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Growth rate and control of development of the photosynthetic apparatus in *Chloroflexus aurantiacus*

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Abstract. *Chloroflexus aurantiacus* was grown photoheterotrophically in a chemostat in order to study the influence of growth rate on the formation of bacteriochlorophyll a (Bchl a) which represents the membrane-bound photosynthetic pigment complexes, and of Bchl c which represents the light harvesting pigment-proteins of the chlorosome. Steady state cell protein levels as well as specific Bchl a contents increased linearly and specific Bchl c contents exponentially when the dilution rate, representing growth rate, was decreased. In spite of differences in the light intensities, continuous cultures growing at comparable growth rates and densities exhibited comparable specific contents of both Bchls and largely identical molar ratios of Bchl c/Bchl a. The growth rate of constantly illuminated batch cultures was varied by changing the concentration of growth-limiting nutrients. Cultures growing at higher growth rates showed higher cell densities but lower specific Bchl levels as well as lower molar ratios of Bchl c/Bchl a than cultures growing at low growth rate. Determination of the light energy flux required for half-maximal saturation of photosynthetic activity (light dependent proton extrusion) by chemostat cultures showed a dependency of that activity by the content of cellular Bchl c. In summary, the results suggest that, growth rate or a factor regulating growth rate, rather than light affected specific Bchl levels and because of the increasing molar ratio of Bchl c to Bchl a, the light harvesting capacity and photosynthetic efficiency of the photosynthetic apparatus.

Key words: *Chloroflexus aurantiacus* – Chemostat – Bacteriochlorophylls a and c – Light harvesting capacity – Control of growth

The thermophilic phototrophic bacterium *Chloroflexus aurantiacus* is able to synthesize simultaneously two different bacteriochlorophyll derivatives, Bchl a and Bchl c (Pierson and Castenholz 1974). Both pigments are chromophores of structurally and functionally defined holochromes of the photosynthetic apparatus (Fuller and Redlinger 1985). Bchl c-protein complexes, fulfilling light-harvesting functions, are exclusively located in the chlorosome, which are cytoplasmic entities attached to the cell membrane. Bchl a-protein complexes, on the other hand, constitute a unit composed at a constant proportion of light-harvesting as well as photochemical reaction centers as an integral part of the cell membrane (Feick et al. 1982).

Since the first investigation on the development of the photosynthetic apparatus in *C. aurantiacus* it has been known that light and oxygen are involved in the control of development by influencing the molar ratio of the two Bchls and, consequently, the ratio of chlorosomes per membrane-bound photosynthetic units (Pierson and Castenholz 1974; Schmidt et al. 1980; Fuller and Redlinger 1985; Foster et al. 1986). Apart from other external factors, Pierson and Castenholz (1974) mentioned the possibility that growth rate may affect the composition of the photosynthetic apparatus. This latter observation, however, has not been considered in subsequent investigations, probably because growth rate is a complex physiological parameter, which may depend on many factors, including light. However, growth rate can be strictly controlled if the organisms are grown in continuous culture in a chemostat rather than in batch culture. In the course of the present investigation *C. aurantiacus* was grown in chemostat, in order to examine the participation of growth rate in the development of the photosynthetic apparatus. It will be shown that under these conditions there was no effect of light on development. Instead, previously observed effects of light can be explained at least in part on the basis of the growth rate of the organism.

Materials and methods

Organism and growth conditions. Chloroflexus aurantiacus strain J-10-fl isolated by Pierson and Castenholz (1974) was grown in medium D (Castenholz 1969) supplemented with (w/v) 0.1% yeast extract, 0.25% casamino acids and 0.08% glycylglycine at pH 8.3. Cultures for the inoculation of continuous chemostat cultures were grown anaerobically in the light in screw-capped bottles at 55° C. For continuous cultivation jacketed culture vessels (Wheaton-Celstir), equipped with a stirring bar and two lateral inlets, were connected to a thermostat. Each of the lateral ports was equipped with a pipette, one of which was connected with the reservoir in order to add fresh medium and the other was used to withdraw culture suspension. Both the inlet as well as the outlet were operated via a dual-channel peristaltic pump, so that the culture volume was kept constant at one liter. In addition to the medium supply the inlet was connected to tank nitrogen, in order to keep the culture anaerobic by a constant slight stream of nitrogen. The culture vessel gassed with nitrogen was used, as well, for batch culture experiments.

