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Photosynthesis, lipids and proteins in the cyanobacterium *Synechocystis* PCC 6803 as affected by temperature

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Abstract

The cyanobacterium *Synechocystis* PCC 6803 was grown photoautotrophically in an inorganic medium at constant growth temperatures of 20, 38 (control) or 43 °C for 9 h. The up and down-shift of cultivation temperature decreased the growth as measured by culture absorbance and chlorophyll *a* content. However, high temperature slightly increased the oxygen evolution while temperature lower than control inhibited oxygen evolution during the whole incubation period. The protein synthesis studied by ^{14}C-labeled protein declined under low temperature by about 50 %. The fatty acid pattern is characterized as lacking in C_{20}/C_{22} acids but containing large amounts of C_{16} and C_{18} polyunsaturated fatty acids, 16:2 and 18:3 in particular. The lower temperature increased the percentage of monounsaturated fatty acids while higher temperature increased the saturated fatty acid content in total lipids and lipid classes studied.

Key words: chlorophyll, fatty acids, oxygen evolution, phospholipids

Introduction

Cyanobacterium *Synechocystis* PCC 6803 is a prokaryotic cell with two membrane systems. One is an envelope, composed of outer and inner (plasma) membranes separated by a peptidoglycan layer, and the other is an intracellular photosynthetic membrane system called thylakoid membrane. These organisms are similar to that of the eukaryotic plant chloroplast (Stanier and Cohen-Bazire 1977) in respect to lipid composition being characterized by an abundance of galactolipids as well as by a high content of α-linolenic acid (Murata and Nishida 1987). This may open a molecular-biological approach to the changes of the growth temperature problems. Environmental temperature is an important factor in the life of plants and animals. Several authors (Knipprath and Mead 1966, Farkas and Csengeri 1976) have reported

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Abbreviations: Chl - chlorophyll; MGDG - monogalactosyl diacylglycerol; OGDG - digalactosyl diacylglycerol; PL - phospholipids; TCA - trichloroacetic acid.

that poikilothermic animals respond to lower environmental temperature by the accumulation of high percentage of unsaturated fatty acids in their bodies. Changes in membrane lipid fatty acid composition as a function of the temperature of growth are particularly evident in the prokaryotic microorganisms such as *Tetrahymena* (Thompson and Nozawa 1977), *Anacystis nidulans* (Sato *et al.* 1979), *Bacillus stearothermophilus* (McElhaney and Souza 1976) and *Escherichia coli* (Okuyama 1969). The temperature effects are studied in two ways: variation in fatty acids composition in isothermally growing organisms, and transient changes in fatty acids composition upon a growth-temperature shift (Okuyama 1969). An increase in environmental temperature typically resulted in the production of membrane lipids containing a relatively higher proportion of saturated fatty acids and lower proportion of unsaturated fatty acids. In addition, an increase in the growth temperature often results in the production of membrane lipids whose hydrocarbon chains have a great average length (Chapman 1975). These changes in fatty acids composition are interrupted in terms of the regulation of membrane fluidity for the proper functioning of biological membranes (Thompson and Nozawa 1977). In the present investigation, we study the variation in the fatty acid composition of lipids and some physiological processes after downward and upward shift of growth temperature.

Materials and methods

Algae and cultivation: *Synechocystis* PCC 6803 (high temperature tolerant strain) was obtained from the Pasteur Culture Collection, Paris, France. The cells were grown photoautotrophically in an inorganic medium (Allen 1968) at constant temperature 20, 38 and 43 °C for 9 h. The cultures were kept under continuous light (120 W m^2) and supplied with a mixture of 97 % air and 3 % CO_2. Growth of the cultures was monitored spectrophotometrically by measuring the absorbance at 800 nm and by measuring the chlorophyll *a* content as described by MacKinney (1941).

Oxygen evolution was measured using the intact cells without exogenous added electron donor or acceptor by means of a Clark-type electrode in 3 cm^3 samples in a thermostated closed perspex cuvette at 25 °C under saturated light.

