Variation in Photosynthetic Membrane and Pigment Content with Light Intensity for *Anacystis nidulans* Grown in Continuous Cultures

By HANS CHRISTIAN UTKILEN,* TORMOD BRISEID AND BENNY ERIKSSON

Botanical Institute, University of Oslo, P.O. Box 1045, Blindern, Oslo 3, Norway

(Received 10 November 1983)

Photosynthetic membrane and chlorophyll content of the cyanobacterium *Anacystis nidulans* were determined after growth at different light intensities in Mg^{2+} -limited and non- Mg^{2+} -limited continuous cultures. Decreasing the light intensity from 445 to $31 \mu\text{E m}^{-2} \text{s}^{-1}$ resulted in a threefold increase of chlorophyll per unit cell volume. The photosynthetic lamellar structure, on the other hand, increased twofold under the same conditions. The specific chlorophyll content of the photosynthetic membrane increased when the light intensity was decreased from 445 to $282 \mu\text{E m}^{-2} \text{s}^{-1}$ and was constant at lower light intensities. The results indicate that the maximum chlorophyll content in the thylakoids of *A. nidulans* was about $1.5 \text{ fg } \mu\text{m}^{-2}$, which means that the membrane contained 1 chlorophyll molecule per nm^2 under these conditions.

INTRODUCTION

Allen (1968) showed that the amount of photosynthetic lamellar structure in *Anacystis nidulans* was directly proportional to the chlorophyll content, when light intensities of 100 or 1000 ft-candle were used. By using different light intensities and qualities for growth of *A. nidulans* in K^{+} - or Mg^{2+} -limited chemostats, Briseid (1980) obtained results indicating that the membrane chlorophyll content might vary in this organism at higher light intensities.

The present study evaluates the chlorophyll content of the photosynthetic lamellar structure in *A. nidulans* grown in continuous cultures fed with 5 or $10 \mu\text{M-Mg}^{2+}$ and light intensities ranging from 31 to $445 \mu\text{E m}^{-2} \text{s}^{-1}$ (roughly 186 to 2975 ft-candle). The chlorophyll density in the membranes was estimated by a combination of quantitative biochemical and ultrastructural techniques.

METHODS

Organism. *Anacystis nidulans* strain UTEX 625 of the Culture Collection of Algae, Department of Botany, University of Texas, was used.

Growth conditions. The organism was grown in continuous cultures fed with a modified medium C (Utkilen, 1982), containing 5 or $10 \mu\text{M-Mg}^{2+}$, at a dilution rate (D) of 0.09 h^{-1} . The culture fed with $5 \mu\text{M-Mg}^{2+}$ was Mg^{2+} -limited, while the culture fed with $10 \mu\text{M-Mg}^{2+}$ was not Mg^{2+} -limited (Utkilen, 1982).

The culture vessels were placed in a waterbath (40°C) between two ranks of four fluorescent tubes (Philips TL AK 40/W 33). This gave an incident irradiation of $445 \mu\text{E m}^{-2} \text{s}^{-1}$ from each side. The light intensities were measured with a LI 1854 Quantum/Radiometer/Photometer (Lambda Instruments Corporation, U.S.A.). Different light intensities were obtained by placing sheets of white paper between the waterbath and the fluorescent tubes. All light intensities given in this paper are those obtained from one side.

Estimation of chlorophyll density in the membranes. The cells were prepared for electron microscopy by a modification of the procedure used by Allen (1968). They were first treated with unbuffered KMnO_4 (1.5%, w/v) for 10 min. After rinsing with distilled water and dehydration in a graded series of ethanol/water, the samples were treated with propylene oxide for 2×5 min and left overnight in propylene oxide/Spurr (3:1) (Spurr, 1969). Next day this was exchanged for pure, fresh Spurr, which was polymerized at 70°C . Sections 70 nm thick, made by an

Ultratome 111 (LKB, Bromma, Sweden) using a diamond knife, were placed on 25 mesh carbon-coated grids and examined in a Jeol 100 C electron microscope (JEOL Ltd, Japan) at 80 kV.

Electron micrographs of $30000\times$ magnification were used to estimate the relation between the surface area of photosynthetic membrane (A_{Phm}) and cell volume (V) as described by Weibel (1969). A simple square lattice consisting of 10 horizontal and 14 vertical lines (test lines) at a distance of 15 μm was placed, in a fixed position, on each micrograph. The surface of this lattice was always smaller than the area of the micrographs.

