

Desaturation of fatty acids as an adaptive response to shifts in light intensity

Geda L. Klyachko-Gurvich^{a,*}, Lev N. Tsoglin^a, Jiří Doucha^b, Jiří Kopetskii^b, Irina B. Shebalina (Ryabykh)^a and Victor E. Semenenko^a

^aTimiryazev Institute of Plant Physiology, Russian Academy of Sciences, ul. Botanicheskaya 35, Moscow, 127276 Russia

^bInstitute of Microbiology, Czech Academy of Sciences, Department of Autotrophic Microorganisms, Opatovicki Mlyn, Třebon, 37901 Czech Republic

*Corresponding author, e-mail: ifr@ippras.ru

Received 26 May 1999

The comparative study of various unicellular algae, characterised by different carbon chain lengths and different numbers of double bonds per fatty acid (FA) chain, exhibited some similarity in the mechanisms of their response to changes in light conditions, in terms of FA metabolism. In all cases, the optimisation of photosynthetic process resulted in some increase in the relative content of the most unsaturated FA, i.e. C16:3 ω 3 and C18:3 ω 3 acids in *Chlorella* cells, C16:4 ω 3 and C18:3 ω 3 in *Dunaliella* and *Chlamydomonas*, C20:5 ω 3 in *Porphyridium*, and C18:2 ω 6 in *Synechocystis* sp. As a rule, these FA were esterified to monogalactosyldiacylglycerols (MGDG), the predominant lipids of thylakoid membranes. Such an increase in the relative content of the polyunsaturated FA usually occurred during the period when the photosynthesis, as well as the biosynthesis of FA de novo, were transiently inhibited following shifts in environmental conditions even at their optimisation. The increase in the relative content of the most unsaturated FA could be performed via desaturation of

their immediate precursors. In turn, the deterioration of life conditions (decrease in the light intensity, ageing of cells or cultures, and others) resulted in the accumulation of these precursors. As a result, the cell could change its FA composition without alteration of the whole multistage process but only at the rate of this end reaction of polyunsaturated FA biosynthesis. In the majority of algae, these polyunsaturated acids were ω 3-homologues, regardless of the difference in their structures, but in some cyanobacteria (e.g. *Synechocystis*) the relative content of ω 6-FA increased. The acceleration of ω 3-FA biosynthesis could be observed, regardless of changes in the total index of unsaturation. This FA desaturation was shown to correlate with the activity of photosystem I (PSI). The specificity of this reaction enables us to assume it to be an adaptive response which provides alterations to lipid-protein interactions in the membrane that may be important for the self-assembly of active chlorophyll-protein complexes for photosynthetic apparatus.

Introduction

The adaptive increase in lipid desaturation as a response to a drop in temperature is a well known phenomenon intrinsic to various organisms (Hitchcock and Nichols 1971, Kreps 1981). The desaturation is suggested to increase the fluidity of membranes that compensate the temperature-induced shifts and is thought to be one of the generally accepted mechanisms of low-temperature acclimation. This adaptive response has been extensively studied at enzymatic and gene levels (Kreps 1981, Los 1997).

In contrast, the change in fatty acid (FA) desaturation, as a response to the alteration of light conditions, is a less comprehensive process. An increased level of FA desaturation induced by an increase in light intensity has been observed in many studies; however, there are many contradictions concerning this phenomenon and its interpretation (Hitchcock and Nichols 1971, Trémolières et al. 1973, Klyachko-Gurvich et al. 1980, Harwood and Jones 1989). The possible role of fluctuations in the fluidity is less understand-

Abbreviations – FA, fatty acids; GL, glycolipids; LHC, light-harvesting complex; MGDG, monogalactosyldiacylglycerols; NL, neutral lipids; PL, phospholipids; PS, photosystem(s). *Fatty acid nomenclature* – The double bond location is designated as ' ω x', that is, the nearest to the methyl terminus double bond is located at 'x' carbon atom from this end (for example, C16:4 ω 3 means the location of double bonds at 4, 7, 10, and 13 carbon atoms starting from the carboxyl group).

able with respect to light than to temperature acclimation, although it is evident that it can facilitate electron and ion transport within a membrane as an inevitable component of the photosynthetic process. Moreover, the data recently reported have demonstrated that the increase in the membrane fluidity due to FA desaturation is not the only mechanism in terms of FA metabolism involved in the response to low temperatures (Kreps 1981, Ohlrogge and Browse 1995, Klyachko-Gurvich et al. 1997). In the majority of higher plants, it can hardly be an important mechanism of light or temperature adaptation as the α -linolenic acid (C18:3 ω 3) is the predominant constituent of membrane lipids, comprising up to 90% of total FA (Hitchcock and Nichols 1971, Harwood and Jones 1989). However, the algae of various taxa are characterised by the different chain lengths (usually 14–22 C atoms) and different numbers (1–6) and positions of double bonds at the carbon chain of their predominant FA (Hitchcock and Nichols 1971, Sud'ina and Lozovaya 1982). The variability of FA composition is also detected, step by step, at the screening of higher plants for these components. In such a case, the alteration of FA composition may be of greater importance.

The analysis of results obtained in numerous experiments on *Chlorella* cells allowed us to suggest (Klyachko-Gurvich et al. 1980) that in this organism, the light could control the end step of the trienoic (C16 and C18) acid biosyntheses via desaturation of the corresponding dienoic acids. The acceleration of this reaction increased the level of trienoic acids, and vice versa, its block resulted in the accumulation of dienoic acids as their biosynthetic precursors. As a result of such regulation, the cell changed its FA composition without alteration of the multistage process of polyunsaturated FA biosynthesis, but only in the rate of the final reaction, i.e. the introduction of a double bond at the ω 3-position of dienoic acids. Evidently, this is a relatively fast response to a shift in light conditions. These alterations initially occurred in the pool of FA esterified to monogalactosyldiacylglycerols (MGDG) (Trémolières et al. 1973, Klyachko-Gurvich et al. 1980, 1981).

In the experiments on the synchronous culture of *Chlorella*, the rapid increase in ω 3-trienoic and decrease in ω 6-dienoic FA esterified to MGDG was observed at the early stage of cell development (Klyachko-Gurvich et al. 1981). At this stage, the total FA content diminished, suggesting that the synthesis of trienoic acids is via desaturation of pre-existing dienoic acids. The cells at this stage of development are known to be incompetent to efficient photosynthesis but they are capable of ATP production and NADPH reduction, due to cyclic electron transport around photosystem I (PSI) (Chemersis et al. 1979, Tsoglin and Klyachko-Gurvich 1980).

The problem is whether the regularities, mentioned above for *Chlorella* cells, exist in the other types of algae characterised by the different structure of FA chains and, if so, what is the significance of this ω 3-FA formation?

In the present study, an attempt was made to consider these problems by studying the adaptive responses of various algae to the shifts of light intensity in physiological

ranges, or more exactly, to consider the response of algae in terms of FA biosynthesis to alterations in the factors significant for the processes of photosynthesis and growth, as light, evidently, does not directly affect the lipid metabolism.