Lipids were extracted from 10 cm^3 aliquots with absorbance of 0.8 by the method of Bligh and Dyer (1959). The lipid classes were separated by thin-layer chromatography on precoated silica-gel plates (*5721-Merck*, Darmstadt, Germany) with a mixture of benzol:acetone:distilled water (30:91:8 v/v) as the mobile phase for separation galactolipids (digalactosyl diacylglycerol and monogalactosyl diacylglycerol) and mixture of petroleum ether:diethyl ether:acetic acid (85:15:1 v/v) for separation of total phospholipids according to Malins and Mangold (1960). After development, the plates were dried in a stream of CO_2 and the lipid classes were identified using 8-anilino-1-naphthalene-sulphate (ANS) fluorescence elution and phosphatidyl choline, phosphatidyl glycerol, sulphoquinovosyl diacylglycerol, diagalactosyl diacylglycerol and monogalactosyl diacylglycerol (*Seradary Research*

Laboratory, Novato, U.S.A.) as standards. The separated lipids were taken into ampoules, containing 5 % HCl (m/v) in dry methanol and transesterified at 80 °C within 2 - 3 h under N_2. The methylesters of the fatty acids were extracted into n-hexane from esterification mixture after dilution with an equal volume of distilled water.

Gas chromatography: Methyl esters were analyzed on a gas liquid chromatographic system (*Hewlett Packard 5890 Series II*, Palo Alto, U.S.A.) equipped with a capillary column coated with SP 2330 of 0.25 μm thickness (0.25 mm i.d. × 30 mm, *CPS-Li Quardex*, New Haven, U.S.A.). High purity nitrogen was applied at a pressure 230 kPa, hydrogen 100 kPa and oxygen 280 kPa. The dual column system was programmed from 160 °C to 200 °C to give partial separation of 18:3 at the rate 0.04 °C s^{-1}. The detector and injector temperature were 220 °C. Identification of the peaks was made using linoleonic standard and by plotting logarithm of relative elution temperature versus the number of carbon atoms (Schmidt and Wynne 1967). To calculate the percentage composition using *Hewlett-Packard 3396 A* integrator, all peaks emerging between lauric (12:0) and linolenic (18:3) were included in calculations.

Total phospholipids: Phosphorus content from the lipid extract was determined according to the method of Rouser *et al.* (1970), spectrophotometrically at 790 nm. The amount of phospholipids was derived from lipid phosphate as μmol mg^{-1} (protein).

Protein labelling: To determine the rate of protein synthesis 1 cm^3 aliquots of the cultures was removed at the designated intervals, pulse labelled with 0.5 MBq of ^{14}C-uniformaly labelled proteins hydrolysate for 1 h and immediately precipitated with 10 % trichloroacetic acid (TCA). The precipitates were collected by centrifugation and washed with cold 5 % trichloroacetic acid and ethanol. The radioactivity was measured in a liquid scintillation spectrometer.

Results

The change in temperature exerted an immediate response on growth of *Synechocystis* cells (Fig. 1a). Increasing temperature up to 43 °C slightly decreased, and low temperature (20 °C) completely inhibited the cell growth. The high and low temperature declined Chl *a* content as compared with control cells. After 9 h, Chl *a* content was lowered by 43 % and 74 % at 43 °C and 20 °C, respectively (Fig. 1b). Similarly, the high temperature (43 °C) slightly increased the oxygen evolution after 6 h growth, while low temperature (20 °C) severely inhibited the oxygen evolution during the whole incubation period. Protein synthesis was progressively reduced by about 50 % at low temperature (Fig. 3). Growth temperature 43 °C induced a maximum level of protein synthesis after 3 h, then decreased it by about 30 % as compared with the control growth temperature (38 °C).

The major fatty acids of *Synechocystis* PCC 6803 were identified as 16:0, 16:1, 16:2, 18:0, 18:1, 18:2 and 18:3. Traces of 14:0 and other unidentified acids were

