Photorespiration in continuous of Dunaliella tertiolecta (Chlorophyta) : relationships between serine, glycine, and extracellular glycolate

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NOTE

PHOTORESPIRATION IN CONTINUOUS CULTURE OF *DUNALIELLA TERTIOLECTA* (CHLOROPHYTA): RELATIONSHIPS BETWEEN SERINE, GLYCINE, AND EXTRACELLULAR GLYCOLATE[1]

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ABSTRACT

The concentrations of extracellular glycolate and intracellular free pools of serine and glycine were monitored in nitrogen-limited continuous cultures of Dunaliella tertiolecta *(Butcher) UTEX LB999, grown at two different irradiances on a light:dark cycle. Under steady-state conditions, this microalga excreted into the medium a large amount of glycolate during the light phase, up to 100 nmol·(10^6 cells)$^{-1}$ for a cell concentration of around 1.5 10^8 cells·L^{-1}, but glycolate disappeared from the dissolved phase in the dark. Cells grown at 70 and those grown at 430 μmol photons·m^{-2}·s^{-1} differed in maximal glycolate concentration, intracellular serine and glycine concentrations, and serine:glycine ratio. Reversal of these photon flux densities to which the cultures were exposed caused rapid modification of the extracellular glycolate and intracellular serine and glycine pools. These results suggest that photorespiratory metabolism in* D. tertiolecta *could be approximately quantified by measuring the changes in dissolved glycolate and intracellular serine and glycine concentrations, extending previous results from cultured phytoplankton and suggesting methods for field studies.*

Key index words: Dunaliella tertiolecta*; glycine; glycolate; photorespiration; phytoplankton; serine*

Abbreviations: CCM, carbon concentrating mechanisms; CLMW, low molecular weight organic carbon; DFAA, intraparticular dissolved free amino; DGA, dissolved glycolate; DOC, dissolved organic carbon; exHL and exLL, continuous cultures HL and LL after light regime inversions; GLY, glycine; HL, continuous culture under high light conditions; HPLC, high performance liquid chromatography; LL, continuous culture under low light conditions; PCOC, photosynthetic carbon oxidation cycle; PCRC, photosynthetic carbon reduction cycle; RUBISCO, ribulose 1,5-bisphosphate carboxylase/oxygenase (E.C. 4.1.1.39); SER, serine

Glycolate, which is produced during photorespiration in photosynthetic metabolism, has often been shown to be one of the organic compounds released in the culture medium of actively photosynthesizing algae (e.g. Hellebust 1965). This pattern was observed even in species where PCOC, or alternative pathways (Winkler and Stabenau 1995), were able to reincorporate glycolate carbon into the PCRC, involving GLY and SER. We hypothesize that, even if CCM are able to limit phosphoglycolate synthesis or PCOC is able to divert phosphoglycolate, limiting further release of glycolate, there could exist a measurable DGA release in phytoplankton cultures, as demonstrated in the field (e.g. Al-Hasan and Fogg 1987, Leboulanger et al. 1997).

We measured the dissolved glycolate and the intracellular pools of free SER and GLY in two continuous cultures of *Dunaliella tertiolecta* (Butcher), together with a large suite of variables for several weeks during a multiparametric experiment described elsewhere (Sciandra et al. 1997). Growth of these cultures was limited by nitrogen availability, and biomasses were comparable from one to the

