Effect of Light Intensity on Photosynthetic $^{14}CO_2$ Fixation of *Anabaena flos-aquae*

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The cyanobacterium *Anabaena flos-aquae* (strain 1444) grown at different intensities of white light (900, 3500 and 30000 lux) showed changes in the content and composition of the pigments. Phycocyanin was more affected by high light conditions during growth than chlorophyll *a*. In comparison to in low white light grown cyanobacteria number of phycobilisomes and thylakoids decreased under strong light. A diminution of $^{14}CO_2$ fixation, total amino acid content, glutamic acid and glutamine pools was found in strong white light grown cells. Under these conditions the majority of ^{14}C-labelling was measured in sugar phosphates. After pressure treatment a marked increase of ^{14}C-incorporation into amino acids could be obtained. Results were discussed with reference to regulation of buoyancy in *Anabaena flos-aquae*.

Introduction

The cyanobacterium *Anabaena flos-aquae* possesses gas vacuoles and is able to regulate the buoyancy of the cells by variation of the cell turgor pressure. This could be demonstrated and measured in other prokaryotes, too [1, 2]. The cell turgor pressure was determined as the difference between the critical pressure to collapse the gas vacuoles of the cells in a hypertonic medium and the pressure of cells suspended in water. Walsby [1] found with the planctonic *Anabaena flos-aquae* that the turgor pressure was higher under high light intensities than exposed to low light conditions. A part of this rise can be caused by accumulation of soluble photosynthates [3]. On the other hand, it was found that a light-stimulated uptake of K^+ is participating in the regulation of buoyancy by light intensity [2]. The gas vacuoles are hollow, cylindrical structures (< 100 nm diameter) with a wall of protein which is impermeable to water [4, 5].

This study presents data of ^{14}C-labelled photosynthetic products of *Anabaena flos-aquae* grown at different light intensities using several light conditions during experiments. The aim of the investigation was to obtain more information on the contribution of photosynthates to the turgor pressure increase in *Anabaena flos-aquae*.

Abbreviations: LWL, low white light; NWL, normal white light; SWL, strong white light.

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Materials and Methods

Anabaena flos-aquae (strain 1444) of the algae culture collection Bloomington, Indiana, USA, was grown at +18 °C in a nutrient medium of Walsby and Booker varied according to Huges *et al.* [6] under normal air conditions (0.035 vol.% CO_2). Further culture conditions were a light/dark rhythm of 16:8 h and different light intensities (900, 3500 and 30000 lux). Experiments were carried out under similar conditions as described for growth.

Estimations of dry weight and pigment contents (chlorophyll *a*, phycocyanin, carotenoids) were carried out after the procedures described by Döhler [7]. Protein content was determined according to Bradford [8]. Free amino acids of *Anabaena* extracts were separated and analyzed by reverse-phase high performance liquid chromatography (HPLC) using a gradient solvent system published in more details by Döhler and Zink [9]. For ultrastructural studies *Anabaena* cells were preparated after the method of Karnovsky [10] and embedded in ERL. Pressure treatment was performed at 200 kPa in an Erlenmeyer flask for 15 min using a diaphragm compressor (DV 15/2, Seitz, Bad Kreuznach) according to the procedure described by Walsby [11].

Cyanobacteria were harvested during exponential growth concentrated by centrifugation and resuspended in fresh nutrient solution. 9.5 ml of this *Anabaena* suspension were brought into a special assimilation chamber of plexiglass and adapted at the special light intensity for 20 min at +18 °C, bubbling with normal air (0.035 vol. CO_2). During

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photosynthetic "steady state" conditions 0.5 ml [^{14}C]bicarbonate (specific activity: 2.14 GBq · mmol^{-1}; 740 kBq · ml^{-1}) was added to the *Anabaena* suspension. ^{14}C-tracer experiments were carried out using the procedure described by Döhler [12] in more detail. After different photosynthetic periods samples of 1 ml were removed from the suspension with a syringe and extracted with ethanol. ^{14}C-labelled products were separated according to the method of Schürmann [13]. For more details see [12, 14].