Materials and methods

Algal strains

Algae were obtained from the collection of microalgal strains and mutants at the Institute of Plant Physiology of the Russian Academy of Sciences (IPPAS) (Catalogue of Microalgal Cultures 1991). Algae of various taxa were used: *Porphyridium cruentum* P-273, *Dunaliella salina* D-209, *Chlorella* sp. C-1, *Chlorella pyrenoidosa* C-26, *Synechocystis* sp. B-283. *Chlamydomonas reinhardtii* K⁺ mutants with different compositions of lipid-protein complexes were obtained from Dr V. G. Ladygin (Institute of Soil Science Photosynthesis RAS, Pushchino-on-Oka, Russia).

Culture conditions

In the majority of experiments, the algae were grown under conditions of constant temperature, continuous illumination, and aeration with air containing 2% CO₂. The control on the growth parameters and the content of proteins and pigments were carried out as described previously (Vladimirova and Semenenko 1962).

The red alga *P. cruentum* P-273 was grown as a batch culture on the Brody-Emerson medium or on seawater at 25–28°C (Klyachko-Gurvich et al. 1985). In other experiments, *P. cruentum* was grown as a continuous culture with the constant optical density of suspension corresponding to a level of dry biomass of about 3 mg ml⁻¹. The Brody-Emerson medium, supplemented with the microelements in accordance with Šetlik (Řezanka et al. 1987), was used. The culture was stabilised at a light intensity of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and then exposed to 500, 750, and again 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The exposure to each light intensity lasted 72 h. The biomass for analyses was sampled several times during the first day following the light change and later at intervals of 24 h.

The green alga *D. salina* D-209 was cultivated as a batch culture using the Semenenko-Abdullaev (Semenenko and Abdullaev 1980) nutrient medium at 28°C and continuous illumination with fluorescent tubes (300–360 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Vladimirova and Semenenko 1962).

The photosynthetic mutants of *C. reinhardtii* K⁺ were maintained in Petri dishes on the agarised medium supplemented with acetate at a light intensity of 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The cells were fixed with liquid nitrogen and then stored at –70°C (Pronina et al. 1990).

The synchronous culture of *Chlorella* sp. C-1 was grown in a cultivator with the plane-parallel walls (10 mm between the walls) illuminated with a xenon lamp, DKSTW-6000 (Russia). The light intensity was 2200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on the surface of the cultivator. The density of the suspension at inoculation was 0.15–0.2 mg ml⁻¹, to avoid the self-

shading of cells. Two preliminary cycles were carried out with light/dark periods (7/4 h respectively). The samples for analyses were withdrawn during the third cycle without the dark period. The culture was grown under continuous aeration with 2% CO₂ in air at 37°C (Tsoglin and Klyachko-Gurvich 1980). *C. pyrenoidosa* C-26 was grown as a batch culture under routine conditions at 26°C (Vladimirova and Semenko 1962).

The cyanobacterium *Synechocystis* was grown at 26°C on the N6 medium (Vladimirova and Markelova 1983, Klyachko-Gurvich et al. 1988). The cells were grown at a light intensity of 450 µmol m⁻² s⁻¹ and then transferred to different light conditions (130, 450, and 800 µmol m⁻² s⁻¹) for 46 h.

Analysis of lipids and fatty acids

Lipids were extracted with a mixture of chloroform and methanol (1:1, v/v) and re-extracted with chloroform. The fractions of glycolipids (GL), phospholipids (PL), neutral lipids (NL), and MGDG were isolated using the adsorptive and distributive column chromatography on silica gel (Whoelm, Eschwege, Germany) under low nitrogen pressure, providing an inert atmosphere during lipid separation, with the addition of ionol as an antioxidant to the solvents (Klyachko-Gurvich et al. 1980, 1985). The preparative and analytical precoated plates (Merck, Darmstadt, Germany) were also used for the isolation and identification of lipids. The FA composition of lipids was estimated by GLC using a Chrom-5 chromatograph equipped with a CI-100 integrator (Laboratory pristroje, Prague, Czech Republic) and a column packed with 3% (w/w) polyethyleneglycoladipate on celite-545 (Reachim, Russia). The FA were separated as methyl esters at a column temperature of 185–190°C. Identification of FA was performed using the authentic standards. The quantitation of FA content was carried out using 15:0, 17:1, or 20:0 acids as the internal standards. The calculation of FA was carried out using 2–3 chromatograms from each of 2–3 analytical replicates.

The relative standard error was 2–5% for major FA and not more than 10% for the minor constituents. To obtain the significant value differences, the number of FA assays was calculated, taking into account the deviations of the general population, and comprised 4–9 assays.

Results

Localisation of polyunsaturated fatty acids in the lipids of *Porphyridium cruentum*

In the red alga *Porphyridium*, the long-chain polyunsaturated FA of the C20 series are the predominant components of lipids (Hitchcock and Nichols 1971, Sud'ina and Lozovaya 1982, Harwood and Jones 1989). It was found that the GL contribute about 42% of the total pool of FA in *Porphyridium cruentum* (Table 1). MGDG comprised 30–50% of this pool. About 28 and 30% of the total FA were esterified to NL and PL, respectively. At the same time, up to 78% of eicosapentaenoic acid (C20:5ω3) was located in

the GL. Its concentration was only 15% in the PL and about 7% in the NL. In contrast, up to 66% of arachidonic acid (C20:4ω6) was located in the PL. The linoleic (C18:2ω6) acid mainly contributed to the NL (about 50% of total) (Klyachko-Gurvich et al. 1985; M. I. Yur'eva 1988. Thesis, Inst. Plant Physiol., RAS, Russia). Such a drastic difference is not a common phenomenon. Nevertheless, this trend can be observed to some degree in various algae (Harwood and Jones 1989).