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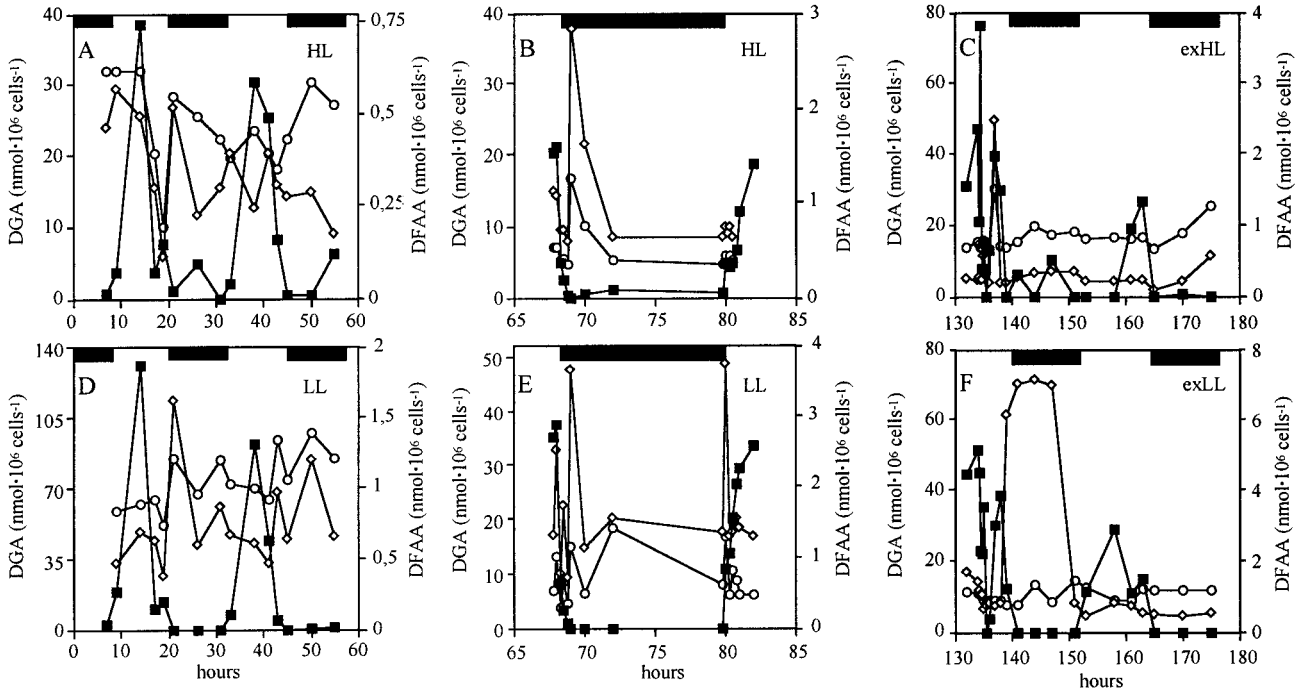

FIG. 1. Variations of dissolved glycolic acid (DGA, black squares, solid line) and intraparticular amino acids (SER, open diamonds; GLY, open circles) in the cultures of *Dunaliella tertiolecta* strain UTEX LB999. All data are expressed as nmol·(10^6 cells)$^{-1}$. Upper panel (A, B, C): High light initial conditions, cyclostat HL. Maximal errors (SE) for this set are ±4 nmol·(10^6 cells)$^{-1}$ for DGA (n = 6), ±0.14 nmol·(10^6 cells)$^{-1}$ for SER, and ±0.62 nmol·(10^6 cells)$^{-1}$ for GLY (replicates). Lower panel (D, E, F): Low light initial conditions, cyclostat LL. Maximal errors (SE) for this set are ±7.5 nmol·(10^6 cells)$^{-1}$ for DGA, ±0.03 nmol·(10^6 cells)$^{-1}$ for SER, and ±0.11 nmol·(10^6 cells)$^{-1}$ for GLY. Dark periods are represented by shaded areas. The two left figures (A, D) show the steady-state condition, and the two middle figures (B, E) show the rapid variations of the three parameters. The two right figures (C, F) show the evolution when the light was inverted between HL and LL, at t = 136 h, each cyclostat becoming exHL and exLL, respectively.

other, the only difference between them being light availability. The aim of this work was to provide evidence for field ecologists that healthy cells were able to release glycolate into their environment, depending, for example, on carbon, light, and nitrogen resources.