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Unless stated otherwise, the culture were stirred at 100 rpm and illuminated with 240 Wm^{-2} of incandescent light measured at the surface of the culture vessel. Changes in growth rate were always done by increasing the rate of medium supply, i. e., by increasing the dilution rate (D). At higher D-values the bacteria began to aggregate and became attached to the wall of the culture vessel. Therefore, and because of the fact that the most significant changes in Bchl levels of cells were observed at lower growth rates, chemostats were run at growth rates below about 0.12 h^{-1}. All of the measurements were done with steady-state cultures.

Chemical analyses. Bchl a and Bchl c contents of cell suspensions were determined after extraction into acetone: methanol (7:2 v/v) at room temperature in the dark for 20 min. After centrifugation absorption spectra of extracts were recorded from 800 to 600 nm. Bchls were estimated by using the molar absorption coefficients ε_{767} = 76 mM^{-1} cm^{-1} for Bchl a (Clayton 1966) and ε_{666} = 74 mM^{-1} cm^{-1} for Bchl c Feick et al. (1982). Protein of cells was quantified according to Lowry et al. (1951).

Light-induced proton extrusion. Light saturation of the rate of light-induced proton extrusion by whole cells was determined at different energy fluxes of incandescent light as described before (Oelze and Fuller 1983). From double reciprocal plots the light energy fluxes required for half-maximal saturation of the reaction were extrapolated.

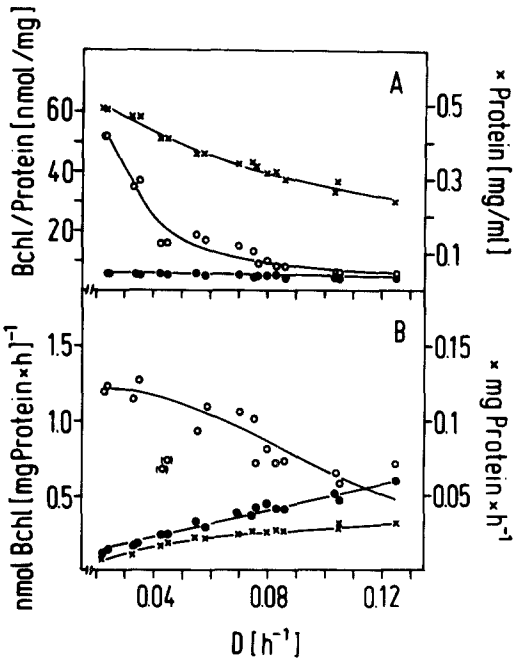

Fig. 1. **A** Steady state protein concentrations (×) as well as specific contents of bacteriochlorophyll (Bchl) a (●) and Bchl c (○) in continuous chemostat culture of *Chloroflexus aurantiacus* growing phototrophically at different dilution rates (D) i.e. growth rates (μ). The temperature was 60°C and the incident light intensity was 240 Wm^{-2}. **B** Rate of protein synthesis (×) as well as specific rates of Bchl a (●) and Bchl c (○) synthesis of cultures described above (**A**)

