Kinetics of photoacclimation in cultures of *Chromatium vinosum* DSM 185 during shifts in light irradiance

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Continuous cultures of *Chromatium vinosum* DSM 185 were shifted from a high to a low irradiance (67 to 4 $\mu\text{E m}^{-2} \text{s}^{-1}$) and vice versa (4 to 67 $\mu\text{E m}^{-2} \text{s}^{-1}$). The kinetics of photoacclimation of the cultures were analysed during these transitions until steady state was reached. When irradiance was shifted from 4 to 67 $\mu\text{E m}^{-2} \text{s}^{-1}$, bacteriochlorophyll synthesis halted for 4 h. During this period, pigments were progressively diluted in the newly formed biomass, resulting in a lower specific pigment content. The specific growth rate of the organisms did not change immediately after the shift, but rather underwent a gradual increase during the following 10 h. This transition was accompanied by a transient increase in the levels of glycogen, indicating that CO_2 fixation rates increased immediately after the shift, and that unused photosynthate was stored as glycogen. The shift from a high to a low irradiance was characterized by an immediate drop in the specific growth rate to virtually zero, and by comparatively sharp decreases in the specific rates of sulfur and sulfide oxidation and in the specific rate of glycogen accumulation. The specific content of bacteriochlorophyll *a* increased during the first 10 h. During the same period the specific content of glycogen decreased.

Keywords: purple sulfur bacteria, photoacclimation, transition

INTRODUCTION

Phototrophic sulfur bacteria develop in illuminated planktonic and benthic environments wherever reduced sulfur compounds are available. These ecosystems are subject to dynamic processes such as vertical mixing or diurnal fluctuations in which phototrophic organisms are exposed to changes in the light climate over a wide range of time scales.

While it has been well described how phototrophic micro-organisms are able to adapt to low-light conditions, little is known about their adaptation to fluctuating irradiances. Changes in the photosynthetic response, specific growth rate, cell volume and concentration of nucleic acids have been observed in light-limited cultures of phototrophic organisms such as the green alga *Scenedesmus protuberans* (Gons & Mur, 1979), the cyanobacterium *Microcystis aeruginosa* (Zevenboom & Mur, 1984) and the purple nonsulfur bacterium *Rhodobacter capsulatus* (Aiking & Sojka,

1979). Exposure of the purple sulfur bacterium *Chromatium vinosum* to growth-limiting irradiances has been shown to induce similar changes in the physiological state of this organism (Sánchez *et al.*, 1998a, b). The changes observed include variations in pigment content, absorption coefficient of cell suspensions, specific content of storage compounds (glycogen and sulfur), cell volume, specific growth rate, and metabolic rates of sulfide and sulfur oxidation and glycogen accumulation.

These studies, however, fail to answer a number of questions related to whether adaptation actually occurs in natural environments where constant conditions are an exception and fluctuations prevail. In order to predict how an organism will respond to a fluctuating light environment, we need to know the time required to adapt to the new conditions. Also, because adaptation includes changes in different cell parameters, it would be interesting to know whether all of these parameters change at the same rate. Analysis of the sequence of changes in the cell variables may help us to understand the relevance of these physiological events for cell adaptation.

Abbreviation: BChla, bacteriochlorophyll *a*.

In the work reported here we studied the variations in cellular composition and metabolic activity which occur in *C. vinosum* during the transition from high to low irradiance and vice versa. We attempted to elucidate the time scale of photoacclimation, as well as the sequence of events and the mechanisms involved in the photo-adaptive changes. The results obtained will help to predict the minimum time required for photoacclimation in natural environments subject to fluctuations in light irradiance.

