Lipid and Fatty Acid Composition of Synchronized *Synechococcus leopoliensis*

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The cyanobacterium *Synechococcus leopoliensis* (*Anacystis nidulans*, strain L 1402-1) grown at 39 °C and 2 vol. % CO_2 could be synchronized by a light/dark regime of 3:5 h (white light intensity 1.5×10^4 erg cm^{-2} sec^{-1}). Content of pigments (chlorophyll a, phycocyanin and carotenoids), RNA and proteins increased linearly up to 100% at the end of the light period while DNA synthesis was lower. Chlorophyll a synthesis was correlated to the photosystem I activity of the isolated thylakoids and to the formation of MGDG. Galacto lipids were synthesized in the light period, only. A lag phase of 2 h was observed in the biosynthesis of SQDG and PG. No significant differences were found between the cell and thylakoid fractions. Palmitic ($C_{16:0}$), hexadecenoic ($C_{16:1}$) and octadecenoic ($C_{18:1}$) acid as major components accounted for more than 90% of total fatty acids in MGDG, DGDG and SQDG. PG contains a small amount of stearic ($C_{18:0}$) and heptadecenoic ($C_{17:1}$) acid. No significant variations in the fatty acid distribution of all lipids could be detected in the cell fraction during the division cycle. Changes in the ratio of saturated to unsaturated fatty acids were found in isolated thylakoids, only. In experiments with [^{14}C]bicarbonate main radioactivity was measured in galacto lipids while using [^{14}C]acetate SQDG and PG were markedly [^{14}C]labelled. Results were discussed with reference to the findings of eucaryotic algae and the formation of photosynthetic membranes.

Introduction

The influence of several parameters on lipids and fatty acids of cyanobacteria has been investigated in a number of laboratories (Murata and Nishita, [1] and references therein). The main acyl lipids (MGDG, DGDG, SQDG and PG) of cyanobacteria are also found in the thylakoid membranes of the chloroplasts from eucaryotic organisms. The diversity in diglyceride portions of lipids in procaryotic cells is reduced in comparison to the eucaryotic organisms [2]. The effect of temperature on lipid and fatty acid composition in cyanobacteria was first studied by Holton *et al.* [3] who found an increase of saturated fatty acids at higher temperatures. Fork *et al.* [4] could confirm these results using a thermophilic *Synechococcus lividus* strain. When the growth temperature was lowered from 55 to 38 °C, the amount of the saturated fatty acid 18:0 decreased whereas the unsaturated fatty acids 18:1 and 16:1 of SQDG and PG increased. A number of physiological activities related to the photosynthesis in relationship to the physical state of thylakoid lipids were studied in dependence on the growth temperatures of several cyanobacteria and algae species [1, 5–7]. A temperature dependent variation in acyl lipid composition occurred in *Anabaena* and *Synechococcus (Anacystis)* cells [8]. Studies on *Synechococcus (Anacystis)* grown to obtain different pigment ratios, showed that light influences the acyl lipid composition as well as the fatty acid distribution of whole cells [9] and phycocyanin-free lamellae [10]. High white light conditions caused in a fatty acid composition which was similar to that from cyanobacteria grown at high temperatures. A few publications are known only about variations in acyl lipid and fatty acid composition during the division cycle of algae [11–14]. This paper presents data from the acyl lipid and fatty acid composition during the cell cycle of *Synechococcus leopoliensis*.

Abbreviations: MGDG, DGDG, SQDG, monogalactosyl-, digalactosyl-, sulphoquinovosyl diacylglycerol; PG, phosphatidylglycerol.

