Cyanophora paradoxa: Fatty Acids and Fatty Acid Synthesis *in vitro*

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Dedicated to Professor Hans Grisebach on the occasion of his 60th birthday

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The fatty acid pattern of *Cyanophora paradoxa* membrane lipids is highly unusual with 16:0, 20:3 and 20:4 as the main acids. The 20:4 acid is preferentially distributed among the cyanelle lipids. In isolated cyanelles a relatively low *in vitro* synthesis of only saturated and monounsaturated fatty acids from [1-^{14}C]acetate was observed which corresponds to the relatively low photosynthetic oxygen evolution.

Introduction

Cyanophora paradoxa is a unicellular eukaryotic alga containing one to several so-called cyanelles. These cyanelles are considered to be endosymbiotic cyanobacteria developing to chloroplasts (*e.g.* [1]). This view is corroborated *e.g.* by the fact that the cyanelle DNA molecules are of the same size as plastid DNA molecules from plant cells (for review see [2]).

Cyanobacteria contain within their membrane lipids either mainly monounsaturated (16:1) fatty acids or, especially filamentous forms, also polyunsaturated (18:2 and 18:3) acids (*e.g.* [3]). In the latter case, therefore, they show a close relationship to chloroplasts where 18:3 acids predominate. The *in vitro* synthesis of fatty acids in cyanobacteria [4, 5] and in chloroplasts (for review see [6]) has been achieved.

In view of the endosymbiotic nature of the *Cyanophora cyanelles,* their apparent similarity to chloroplasts and their possible cyanobacterial origin, an analysis of the cellular and cyanelle fatty acid patterns and their *in vitro* synthesis is presented.

Materials and Methods

A strain of *Cyanophora paradoxa* (Korschikoff) was kindly provided by Dr. H. Bothe (Botanisches Institut, Universität Köln). The organism was grown as described by Marten *et al.* [7].

Cells were harvested at room temperature ($400 \times g$, 5 min) and resuspended in medium I containing 0.4 M sucrose, 0.05 M Tricine, pH 7.8, in the cold and centrifuged again. The cell pellet was then resuspended at room temperature in medium II containing 0.22 M sucrose, 0.05 M Tricine, 0.5 mM EDTA, pH 7.8, and the suspension was allowed to rest for 5–10 min. By this treatment 60 to 70% of the cells released their cyanelles in intact form as revealed by phase contrast microscopy. All the following steps were done in the cold. After centrifugation and an additional washing in medium II, the crude cyanelle fraction was loaded on top of a discontinuous Percoll-gradient (80% and 100% Percoll in medium II). During centrifugation ($1600 \times g$, 15 min) the cyanelles were concentrated at the 80/100% interphase. They were carefully removed from the gradient, diluted by 10 vol medium II and centrifuged. After this procedure the cyanelles remained nearly 100% intact.

Chloroplasts from spinach leaves were isolated as described [8].

For fatty acid synthesis *in vitro* the cyanelles and chloroplasts were incubated for 40 min in the following incubation medium: 50 mM PIPES, 5 mM KH_2PO_4, 10 mM $NaHCO_3$, 1 mM $MgCl_2$, 0.33 M sorbitol, 0.5 mM CoA, pH 7.8. [1-^{14}C]acetic acid (2.0 GBq mmol^{-1}, Amersham Buchler, Braunschweig) was used as a labelled substrate. Incubations were done at 25 °C and about 7000 lux.

The fatty acid moieties of lipids were analyzed after saponification and methylation using diazomethane by argentation thin layer chromatography [9] and by gas liquid chromatography using a 6 ft. glass column (2 mm i.d.) packed with EGSSX, 10% on Gas-Chrom P (Serva, Heidelberg). The analysis was performed on a Packard Mod 437 gas chromatograph at a constant oven temperature of 215 °C and a

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He-flow of 16 ml min^{-1}. Hydrogenation of double bonds was done using a platin catalyst.

Oxygen evolution of cells and isolated cyanelles was measured using a Clark-type electrode system at 25 °C in a final volume of 2 ml at 10000 lux. For these measurements, intact cells were incubated in culture medium and isolated cyanelles in 0.05 M Tricine, 0.1 M sorbitol, 0.5 mM Na_2HPO_4, pH 7.8.