Cyclostats and cultures. A precise description of protocol and material was published in Malara and Sciandra (1991) and the main results of experiment for the present paper in Sciandra et al. (1997). *Dunaliella tertiolecta* (strain UTEX LB999) was cultivated on f/4 medium according to Sciandra et al. (1997). Fresh medium was supplied at 2.5 L·day^{-1}, giving a dilution rate of 0.5 day^{-1}. No combined nitrogen was detected in the effluent, and cultures were clearly limited by nitrogen. For the first 5 days, the cell density in the HL cyclostat remained stable, with a growth rate of 0.5 day^{-1}. In contrast, the cell density in LL decreased regularly, and light deficiency induced a supplementary growth limitation at 0.45 day^{-1}. Total dissolved inorganic carbon concentrations were similar in both cultures, with pCO_2 oscillating between 150 and 350 ppm as a function of diel light cycles, compared to surface water pCO_2 in the oceans of 355 ppm (Riebesell et al. 1993). In the middle of the light phase of day 5 after the beginning of the experiment, the irradiances of the cyclostats HL and LL were inverted. Cyclostats were then named exHL and exLL, respectively.

Intracellular amino acids determination. Amino acids determination was performed by HPLC according to the method of Lindroth and Mopper (1979) and Martin-Jézéquel et al. (1988). The absolute error on culture samples never exceeded 3.5% for SER and 12% for GLY determinations (based on replicate sampling and replicate measurements).

Dissolved glycolate analysis. Analysis was performed by HPLC according to Leboulanger et al. (1994), after standardization with 10–1000 μg·L^{-1} glycolate in 25 mL f/4 media. The precision of the analysis was always better than 20% absolute error (with n = 6–9), except for values below 100 μg·L^{-1}, where absolute error could equal the mean measurement.

Steady state. The steady-state condition was monitored for 2 days (from day 1, 7 h, to day 3, 8 h), and results are presented in Figure 1A, D (HL and LL). In each culture, a large amount of DGA appeared in the medium soon after transfer to light (time scale of less than 1 h for a detectable change in DGA), and greater maximum levels were found in LL (more than 100 nmol [106 cells]$^{-1}$) relative to HL (around 35 nmol·[10^6 cells]$^{-1}$). DGA concentration changes in each culture showed a trend related to the light cycle: When the light was switched on (8 h), the compound

TABLE 1. *Compiled data for glycolate release and physiological state of* D. tertiolecta *cells (data from Sciandra et al. 1997). Modeled values are calculated according to Yokota et al. (1987) for* Chlamydomonas reinhardtii. *Errors are given as ±SD (pseudoreplicates, n = 3).*

Culture	Glycolate release ($nmol \cdot h^{-1} \cdot 10^6\ cell^{-1}$)	Carbon content ($pmolC \cdot cell^{-1}$)	Growth rate (d^{-1})	Chl *a* per cell volume ($\mu g \cdot mm^{-3}$)	Cell volume ($mm^3 \cdot L^{-1}$)	Cell count ($cell \cdot L^{-1}$)	DIC ($nmol \cdot L^{-1}$)
HL	14 (±2)	3 (±0.3)	1.3	5 (±1.5)	14 (±3)	2×10^8	200–220
LL	30 (±4)	2.2 (±0.3)	1.65	15 (±3)	8 (±3)	1.6×10^8	200–220

	CO_2 fixation ($nmol \cdot h^{-1} \cdot 10^6\ cell^{-1}$)	Modeled CO_2 fixation ($nmol \cdot h^{-1} \cdot 10^6\ cell^{-1}$)	Modeled glycolate release ($nmol \cdot h^{-1} \cdot 10^6\ cell^{-1}$)
HL	75 (±9)	52	2
LL	117 (±11)	116	4

appeared rapidly, and it decreased as early as 17 h. During the dark phase the DGA concentration was mostly undetectable. Glycolate contributed from 4% to 7% of DOC in HL and from 4% to 12.5% in LL (B. Avril, pers. comm.).