The relation between A_{Phm} and V is then given by:

$$A_{\text{Phm}}/V = (2 \times I_{\text{Phm}(\text{total})})/(L \times P_i/P_{\text{total}})$$

where L is the total length of test lines (140 μm in this case), P_i is the number of test points (intersections between test lines) covering the cells, P_{total} is the total number of test points in the lattice (140 in this case), and $I_{\text{Phm}(\text{total})}$ is the total number of intersections between test lines and the photosynthetic membrane.

To compensate for estimation errors caused by the invisibility of membrane profiles oriented obliquely to the axis of the electron beam, the method of Reith (1977), developed for estimation of the relation between surface of cristall-membranes and mitochondrial volume, was used as follows:

$$I_{\text{Phm}(\text{total})} = I_{\text{Phm}(\text{distinct})} \times K$$

where $I_{\text{Phm}(\text{distinct})}$ is the number of intersections between test lines and distinct photosynthetic membrane. The value of K was found by means of the cytoplasmic membrane, on the assumption that the relation between total and distinct photosynthetic membrane is the same as that between total and distinct cytoplasmic membrane. The total intersection between test lines and cytoplasmic membrane can always be found even when it is indistinct, i.e. obliquely oriented, since this membrane entirely surrounds the cell. The value of K found in this way was used to calculate the corrected $I_{\text{Phm}(\text{total})}$.

After determining cell number, mean cell volume (μm^3 per cell) and chlorophyll content (fg per cell), the specific chlorophyll content of the photosynthetic membrane is given by:

$$\text{Chlorophyll}/A_{\text{Phm}} = (\text{chlorophyll} \times L \times P_i)/(V \times 2 \times I_{\text{Phm}(\text{total})} \times P_{\text{total}})$$

About 15 micrographs, each with about 25 cells, were examined from every light intensity.

Analytical methods. These were as previously described (Utkilen, 1982).

RESULTS AND DISCUSSION

When examining the photosynthetic membrane and chlorophyll content of *A. nidulans* grown at different light intensities, two continuous cultures were used. One culture was fed with $5 \mu\text{M-Mg}^{2+}$, and has been shown to be Mg^{2+} -limited at $365 \mu\text{E m}^{-2} \text{s}^{-1}$ (Utkilen, 1982). The other culture, which was fed with $10 \mu\text{M-Mg}^{2+}$, has been shown to be influenced by another limitation, which could be light (Utkilen, 1982). These two cultures were therefore compared in order to see if the Mg^{2+} -limited conditions used would influence chlorophyll and photosynthetic membrane synthesis.

At $445 \mu\text{E m}^{-2} \text{s}^{-1}$ the steady-state dry weight was about $180 \mu\text{g ml}^{-1}$ in the culture fed with $10 \mu\text{M-Mg}^{2+}$ and about $100 \mu\text{g ml}^{-1}$ in the culture fed with $5 \mu\text{M-Mg}^{2+}$. However, at $103 \mu\text{E m}^{-2} \text{s}^{-1}$ the steady-state biomass was about $65 \mu\text{g ml}^{-1}$ in both cultures, and a further decrease in light intensity to $31 \mu\text{E m}^{-2} \text{s}^{-1}$ resulted in the same linear decrease in biomass for both cultures. These results show that growth was limited to the same extent in both cultures below $103 \mu\text{E m}^{-2} \text{s}^{-1}$. If Mg^{2+} was still the limiting factor below this light intensity, one would expect a different steady-state biomass in cultures fed with 5 and $10 \mu\text{M-Mg}^{2+}$. Since this was not the case, and light was the only growth factor that had been changed, it was reasonable to conclude that both cultures were light-limited below $103 \mu\text{E m}^{-2} \text{s}^{-1}$.

At $445 \mu\text{E m}^{-2} \text{s}^{-1}$ the chlorophyll content was 7 fg per unit (μm^3) cell volume regardless of the Mg^{2+} concentration in the medium, and it increased to 22 fg μm^{-3} in both cultures upon decreasing the light intensity (Fig. 1). These results indicate that the maximum chlorophyll content of *A. nidulans* under the conditions used was 22 fg μm^{-3} , since the cultures were light-limited below $103 \mu\text{E m}^{-2} \text{s}^{-1}$ (see above) and the organism therefore should be saturated with respect to chlorophyll. The results also show that the Mg^{2+} -limited ($5 \mu\text{M}$) conditions used did not influence the chlorophyll content, since this was independent of the Mg^{2+} concentration in the feed medium.

Fig. 1.