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Materials and Methods

The cyanobacterium *Synechococcus leopoliensis* (former *Anacystis nidulans*, strain L 1402-1) obtained from the Algenreinkulturensammlung, Göt-

tingen, was grown at 39 °C and 2 vol. % CO_2 using the BG-11 nutrient medium [15]. Synchronization of the cell division could be obtained by a light/dark rhythm of 3:5 h (white light intensity: 1.5×10^4 erg cm^{-2} sec^{-1}) according to the procedure of Lorenzen and Kaushik [16]. Experiments were performed with *Synechococcus* cells harvested from the 3. cycle. Cells were ruptured with a Branson-Sonifier (Model S-75) and phycocyanin-free lamellae were obtained from the homogenates by fractional centrifugation after Löffelhardt [17]. Lipids were extracted according to the procedure of Tevini [18]. The quantities of lipids were determined by estimating glycolipid sugar content [19] and PG-bound phosphorus as described by Debuch *et al.* [20]. Fatty acid composition of the acyl lipids were separated and analyzed by gasliquid chromatography (GC) using a Varian 3700 Model with a 2 m glass column (10% DEGS on chromosorb G, 80–100 mesh). ^{14}C experiments were carried out after the procedures of Döhler [21]. Preparation, extraction and analytical methods were described in detail by Datz and Döhler [10].

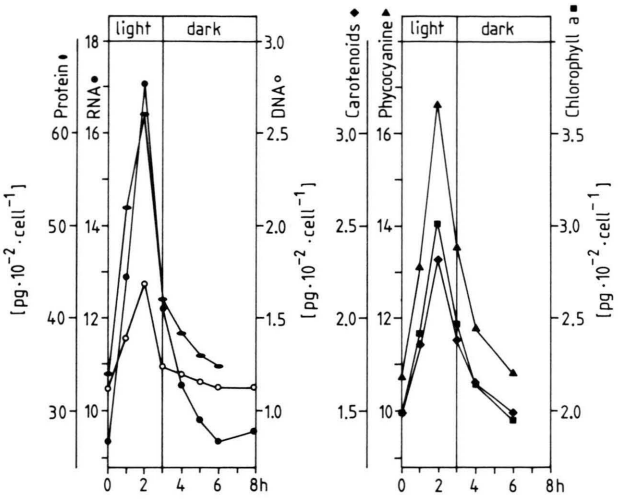

Fig. 1. Changes in pigmentation and the contents of DNA, RNA and protein during the division cycle of *Synechococcus leopoliensis*. Values are calculated for one cell and presented in picogram 10^{-2} per cell, ○——○ DNA, ●——● RNA, —— protein, ■——■ chlorophyll a, ▲——▲ phycocyanin and ◆——◆ carotenoids. Further details in Materials and Methods.

Results

It was found that pigments (chlorophyll a, carotenoids, phycocyanin), RNA and protein content increased linearly up to 100% at the end of the light period. Rate of DNA synthesis was much lower in comparison to the other variables at the same time. Data calculated on the basis of a single cell are shown in Fig. 1. In all cases maximal values could be determined at the beginning of the cell division (2 h light). The generation time of *Synechococcus* was 5 h under the conditions used. No differences in the synthesis of the pigments could be found in cells and thylakoids. Chlorophyll a formation was closely correlated to the photosystem I activity of the isolated thylakoids. The ratio of the pigments was constant during the cell cycle.

The percentage increase of the acyl lipids from the cell and thylakoid fractions during the division cycle of *Synechococcus* can be seen in Fig. 2. The galacto lipids (MGDG and DGDG) were synthesized mainly during the light period. No significant differences between cells and thylakoids could be observed. Data indicate that the formation of DGDG was performed after the biosynthesis of MGDG. However, the biosynthesis of SQDG and PG started with a lag phase of 2 h after formation of the galacto lipids. The

