FATTY ACIDS IN THE THERMOPHILIC ALGA, *CYANIDIUM CALDARIUM*[1]

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SUMMARY: Lipids and fatty acids were compared in 20° and 45° photoautotrophically grown *Cyanidium caldarium*. Glycolipids were increased by 90% in 45° as compared to 20° grown cells. Linolenic acid was the most abundant fatty acid in the 20° grown cells but was very low in the 45° cultures. The replacement of linolenic acid in glycolipids might likely be met in 45° grown cells by linoleic acid since linoleic acid was increased several fold. If polyunsaturated fatty acids play a special role in photosynthesis, it would appear that linoleic acid has assumed this role in high temperature grown cells. On the other hand, phospholipids were decreased by 40% in 45° as compared with 20° grown cells. Yet, the marked decrease in linolenic acid in phospholipids is not replaced by linoleic acid.

Several different factors have been proposed which could account for the ability of cells to survive large changes in growth temperature. Proteins from thermophilic organisms have been found generally to be more heat stable than the proteins from mesophiles which seems to be due to the primary sequence of the proteins (Josse and Harrington, 1964; Campbell and Manning, 1961; Devanathan *et al.*, 1969; and Krausz and Becker, 1968). Stabilization of proteins against heat denaturation by fatty acids has been reported (Boyer *et al.*, 1946), while polyamines have been reported to stabilize ribosomes against denaturation (Datta *et al.*, 1969). The nature of ribosomal protein has also been reported to be dependent upon the temperature for optimum growth of certain organisms (Kang, 1970; Mangiantini *et al.*, 1965; and Flaks *et al.*, 1966). The

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fact that the melting points of fatty acids are markedly influenced by the degree of unsaturation correlates with reports that the amount of unsaturated fatty acids incorporated into phospholipids of organisms is dependent upon their growth temperature (Esfahani *et al.*, 1969; and Rao, 1967).

It has been proposed that linolenic acid is an essential component of the photosynthetic oxygen evolving reaction chain (Erwin and Bloch, 1963). This has been supported by the finding that the concentration of polyunsaturation especially in the galactosyldiglycerides is a function of the cell chlorophyll content and that often linolenic acid is the major fatty acid in these glycolipids (Wolf *et al.*, 1966; Melandri *et al.*, 1970; and Crombie, 1958). It is important to note that the galactolipids are associated almost exclusively with the chloroplast lamellae (Wintermans, 1960; and Allen *et al.*, 1966). Because a marked decrease in glycolipids and a disappearance of linolenic acid in heterotrophically grown *Cyanidium caldarium* at high growth temperature (55°C) was accompanied by bleaching (Kleinschmidt and McMahon, 1970), it was thought that a photoautrophic growth of this alga might not experience a decrease in either glycolipid or linolenic acid even at a high temperature. This investigation was done to clarify this point and to further elucidate the role of fatty acids in contributing to the ability of living organisms to withstand large variations in growth temperature.

METHODS

The alga obtained from hot springs in Yellowstone National Park was grown under approximately 300 ft-c illumination on a medium consisting of double the concentration of Allen's medium AA (Allen, 1963). Either air or 100% CO_2 was introduced into the growing culture after filtration through glass wool filters. The temperature was maintained by immersing the growth tanks in a temperature-controlled bath of water overlaid with mineral oil. Cells were harvested after several weeks of growth and freeze-dried. Weighed portions of the dried cells were extracted with chloroform for 20 hours in a Soxhlet extractor. Lipid samples of 12 mg or less were applied to silicic acid

columns for separation into classes on the basis of their polarity as described previously (Kleinschmidt and McMahon, 1970; and Lis *et al.*, 1961). The eluted samples were dried under nitrogen and weighed. The lipid samples were saponified, the free fatty acids isolated and converted to methyl-esters (Morrison and Smith, 1964). The esters were analyzed by gas-liquid chromatography. Diethylene glycol succinate stabilized on Anakrom ABS, 80/90 mesh was used as the stationary phase and the column was maintained at 190° C. Known fatty acid standards (Applied Science) were used for comparison of retention time and quantitive analysis of the algal fatty acids.

Pre-coated silica gel plates (Brinkman Instruments Co.) dipped in silver nitrate solution [$AgNO_3$:methanol:water (10:90:25) w/v/v] were used to confirm the degree of unsaturation and configuration of the fatty acids (Wood and Snyder, 1966).

RESULTS

The total amount of chloroform extractable lipid shown in Table I obtained from the 45° air-grown cells was one-third higher than that obtained from the 20° air-grown cells. The glycolipid content (fraction III) of the 45° cells was 90% higher than that in the 20° cells. The temperature induced phospholipid change (fraction IV), however, was opposite to the glycolipid change in that there was 40% more phospholipid in the 20° air-grown cells than in the 45° air-grown cells. The lipid fractions from the 100% CO_2-grown cells follows similar in quantity to the lipid classes of the 45° air-grown cells.