Fig. 1. Variation with light intensity in chlorophyll per unit cell volume, for *A. nidulans* grown in continuous cultures ($D = 0.09 \text{ h}^{-1}$) fed with (a) $10 \mu\text{M-Mg}^{2+}$ or (b) $5 \mu\text{M-Mg}^{2+}$. The data are average values obtained from three samples.

Fig. 2.

Fig. 2. Variation with light intensity in photosynthetic membrane area per unit cell volume, for *A. nidulans* grown in continuous cultures ($D = 0.09 \text{ h}^{-1}$) fed with (a) $10 \mu\text{M-Mg}^{2+}$ or (b) $5 \mu\text{M-Mg}^{2+}$. The bars represent S.D.

Fig. 3. Variation with light intensity in specific chlorophyll content of the photosynthetic lamellar structure in *A. nidulans* grown in continuous cultures ($D = 0.09 \text{ h}^{-1}$) fed with (a) $10 \mu\text{M-Mg}^{2+}$ or (b) $5 \mu\text{M-Mg}^{2+}$. The bars represent S.D.

The culture fed with $10 \mu\text{M-Mg}^{2+}$ reached the maximum chlorophyll content at higher light intensities than the culture fed with $5 \mu\text{M-Mg}^{2+}$ (Fig. 1). This was presumably due to the fact that its biomass was higher than for the culture fed with $5 \mu\text{M-Mg}^{2+}$ and therefore the light conditions in the $10 \mu\text{M-Mg}^{2+}$ culture were actually different from those in the $5 \mu\text{M}$ culture.

The relation between photosynthetic membrane area and mean cell volume increased up to a factor of 2 with decreasing light intensity for both cultures (Fig. 2). As observed for chlorophyll, the maximum membrane content was reached at higher light intensities in the culture fed with $10 \mu\text{M}$ than in the one fed with $5 \mu\text{M-Mg}^{2+}$.

Since there was a threefold increase in chlorophyll per unit cell volume while the membrane content was only doubled, there must have been an increase in chlorophyll per unit membrane area. The relation between chlorophyll and membrane content (Fig. 3) revealed that the increase in chlorophyll per unit membrane area occurred between 445 and 282 $\mu\text{E m}^{-2} \text{ s}^{-1}$ only, while it was constant at lower light intensities. The results (Fig. 3) indicate that the maximum amount of chlorophyll per unit membrane area was about 1.5 fg μm^{-2} . From this value the maximal density of chlorophyll molecules per unit membrane area was calculated to be 1 chlorophyll molecule per nm².

Allen (1968) found a constant chlorophyll content in the photosynthetic membrane below 145 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (1000 ft-candle), which is in accordance with our results, but she did not examine the effect of higher light intensities. Our results show that *A. nidulans* is able to vary the specific chlorophyll content of the membrane to a large extent at high light intensities. It increased by about 70% in the culture fed with 10 $\mu\text{M-Mg}^{2+}$ when the light intensity was decreased from 445 to 282 $\mu\text{E m}^{-2} \text{ s}^{-1}$, and by about 40% in the other culture. The large discrepancy between the two cultures is most probably caused by the large errors in the estimation of specific membrane content (Fig. 2). In spite of this, the conclusion from these results is that *A. nidulans* varies the specific chlorophyll content in the thylakoids at high light intensities, while there is a constant content at moderate or low light intensities.

Thus *A. nidulans* seems to decrease its chlorophyll content by first decreasing the photosynthetic lamellar structure to a certain level, and then, when this membrane level is reached, the chlorophyll content in the membrane decreases. The same mechanism might also work for other cyanobacteria.

The authors thank Professor P. Halldal and Dr T. Barnard for critical reading of the manuscript.

REFERENCES

- ALLEN, M. M. (1968). Photosynthetic membrane system in *Anacystis nidulans*. *Journal of Bacteriology* **96**, 836–841.
- BRISEID, T. (1980). *Kalium begrenset vekst av Anacystis nidulans*. Cand. real. thesis, University of Oslo, Norway.
- REITH, A. (1977). Problems in the estimation of surface densities (S.D.) of biomembranes. *Mikroskopie* **33**, 80–87.
- SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **26**, 31–43.
- UTKILEN, H. C. (1982). Magnesium-limited growth of the cyanobacterium *Anacystis nidulans*. *Journal of General Microbiology* **128**, 1849–1862.
- WEIBEL, E. R. (1969). Stereological principles for morphometry in electron microscopy cytology. *International Review of Cytology* **26**, 235–302.