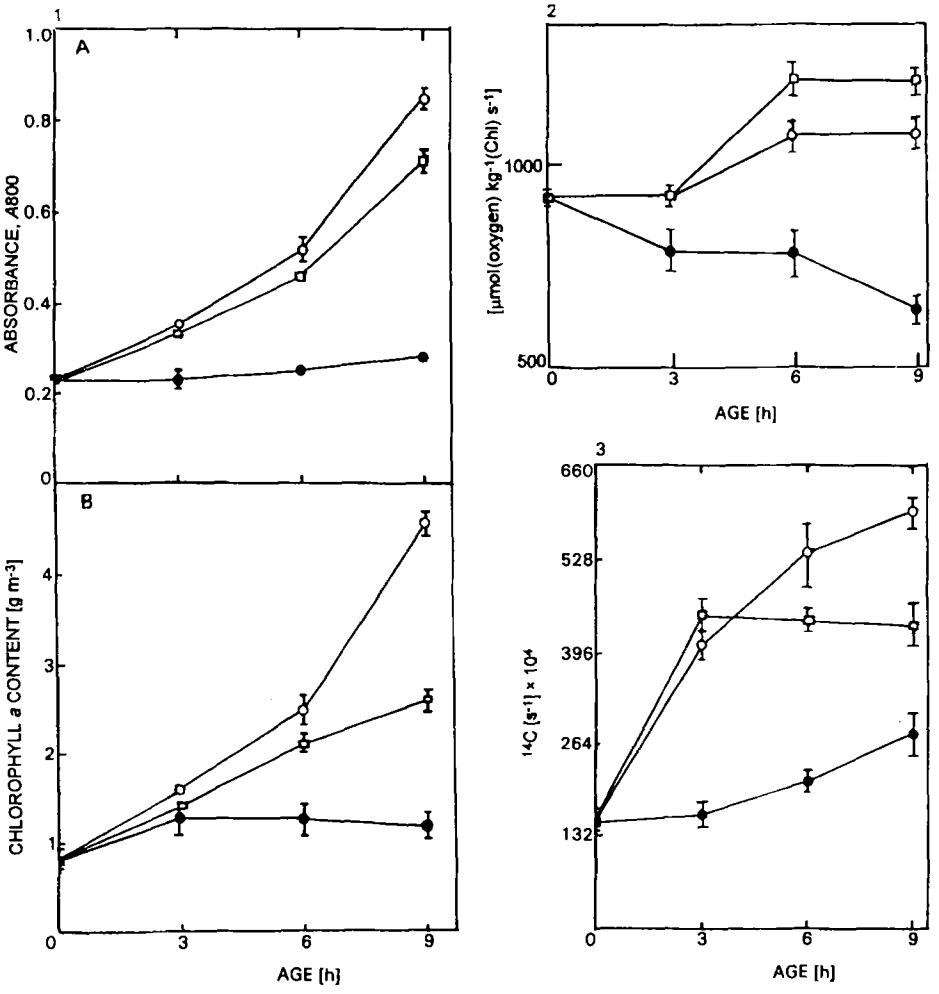

Fig. 1. Effect of culture temperature on the growth of *Synechocystis* PCC 6803. (*A*) Absorbance measured at 800 nm, (*B*) chlorophyll *a* content. Control 38 °C (*open circles*), 20 °C (*closed circles*) and 43 °C (*squares*). The bars indicate the standard deviation of the means.

Fig. 2. Effect of culture temperature on the photosynthetic activity measured as oxygen evolution of the intact cells of *Synechocystis* PCC 6803. Control 38 °C (*open circles*), 20 °C (*closed circles*) and 43 °C (*squares*). The values are the means of three measurements.

Fig. 3. Effect of up and down-shift of culture temperature on protein synthesis measured by ^{14}C-protein labelling in *Synechocystis* PCC 6803. Control 38 °C (*open circles*), 20 °C (*closed circles*) and 43 °C (*squares*). The values are the means of three measurements.

detected, but they accounted for less than 1 % of the total fatty acids and were not further studied. Table 1, indicated that at high temperature, the saturated fatty acids made up an increased percentage of the total fatty acids.

Table 1. Effect of culture temperature on the relative proportion of various fatty acids of total lipids and phospholipids in *Synechocystis* PCC 6803.

Fatty acid	[% of total lipids]			[mol kg^{-1}(phospholipids)]		
	20 °C	38 °C	43 °C	20 °C	38 °C	43 °C
16:0	40.0	70.2	72.0	28.8	49.5	52.6
16:1	19.0	8.2	6.5	27.0	29.0	21.3
16:2	-	-	-	13.9	5.8	9.7
18:0	6.0	8.0	13.0	9.2	3.8	5.3
18:1	21.0	10.0	5.2	8.3	8.1	6.2
18:2	8.7	2.0	2.5	7.4	3.6	3.6
18:3	4.7	1.0	5.3	5.3	0.4	-

At low temperature (20 °C), the percentage of monounsaturated fatty acids increased. These increases were balanced by a significant decrease in unsaturated fatty acids. Concerning phospholipids, the linolenic acid increased under low temperature (5.3 %) and could not longer be detected under high temperature. As a result, the average number of double bonds in PL molecule increased from 54 to 93 upon lowering the growth temperature from 38 °C to 20 °C. In order to determine which particular lipids are responsible for the variation in the fatty acid composition with growth temperature, we examined the fatty acid composition of individual lipid classes (Table 2). At low temperature, hexadecadienoic acid increased significantly in DGDG, while linoleic acid increased in MGDG. Generally, in all lipid classes studied as well as total lipids the unsaturated fatty acids increased at lower temperature while, they decreased under high temperature.