Results

The operation of chemostat cultures is based on the limitation of growth by a nutritional factor (Herbert et al. 1956). Growth limitation includes both the amount of biomass produced as well as the rate at which biomass is produced, i. e. the growth rate. While biomass production depends on the concentration of a limiting factor added to the culture, growth rate depends on the rate at which the limiting factor is added. In order to quantify growth parameters on the basis of the utilization of the limiting factor by the culture it is necessary to define this factor. In the case of *Chloroflexus aurantiacus* this raises some problems because this organism grows best on a complex nutritional medium and it is very sensitive towards slight changes in growth conditions (Pierson and Castenholz 1974; Schmidt 1980). Although Madigan et al. (1974) described the growth of *C. aurantiacus* on defined synthetic media, continuous cultivation of the organisms under these conditions has not been possible. The complex medium containing yeast extract as well as casamino acids (Pierson and Castenholz 1974) was required in order to obtain sufficiently high growth rates to adequately maintain chemostat cultures. Moreover, no single amino acid has been identified which stimulated Bchl c formation comparably to casamino acids. But, since we wanted to know whether the growth rate was involved in the control of the composition of the photosynthetic apparatus in *C. aurantiacus*, we used the chemostat in order to adjust the cultures to defined growth rates.

A dependency of steady state cell protein levels as well as specific Bchl a and Bchl c contents of cells on the dilution rate (D = growth rate) is shown in Fig. 1A. Both protein and specific Bchl a contents increased linearly with decreasing D-values. Specific Bchl c contents, however, increased exponentially, which means that the molar ratio of Bchl c/Bchl a increased exponentially as well.

Multiplication of steady state Bchl and protein levels by the growth rate (i. e. the dilution rate), yields the specific rates of synthesis (Fig. 1 B). It is evident that the specific rate of Bchl c synthesis decreased when the growth rate was increased, while the specific rate of Bchl a synthesis as well as the rate of cell protein formation increased almost in parallel.

Cultures grown at different temperatures always showed a comparable response toward changes in growth rate, i.e., protein levels and specific Bchl contents increased when the growth rate was decreased. However, the extent of such changes in specific Bchl contents as well as the Bchl c to Bchl a ratio exhibited significant temperature dependency. This is demonstrated in Fig. 2 which depicts specific Bchl c and Bchl a contents as functions of the steady state cell protein concentration of cultures grown at several temperatures. Evidently, specific Bchl a contents increased linearly with increasing protein concentration. But specific Bchl c levels increased exponentially with linear increases of protein concentrations, where the exponents decreased when the temperature was raised. All of the values presented in Fig. 2 were determined with cultures grown in steady states at defined growth rates. By comparison with Fig. 1 this means that at a given temperature the lower specific Bchl contents and protein levels were obtained at higher growth rates and vice versa.

On the basis of previous work on the control of Bchl synthesis by light, the exponential increase of Bchl c content of cells appeared to be related to decreasing light intensity within the culture due to increased shading at higher culture

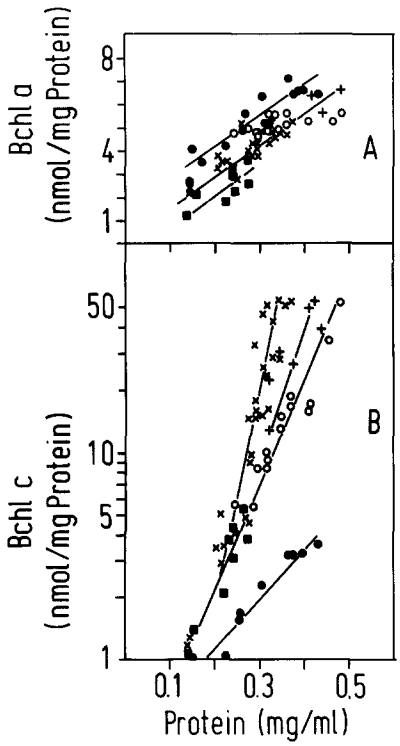

Fig. 2 A, B. Steady state specific bacteriochlorophyll (Bchl) a (**A**) and Bchl c (**B**) contents versus steady state protein levels of chemostat cultures of *Chloroflexus aurantiacus* growing at 240 Wm^{-2} and the following temperatures: 46° C (■); 52° C (×); 56° C (+); 60° C (○) 62° C (●). Variation of the data at a given temperature resulted from variation of the dilution rate. For further details see Fig. 1