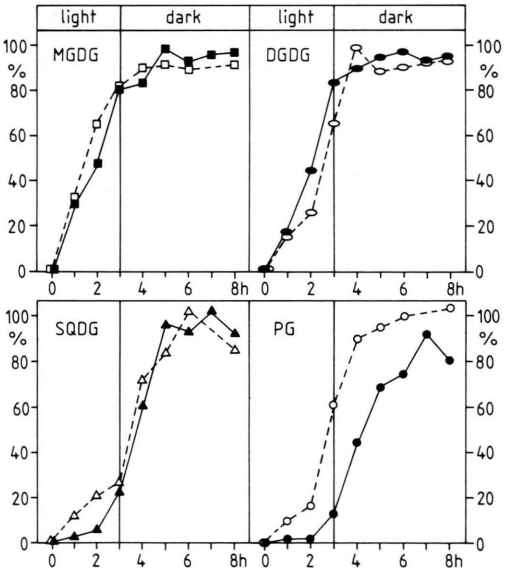

Fig. 2. Percentage increase of the acyl lipids in the cell and thylakoid fractions during the cell cycle of *Synechococcus leopoliensis*. ——— values of the cell and – – – – thylakoid fractions. Further details in Materials and Methods.

increase in production of the thylakoid-bound phospholipid was generally earlier than that of the cells. The variations in the percentage proportion of the individual acyl lipids of the cell and thylakoid fractions are shown in Fig. 3A. Maximum of the MGDG content of both the cells and the thylakoids was found after 2 h in the light while maximum values of DGDG could be determined after 3 and 4 h in the cell cycle from the cell and thylakoid fractions, respectively. The SQDG and PG reached maximum amounts in the dark period and no differences between the cell and thylakoid fraction were detectable. A correlation of the chlorophyll a data exists to the galacto lipids, only.

The analysis of the fatty acids of all tested acyl lipids showed that palmitic ($C_{16:0}$), hexadecenoic ($C_{16:1}$) and octadecenoic ($C_{18:1}$) acid as major components accounted for more than 90% of total fatty acids in MGDG, DGDG and SQDG. PG contains also small amounts of stearic ($C_{18:0}$) and heptadecenoic ($C_{17:1}$) acid. Content of $C_{18:1}$ of all acyl lipids did not vary much during the cell cycle. The amount of $C_{16:0}$ and $C_{16:1}$ of the cell fractions showed also no significant changes. Variations in the fatty acid distribution could be observed mainly in acyl lipids of the thylakoid fractions. Fig. 4 contains data showing the ratio of saturated to unsaturated fatty acids calculated from the major fatty acids of the cell and thylakoid fractions during the division cycle of *Synechococcus*. A decrease of the $C_{n:0}/C_{n:1}$ ratio during the light period could be detected in the thylakoid-bound acyl lipids followed by an increase in the dark. Similar results were obtained from the DGDG fraction of the cells, only. In spite of variations after the cell division the ratio of saturated to unsaturated, fatty acids was nearly identical with that at the beginning of the cycle.

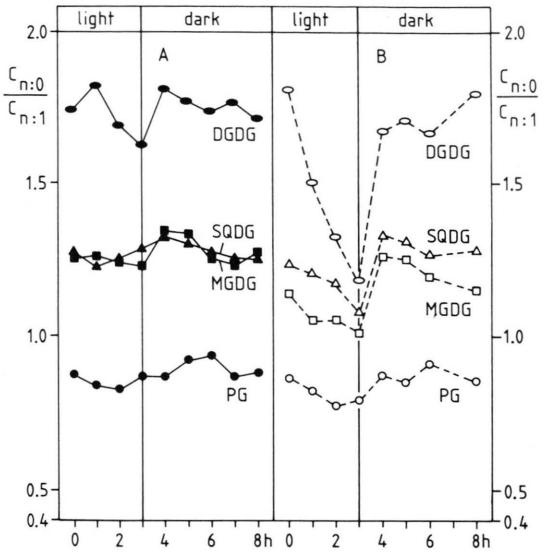

Fig. 4. Changes in the ratio of saturated to unsaturated fatty acids of the acyl lipids during the cell cycle of *Synechococcus leopoliensis*. *A* cell and *B* thylakoid fraction. Further details in Materials and Methods.