METHODS

Organism and growth conditions. *Chromatium vinosum* DSM 185 was grown at 25 °C in a redox-controlled sulfidostat, which allowed the dilution rate to be adjusted automatically to the growth rate of the organism after changes in irradiance (Sánchez *et al.*, 1996). This culture set-up was designed to maintain a constant concentration of hydrogen sulfide inside the culture vessel despite variations in the light supply. The medium contained carbonate as the only carbon source and hydrogen sulfide as electron donor (Mas & Van Gemerden, 1987). The concentration of sulfide in the reservoir of the continuous culture ($S_{R-sulfide}$) was set to 2 mM. Continuous illumination was provided by incandescent light bulbs placed at one side of the culture vessel. Incident irradiances of 67 and 4 $\mu E m^{-2} s^{-1}$ were obtained by varying the power and the distance of the vessel from the light source. In steady state, these irradiances resulted in specific rates of light uptake (q_p) of 32.2 and 4.3 $\mu E (mg \text{ protein})^{-1} h^{-1}$ respectively. The specific rate of light uptake was calculated as the ratio between the total amount of light absorbed by the culture vessel per time unit and the biomass of the culture (Sánchez & Mas, 1996).

Sampling and analyses. After a change in irradiance, samples were collected at 30 or 60 min intervals from the culture overflow, and maintained in the dark on ice. The samples were then centrifuged; after discarding the supernatants, the pellets were frozen and kept at -20 °C until the analyses were performed.

The concentration of residual hydrogen sulfide was measured in 2 ml samples taken directly from the culture vessel with a syringe and fixed immediately with 20 ml zinc acetate (2%, w/v). Sulfide was then assayed according to Pachmayr (1960), as described by Trüper & Schlegel (1964).

Bacteriochlorophyll *a* and sulfur were measured spectrophotometrically at 770 nm and 265 nm respectively, in methanol extracts. The actual concentrations of both substances were calculated using the absorption coefficients provided by Stal *et al.* (1984). Proteins were determined by the Lowry method after extraction of the pellet with methanol to remove sulfur, and subsequent solubilization of the samples in 1 M sodium hydroxide for 10 min at 100 °C (Herbert *et al.*, 1971). Total sugars were assayed with the anthrone reagent (Herbert *et al.*, 1971) in samples in which sulfur had been previously extracted with methanol. The values obtained were used to estimate the concentration of glycogen after subtracting the amount of structural sugars (0.02 mg structural sugars per mg protein, measured in glycogen-depleted cultures of the same organism).

Calculation of metabolic rates. The specific growth rate (μ), the specific rates of sulfide oxidation (q_1) and sulfur oxidation (q_2), the metabolic rates of sulfur and glycogen accumulation (q_{s^0} and q_G) and the specific rate of pigment synthesis (q_{BChla}) were calculated from the dilution rate D , and the concentrations of sulfide (S^{2-}), sulfur (S^0), glycogen (G), bacterio-

chlorophyll *a* (BChla) and protein (X) using the following equations:

$$\mu = \frac{dX}{X \cdot dt} + D$$

$$q_1 = \frac{D \cdot (S_r - S^{2-}) - \frac{dS^{2-}}{dt}}{X}$$

$$q_2 = \frac{D \cdot S^0 + \frac{dS^0}{dt}}{X} - q_1$$

$$q_{s^0} = \frac{\frac{dS^0}{dt} + D \cdot S^0}{X}$$

$$q_G = \frac{\frac{dG}{dt} + D \cdot G}{X}$$

$$q_{BChla} = \frac{\frac{dBChla}{dt} + D \cdot BChla}{X}$$

The rates were calculated for 0.5 h time intervals throughout the duration of the transient states. In order to avoid the errors introduced by the variability of the data points, a smoothed curve was first fitted to the primary data (concentrations of protein, BChla, glycogen and sulfur). The curves were later used to calculate the specific rates of change of the variables mentioned above over each of the 0.5 h time intervals.

RESULTS

In the experiments described below, steady-state continuous cultures of *C. vinosum* were subjected to shifts in light irradiance. During the resulting transient states, several variables were monitored, related both to the culture (dilution rate, and concentration of sulfide, elemental sulfur, BChla, protein and glycogen), and to the physiological state of the organism (specific content of BChla, sulfur and glycogen). The data obtained allowed the calculation of the metabolic rates of sulfide and sulfur oxidation, sulfur and glycogen accumulation, BChla synthesis, and growth.