Light-dependent changes in lipid and FA metabolism of *Porphyridium cruentum*

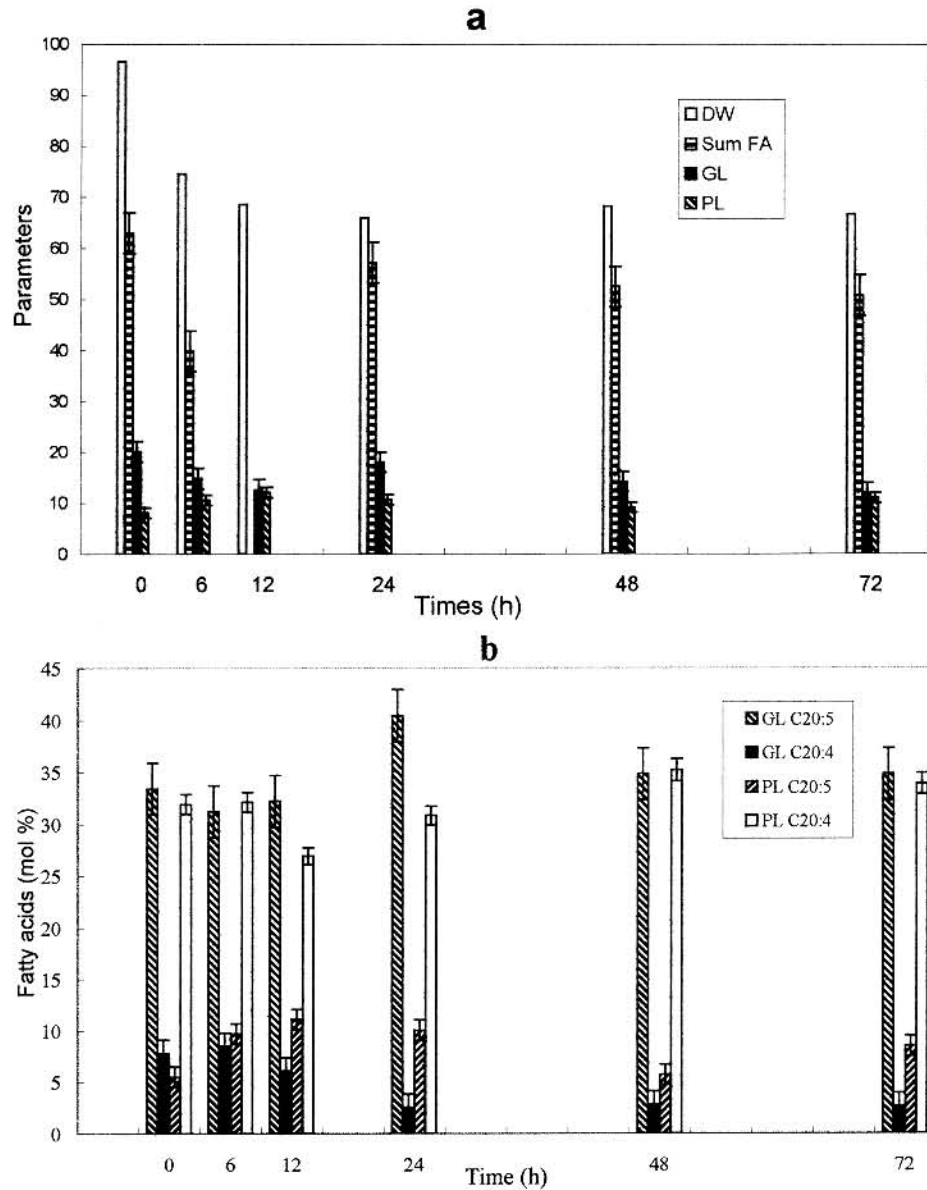
The study of light effects on the lipid composition of algae in a batch culture faces some disadvantages. The cultures grown at different light intensities differ in growth rate and in the amount of algal cells in the suspension. These change the optical density and, consequently, the real light conditions in cultures (M. I. Yur'eva 1988. Thesis, Inst. Plant Physiol., RAS, Russia). We made an attempt to study the time-course of alterations in the FA composition using the continuous culture of *P. cruentum* to avoid the influence of culture density. In these experiments, the level of optical density of the suspension was maintained for 72 h after the shift in light intensity from 250 to 500, thereafter to 750 and finally back to 250 µmol m⁻² s⁻¹. The fluctuations in the dry weight of 1 ml suspension did not exceed 10%, as compared to its initial level. However, the number of cells increased and the weight of individual cells decreased over 24 h after the light increase up to 500 µmol m⁻² s⁻¹, and then both stabilised at the new levels (Fig. 1). A substantial decrease was noticed in the content of Chl *a* and porphyrines at this light intensity, as compared to that of 250 µmol m⁻² s⁻¹. However, the content decreased slightly with a further elevation in light level (data not presented). This indicated changes in the structure of the population as had previously been noted for *Chlorella* cells (Tsoglin and Klyachko-Gurvich 1980). Thus, we failed to carry out a one-factor experiment on both light intensity and population structure. The proportion of the cells at different ages could be suggested to have an influence on the lipid content and fatty acid composition.

Rather complicated transient alterations occurred in the lipid metabolism during the first period (0–24 h) at higher light intensity. It should be noted that the total content of

Table 1. Distribution of predominant fatty acids among the lipid fractions of *Porphyridium cruentum*. The algae were grown as a batch culture at continuous illumination (550 µmol m⁻² s⁻¹) and aeration with CO₂-enriched air (1.7%) at 26°C. NL, neutral lipids; GL, glycolipids; PL, phospholipids.

Fatty acids	Fatty acids, % of total pool in lipid extract		
	NL	GL	PL
C16:0	21.3 ± 0.1	54.4 ± 0.1	24.3 ± 0.3
E-C16:1ω13	–	–	100.0
C18:2ω6	49.1 ± 0.3	32.2 ± 0.3	18.7 ± 0.7
C20:4ω6	23.3 ± 0.7	10.9 ± 0.6	65.9 ± 0.7
C20:5ω3	6.5 ± 0.1	78.2 ± 1.2	15.3 ± 0.3
Total	27.4 ± 0.1	42.1 ± 0.6	29.5 ± 0.4

Fig. 1. The time-course changes of some parameters in the continuous culture of *Porphyridium cruentum* under shifts in light intensity from 250 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. (a) Cell dry weight (pg cell^{-1}) and the content of total lipids, glyco- and phospholipids ($\text{mg g}^{-1} \text{DW}$). (b) The relative content (mol %) of eicosapentaenoic ($\text{C}_{20:5\omega3}$) and arachidonic ($\text{C}_{20:4\omega6}$) acids in glyco- and phospholipids.



$\text{C}_{20:5\omega3}$ only slightly decreased this time in contrast to all other acids (Table 2). Then, by 72 h, the lower content of total lipids and GL but the same of PL on a dry weight basis was adjusted in the cells (Fig. 1a). The relative content (mol %) of $\text{C}_{20:5\omega3}$ approached the initial level in the GL but somewhat increased in the PL, while that of $\text{C}_{20:4\omega6}$ steadily diminished in the GL but retained the same level in the PL by 72 h (Fig. 1b). As a result, the ratio of $\text{C}_{20:5\omega3}$ to $\text{C}_{20:4\omega6}$ augmented in the GL, mainly due to some decrease in the amount of $\text{C}_{20:4\omega6}$ acid (Fig. 2). This ratio remained practically unchanged in the pool of total lipids and decreased in the PL after a short-term elevation.

The next increase in light intensity up to 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (slightly photoinhibitory conditions) resulted in a further decrease in the content of all lipids (Table 2). The relative content of $\text{C}_{20:5\omega3}$ and its ratio to $\text{C}_{20:4\omega6}$ were also diminished in this case. It is of interest to note the rather constant level of *trans*-3-hexadecenoic acid (E-

$\text{C}_{16:1\omega13}$) and a slight accumulation of $\text{C}_{20:3\omega6}$ after initial transient decrease (Table 2). The return to 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ caused the restoration of the initial parameters of culture. Some discrepancy in parameter characteristics, in particular the lower amount of $\text{C}_{20:5\omega3}$ and higher content of some minor components as compared to their initial level, could be explained by the ageing of culture. Thus, the experiments on the continuous culture revealed the pattern of significant light-dependence of FA composition but did not allow us to distinguish between the light effect on the FA metabolism via photosynthesis or/and population structure.