Results

Variation in light conditions during growth results in a decrease of chlorophyll *a* and phycocyanin contents as light intensity arose. Diminution of phycocyanin was more pronounced than that of chlorophyll *a*. A slight decrease of protein and dry weight values was observed, too. The ratio of chlorophyll *a* to phycocyanin to carotenoids varied from 1:8.3:0.5 in low white light grown cells to 1:4.8:0.6 in cells grown in "normal" white light and to 1:1.6:0.9 in cultures exposed to strong white light. Similar findings were published for *Anabaena cylindrica* [14] and *Anacystis nidulans* [7].

In strong white light grown cells are characterized by an increase in the number of carboxysomes and a reduction of the number of thylakoids, gas vacuoles and phycobilisomes. Similar results were published for *Anacystis nidulans* [15].

Variation of light intensities during growth changed the pattern and content of free amino acids of *Anabaena flos-aquae*. After pressure treatment the percentage proportion of glutamic acid and glutamine rose while pools of aspartic acid, serine glycine, alanine and tyrosine decreased.

Photosynthetic $^{14}CO_2$ fixation of *Anabaena flos-aquae* grown at different light intensities (900, 3500 and 30000 lux) were studied at several light conditions (2000, 8000 and 60000 lux) during experiments. $^{14}CO_2$ assimilation rate of in low white light (LWL) grown cells decreased as light intensity arose during experiment: Cyanobacteria exposed to LWL during experiments showed in all cultures the highest $^{14}CO_2$-incorporation rates.

It could be shown that rise in turgor pressure of the cells may be partly due to the accumulation of soluble photosynthates of low molecular weights [3]. Therefore we have estimated ^{14}C-radioactivity of photosynthetic products of *Anabaena flos-aquae* under several light conditions during growth and experiments. The majority of radioactivity of photosynthetic $^{14}CO_2$ fixation of cyanobacteria grown in relative high ("normal", 3500 lux) and strong (35000 lux) white light – exposed to high light intensities (8000 and 60000 lux) during experiments – was found in sugar monophosphates, 3-phosphoglyceric acid and amino acids. Short-term kinetics experiments showed that under these conditions until a photosynthetic period of 10 min about 50% of [^{14}C]bicarbonate was incorporated into sugar monophosphates (Fig. 1). Percentage proportion of total amino acids arose to 25–30% after 10 min photosynthesis; mainly aspartic acid, glutamic acid and glutamine were ^{14}C-labelled. Ultrastructure studies showed that these *Anabaena* cells exhibit a small number of gas vacuoles, only (Fig. not shown).

Similar to the results of in SWL grown *Anabaena* cells sugar monophosphates were mainly ^{14}C-labelled in cells cultured in LWL. Proportion of ^{14}C-labelled photosynthetic products varied after pressure treatment (Fig. 2b). Radioactivity of amino acids (Aa)

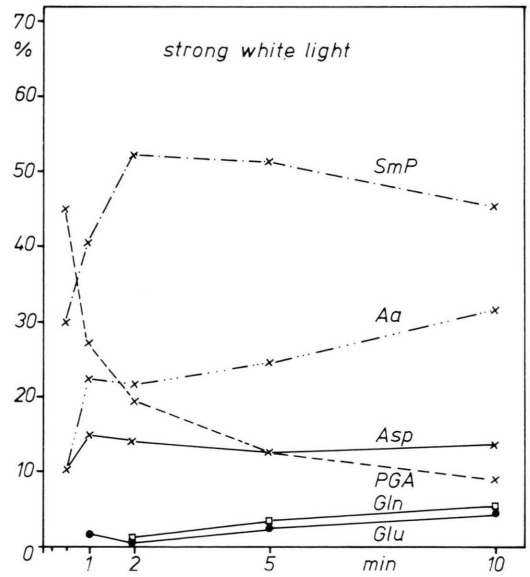

Fig. 1. Kinetics of ^{14}C-incorporation into several photosynthetic products (percentage proportion) of *Anabaena flos-aquae (strain 1444)* grown at +18 °C under normal air conditions (0.036 vol.% CO_2). Cyanobacteria were irradiated during growth and experiment with strong white light. Radioactivity present in total amino acids (Aa), asparagine (Asn), aspartate (Asp), glutamate (Glu), glutamine (Gln), 3-phosphoglyceric acid (PGA) and sugar monophosphates (SmP).

