Influence of Temperature and Salinity on Carbon and Nitrogen Content in *Dunaliella viridis* Teodoresco under Nitrogen Sufficiency

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**Abstract**

Cell carbon and nitrogen in *D. viridis* are strongly dependent on the culturing conditions. Both elements increase with increasing salinity. At 31°C cell carbon is maximum and cell nitrogen minimum. This temperature was described previously (Jiménez, C., Niell, F. X. & Fernandez, J. A. (1990). *Hydrobiologia*, 197, 165-72) as the optimal one for achieving the maximum oxygen evolution. These results point out a possible competence for the reducing power during carbon and nitrogen assimilation processes, and under conditions of high photosynthesis (carbon assimilation) there is a partial inhibition of nitrate reduction, making C:N ratio maximum under conditions of maximum net photosynthesis.

The study of cell glycerol, nitrate, structural proteins and free amino acids indicates that all of these solutes accumulate in the cells as a result of the high salinity adaptation.

**Key words:** *Dunaliella viridis*, cell carbon and nitrogen, salinity, temperature, C:N evolution.

**INTRODUCTION**

Unicellular green flagellates of the genus *Dunaliella* are among the most important phytoplanktonic algae in hypersaline habitats. Their ability to accumulate large amounts of glycerol as compatible solute (Wegmann, 1971; Ben-Amotz & Avron, 1973, 1980a, b; Borowitzka & Brown, 1974; Ben-Amotz, 1975; Borowitzka et al., 1977) allows them to maintain their protoplasm iso-osmotic with the external solution, except at extremely low salinities (Ben-Amotz, 1975). In past years some authors have proposed that glycerol is not the only solute responsible for the iso-osmolarity of the cells and that carbohydrates, inorganic ions and free amino acids are also important in high salinity adaptation processes (Gimmler et al., 1981; Ahmad & Hellebust, 1986, 1988; Pick et al., 1986) but their role is still uncertain. This accumulation of solutes leads to a cell increase of some elements as a result of increasing salinity; among them carbon and nitrogen have great ecological implications because of their importance in the physiological state of the plants (Niell, 1976; Hanisak, 1979).

In the present work we have studied the influence of two variables of control, temperature and salinity, in the evolution of both elements, carbon and nitrogen, in *Dunaliella viridis* cells and their implication for C:N ratio variations.

**METHODS**

*D. viridis* cells used in the experiments were isolated from lake Fuente de Piedra (Málaga, Southern Spain; 37°06'N, 04°45'W) and grown in chemostats in a strictly inorganic medium (Johnson et al., 1968), under a constant light intensity of 150 μmol m⁻² s⁻¹ at a dilution rate of 0.25 day⁻¹; final NO₃ concentration was 10 mM.

A combination of three salinities (1 M, 2 M and 3 M NaCl) and five temperatures (15, 23, 31, 38 and 42°C) has been used. After each treatment total cell carbon and nitrogen, glycerol, internal nitrate and total free amino acids and proteins were determined.

In order to obtain the total cell carbon and nitrogen, a sample of 5 ml was filtered through a Whatman GF/C filter and dried at 60°C for 6 h. Carbon and nitrogen content was estimated by means of a C:N:H Perkin-Elmer 240C elemental
analyser. Another filter was dried and crushed so that we could estimate cell nitrate (Shinn, 1941) by means of a Technicon AII autoanalyser.

Glycerol was determined by periodate oxidation followed by treatment with acetylacetone (Ben-Amotz & Avron, 1978); total free amino acid concentration was estimated using the ninhydrine–ascorbate method (Pesez & Bartos, 1974) and structural proteins by means of Bradford (1976) methodology.

RESULTS

Temperature and salinity introduce significant changes in total cell carbon and nitrogen in D. viridis (Fig. 1). The general pattern for total carbon evolution shows an increase of this element with increasing salinity; the response against temperature shows a maximum at 31°C, which agrees with the temperature for maximum net assimilation rate for this species (Jiménez et al., 1990).

The same as in carbon, the total cell nitrogen also increases with salinity but, on the other hand there is a minimum of cell nitrogen at 31°C, where net assimilation rate is a maximum. These results induce us to think of a possible competition for the reducing power during carbon and nitrogen assimilation (Falkowski & Stone, 1975); CO₂ assimilation needs less reducing power than NO₃, and under conditions of high photosynthesis (carbon assimilation) there is a partial inhibition of nitrate reduction.

This evolution of total cell carbon and nitrogen as a response to changing temperature and salinity also makes the C:N ratio evolve. The highest C:N ratio has been found at a salinity of 2 M NaCl and a temperature of 31°C; combination of these treatments was described previously (Jiménez et al., 1990) as the optimal culturing conditions for the maximum net assimilation in D. viridis. Sub-optimal temperatures or salinities induce a general decrease in the C:N ratio.

It can be seen that the highest nitrogen and carbon concentrations are found at 3 M NaCl, but at 2 M NaCl there is more carbon related to cell nitrogen, as a result of very active photosynthesis. General increase in total cell carbon following an increase in the salinity of the medium is due to the accumulation of internal glycerol in order to compensate for increasing external osmolarity (Fig. 2(a)). Glycerol synthesis is the major way for carbon in Dunaliella cells growing at high salinities. A general increase in internal NO₃ (Fig. 2(b)), structural proteins (Fig. 2(c)) and total free amino acids (Fig. 2(d)) with increasing salinity has also been found which could explain the high increase in total cell nitrogen described in Fig. 1.