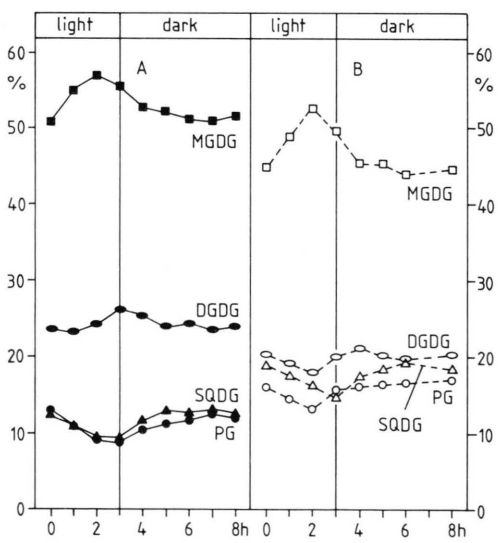

Fig. 3. Variations in the percentage proportion of the acyl lipids of the cell and thylakoid fractions during the division cycle of *Synechococcus leopoliensis*. *A* cell and *B* thylakoid fraction. Further details in Materials and Methods.

In another series of experiments the [^{14}C]incorporation into the acyl lipids of synchronized *Synechococcus* cells has been investigated after application of [^{14}C]bicarbonate and [^{14}C]acetate. Radioactivity could be measured after a relative short time in the lipid fraction of both the cells and the thylakoids. [^{14}C]labelling of the individual acyl lipids was in agreement with the result of the quantitative percentage distribution of the glycolipids. However, the [^{14}C]label of the phospholipid was very low compared to the percentage proportion (Table I). Time course of [^{14}C]incorporation into the acyl lipids during the division cycle after adding [^{14}C]bicarbonate showed – in agreement with the findings of the quantitative estimations – an increase in the light and decrease in the dark period. Main part of

Table I. Changes in the [^{14}C]incorporation into the acyl lipids of the cell and thylakoid fraction during the cycle of *Synechococcus leopoliensis* after 10 and 20 min photosynthesis. Values are presented as dpm ml^{-1}. [^{14}C]bicarbonate (111 kBq ml^{-1}; specific activity 2.07 GBq nmol^{-1}) was used. Further details in Materials and Methods.

Time of sample	Lipid	Cell fraction fixation time 10	20	Thylakoid fraction fixation time 10	20
0	MGDG	471	1604	346	632
	DGDG	339	1074	190	350
	SQDG	295	919	160	252
	PG	35	94	13	15
1.5	MGDG	682	2261	475	1048
	DGDG	406	1179	272	542
	SQDG	219	658	137	282
	PG	61	125	18	39
3	MGDG	1002	2680	579	1851
	DGDG	589	1608	393	960
	SQDG	369	858	180	468
	PG	84	178	27	42
4.5	MGDG	1192	2313	569	1118
	DGDG	710	1535	346	655
	SQDG	676	1084	299	404
	PG	129	182	20	33
6	MGDG	880	1982	741	1143
	DGDG	606	1381	378	594
	SQDG	632	1051	382	480
	PG	108	163	28	48

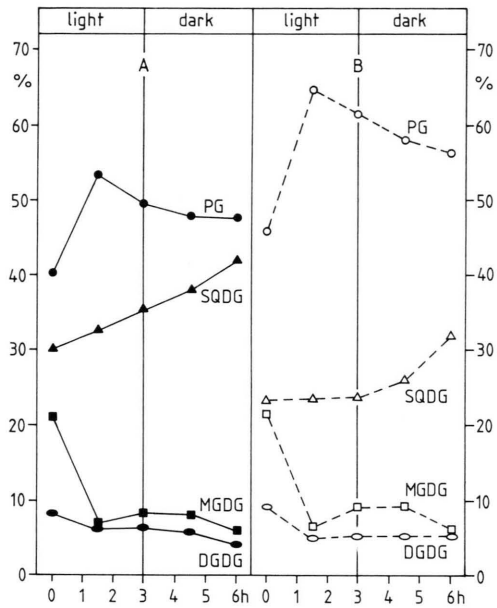

Fig. 5. Percentage distribution of [^{14}C]label after 10 min application of [^{14}C]acetate during the cell cycle of *Synechococcus leopoliensis*. *A* cell and *B* thylakoid fraction. Further details in Materials and Methods.