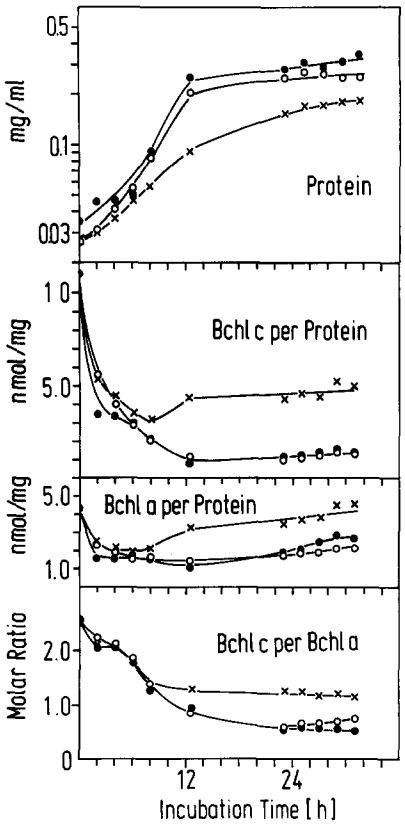

Fig. 3. Growth, specific bacteriochlorophyll (Bchl) contents, and molar ratios of Bchl c/Bchl a in phototrophic batch cultures of *Chloroflexus aurantiacus* growing at 56° C, 500 Wm^{-2} of incandescent light and at (A) 1/3 concentrated growth medium (Pierson and Castenholz, 1974) (×), supplemented with the full concentration of either (B) yeast extract (●) or (C) casamino acids (○)

Table 1. Steady state value of cell protein, of specific bacteriochlorophyll (Bchl) a, and Bchl c contents as well as of molar ratios of Bchl c/Bchl a in continuous cultures of *Chloroflexus aurantiacus* growing at a dilution rate of 0.032 h^{-1}. The concentration of the inflowing medium was diluted so that protein levels became comparable during growth at different light energy fluxes (Wm^{-2}) as indicated

Wm^{-2}	Protein (mg ml^{-1})	Bchl a (nmol mg^{-1} Protein)	Bchl c (nmol mg^{-1} Protein)	Bchl c/Bchl a (molar ratio)
75	0.29	5.2	34.4	6.6
100	0.24	7.7	47.6	6.2
240	0.29	6.1	36.8	6.0
290	0.32	7.0	47.1	6.7

densities. Alternatively, the dependency of Bchl c contents on the dilution rate (D) independent of light intensity suggested a relationship between growth rate and Bchl c synthesis. In order to test this hypothesis, chemostat cultures were grown at comparable densities and growth rates but varying light intensities, and secondly, batch cultures were grown at constant light intensity but different growth rates obtained by varying concentrations of casamino acids. The results of the first experiment (Table 1) revealed that light had no significant effect on the steady state levels of the two Bchls or on the molar ratio of Bchl c to Bchl a. In the second experiment, batch cultures were employed which were grown at 500 Wm^{-2} of incandescent light on medium diluted to one third of its original composition. This dilute medium was used either directly (culture A) or supplemented with the full concentrations of either casamino acids (culture C) or yeast extract (culture B). Figure 3 demonstrates that culture A grew with a lower growth rate than cultures B and C. During the first six hours of incubation specific Bchl contents of all three cultures decreased. From 6 to 12 h, however, specific Bchl contents of culture A increased while they decreased further in cultures B and C. This shows that, although subjected to less self-shading, culture A with the lower growth rate exhibited not only higher specific Bchl contents, but also higher ratios of Bchl c to Bchl a.