DISCUSSION

C:N ratio

It has been previously reported that C:N ratio is maximum in steady-state cultures or in the less active parts of the plants (Fogg, 1959; Niell, 1976; Hanisak, 1979). In this way cultures under very active photosynthesis and meristematic tissues had the highest nitrogen concentrations and so the C:N ratio was minimum; nitrogen concentrations seemed to control C:N ratio evolution (Hanisak, 1979). On the other hand it has been proposed

![Fig. 1. Variation of carbon and nitrogen in D. viridis cells as a function of temperature and salinity.](image)

![Fig. 2. Evolution of internal glycerol (a), nitrate (b), structural proteins (c) and total free amino acids (d) in D. viridis cells as function of the salinity, 15°C.](image)
Effect of temperature and salinity on *D. viridis* (Jackson, 1977) that total nitrogen remains almost constant in every tissue and that carbon is the only one responsible for C:N ratio variations. In a previous report (Niell & Mouriño, 1981) one of us postulated that the C:N ratio could evolve if total cell carbon and/or nitrogen varied. Our present results agree with that hypothesis; it appears that the C:N ratio evolution in *D. viridis* cells is due to carbon and nitrogen variations. It has been shown that *D. viridis* increases total cell carbon and nitrogen following an increase in the salinity of the medium, and that also temperature controls both total carbon and nitrogen evolution.

A very active photosynthesis in optimal culturing conditions can lead to a global carbon accumulation because CO$_2$ assimilation is quicker and less expensive than NO$_3$ assimilation (Losada *et al.*, 1981), thus protein synthesis can be lowered. So competition for the reducing power between CO$_2$ and NO$_3$ assimilation systems (Falkowski & Stone, 1975; Elrifi & Turpin, 1985) can result in a situation of maximum C:N ratio in plants under optimal culturing conditions.

It has been described that an increase of the photosynthetic rate can promote a net carbohydrate accumulation (Acock & Allen, 1985; Curtis *et al.*, 1989), and that an inhibition of nitrate uptake, in *Monochrysis lutheri*, could lead to an increase of the growing rate (Laws & Caperon, 1976); also a quicker assimilation of carbon than nitrogen into organic matter, under conditions of high photosynthesis, results in an increase of C:N ratio (Niell & Mouriño, 1981). On the other hand, most of the carbon photo-assimilated by the phytoplankton is incorporated into carbohydrates during the exponential growth phase and to amino acids at the stationary one (Morris *et al.*, 1974; Mukerji *et al.*, 1978; Fabregas *et al.*, 1986); this shift may be the result of a partial inhibition of nitrogen uptake during the active growth phase. As growth rate slows nitrogen uptake and protein synthesis increase. Our results confirm an interaction between carbon and nitrogen assimilation systems; a high carbon uptake leads to a partial inhibition of nitrate reduction and as it was proposed previously, most of the carbon can be incorporated into carbohydrates; a reduction in carbon incorporation allows the algae to use part of the reducing power for nitrogen uptake.

**Glycerol, proteins and amino acid accumulation**

It is well known that the species of the genus *Dunaliella* accumulates glycerol as ‘compatible solute’ (Brown & Simpson, 1972; Borowitzka & Brown, 1974) responsible for the maintenance of the iso-osmolarity between the cell and the medium when salinity increases (Wegmann, 1971; Ben-Amotz & Avron, 1973, 1980a,b; Ben-Amotz, 1975; Borowitzka *et al.*, 1977; Ginzburg, 1987); this causes the total cell carbon to increase (Ahmad & Hellebust, 1986). An increase in cell nitrogen after an increase of the salinity of the medium has also been described due to an accumulation of proteins and free amino acids in *Chlamydomonas pulsatilla* (Ahmad & Hellebust, 1988) or free amino acids in *Ulva lactuca* (Patil & Joshi, 1970). In the present report an almost linear increase of structural cell proteins and free amino acids in a salinity range of 1 M to 3 M NaCl has been detected. Total free amino acids and glycerol are similar in concentration at low salinity (~ 15 pg cell$^{-1}$ at 1 M NaCl) but amino acids are only 25% in weight of total glycerol at high salinity (3 M NaCl). As was proposed previously (Ahmad & Hellebust, 1986), glycerol plays a major osmoregulatory role at high salinities and other osmoregulatory solutes such as amino acids, inorganic ions and carbohydrates make a minor contribution to cell osmolarity; our results indicate that free amino acids can account for a big contribution to cell iso-osmolarity at low salinities. As they accumulate less than glycerol as salinity increases their importance in cell iso-osmolarity becomes small at high salinities.

In recent years, solutes other than glycerol (amino acids, carbohydrates, inorganic ions) are becoming very important in the description of osmoregulatory processes in halotolerant algae, but the extent to which these solutes contribute to the intracellular osmolarity is still uncertain (Ahmad & Hellebust, 1986).

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