Intracellular free SER and GLY mean concentrations were relatively higher in LL cultures (values mostly 0.4–1.4 $nmol \cdot [10^6\ cells]^{-1}$) than in cells from HL (values mostly 0.2–0.6 $nmol \cdot [10^6\ cells]^{-1}$). During steady-state condition, the mean ratio SER:GLY was around 0.6. The concentrations of both amino acids increased at the beginning of each dark phase in the two cultures.

During the day-to-night transition (day 3, 20 h), DGA disappeared completely within 1 h (day 3, 21 h) in both HL and LL, with a rate of decrease of about 400 $\mu g\ glycolate \cdot L^{-1} \cdot h^{-1}$, which was much greater than the rate of medium dilution of 0.5 d^{-1} (Fig. 1B, E). The decrease of DGA was not induced but only accelerated by darkness. During the night-to-day transition (day 4, 8 h), DGA appeared rapidly (after 15 min) and then increased at the same rate in HL and LL, at about 350 $\mu g\ glycolate \cdot L^{-1} \cdot h^{-1}$. We observed a higher cell-specific rate of DGA excretion in LL (i.e. 15 $nmol \cdot h^{-1} \cdot [10^6\ cells]^{-1}$) than in HL (8 $nmol \cdot h^{-1} \cdot [10^6\ cells]^{-1}$). In the dark, cell-specific disappearance of glycolate was also faster in LL than in HL (respectively, 33 and 26 $nmol \cdot h^{-1} \cdot [10^6\ cells]^{-1}$).

In both cultures, the decrease of DGA in the beginning of the dark phase coincided with a transient increase of SER and GLY. As in the steady state of the same experiment, SER in HL (0.6 ± 0.02 $nmol \cdot [10^6\ cells]^{-1}$) was lower than in LL (1.5 ± 0.18 $nmol \cdot [10^6\ cells]^{-1}$), although transitory peaks of SER were similar (2.3–2.9 $nmol \cdot [10^6\ cells]^{-1}$, produced in 15 min).

Effects of light regime inversions. Irradiance was changed at 14 h on day 6; a strong perturbation in DGA concentration occurred during the hours following the perturbation (Fig. 1C, F). Forty-five minutes after light inversion, DGA concentration fell to undetectable levels during the light phase (day 6, 14 h 45 min). Then a rapid rise occurred until day 6, 18 h, followed by a decrease to undetectable levels in the beginning of the dark phase, in the same way as during the steady state. During stabilization of the two cultures (day 6), the importance of DGA excretion seemed to be minimal relative to previous days, with midday peaks reaching only 38 ± 9 $nmol \cdot [10^6\ cells]^{-1}$ for exLL and 26 ± 8 $nmol \cdot [10^6\ cells]^{-1}$ for exHL. The difference in maximal concentrations between the two cultures was smaller than in the steady state. The SER concentration decreased in exLL, falling to the level of HL under high light (<1 $nmol \cdot [10^6\ cells]^{-1}$). In exHL, SER concentration did not rise after the perturbation but closely followed the change in DGA.