Results and Discussion

The fatty acid pattern of the main cellular and cyanelle lipids of *Cyanophora paradoxa* is very unusual, as can be seen from Table I. Within the typical cyanelle lipids phosphatidylglycerol, monogalactosyldiacylglycerol and digalactosyldiacylglycerol 16:0 and 20:4 acids predominate, whereas phosphatidylethanolamine which occurs exclusively in cellular membranes contains 16:0, 18:0, 18:1, 20:3 and 22:0. Phosphatidylcholine which is found in both, cellular and cyanelle membranes, shows an intermediate pattern. From these results a direct relationship to the fatty acid pattern of any cyanobacterial or higher plant cell so far analyzed can not be established, whereas some marine and freshwater algae do contain minor amounts of 20:3 and 20:4 acids (for review see [10]). A certain parallelism to plant cells, however, may be stated in that the most unsaturated acids, 20:4 in *Cyanophora* and 18:3 in plant cells, are most abundant within the cyanelle and chloroplast lipids, respectively. The unexpected findings presented in Table I raise questions on the biosynthesis and significance of these highly unsaturated C20 acids; a comparative analysis of similar organisms such as *Glaucocystis* and *Gloeochaete* would be desirable.

Isolated, morphologically intact cyanelles incorporate [1-^{14}C]acetate into fatty acids when incubated in the light. The incubation rate, however, was relatively low in comparison with isolated spinach chloroplasts. During the standard incubation time of 40 min the following values were obtained (values in brackets are for spinach chloroplasts): 2 nmol mg^{-1}chlorophyll (7 nmol mg^{-1}chlorophyll) at an acetate concentration of 0.034 mM in the incubation medium and 10 nmol mg^{-1}chlorophyll (100 nmol mg^{-1}chlorophyll) at an acetate concentration of 0.2 mM. This relatively low incorporation in the cyanelles corresponds with the likewise low photosynthetic oxygen evolution of 25 μmol h^{-1} mg^{-1}chlorophyll which was linear maximally up to 10 min. This value is in accordance with that measured *e.g.* by Floener and Bothe [11].

Argentation thin layer chromatography of the total fatty acids following the incubation in the presence of [1-^{14}C]acetate and saponification revealed that the acids synthesized *in vitro* were saturated (about 80%) and monounsaturated (about 20%). This corresponds with the results obtained using chloroplasts where also saturated and monounsaturated acids were the only products formed *in vitro*.

The distribution of the radiolabelled acids among the cyanelle lipids is shown in Table II. All fatty acid

Table I. Fatty acid patterns of membrane lipids from *Cyanophora paradoxa*.

Fatty acid	Fatty acid pattern [%]				
	Phosphatidyl-glycerol	Monogalactosyl-diacylglycerol	Digalactosyl-diacylglycerol	Phosphatidyl-choline	Phosphatidyl-ethanolamine
14:0	4.5	4.0	tr.	tr.	tr.
16:0	62.2	35.6	38.6	49.2	36.1
16:1	tr.	tr.	tr.	tr.	tr.
18:0	0.7	0.8	tr.	2.2	8.0
18:1	2.3	2.6	2.4	7.1	12.8
18:2	0.8	2.0	tr.	7.9	5.6
18:3	tr.	3.0	5.8	tr.	tr.
20:0	tr.	tr.	tr.	2.5	2.6
20:3	4.4	7.6	1.9	14.5	18.4
20:4	25.0	44.3	51.2	16.5	4.9
22:0	tr.	tr.	tr.	tr.	11.5

tr. = trace amounts only.

Table II. Fatty acid synthesis in isolated cyanelles from *Cyanophora paradoxa*. Distribution of radiolabelled acids among the cyanelle lipids.

Lipids	Radioactivity [% of total]
Acyl-CoA	9.7
Phosphatidylglycerol	12.1
Monogalactosyldiacylglycerol	4.5
Digalactosyldiacylglycerol	16.5
Sulfoquinovosyldiacylglycerol	2.1
Phosphatidylcholine	21.7
Free fatty acids	14.3
Diacylglycerol	17.3
Unidentified	1.8

containing lipids were labelled without any preferential incorporation. The presence of exogenously applied ATP in the incubation medium had no significant influence on this labelling pattern.

Further analysis of the fatty acid synthase system and of the general capacities of lipid synthesis in isolated *Cyanophora* cyanelles and their cooperation in this respect with the cell itself would be very interesting when the energy and reducing equivalents providing photosynthetic activities of these cyanelles *in vitro* are improved. Work in this direction is in progress.

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