Irradiance shift-up

When the transition was carried out from a low to a high irradiance (4 to 67 $\mu E m^{-2} s^{-1}$) the response of the culture was virtually instantaneous. First, the dilution rate of the system (Fig. 1) increased from 0.02 to 0.09 h^{-1} in somewhat less than 3 h. After this initial increase the dilution rate decreased to 0.07 h^{-1} and from then on, it started a slow increase which brought it up to 0.13 h^{-1} . The whole transition lasted approximately 50 h. This observed transient decrease in the dilution rate coincided with a sharp reduction in the rates of sulfide and sulfur oxidation (Fig. 2c). Despite the changes in the dilution rate of the culture, the specific growth rate of the organisms displayed a remarkably steady increase,

Fig. 1. Dilution rate (D) and specific growth rate (μ) as a function of time in cultures of *C. vinosum* after a shift-up in irradiance from 4 to $67 \mu\text{E m}^{-2} \text{s}^{-1}$.

Fig. 2. (a) Concentrations of protein and glycogen, (b) concentration of BChla and washout kinetics of the system (dotted line), and (c) concentrations of sulfide and sulfide, and specific rates of sulfide (q_1) and sulfur (q_2) oxidation in cultures of *C. vinosum* after a shift-up in irradiance from 4 to $67 \mu\text{E m}^{-2} \text{s}^{-1}$.

Fig. 3. Specific contents of BChla (a), sulfur (b) and glycogen (c), and specific rates of BChla synthesis (q_{BChla}) and sulfur and glycogen accumulation (q_s^0 and q_g) in cultures of *C. vinosum* after a shift-up in irradiance from 4 to $67 \mu\text{E m}^{-2} \text{s}^{-1}$.

which stabilized after only 8 h. This means that the organisms achieved their maximum possible specific growth rate long before the culture was in steady state. The biomass of the culture, represented by the concentration of protein in Fig. 2(a), remained constant during the first 3 h, for as long as D and μ increased in parallel and growth and washout compensated. After this apparent lag and coinciding with the drop in dilution rate mentioned above, the biomass of the culture started to increase, reaching stable values after 50 h. The concentration of glycogen increased fourfold, showing a transient maximum 7 h after the onset of the transition (Fig. 2a).

The concentration of BChla was also determined (Fig. 2b). During the first 8 h the concentration of BChla decreased, following kinetics closely matching the washout kinetics of the system, an observation that suggests the absence of active pigment synthesis or degradation. After this initial decrease, BChla started to accumulate, reaching stable levels between 40 and 50 h after the change of irradiance.

The time course of the concentrations of elemental

Fig. 4. Dilution rate (D) and specific growth rate (μ) as a function of time in cultures of *C. vinosum* after a shift-down in irradiance from 67 to 4 $\mu\text{E m}^{-2} \text{s}^{-1}$.

sulfur and hydrogen sulfide is shown in Fig. 2(c). Sulfur showed a slight increase during the first 10 h. Hydrogen sulfide, owing to the characteristics of the culture setup (see Methods) was kept constant at 30 μM throughout the transition. The specific rates of sulfide and sulfur oxidation (q_1 and q_2), also shown in Fig. 2(c), showed a sharp increase during the first 3 h with a transient overshoot before stabilizing at the steady-state values in less than 10 h.

Using these data, we calculated the specific contents of BChla, glycogen and sulfur, which provide a good image of the physiological state of the cell at a given time. The specific rates of glycogen and sulfur storage, and of BChla synthesis, were also calculated. The results are shown in Fig. 3 as a function of time.

During the first 8 h of the transition, the specific content of BChla (Fig. 3a) decreased from 85 to 46 $\mu\text{g BChla (mg protein)}^{-1}$. The specific rate of BChla synthesis dropped from 1.5 $\mu\text{g BChla (mg protein)}^{-1} \text{h}^{-1}$ to zero immediately after increasing the irradiance, and remained at zero for the next 4 h. From then on it started to increase again until stabilizing at 6.5 $\mu\text{g BChla (mg protein)}^{-1} \text{h}^{-1}$. These results indicate quite clearly that *C. vinosum* does not require pigment degradation in order to lower the pigment content, but merely stops pigment synthesis while continuing growth at an even higher rate (Fig. 1). In this way, the pigments already present are diluted in the newly formed cell material and the content decreases.