The maximal contribution of DGA to DOC in the culture supernatant never exceeded 12.5%, but this organic pool was very variable and was readily usable for heterotrophic growth in marine waters (Edenborn and Litchfield 1987, Gomes et al. 1991, Jones and Cannon 1986). We hypothesize that the disappearance of DGA in the culture media during the night is due mainly to reassimilation and metabolism by *D. tertiolecta,* as previously demonstrated in *Euglena* (Yokota and Kitaoka 1987, Yokota et al. 1990) and *Chlamydomonas reinhardtii* (Yokota et al. 1987). The observed release of DGA in this work may be compared to calculated predictions. By using the equations and estimates of Bjornsen (1988), with a permeability coefficient of $10^{-9}\ cm \cdot s^{-1}$ for glycolate (arbitrary value) and a surface:volume ratio around 0.7 μm^{-1} for this organism (Mullin et al. 1966), we obtain a theoretical mean daily release of 60% of the intracellular low molecular weight carbon. In our experiment, the total intracellular carbon stock varied between 2.3 and 4.41 pmol $C \cdot cells^{-1}$ (Sciandra et al. 1997), which was assumed to contain 10%–20% of C_{LMW} (Bjornsen 1988). We obtain a theoretical range for the daily total release of C_{LMW} from 140 to 500 nmol C_{LMW} $(10^6\ cells)^{-1} \cdot d^{-1}$. In results presented here, DGA amounts are up to 100 $nmol \cdot (10^6\ cells)^{-1} \cdot d^{-1}$, which equals 200 nmol $C_{LMW} \cdot (10^6\ cells)^{-1} \cdot d^{-1}$. In such a case, and considering the above hypothesis, glycolate could be considered the major C_{LMW} compound released by *D. tertiolecta.*

By using the model of Yokota et al. (1987) established for *Chlamydomonas reinhardtii* and the data set for our experiment (Table 1), we can compare the predicted and the observed glycolate release and inorganic carbon net fixation. The estimated DGA release for the *Chlamydomonas* model was, respectively, 2

and 4 nmol $C \cdot h^{-1} \cdot (10^6 \text{ cells})^{-1}$ in HL and LL chemostat. In our experiment, *D. tertiolecta* released 14 and 30 nmol $C \cdot h^{-1} \cdot (10^6 \text{ cells})^{-1}$ in HL and LL chemostat, values that correspond to about seven times the predictions in both cultures. Adding the C lost as glycolate to the C increase in particulate organic matter yields a loss of glycolate around 16 for HL to 20% for LL of the total C fixation rate by *D. tertiolecta*.

Part of this released DGA seems to be reassimilated, as previously demonstrated in other species in laboratory cultures (e.g. Yokota et al. 1990 for *Euglena gracilis*), and up to 16% of added dissolved glycolate has been found to be incorporated into stable products of *D. tertiolecta* cells in batch cultures (Leboulanger et al. 1995). Dissolved free amino acids—SER and GLY—are higher in cells from LL cyclostat than in cells from HL, possibly because of the relative nitrogen depletion in HL, where growth rate and average cell density are higher than in LL. Nitrate limitation in HL appears to enhance the carbon concentrating mechanism (Beardall et al. 1991), and the subsequent increase of the intracellular partial pressure of CO_2 represses the oxygenase activity of RUBISCO, so that glycolate is produced at a lower rate than under LL conditions.

Dunaliella tertiolecta cells allocate up to 20% of total reduced CO_2 into extracellular glycolate, even in active growth. This apparent release, a possible source of error in estimating primary production, should be considered the result of conditions in which cells have grown. Photorespiration has been considered as O_2 uptake and CO_2 and NH_3 evolution associated with the intracellular metabolism of phosphoglycolate, but this function needs to be explored in terms of energy and matter fluxes, for example, by CO_2 exchange and fluorescence measurements (review for higher plants in Leegood et al. 1995). As with phosphate depletion in cultured *Chlorella* (Kozlowska and Maleszewski 1994), inadequate nitrate and light supplies enhance the release of glycolate by *D. tertiolecta* in our experiment. Better estimates of photorespiratory losses must employ simultaneous DGA and intraparticular free SER and GLY measurements to allow a better estimation of C and N fluxes though the photorespiratory pathway in microalgae. In such a case, the use of tracers, especially ^{18}O, could achieve quantification of total carbon fluxes through photorespiration and glycolate relase (Raven 1990). A precise understanding of such fluxes and regulations is needed to ensure a better understanding of the results obtained on natural phytoplankton communities.

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