Age-dependent changes in the FA composition of the cell

It seemed possible to distinguish between these processes in the experiments with the synchronous culture of *Chlorella*. The comparison of young and mature cells showed a higher

Table 2. Light-dependent changes in the content of predominant fatty acids in *P. cruentum* (mg 100 g⁻¹ DW). Continuous culture of *P. cruentum* of constant suspension density at sequential changes in light intensity. The content of lipid FA after 72 h exposure to the indicated light intensity.

Fatty acid	Light intensity, $\mu\text{mol m}^{-2} \text{s}^{-1}$			
	250	500	750	250
C16:0	2 192 ± 59	1 661 ± 80	1 498 ± 23	2 316 ± 250
E-C16:1 ω 13	120 ± 4	108 ± 1	121 ± 2	124 ± 27
C18:2 ω 6	1 027 ± 15	793 ± 37	634 ± 10	1 128 ± 46
C20:3 ω 6	55 ± 1	48 ± 6	71 ± 1	96 ± 1
C20:4 ω 6	1 702 ± 39	1 307 ± 27	1 128 ± 14	1 923 ± 158
C20:5 ω 3	991 ± 55	912 ± 6	697 ± 8	564 ± 89
Sum of FA in total lipids	6 294 ± 145	5 085 ± 179	4 554 ± 41	6 458 ± 149

ratio of trienoic to dienoic acids in the former. The difference was especially evident for MGDG (Table 3). The highest total MGDG concentration and its higher contribution to the total lipids were observed in the mature cells, and these parameters diminished during further development. However, in the pool of total lipids, the content of trienoic acids on the dry weight basis had an almost constant value throughout the cell cycle. It is worth noting that, in this experiment, the intensity of light was so high and the suspension density was so low (from 0.125 to 1.45 mg ml⁻¹) that there were no external factors limiting the growth. At the same time, the *Chlorella* cells grown as the synchronous cultures under different light intensities demonstrated that the ratio of trienoic to dienoic acids in the young cells was closely related to the light intensity and photosynthetic activity (Klyachko-Gurvich et al. 1981).

Effect of the growth rate of *Dunaliella salina* on fatty acid composition

The distinct dependence of FA composition on the growth rate can be observed in the experiments carried out on the batch culture of *D. salina*. The extremely unsaturated hexadecatetraenoic acid (C16:4 ω 3), esterified almost exclusively to MGDG, is known to be intrinsic to this alga and to *Chlamydomonas* species. The accumulation of this acid correlates with an active growth and its level drops during density increase in the batch cultures of these algae (Fried et al. 1982, Petkov et al. 1990). In our experiments, some decrease in the relative content of saturated and monounsaturated acids, with a distinct increase in the content of polyunsaturated acids, especially C16:4 ω 3, occurred at the culture transition from the lag to the linear phase of culture growth. The opposite changes were exhibited during the period of retardation of growth and photosynthesis at the beginning of the stationary phase (Fig. 3).

Polyunsaturated FA synthesis in *Chlamydomonas* mutants

The correlation between desaturation of the fatty acids esterified to MGDG and the activity of photosystems (PS) was studied using photosynthetic mutants of *Chlamydomonas reinhardtii* (Table 4). These mutants retained the core complexes of PSI, PSII (or both) but have no light-harvesting complexes (LHC) (Ladygin 1970). In the mutant, with retained activity of both PSI and PSII, the total lipid

content was lower but the relative contents of C16:4 ω 3 and C18:3 ω 3, the predominant fatty acids of MGDG in this organism, exceeded their level in the wild strain *C. reinhardtii* K⁺. As compared to this strain and to the cells of wild type, the mutant with the active PSI was characterised by almost the same relative content of polyunsaturated FA but contained practically no E-C16:1 ω 13. By contrast, the mutant with the active PSII was characterised by relatively high content of this acid, whereas the C16:4 ω 3 and C18:3 ω 3 acids were found in significantly lower proportion.

Synthesis of ω 3-FA in the presence of cerulenin

The synthesis of polyunsaturated FA proceeds as a sequential introduction of double bonds in a molecule. Therefore, the majority of FA can be considered as intermediate products and their amount depends on the balance between their formation and expense for a further desaturation (or for some other metabolic processes). In the experiments with the inhibitor of fatty acid biosynthesis cerulenin, it was demonstrated that in *C. pyrenoidosa* C-26, the formation of ω 3-series trienoic acids concentrated in MGDG could be in

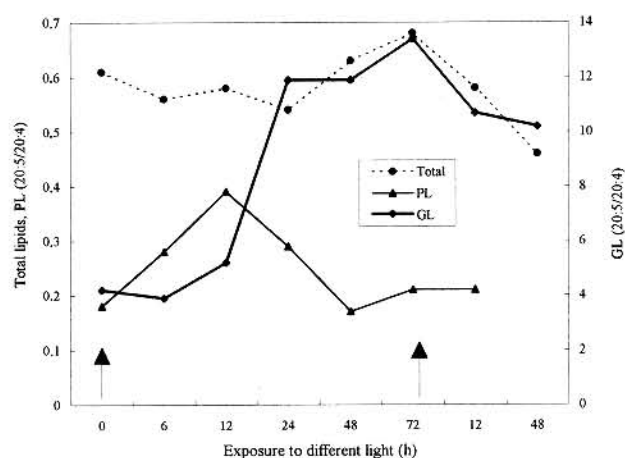


Fig. 2. Light-dependent changes in the ratio of eicosapentaenoic (C20:5 ω 3) to arachidonic (C20:4 ω 6) acid in the pool of total lipids and in the fractions of glyco- and phospholipids in the continuous culture of *Porphyridium cruentum* after light increase from 250 to 500 and 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The arrows designate the time of light changes.

Table 3. Age-dependent changes in the content of polyunsaturated fatty acids in *Chlorella* cells (nmol mg⁻¹ DW). Synchronous culture of *Chlorella* sp. C-1 (IPPAS) was grown at a light intensity about 2 200 μmol m⁻² s⁻¹. Cell ages: 1.5 h, young; 4.5 h, mature; and 7.5 h, cells immediately before division.