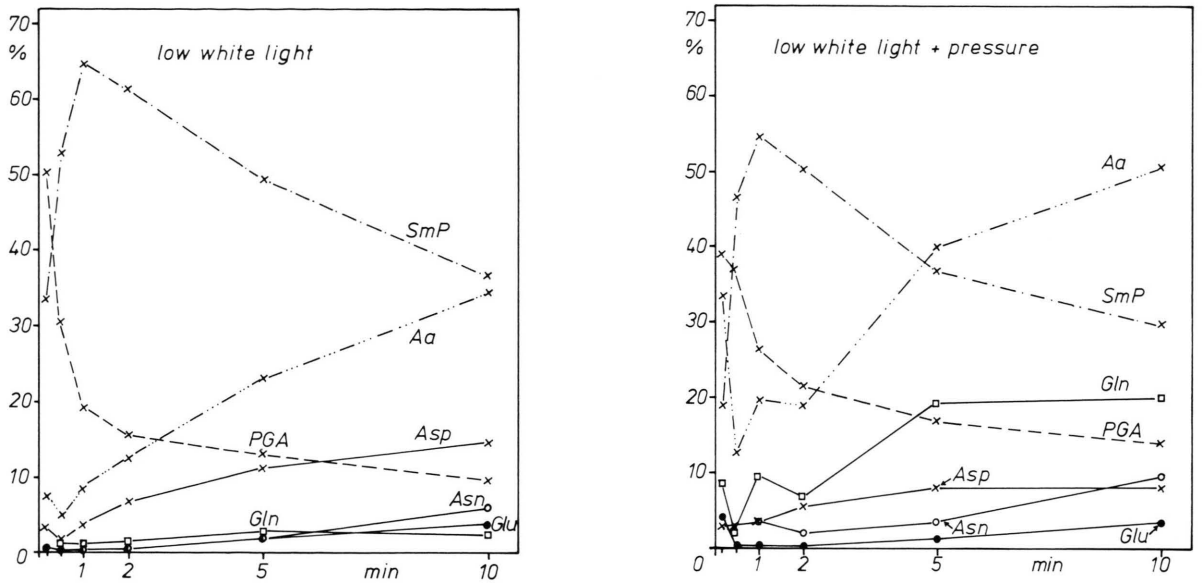

Fig. 2. Kinetics of ^{14}C-incorporation into several photosynthetic products (percentage proportion) of *Anabaena flos-aquae* grown under low white light conditions and exposed to pressure treatment.

increased markedly usually that of glutamine. These cells exhibit practically no gas vacuoles. Similar results – high amino acid labelling – were obtained when LWL grown cyanobacteria were exposed to NWL. Summarizing, after destruction of the gas vacuoles an enhancement of amino acid biosynthesis was observed.

Discussion

Recent studies on CO_2 assimilation demonstrated the dominant role of the Calvin cycle pathway in cyanobacteria [7, 14, 16]. However, a light triggered phosphoenolpyruvate carboxylation reaction was observed under special conditions [7, 14]. After pressure treatment significant changes were found: ^{14}C-radioactivity of sugar phosphates decreased to about 40% of total photosynthetic products while ^{14}C-labelling of total amino acids arose to 40–45%. This indicates an enhanced biosynthesis of amino acids. In longterm kinetics experiments – 20 min photosynthetic $^{14}CO_2$ fixation – a high percentage proportion of sugar phosphates and a low of total amino acid ^{14}C-labelling could be found under high light conditions. Grant and Walsby [3] showed that the rise in turgor pressure is partly due to the accumulation of soluble photosynthates of low molecular weights. It can be suggested that a partly collapse of the gas vacuoles of the cyanobacteria exposed to high light may be due to the enhanced synthesis of sugar monophosphates (see Figs. 1 + 2). Further studies must show the special role of photosynthates at the turgor pressure rise in *Anabaena flos-aquae.*

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