radioactivity was found in MGDG (26%) and DGDG (25%). This indicates a [^{14}C]incorporation into the lipids *via* the sugar metabolism and less *via* glycerol or fatty acid chain.

In another series of experiments, distribution of [^{14}C]label in the individual acyl lipids after addition of [^{14}C]acetate was studied during the cell cycle of *Synechococcus* (Fig. 5). [^{14}C]incorporation into the acyl lipids of the thylakoids was lower than of the cell fraction. A significant high [^{14}C]labelling could be measured in SQDG and PG of both fractions especially in the thylakoids. Radioactivity in PG increased during the first 2 h of the light period while a decrease in MGDG was found; antiparallelity exists in [^{14}C]label of these both lipids. A nearly linear increase of [^{14}C]incorporation into SQDG could be determined during the cell cycle whereas [^{14}C]label in DGDG slightly decreased. Our results indicate a correlation between the cell division and the synthesis of SQDG and PG as well as the pools of both acyl lipids out of the thylakoids.

Discussion

Our findings showed that doubling of the cells and replication of DNA are finished in the dark period (4 h after start of illumination). In agreement with other authors, a close correlation between synthesis of DNA and the cell division of synchronized *Synechococcus* exists. Rate of DNA formation reached 50–65% at the beginning of cell division [16, 22, 23]. Studies using mitomycin – an inhibitor of the DNA synthesis – showed that the beginning of replication is an important trigger for the cell division [24]. Synthesis of RNA and proteins takes place only during the light period and runs parallel to the formation of DNA (see Fig. 1) which is in agreement with studies on *Escherichia coli* [25] and in contradiction to the findings with eucaryotic organisms [26]. Synthesis of the proteins and pigments of *Chlamydomonas* and *Euglena* cells could be observed in the light period, only [11, 27].

Activity of photosystem I and II of the isolated thylakoid membrans during the cell cycle of *Synechococcus* (76 and 172 μM O_2) was nearly identical with estimations of whole cells by Lorenzen and Kaushik [16]. Our results showed that the biosyn-

thesis of the galacto lipids (MGDG and DGDG) and of chlorophyll a was parallel at the light period. The ratio of MGDG to chlorophyll remained constant during the division cycle of *Synechococcus*, only. Similar results have been obtained from synchronized *Chlamydomonas* cells [11]. This indicates a relationship between the galactolipids and the orientation of the pigments in the thylakoid membrane. Results of ^{14}C experiments showed that DGDG was produced earlier in the cells than in the thylakoids which can be attributed to a galacto lipid synthesis in the plasma membrane.

Significant variations in the ratio of saturated to unsaturated fatty acids during the cell cycle of *Synechococcus* could be observed in isolated thylakoids, only (see Fig. 4). Changes in the porportion of unsaturated fatty acids of PG and SQDG differs with the de novo synthesis of these lipids. This indicates an intermediary function in the lipid metabolism which could be demonstrated by experiments using [^{14}C]acetate. Main part of radioactivity was found in thylakoid-bound PG (73.5%) and SQDG (77.9%) within 10 min photosynthesis. Similar high rates of [^{14}C]incorporation were measured in *Anabaena cylindrica* and *Chlorella vulgaris* [28]. Results of ^{14}C experiments can be explained by a SQDG synthesis out of the photosynthetic membranes and by a multistep process for the formation of the membranes.

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