Fatty acid	Cell age, h					
	1.5	4.5	7.5	1.5	4.5	7.5
	Total lipids			MGDG		
C16:2ω6	13.0 ± 0.9	13.8 ± 0.4	16.2 ± 1.3	4.8 ± 0.1	7.9 ± 0.4	5.1 ± 0.3
C16:3ω3	60.1 ± 2.6	48.3 ± 2.1	53.9 ± 3.6	22.2 ± 0.7	35.0 ± 1.2	17.9 ± 0.4
C16:3ω3/C16:2ω6	4.61	3.50	3.32	4.62	4.42	3.50
C18:2ω6	40.1 ± 1.3	32.9 ± 6.1	42.5 ± 1.9	2.6 ± 0.2	7.0 ± 0.4	4.1 ± 0.0
C18:3ω3	86.7 ± 4.8	76.8 ± 5.8	81.6 ± 4.7	20.7 ± 0.5	32.3 ± 1.5	16.6 ± 0.4
C18:3ω3/C18:2ω6	2.16	2.33	1.92	7.96	3.96	4.05
All fatty acids	528.8 ± 42.3	273.5 ± 18.9	274.6 ± 20.0	124.8 ± 6.2	134.0 ± 2.8	93.0 ± 2.4
MGDG, % of total	-	-	-	23.6	57.0	33.9

progress even though the synthesis of FA de novo was blocked and the content of ω6-series acids, as the intermediates of trienoic acid biosynthesis, decreased (Table 5). It is worthwhile to note that the increase in the trienoic acid content correlated neither with the significant alteration in the total level of FA nor with the changes in their index of unsaturation. Similar accumulation of intermediate products of polyunsaturated FA biosynthesis was observed in various algae at irradiance decrease to a sub-optimal level or at any other impairments in the culture state (Klyachko-Gurvich et al. 1985, Řezanka et al. 1987, Petkov et al. 1990).

Light-dependent changes in FA composition of *Synechocystis* B-283

In the cyanobacterium *Synechocystis* B-283 (IPPAS), dienoic acids (C16:2ω6 and C18:2ω6) were found to be the most unsaturated. We studied the FA of this alga, grown under optimal light conditions, after cell exposure to the optimal, superoptimal, and suboptimal illumination for 46 h (Fig. 4). At the optimal light, the content of C18:2ω6 acid in the total lipid pool increased almost entirely due to the accumulation of that esterified to MGDG. A decrease in light intensity led to the retardation of lipid biosynthesis, to the blocking of MGDG synthesis and possibly to some destruction of C18:2ω6 in the pool of MGDG. The accumulation of palmitic (C16:0) and oleic (C18:1ω9) acids proceeded, evidently, in lipids other than MGDG. The increase in light intensity over the optimal value caused a slight decrease in the level of both, total lipid and MGDG. The rate of C18:2ω6 formation increased in total lipids and remained unchanged in MGDG, the rate of C18:1ω9 accumulation decreased in both. Thus, in this case, the transformation of monoenoic to dienoic acid took place and the FA belonging to ω6-but not to ω3-series were accumulated under optimal light intensity. Nevertheless, these FA were also the most unsaturated and were associated with MGDG. It is of interest that the relative content of Z-C16:1, the specific isomer of *cis*-hexadecenoic acid detected in the MGDG of this alga, was light-independent, but its content on a dry weight basis changed along with that of the MGDG (Klyachko-Gurvich et al. 1988).

Discussion

Unlike the majority of higher plants, where C18:3ω3 is the predominant constituent of thylakoid lipids, algae exhibit a great diversity in their polyunsaturated FA nature (Hitchcock and Nichols 1971, Sud'ina and Lozovaya 1982, Harwood and Jones 1989). The investigation of various algae, which belong to different systematic groups, indicates that the optimisation of photosynthetic processes correlates with some increase in the relative content of the FA of the highest degree of unsaturation (Fig. 2, Table 3). As a rule, these FA are esterified to and concentrated in MGDG, the predominant lipids of thylakoid membranes in the majority of plant organisms from cyanobacteria to higher plants. Most commonly, these polyunsaturated FA are the ω3-homologues, i.e. they had a double bond at the C-3 atom relative to the methyl terminus of the chain, regardless of the differences in the number of C atoms and double bonds. Some cyanobacteria, e.g. *Synechocystis* B-253 (Fig. 4) and *Spirulina*, containing γ-linolenic (C18:3ω6) acid, were the only exceptions among the studied species. The screening of literature for the data concerning the FA content in a wide range of algae completely confirms the predominant location of ω3-acids in MGDG (Hitchcock and Nichols 1971, Sud'ina and Lozovaya 1982, Harwood and Jones 1989).

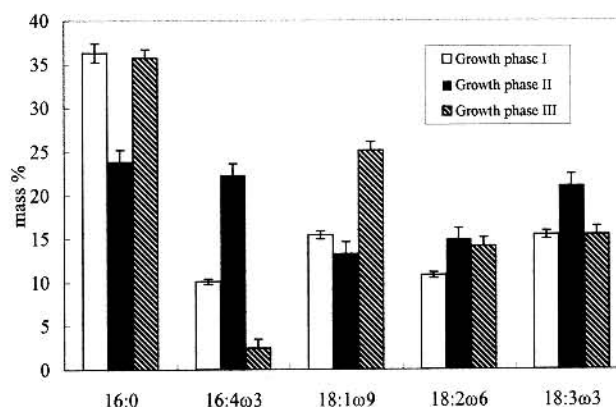


Fig. 3. The correlation of specific growth rate (μ) and the relative content (mass %) of C16:4ω3 acid in a batch culture of *Dunaliella salina* D-209 (IPPAS) at different growth phases: I, lag-period ($\mu = 0.067$ day⁻¹); II, linear phase ($\mu = 0.397$ day⁻¹); III, the beginning of stationary phase ($\mu = 0.207$ day⁻¹).

Table 4. The content of fatty acids (ng per 100 cells) in the cells of *Chlamydomonas reinhardtii* photosynthetic mutants with retained definite complexes of photosystems. *The position of the double bond is not estimated.

Fatty acid	K'	PS (I+II)	PSI	PSII	PSI/PSII, %
C16:0	431.0 ± 17.7	166.3 ± 17.6	190.3 ± 20.0	200.9 ± 8.8	94.7
C16:1*	67.5 ± 8.3	6.0 ± 0.4	15.6 ± 1.2	29.9 ± 2.2	52.2
E-C16:1ω13	9.5 ± 0.9	7.7 ± 0.1	0.5 ± 0.05	6.3 ± 0.2	7.8
C16:4ω3	122.6 ± 8.3	78.0 ± 4.1	51.4 ± 2.2	44.7 ± 1.2	115.0
C18:0	18.0 ± 1.0	8.8 ± 0.5	10.4 ± 3.6	14.2 ± 0.8	73.2
C18:1ω6	128.0 ± 5.5	31.4 ± 1.8	35.6 ± 0.5	87.2 ± 2.2	40.8
C18:2ω6	33.2 ± 1.4	12.4 ± 0.1	10.4 ± 0.1	24.8 ± 0.6	41.9
C18:3ω6	70.1 ± 0.1	34.3 ± 0.2	35.6 ± 1.7	85.1 ± 1.1	41.8
C18:3ω3	183.2 ± 11.4	137.7 ± 3.6	121.3 ± 7.0	80.7 ± 1.0	150.3
Sum	1 080.6 ± 44.3	494.7 ± 6.9	473.8 ± 29.4	587.2 ± 10.6	80.7

The data on the light-dependent changes in FA desaturation available in the literature are rather contradictory. This can be related to the non-linear light-dependence of FA composition in the same manner as is well known for chlorophyll content. The dynamics of the FA desaturation level following a shift in light intensity has also a non-linear character. As a rule, there is a brief initial period when the photosynthesis is inhibited even at the transition to more optimal conditions (Klyachko-Gurvich et al. 1980). A similar transient period is found at low-temperature acclimation (Porankiewicz et al. 1998). It has been shown previously that the process of desaturation can proceed at this time due to the transformation of pre-existing molecules that can increase the ratio of the most unsaturated FA to their biosynthetic precursors or competitors. Moreover, in this situation, the desaturation can be revealed more distinctly as it is not hidden by the de novo synthesis of FA. Such a phenomenon has been demonstrated in many studies (Sato and Murata 1981, Farkaš and Lechozki 1984, Cohen et al. 1995).