Table 2. Effect of culture temperature on the relative proportion of the fatty acids of galactolipids of *Synechocystis* PCC 6803.

Fatty acid	[% of MGDG]			[% of DGDG]		
	20 °C	38 °C	43 °C	20 °C	38 °C	43 °C
16:0	37.5	55.2	58.1	37.1	55.1	52.8
16:1	14.1	24.6	27.1	4.9	18.9	15.2
16:2	13.5	7.0	4.8	29.7	9.3	7.8
18:0	5.9	6.2	3.9	13.9	12.5	9.4
18:1	8.7	5.6	4.8	9.4	8.0	12.3
18:2	18.9	1.1	1.0	3.7	3.3	1.9
18:3	1.6	6.3	0.2	1.2	0.8	0.5

Discussion

Changes in glycerolipid and degree of unsaturation of fatty acids with altered growth temperature have already been investigated in many organisms (Süss and Yordanov 1986, Thomas *et al.* 1986) and in *Synechocystis* PCC 6803 (Wada and Murata 1989). The desaturation is lower when the organisms are grown at higher temperature, because the cells in cold environment need unsaturated fatty acids of lower melting point to prevent increased rigidity of cellular membrane and *vice versa* (Okuyama 1969). The present data indicate that the same relationship is observed between growth temperature and desaturation of fatty acids of total fatty acids and its fractions in *Synechocystis* PCC 6803. From this point there was apparently a linear decrease in the number of double bonds in the fatty acids and increase in the number of carbon atoms of the fatty acids (Ono and Murata 1981). The increase in desaturation in *Synechocystis* and the decrease in average chain length of fatty acids on lowering the growth temperature predict a downward shift of temperature for the transition between the liquid-crystalline and phase separation states of the membrane lipids (Sinesky 1974). Further increase in the growth temperature resulted in an increase of O_2 generation photochemically, which induces lipid peroxidation, membrane damage and kills cells (Michelson and Buckingham 1974).

We propose also that, high temperature acclimation phenomenon of *Synechocystis* thylakoids could be characterized by the operation of two-phase mechanism. An elevated level of lipid saturation appears to be the main factor govering the slow, long-term adaptation phase.

A remarkable increase in the ratio of protein could also be detected upon high temperature acclimation of thylakoids derived from *Synechocystis* PCC 6803 (Vigh *et al.* 1990). Both tendencies act towards reduction of the overall level of membrane fluidity and prevention of non-bilayer lipid structure in heat exposed thylakoid (Horvath *et al.* 1986, Schlame *et al.* 1990).

Photosynthetic oxygen evolution has long been known to be particularly sensitive to heat damage (Berry and Björkman 1980, Vigh *et al.* 1989). The results of this investigation demonstrated that increasing growth temperature to 43 °C, a slight increase in oxygen evolution was obtained. This increase may be due to enhancement of the electron transport process which leads to generation of oxygen (Fig. 2). According to Fork *et al.* (1987), below the critical temperature heat pretreatment had little or no effect and above which there is a dramatic reduction in oxygen evolution efficiency. It is suggested that high temperature adaptation induced reduction of heat susceptible tested on terms of recovery and capacity for resuming cell growth and associated with immediate damage to photosynthetic apparatus resulted from heat-treatment leading to decrease chlorophyll formation, followed by decreasing in growth rate (Berry and Björkman 1980).

Elevation of growth temperature of the cells greatly increased incorporation of ^{14}C-amino acids into TCA precipitable proteins at temperature in the range of 20 to 38 °C. These data may be explained either by the presence of several amino acid pools with more label entering the pools for protein synthesis, or by increasing rates of protein synthesis under heat-stress conditions in cells preadapted to higher growth

temperature (Ballinger and Pardue 1983). It is suggested that less thermotolerant, low temperature adapted cells labelling for proteins gradually diminished.

In summary, it is evident that the cyanobacterium *Synechocystis* PCC 6803, responds to low temperature by desaturating the fatty acids of membrane lipids. The lipid fatty acid desaturation is regarded as an emergency acclimation to compensate for the decrease in membrane fluidity due to a decrease in temperature. On the other hand, high temperature increased the saturated fatty acids. The conversion of saturated to unsaturated fatty acids is promoted by low growth temperature in *Synechocystis*.

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