The specific contents of storage compounds (intracellular sulfur and glycogen) also showed marked changes during the transition to a higher irradiance. Sulfur (Fig. 3b), which was present at 50 $\mu\text{mol (mg protein)}^{-1}$, remained at that level during the 4 h following the change in illumination. After that it slowly decreased to 20 $\mu\text{mol (mg protein)}^{-1}$. Glycogen (Fig. 3c),

Fig. 5. (a) Concentrations of protein and glycogen, (b) concentration of BChla, and (c) concentrations of sulfur and sulfide, and specific rates of sulfide (q_1) and sulfur (q_2) oxidation in cultures of *C. vinosum* after a shift-down in irradiance from 67 to 4 $\mu\text{E m}^{-2} \text{s}^{-1}$.

in contrast, increased twofold, showing a transient accumulation which reached a maximum of 580 $\mu\text{g (mg protein)}^{-1}$ 7 h after the shift. The specific rates of both sulfur and glycogen accumulation had very high values during the first hours of the transition, only to fall back later to values somewhat higher than the original.

Irradiance shift-down

The shift-down in irradiance was characterized by an immediate drop in the dilution rate (Fig. 4), which from then on started a slow recovery up to a stable value (0.02 h^{-1}). The specific growth rate, which went down to zero at the moment of the shift-down, showed a similar behaviour, stabilizing in approximately 40 h.

The decrease in irradiance resulted in a steady reduction in the biomass of the culture, as can be seen in Fig. 5(a), where protein concentration is plotted as a function of time. The concentration of glycogen, also shown in Fig. 5(a), decreased during the first 5 h, parallel to the increase in the concentration of BChla (Fig. 5b) during the same period. This suggests a significant role of

Fig. 6. Specific contents of BChla (a), sulfur (b) and glycogen (c), and specific rates of pigment synthesis (q_{BChla}) and sulfur and glycogen accumulation (q_{S^0} and q_{G}) in cultures of *C. vinosum* after a shift-down in irradiance from 67 to $4 \mu\text{E m}^{-2} \text{s}^{-1}$.

glycogen as an energy source during the process of photoacclimation to low irradiances. The concentration of sulfide, represented in Fig. 5(c), remained constant at $30 \mu\text{M}$ for the duration of the experiment, as in the previous transient state. The concentration of sulfur increased gradually, reaching stable levels in 80 h. The specific rates of sulfide and sulfur oxidation (Fig. 5c) reached very low values immediately after the transition, but recovered quite quickly (approx. 10 h), probably owing to the enhanced light-harvesting capacity derived from the increase in the specific pigment content.

The specific content of BChla (Fig. 6a) changed in two phases, with a sharp increase during the first 10 h and a slower continuous rise during the following 80 h. On the other hand, the sulfur content (Fig. 6b) increased continuously during this period, reaching a value of $49 \mu\text{mol S}^0 (\text{mg protein})^{-1}$. The specific content of glycogen decreased markedly, reaching its minimum level after 5 h (Fig. 6c). However, it then showed a constant increase to the steady-state level [$67 \mu\text{g} (\text{mg protein})^{-1}$], which took about 80 h to complete.

All the metabolic rates suffered a sharp drop after the

shift-down in irradiance (Fig. 6), and in most cases they showed a progressive recovery, reaching steady-state values lower than those found under high-light conditions. It is worth mentioning that the specific rate of glycogen accumulation becomes negative during the first 7 h, indicating that during this period glycogen was actively degraded.

DISCUSSION

The results of this study show that *C. vinosum* adapts to changes in light irradiance by altering the cellular composition as well as the rates of several metabolic processes. The transition from a low to a high irradiance was characterized by a swift increase in glycogen content. This increase occurred during the period of growth acceleration, when CO_2 fixation was already proceeding at a very high rate. The carbon fixation products of photosynthesis could not yet be invested in balanced cell growth and thus were accumulated as glycogen. Such a lag between nutrient uptake, or in this case CO_2 fixation, and growth is not uncommon in bacteria, being a consequence of the time required to assemble the protein-synthesis machinery required to grow at a high rate (Tempest & Neijssel, 1978). Three hours after the shift in irradiance, approximately 32% of the reducing power used by the cells was being stored as glycogen. Twelve hours later, once the culture was growing at a stable μ , only 7% of the reducing power used by the cell was stored as glycogen. This indicates that glycogen accumulation constitutes a buffering mechanism for the excess energy absorbed by the cell during high illumination transients. The maximum rate of glycogen accumulation [$126.5 \mu\text{g glycogen} (\text{mg protein})^{-1} \text{h}^{-1}$] is virtually identical to the maximum potential rates of glycogen synthesis previously reported in cultures of the same organism in which growth was inhibited by the addition of chloramphenicol (Beefink & Van Gernerden, 1979).