The results of numerous experiments with *Chlorella* cultures suggest that light can control the last step of trienoic acid formation. As a result of such a regulation, the cell changes its fatty acid composition without alteration in the whole multistage process of polyunsaturated FA biosynthesis, but only in the rate of this end reaction, namely, in the desaturation of dienoic acids at the ω3-position. The acceleration of this reaction increases the level of trienoic acids, and vice versa. Its arrest results in the accumulation of dienoic acids as the intermediates of the trienoic acid biosynthesis (Klyachko-Gurvich et al. 1980, 1981, 1985).

The further study of various algae demonstrated the light-induced acceleration of double bond incorporation at the ω3-position in the majority of organisms. But in algae other than *Chlorella*, containing long-chain FAs with a high number of double bonds, the pattern can be more complicated. For example, linoleic acid is suggested to be the key unit in the chain of intermediate products being the point of bifurcation for the pathways of C20:4ω6 and C20:5ω3 biosynthesis in *Porphyridium* cells (Hitchcock and Nichols 1971, Cohen et al. 1995; M. I. Yur'eva 1988. Thesis, Inst. Plant Physiol., RAS, Russia). However, studying the effect of the environment and screening the various species of *Porphyridium*, we faced the accumulation of various products at an inhibition of the C20:5ω3 biosynthesis. This

enables us to hypothesise that light can regulate the pathway of this acid synthesis at different points (Klyachko-Gurvich et al. 1994; M. I. Yur'eva 1988. Thesis, Inst. Plant Physiol., RAS, Russia). Similar conclusions were made in the report of Cohen et al. (1995).

The adaptive response to a shift in light intensity, in terms of lipid metabolism, is a rather complicated process, which is dependent on various factors. Evidently, the ω3-desaturation is a fast response. The slower adaptive response can be related to the alteration in lipid biosynthesis, in particular MGDG. The sharp drop in light intensity was shown to induce cell division in the synchronous *Chlorella* culture (Klyachko-Gurvich et al. 1980, 1981) and, hence, some increase in the content of trienoic acids in accordance with their higher level intrinsic to the young cells (Table 3). The experiments carried out using *Arabidopsis* mutants defective in different desaturase genes, revealed the potential for organisms to form the polyunsaturated FA by the different (eukaryotic and prokaryotic) pathways (Ohlrogge and Browse 1995). The time-course of changes in FA composition of *Porphyridium* cells under light shift allowed us to suggest that the transient increase in the relative content of PL C20:5ω3 (Fig. 2) could be related to the temporary acceleration of the eukaryotic pathway of FA biosynthesis during the culture adaptation to new light conditions.

Table 5. The effect of cerulenin on the lipid fatty acid content (mg 10⁻⁸ cells) and composition in *Chlorella* cells. Batch culture of *C. pyrenoidosa* C-26 (IPPAS) was grown at 26°C, light intensity of 360 μmol m⁻² s⁻¹. The cells were treated with cerulenin (4 mg ml⁻¹) for 24 h. The cerulenin treated culture accumulated about 20% of biomass as compared to control culture, but weight of the individual cells was 2.5 times higher than that in control culture. I_{un}, index of unsaturation.

Fatty acid	Control	Cerulenin
C16:0	10.2 ± 0.7	13.7 ± 1.1
C16:1ω7(9)	1.4 ± 0.1	2.1 ± 0.2
E-C16:1ω13	1.1 ± 0.1	0.8 ± 0.1
C16:2ω6	6.0 ± 0.4	2.4 ± 0.2
C16:3ω3	2.3 ± 0.1	6.5 ± 0.3
C18:0	0.8 ± 0.1	1.4 ± 0.1
C18:1ω9	1.6 ± 0.1	3.5 ± 0.3
C18:2ω6	10.1 ± 0.5	4.2 ± 0.2
C18:3ω3	4.4 ± 0.2	9.5 ± 0.3
Total	37.9 ± 1.5	44.1 ± 1.1
C16:3ω3/C16:2ω6	0.38	2.71
C18:3ω3/C18:2ω6	0.44	2.26
I _{un}	1.49	1.57

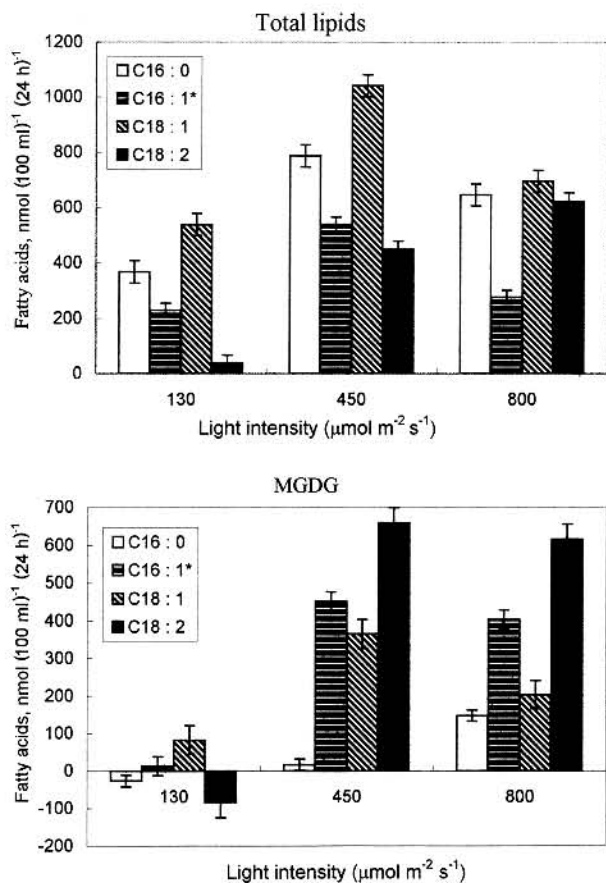


Fig. 4. Light-dependent accumulation of predominant fatty acids in the pools of total lipids and MGDG in the cells in a batch culture of *Synechocystis* sp. B-283 (IPPAS). The cells adapted to light of $450 \mu\text{mol m}^{-2} \text{s}^{-1}$ were transferred for 46 h to a light intensity of 130, 450, or $800 \mu\text{mol m}^{-2} \text{s}^{-1}$. The rate of MGDG accumulation comprised 0.7, 60.7, and 70.7% of the total lipids, respectively. C16:1*, Z-isomer of hexadecenoic acid, the position of double bond is not estimated.

