

VARIATIONS OF THE LIPID COMPOSITION DURING THE FORMATION OF CYSTS IN THE GREEN ALGA *PROTOSIPHON BOTRYOIDES*

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(Received 27 December 1974)

Key Word Index—*Protosiphon botryoides*; Chlorophyceae; green algae; lipid composition; unsaturated fatty acids; carotenoids; chlorophyll; mineral nutrition.

Abstract—The formation of cysts in the alga *Protosiphon botryoides*, grown in a poor medium, is accompanied by important changes of the lipid composition of the total homogenate. Comparative to the green alga, grown in normal conditions, the following differences were observed in the homogenate and in the red globules which appear in the cytoplasm: an increase in the total fatty acids and carotenoids and a decrease in the chlorophyll content, an increase in the neutral lipids content and a higher amount of polyunsaturated fatty acids. These biochemical changes are discussed in relation to the cytological modifications observed previously.

The formation of cysts in the green alga *Protosiphon botryoides* grown under conditions of progressive exhaustion of nutrients is accompanied by many ultrastructural changes described previously [1]; the cytoplasm is invaded by dense globules; the volume of the plastids decreases and the structure of the intraplastidial membrane is modified. It was of interest to study the changes in lipid composition accompanying these structural changes, and to determine what lipids accumulate in the pigmented globules.

RESULTS

The content of total fatty acids, phospholipids, chlorophylls and keto-carotenoids of the algae grown under normal conditions ('green stage') or in a nutrient-poor medium ('red cysts') is shown in Table 1. It can be seen that growth under deficient conditions is accompanied by a great increase in the amount of total fatty acids and keto-carotenoids, and a marked decrease in the chlorophyll content.

Analysis of the different classes of lipids present (Table 2) revealed further differences between the algae in the green stage and the red cysts. The lipids of the green algae are rich in phospholipids and contain equal proportions of galactolipids and neutral lipids while the lipids of the cysts (both homogenate and globules) are mainly neutral lipids accompanied by a low content of phospholipids and galactolipids. The fact that the lipids of the globules are mainly neutral lipids is in accordance with their low density as shown by the fact that they float on the surface of the supernatant after centrifugation.

Table 1. Lipid and pigment content of the green stage and of the red cysts of *Protosiphon botryoides*

Component	Content (mg/100 mg protein)		
	Green stage	Red cysts	
		Homogenate	Globules
Total fatty acids	1.73	5.27	33.01
Phospholipids	1.16	0.90	1.90
Chlorophylls	0.73	0.05	0.06
Keto-carotenoids	tr	0.59	0.75

Table 2. Relative distribution of the lipid classes in the green stage and in the red cysts of *Protosiphon botryoides*

Lipid classes	% Total lipids		
	Green stage	Red cysts Homogenate	Globules
Phospholipids	48.0	22.3	10.7
Monogalactosyldiglycerides	14.6	6.3	6.2
Digalactosyldiglycerides	13.3	3.4	4.7
Neutral lipids	23.7	68.0	79.0

The fatty acid composition of algae in the green stage and of the cysts showed marked differences (Table 3). The fatty acids of the cysts are characterized by greater amounts of unsaturated fatty acids (oleic, linoleic, linolenic) while palmitic and especially palmitoleic acids are less abundant than in the green algae.

DISCUSSION AND CONCLUSIONS

The results presented here show that the formation of cysts in the alga *Protosiphon botryoides* is accompanied by important modifications in lipid composition. The quantitative variations of the lipid classes (Table 2) are in accordance with the cytological observations, since the ultrastructural changes of the plastidial membranes and the invasion of the cytoplasm by pigmented globules are accompanied by a decrease in the percentage of phospholipids—(essential constituents of the cell membranes)—and galactolipids—(generally considered as uniquely located in the plastids) and an increase in neutral lipids, which may be

regarded as storage lipids. The globules only contain a small quantity of phospholipids and galactolipids; but since they also contain chlorophyll, they must contain some membranes. It is difficult to know, however, whether the phospholipids and the galactolipids observed are really associated with such membranes or are free in the globules.