During the transition from a high to a low irradiance the specific content of BChla increased. This increase occurred at a high rate during the first 10 h, concomitant with a decrease in the glycogen content. Once glycogen was degraded, the pigment content continued to rise during the following 80 h, but at a much slower rate. This observation suggests that glycogen degradation could play an important role in this process, supplying metabolic precursors for the biosynthesis of the photosynthetic apparatus and allowing a faster rate of adaptation. Similar experiments which studied the time course of photoacclimation to changes of growth irradiance in the marine diatom *Thalassiosira weissflogii* (Post *et al.*, 1985) showed that after a shift-down in irradiance the carbohydrate content also decreased.

Our results demonstrate that the carbohydrate pool is highly dynamic and responds quickly to shifts in the light climate, constituting an adaptive advantage for organisms living in a fluctuating environment.

The change in specific pigment content was dependent on the situation. During the shift from a low to a high

irradiance, pigment synthesis stopped and the pigments already present were progressively diluted in the newly formed biomass until stable levels were reached. This resulted in a decrease in pigment concentration which followed the washout curve imposed by the dilution rate of the system (Fig. 2b). In contrast, when the transition was from a high to a low irradiance, the organism was able to increase the pigment content in about 8–10 h, roughly the equivalent of the generation time of the organism under optimum conditions. The time scale involved precludes significant acclimation when the organism faces fluctuations with a shorter period.

When *C. vinosum* was transferred from a high to a low irradiance, the sulfur content increased during a transient period which lasted 90 h. Under light limitation, the amount of energy available for CO₂ fixation is severely reduced, and thus the reducing power which can be obtained from sulfide oxidation cannot be used. In natural environments, sulfur accumulation could be contemplated as a strategy to make a surplus of reducing power unavailable to other organisms. During shift-up transition to a lower sulfur content took about 30 h to complete.

From the data shown above, it is clear that the different variables require different time intervals to adapt to the new situation. In order to compare the time scales we defined a new variable (t_{50}) as the time required to reach 50% of the maximum variation observed. The values of t_{50} differed slightly depending on whether the organisms were subjected to an increase or to a decrease in light irradiance. Thus, the t_{50} values for sulfur and BChl_a during shift-up were approximately 30 h, whereas t_{50} for glycogen was less than 1 h. During shift-down, t_{50} for sulfur was 11 h, for BChl_a 4 h, and for glycogen less than 1 h. These data clearly indicate that *C. vinosum* has mechanisms for adjusting to light changes which involve a rapid mobilization of the carbohydrate pool, while other variables that were measured here change at much lower rates.

If properly calibrated, these variables can be used as physiological markers of the light history of the organism, and thus can provide information about the magnitude and importance of processes such as turbulent motion through the water column or temporal variations in the patterns of light availability.

In planktonic environments, microbial motility is often overcome by turbulent motion. Aquatic systems subject to turbulent mixing, such as lakes, streams or the upper layers of the ocean, constitute an environment in which the light climate of the organisms is periodically altered as a result of vertical transport between high-light and low-light layers. Under these conditions, adaptation to different irradiances will only occur if the time scale of vertical mixing allows enough time at high or at low irradiances for the organism to develop the changes described in the present paper. If the time scale of photoacclimation is shorter than the time scale of the physical processes taking place, we will find a vertical gradient in the physiological state of the population

resulting from acclimation to different irradiances. On the other hand, if the time scale of the mixing process is shorter than the time the cell takes to adapt to light variations, the vertical distribution of the adaptive variable through the water column will be uniform. Several authors have used this type of approach to estimate the rates of vertical mixing in natural populations of phytoplankton (Steemann Nielsen & Hansen, 1961; Falkowski, 1983; Cullen & Lewis, 1988).

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