The active desaturation of FA esterified to MGDG evidently depends on the light intensity, not directly but via its effect on the two reciprocally related processes, photosynthesis and growth (Table 3). In experiments on the synchronous cultures of *Chlorella*, it has been estimated that there exists an initial stage of cell development from autotrophs when the formation of the PS core complexes, but not LHC, proceeds in the cells and ATP and NADPH are produced due to the cyclic electron transport around PSI. However, there are no interactions between the two PS to provide efficient photosynthesis (Chemersis et al. 1979, Tsoglin and Klyachko-Gurvich 1980). Some decrease in total lipids as the respiration substrates is typical at this stage, but in the pool of MGDG, the ω 3-fatty acids are accumulated at the expense of a decrease in the ω 6-acid content. The intensive accumulation of dienoic acids esterified to MGDG occurs later, evidently in the period of maximal photosynthetic activity along with the formation of LHC, plastoquinone pool and the appearance of interaction between both PS (Chemersis et al. 1979, Tsoglin and Klyachko-Gurvich 1980, 1981).

This suggests the different roles of MGDG molecules distinguished in their fatty acid composition in the organisation of the different complexes of photosynthetic apparatus: the relation of the MGDG enriched with trienoic acids to the PS core complexes and the MGDG with the relatively high content of dienoic acids to the formation of LHC. This implies the importance of changes in the FA composition for the arrangement of photosynthetic apparatus.

To test the possible correlation between desaturation of the fatty acids esterified to MGDG and the activity of PSI or PSII, we carried out the determination of fatty acid composition in the photosynthetic mutants of *C. reinhardtii*, which retained the core complexes of PSI, PSII, or both, but not LHC (Ladygin 1970). In the mutant that retained the activity of both PS, the relative content of C16:4 ω 3 and C18:3 ω 3 acids, the predominant fatty acids of MGDG in this organism, exceeded that of the wild strain of *C. reinhardtii* K⁺ (Table 4). Hence, this mutant seemed to be similar to young cells in terms of its FA composition as well as in the structure of its photosynthetic machinery. It should be noted that the mutant with the active PSI was characterised by almost the same relative content of polyunsaturated FA. In contrast, the content of these acids was significantly lower in the mutant with the active PSII.

The use of photosynthetic mutants seems to be the most direct approach to the problem. However, it is important to remember that these mutants require the addition of acetate and this can turn them to photoheterotrophic nutrition. In this case, the FA biosynthesis proceeds via an acetate metabolism that requires only the activity of PSI. Nevertheless, the experiments with photosynthetic mutants, in line with the observations on chloroplast development in synchronous cultures and the response of various algae to the changes in light intensity, suggest that the cyclic electron transport around PSI, providing the photophosphorylation and formation of reduced ferredoxin, is the necessary and sufficient factor for desaturation of the FA esterified to MGDG (Pronina et al. 1990, Klyachko-Gurvich et al. 1991). The changes in the low-temperature fluorescence during the development of *Chlorella* cells and at the decrease in light intensity argued for such a suggestion (Klyachko-Gurvich et al. 1981). The analysis of *P. cruentum* cells by the methods of immunochemistry and spectroscopy revealed an increase in the amount of ATPase and PSI complexes but a decrease in the PSII and LHC content along with an increase in light intensity (Cunningham et al. 1991). These observations are also in accordance with our data.

In terms of contemporary views, the acceleration of cyclic electron transport around PSI (regardless of inducing factors) activates the ω 3-desaturases, the acyl-MGDG enzymes, which are integrated in thylakoid membranes and use the ferredoxin as an electron donor (Wada et al. 1993, Los and Murata 1994, Mustardy et al. 1996, Los 1997). Recently, it has been estimated (Kis et al. 1998) that the activation of desaturases by light occurs via the expression of inducible genes encoding these enzymes as well as in the case of low-temperature acclimation (Los and Murata 1994, Mustardy et al. 1996). Thus, we assume the ω 3-desaturation of the FA esterifying the MGDG to be an adaptive response of the cell to the shift in the growth conditions or in a stage

of development requiring the rearrangement of the structure of thylakoids to provide more efficient photosynthesis. This reaction is suggested to be a rather common, although not singular, mechanism of plant adaptation. We did not observe the same mechanism of adaptation in cyanobacteria but many other properties of their photosynthetic apparatus are also rather specific.

Another aspect of this problem is the importance of the above considered desaturation for the process of photosynthesis. The enhancement of desaturation inevitably increases the membrane fluidity that is a well characterised phenomenon of temperature acclimation. But the specificity of light-induced incorporation of double bonds at the ω 3-position of FA in the majority of organisms and its selection in the evolution as the predominant process in higher plants suggest a more particular role of this reaction. The different trends in alteration of the index of unsaturation and the ratio of the ω 3- to ω 6-acids were observed in all experiments presented in this paper. Thus, it is evident that changes in the membrane fluidity are, if at all, not the only mechanism for interactions between desaturation and photosynthesis. The lipid capability to form hexagonal structures, for example, is of great importance (Murphy 1986). On the other hand, the protein incorporation into the lipid matrix is known to be related to their capability to form α -helices and saturated FA hinders this process. It has been recently shown that the presence of a nonlamellar-prone lipid other than MGDG, phosphatidylethanolamine, enhances the binding of the α -subunit of G-protein to model membranes, especially if it is esterified with polyunsaturated FA (Escribá et al. 1997). It has been demonstrated that the chlorophyll-protein complexes of the photosynthetic apparatus have an oligomeric structure and the formation or dissociation of these oligomers can be important in chloroplast functions (Dreifuss and Thornber 1994a,b). This process has been shown to proceed in the association with thylakoid membranes. Thus, the changes in FA composition can be suggested to affect the interactions of lipids and proteins. The increasing desaturation at the ω 3-position, as one of the primary processes of adaptation to an improvement of conditions for photosynthesis, can be assumed to provide the conformational changes appropriate for the self-assembly of the PS core complexes. We observed that the synthesis of the MGDG enriched with trienoic acids correlated to the formation of the PS core complexes and MGDG with dienoic acids to the formation of LHC in the experiments on the synchronous *Chlorella* culture (Tsoglin and Klyachko-Gurvich 1980, Klyachko-Gurvich et al. 1981). A similar specific role of thylakoid lipids has been rather well estimated for the E-C16:1 ω 13-containing phosphatidylglycerol, which is demonstrated to stabilise the oligomeric structure of LHCII (Garnier et al. 1990). In experiments with *Arabidopsis thaliana* mutants, the stabilising role of DGDG for the water-oxidising complex has recently been demonstrated (Reifarh et al. 1997). There is no reliable information concerning the influence of MGDG on the self-assembling of chlorophyll-protein complexes. Nevertheless, the results of physiological exper-

iments, presented and discussed in this article, argue for such a role of the MGDG.

Acknowledgements – We are grateful to Dr I. Šetlik for much valuable and constructive advice. We are also thankful to Dr V. Ladygin for the submission of *Chlamydomonas* mutants. This work was partly supported by the Russian Foundation for Basic Research (project no. 96-04-49165).