Finally the question was raised whether growth rate dependent changes in the ratio of Bchl c to Bchl a were of significance to photosynthetic activity. It is assumed that light energy trapped by Bchl c of chlorosomes is channeled via base plate Bchl a (P792) to the constantly composed light-harvesting photochemical reaction-center complex housed in the cytoplasmic membrane (Betti et al. 1982). This presumes that light saturation of the photochemical electron-transport depends at least partially on the Bchl c light-harvesting capacity of chlorosomes. In order to test this presumption, the light energy flux was measured, which was required to half-maximally saturate light induced proton extrusion by cells of different Bchl c and Bchl a contents. Through the data plotted in Fig. 4, it becomes evident that light requirements of proton extrusion are dependent

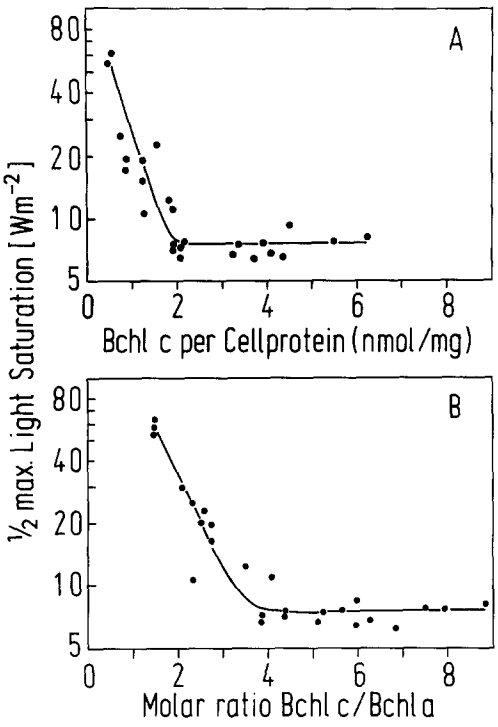

Fig. 4A, B. The dependency of light energy flux required for half-maximal saturation of proton extension by cells of *Chloroflexus aurantiacus* on **A** the specific bacteriochlorophyll (Bchl) c content and **B** the ratio of Bchl c/Bchl a. Cell samples with different Bchl c levels were withdrawn from continuous cultures as described in Fig. 2

on either the Bchl c contents per protein or the Bchl c to Bchl a molar ratio.

Discussion

We have demonstrated that growth rate rather than light intensity affects the content of Bchl proteins of the photosynthetic apparatus in *C. aurantiacus*. This conclusion is derived from the facts that changes in growth rate at constant incident light intensity caused changes in the levels and relative proportion of Bchl c and Bchl a, which could not be observed under different incident light intensities at constant growth rate and cell density. Moreover, the conclusion is corroborated by results from batch culture experiments, which revealed that under constant light intensity changes in the specific contents of both Bchls as well as in their relative proportion were most pronounced with cultures growing at higher growth rates.

The question arises as to whether our data obtained with growth rate controlled chemostats agree with data obtained previously with batch cultures growing at different light intensities. In other words, can previously observed light effects be explained on the basis of growth rate effects? Our batch culture experiments as well as those of Pierson and Castenholz (1974) who showed that variation in incident light intensity resulted in variation not only of Bchl contents but also of growth rate confirm that growth rate is a factor. As a matter of fact, the latter authors showed a dependency of Bchl levels on growth rate which was almost identical with ours. Nevertheless, it should be remembered that the growth rate was varied by light while we kept light constant, and changed the growth rate either by the rate of medium supply (chemostat) or by the concentration of growth limiting substrates (batch culture). Growth rate, as well, may also be the reason for decreased Bchl c contents as seen at elevated temperatures if temperature is a factor stimulating growth rate.

The previous hypothesis of a control of the ratio of light harvesting Bchl c to photochemical reaction center Bchl a, by light alone appears feasible because it is based on the assumption that the cells can adjust their light harvesting capacity to the availability of light. As shown in this communication the light harvesting capacity determined on the basis of light saturation of proton extrusion varied with the growth rate, as well.

Presently, the control of the development of the photosynthetic apparatus is being studied with attention to the basis of differential gene activation as well as control at the level of translation or post translational processing (Fuller and Redlinger 1985; Drews 1986). The present investigation shows that the development in the light can be controlled solely by growth rate and by the medium supply, which might be assumed to influence translational activity. Thus, in order to get further information on the molecular events controlling the photosynthetic apparatus in *C. aurantiacus*, it is necessary to define the factors limiting growth as well as those for the formation of Bchl functional units.

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