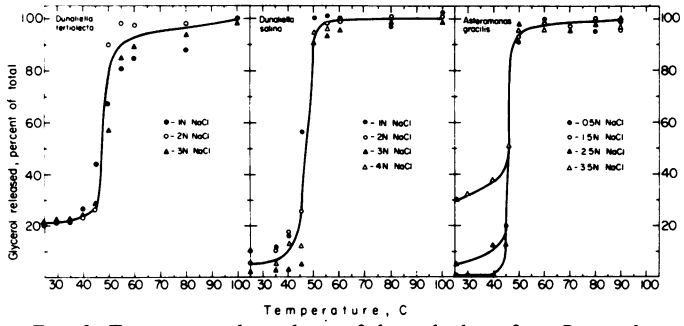

FIG. 3. Temperature dependence of glycerol release from *D. tertiolecta*, *D. salina*, and *A. gracilis*. Procedure was as described for Fig. 1. *D. tertiolecta* grown in 1, 2, and 3 N NaCl, contained 30, 70, and 150 pg glycerol/cell, respectively. *D. salina* grown in 1, 2, 3, and 4 N NaCl, contained 25, 63, 78, and 110 pg glycerol/cell, respectively. *A. gracilis* grown in 0.5, 1.5, 2.5, and 3.5 N NaCl contained 55, 111, 190, and 290 pg glycerol/cell, respectively.

age into the medium. The most likely barrier to such leakage is the cell membrane, which must therefore possess unusual characteristics to enable it to markedly reduce leakage of a small, usually highly permeable, substance like glycerol despite the large concentration gradient ($>10^4$) across it.

The communication here indicates that this property of the membrane is highly temperature-dependent. Essentially no release of glycerol occurs below 40 C; all the glycerol is released above 60 C with 50% release occurring around 50 C. This sharp temperature dependence is very similar for algae grown in a variety of salt concentrations, from 1 to 4 M, or among the several species of the genus *Dunaliella* tested and of *A. gracilis*.

The mechanism underlying this sharp temperature dependence of the ability to retain glycerol is not clear. It may relate to a change in membrane organization due to a melting transition of an unusual membrane lipid component, which is concerned with imparting glycerol impermeability to the membrane, or, more generally, to a conformational change of an essential membrane component with a sharp temperature dependence (10).

A previous study (9) of the temperature resistance of a variety of properties of *Dunaliella parva*, such as CO_2 fixation, photosynthetic activities of isolated thylakoids, and K^+ efflux from the cells, indicated that cells grown at high salt concentration were significantly more thermoresistant than those grown at lower concentration. Thus, the change in property which is responsible for the loss of the ability to retain glycerol may be more specific than that which leads to the inactivation of the other properties studied.

LITERATURE CITED

1. BEN-AMOTZ A, M AVRON 1973 The role of glycerol in the osmotic regulation of the halophilic alga *Dunaliella parva*. Plant Physiol 51: 875–878
2. BEN-AMOTZ A, M AVRON 1978 On the mechanism of osmoregulation in *Dunaliella*. *In* SR Caplan, M Ginzburg eds, Energetics and Structure of Halophilic Microorganisms. Elsevier, Amsterdam, pp 529–541
3. BEN-AMOTZ A, M AVRON 1980 Glycerol, β-carotene, and dry algal meal production by commercial cultivation of *Dunaliella*. *In* G Shelef, DG Soeder, eds, Algae Biomass Production and Use. Proceedings of the International Symposium on Production and Use of Micro-algae Biomass. Elsevier, Amsterdam, In press
4. BEN-AMOTZ A, M AVRON 1980 Osmoregulation in the halophilic algae *Dunaliella* and *Asteromonas*. *In* DW Rains, RC Valentine, A Hollaender, eds, Genetic Engineering of Osmoregulation: Impact on Plant Productivity for Food, Chemicals and Energy. Plenum Press, New York, pp 91–99
5. BOROWITZKA LJ, AD BROWN 1974 The salt relations of marine and halophilic species of the unicellular green alga *Dunaliella*. Arch Microbiol 96: 37–52
6. CRAIGIE J, J MACLACHLAN 1964 Glycerol as a photosynthetic product in *Dunaliella tertiolecta*. Can J Bot 42: 777–778
7. FRANK G, K WEGMANN 1974 Physiology and biochemistry of glycerol biosynthesis in *Dunaliella*. Biol Zentralb 93: 707–723
8. GIMMLER H, R SCHIRLING 1978 Cation permeability of the plasmalemma of the halotolerant alga *Dunaliella parva*. II. Cation content and glycerol concentration of the cells as dependent upon external NaCl concentration. Z Planzenphysiol 87: 435–444
9. GIMMLER H, EM KUHNL, G CARL 1978 Salinity dependent resistance of *Dunaliella parva* against extreme temperatures. I. Salinity and thermoresistance. Z Pflanzenphysiol 90: 133–153
10. MADDEN TD, PJ QUINN 1979 Arrhenius discontinuities of Ca^{2+}ATPase activity are unrelated to changes in membrane lipid fluidity of sarcoplasmic reticulum. FEBS Lett 107: 110–117
11. MULLER W, K WEGMANN 1978 Sucrose biosynthesis in *Dunaliella*. I. Thermic and osmotic regulation. Planta 141: 151–158
12. WEGMANN K 1971 Osmotic regulation of photosynthetic glycerol production in *Dunaliella*. Biochim Biophys Acta 234: 317–323