It is clear from Table 3 that the fatty acid composition of *Protosiphon botryoides* at the green stage is similar to that of other green algae, i.e. *Acetabularia mediterranea* [3] or *Chlorella vulgaris* [4] [5], being rich in palmitic and oleic acids, and containing also unsaturated fatty acids. The cysts and the globules on the other hand are much richer in unsaturated fatty acids; this is similar to the observations of Czygan and Eichenberger [6] on the alga *Ankistrodesmus*. These authors found, in alga grown in a nitrogen-poor medium, a fatty acid composition comparable to that found in *Protosiphon*. Kleinig and Czygan [7], studying the carotenoids of *Protosiphon*, found that these compounds were esterified by saturated fatty acids but since they did not determine the total lipid composition of their alga, it is difficult to compare their results and the data given here. Newman [8], working on various vascular plants, observed an increase in the saturated fatty acid content of senescent leaves relative to young leaves; this discrepancy with our observations is probably due to the fact that this author considered the isolated plastids and not the entire cell.

The increase in the content of unsaturated acids in *Protosiphon* can be compared to the variations

Table 3. Fatty acid composition of the green stage and the red cysts (homogenate and globules)

Main fatty acids	% Total fatty acids			
	Green stage	Red cysts Homogenate	Globules	
C 14:2	1	tr	tr	
C 16	31.4	19.8	21.0	27.5
C 16:1	11.1	2.6	1.8	5.5
C 16:2	2.3	1.5	1.1	—
C 16:3	2.5	2.0	1.7	—
C 16:4 ?	7.4	7.2	6.4	—
C 18	3.0	2.5	2.5	7.0
C 18:1	12.4	24.2	24.2	34.0
C 18:2	6.5	15.1	15.6	12.0
C 18:3 γ	4.3	1.7	2.2	1
C 18:3	14.4	21.2	21.0	4.5
C 20	2.4	1.1	1.6	—

observed during the maturation of certain fruits or seeds [9]; as in the seeds, the lipids accumulated in the globules may constitute a storage pool which can be subsequently used by the alga.

EXPERIMENTAL

Material. Algae used in this work, *Protosiphon botryoides* (Kütz) Klebs, came from Indiana University; the growth medium was that of Bristol [1] slightly modified; when the medium was often changed, the algae grew actively ('green stage') but if it became progressively exhausted, cell divisions stop and, after some weeks, the algae became red ('red cysts').

Preparation of the cell homogenates and isolation of pigmented globules. 'Green algae' or the 'red cysts' were collected and homogenized in a buffered soln of sucrose. Red globules of the cysts were isolated by centrifugation at 20000 *g* for 2 hr when they float to the surface of the supernatant [2].

Extraction and analysis of the lipids. Total lipids of the homogenates or of the globules were extracted according to the technique of Bligh and Dyer [10]. The lipid extract was dissolved in C₆H₆-EtOH (4:1). Methyl extracts of the fatty acids were extracted from 1 ml of lipid extract by using the method of Metcalfe *et al.* [11] and analyzed by GLC [9]; the amount of each fatty acid was determined after addition of an internal standard (methyl heptadecanoate). For the study of the lipid classes, the total lipid extract was separated by TLC on Si gel, using a double development procedure [12, 13]; the zones corresponding to the lipids were scraped from the plates and the fatty acids extracted, methylated and determined by GLC [14].

Quantitative analysis of the constituents. Phospholipid content was determined in the total lipid extract by the technique of Shibuya *et al.* [15]. Proteins were determined by the method of Gornall *et al.* [16] after treatment of the samples with 5% sodium deoxycholate. The amount of chlorophyll was determined by the procedure of Wintermans and De Moto [17]. The approximative quantity of the keto-carotenoids was

estimated by measuring the absorbance at 475 nm and by assuming a $E_{1\%}^{1\text{cm}}$ equal to 2200.

Acknowledgements—The authors are much indebted to Prof. G. Giraud (Director of the Laboratoire de Botanique) and to Prof. P. Mazliak (Director of the Laboratoire de Physiologie Cellulaire) for critical reading of the manuscript.

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