Table II shows the major fatty acids in the glycolipid and phospholipid fractions at the two growth temperatures. The increase in total fatty acids in glycolipids from 20° to 45° air-grown cells is comparable to the glycolipid increase. Yet, the types of fatty acids are markedly changed in the glycolipids with an increase in growth temperature. There is a large decrease of linolenic acid at the high growth temperature. Although linolenic acid is low, it should be noted that linoleic acid is increased by ten-fold which results in net two-

TABLE I

Effect of Growth Conditions on Lipid Distributions into Classes

mg lipid/g dry cell weight

	Air 20°	Air 45°	100% CO_2 45°
Total lipid	45	60	65
Lipid fraction			
I	3.4	2.3	1.8
II	5.8	8.9	5.5
III	21.4	41.0	48.0
IV	11.5	8.3	7.8
Sum eluted	42.1	61.5	62.3
% Recovery	94	102	96

Each fraction from the silicic acid column contains: I: Hydrocarbons, sterol esters and waxes; II: Mono- di- and triglycerides, free fatty acids and free sterols; III: Glycolipids; and IV: Phospholipids.

fold increase in total unsaturated fatty acids in the glycolipids. The saturated fatty acids were increased even more, so that at the high growth temperature the ratio of unsaturation to saturation in fatty acids of the glycolipids decreased from 3:1 in the 20° air-grown cells to 1.7:1 in the 45° air-grown cells. The ratio of 2.3:1 in these fatty acids in the 45°, 100% CO_2-grown cells was intermediate between the ratios at two temperatures of the air-grown cells.

The phospholipid fatty acids did not show an increase in either total fatty acids or unsaturated fatty acids (Table II), but due to the absence of linolenic acid in the 45° grown cells there was a 40% reduction of unsaturated fatty acids of both air- and CO_2-grown cells as compared to the 20° grown cells.

TABLE II

Fatty Acids in Glycolipids and Phospholipids from Chloroform Extracts of Air and 100% CO_2 Grown Cultures of *C. caldarium*. Abbreviations for each of the fatty acids are: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3).

mg fatty acid/gm dry cell weight

	16:0	18:0	18:1	18:2	18:3	Total fatty acid
Growth conditions			Glycolipids			
20° in air	1.44	0.05	0.57	1.03	3.10	6.19
45° in air	5.90	0.18	2.60	7.70	0.21	16.59
45° in 100% CO_2	6.20	0.14	2.90	11.20	0.17	20.61
			Phospholipids			
20° in air	0.48	0.25	0.48	0.78	1.44	3.43
45° in air	0.58	0.26	0.77	0.79	0.33	2.73
45° in 100% CO_2	0.53	0.20	0.90	0.70	0.18	2.51

DISCUSSION

The absence or greatly reduced amounts of linolenic acid in the high temperature cells confirms earlier work (Kleinschmidt and McMahon, 1970) and shows that the concentration of this fatty acid in *Cyanidium* is not a direct function of chlorophyll content as seems to be the case in other plants (Wolf *et al.*, 1966; and Melandri *et al.*, 1970). However, the increase in total glycolipid polyunsaturated fatty acids in the 45° grown cells over that found in the 20° grown cells suggests that there is a requirement for a given amount of polyunsaturation by photosynthesizing chloroplasts in this alga. When the trienoic acid is depleted due to high temperature stress, the possible requirement for polyunsaturated fatty acids may be met by the increase in linoleic acid. Thus there may still be general agreement of this report with the

studies which show that polyunsaturated fatty acid content is a function of chlorophyll concentration. The ratio of unsaturated to saturated fatty acids in the 45°, 100% CO_2-grown cells being intermediate between the ratio in the 45° and 20°, air-grown cells is a further indication of this role. These data support a previous report (Seckbach *et al.*, 1970) that 45°, CO_2-grown cells are more highly pigmented than the 45°, air-grown cells, while at a lower temperature the air-grown cells have a higher pigment content than the CO_2-grown cells. Thus, the change in the relative amount of unsaturation seems to correlate with the change in pigment content under different growth conditions.

The situation in the phospholipids is quite different. The absence of linolenic acid causes a marked decrease in unsaturated fatty acids as well as in total fatty acids. These results are quite interesting when it is noted that the phospholipids are located mainly in the cytoplasmic membrane but the galactolipids are restricted to the chloroplast lamellae (Allen, 1970; Allen *et al.*, 1966; Wintermans, 1960). Thus the phospholipids experience a reduction in unsaturation at the high growth temperature in order to possibly increase the melting point of strategic membrane matrixes, but the galactolipids may have an absolute requirement for polyunsaturated fatty acids, unrelated to melting point but closely connected to photosynthesis. The work of Kleinschmidt and McMahon (1970) taken in conjunction with this report tends to support this concept. They found that a 60% reduction in the glycolipids of thermophilically grown cells on a heterotrophic medium with galactose and malt extract as carbon sources was accompanied by bleaching compared to the glycolipid content in the green mesophilically grown cells on the same medium. When the cells were grown on an autotrophic medium in the present report, both temperature conditions resulted in green cultures, and the 45° grown cells had even more glycolipid than did the 20° grown cells.

Rosenberg (1967) suggested the feasibility of a combination of polyunsaturated fatty acids and chlorophyll by the fact that the methyl groups of the phytol chain of chlorophyll fit in juxtaposition to the double bonds of poly-

unsaturated fatty acids. Gaines *et al.* (1964) have further shown that unsaturated fatty alcohols are miscible with chlorophyll but that saturated alcohols are not.

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