References

- Catalogue of Microalgal Cultures in the Collections of the USSR (1991) Semenenko VE (ed.) ONTI, Puschino.
- Chemers YuK, Grishina NA, Venediktov PS (1979) Difference in the character of chlorophyll *a* and *b* synthesis during life cycle of *Chlorella*. *Sov Plant Physiol* 26: 302–305
- Cohen Z, Khozina I, Shiran D, Norman HA, Pillai P (1995) In vivo and in vitro inhibition of fatty acid desaturation by the antibiotic cerulenin. In: Kader J-C, Mazliak P (eds) *Plant Lipid Metabolism*. Kluwer Academic Publishers, Dordrecht, pp 462–464
- Cunningham FX Jr, Mustardy L, Gantt E (1991) Irradiance effects on thylakoid membranes of the red alga *Porphyridium cruentum*: An immunochemical study. *Plant Cell Physiol* 32: 419–426
- Dreifuss BW, Thornber JP (1994a) Assembly of the light-harvesting complexes (LHCs) of photosystem II. Monomeric LHCIIb complexes are intermediates in the formation of oligomeric LHCIIb complexes. *Plant Physiol* 106: 829–839
- Dreifuss BW, Thornber JP (1994b) Organization of the light-harvesting complex of photosystem I and its assembly during plastid development. *Plant Physiol* 106: 841–848
- Escribá PV, Ozaita A, Ribas C, Miralles A, Fodor E, Farkaš T, Garcia-Sevilla JA (1997) Role of lipid polymorphism in G protein-membrane interactions: Nonlamellar-prone phospholipids and peripheral protein binding membranes. *Proc Natl Acad Sci USA* 94: 11375–11380
- Farkaš T, Lechozki E (1984) Cerulenin-induced changes in composition and physical state of lipids in *Chlorella pyrenoidosa*. In: Siengenthaler P-A, Eichenberger W (eds) *Developmental Plant Biology, Structure, Function, and Metabolism of Plant Lipids*, Vol. 9. Elsevier, Amsterdam, pp 41–44
- Fried A, Tietz A, Ben-Amotz A, Eichenberger W (1982) Lipid composition of the halotolerant alga *Dunaliella bardwili*. *Biochim Biophys Acta* 713: 419–426
- Garnier J, Wu B, Maroc J, Guyon D, Trémolières A (1990) Restoration of both an oligomeric form of the light-harvesting antenna CP11 and a fluorescence state II–state I transition by Δ^3 -trans-hexadecenoic acid-containing phosphatidylglycerol in cells of mutant of *Chlamydomonas reinhardtii*. *Biochim Biophys Acta* 1020: 153–162
- Harwood JL, Jones AL (1989) Lipid metabolism in algae. *Adv Bot Res* 16: 1–53
- Hitchcock S, Nichols BW (1971) *Plant Lipid Biochemistry*. Academic Press, New York, NY, p 387.
- Kis M, Zsiroš O, Farkaš T, Wada H, Nagy F, Gombos Z (1998) Light-induced expression of fatty acid desaturase genes. *Proc Natl Acad Sci USA* 95: 4209–4214
- Klyachko-Gurvich GL, Semenova AN, Semenenko VE (1980) Lipid metabolism of chloroplasts during *Chlorella* adaptation to the decrease in light intensity. *Sov Plant Physiol* 27: 290–297
- Klyachko-Gurvich GL, Tsoglin LN, Semenova AN (1981) On the involvement of monogalactosyldiacylglycerols (MGDG) of various fatty acid composition in organisation of the chloroplast membrane. *Sov Plant Physiol* 28: 358–365
- Klyachko-Gurvich GL, Yurieva MI, Semenenko VE (1985) Specificity of fatty acid composition of acyl lipids in the unicellular red alga *Porphyridium cruentum*. *Sov Plant Physiol* 32: 82–89
- Klyachko-Gurvich GL, Tarkhanova GI, Ryabykh IB, Semenenko VE (1988) Unusual isomer of hexadecenoic acid in monogalactosyldiacylglycerols of the blue-green alga *Synechocystis*. *Sov Plant Physiol* 35: 896–901

- Klyachko-Gurvich GL, Pronina NA, Ryabykh IB, Semenenko VE (1991) Specific composition of fatty acids in lipids of *Chlamydomonas reinhardtii* mutants with different organisation of chloroplast photosystems. *Sov Plant Physiol* 38: 852–859
- Klyachko-Gurvich GL, Doucha J, Kopezkii J, Ryabykh IB, Semenenko VE (1994) Comparative investigation of fatty acid composition in lipids of various strains of *Porphyridium cruentum* and *Porphyridium aerugineum*. *Russ J Plant Physiol* 41: 248–255
- Klyachko-Gurvich GL, Pronina NA, Furnadzhieva S, Ramazanov ZM, Petkov G (1997) Lipid composition and membrane state of *Dunaliella salina* cells subjected to suboptimal temperature. *Russ J Plant Physiol* 44: 183–191
- Kreps EM (1981) *The Lipids of Cell Membranes*. Nauka, Leningrad, p 340.
- Ladygin VG (1970) Pigment mutants of *Chlamydomonas reinhardtii* provoked by nitrosoethylurea and ultraviolet irradiation. *Genetika* 6: 42–50
- Los D, Murata N (1994) The low-temperature-induced accumulation of the *desA* transcript in *Synechocystis* PCC6803 is a result of both, activation of transcription and maintenance of RNA stability. *Russ J Plant Physiol* 41: 147–151
- Los DA (1997) Fatty acid desaturases: Adaptive expression and principles of regulation. *Russ J Plant Physiol* 44: 458–469
- Murphy DJ (1986) The molecular organisation of the photosynthetic membranes of higher plants. *Biochim Biophys Acta* 864: 22–94
- Mustardy L, Los DA, Gombos Z, Murata N (1996) Immunocytochemical localisation of acyl-lipid desaturases in cyanobacterial cells: Evidence that the thylakoid membranes are sites of lipid desaturation. *Proc Natl Acad Sci USA* 93: 10524–10527
- Ohlrogge J, Browse J (1995) Lipid biosynthesis. *Plant Cell* 7: 957–970
- Petkov GD, Klyachko-Gurvich GL, Furnadzhieva S, Pronina NA, Ramazanov ZM (1990) Genotypic differences and phenotype changes of lipid fatty acid composition in the strains of *Dunaliella salina*. *Sov Plant Physiol* 37: 268–272
- Porankiewicz J, Selstam E, Campbell D, Öquist G (1998) Membrane lipid composition and restoration of photosynthesis during low-temperature acclimation in *Synechococcus* sp. strain PCC 7942. *Physiol Plant* 104: 405–412
- Pronina NA, Klyachko-Gurvich GL, Ladygin VG, Semenenko VE (1990) The activity of carbonic anhydrase in *Chlamydomonas reinhardtii* mutants with different organisation of chloroplast photosystems. *Sov Plant Physiol* 37: 899–906
- Reifarth F, Christen G, Seeliger AG, Dörmann P, Benning C, Renger G (1997) Modification of the water oxidising complex in leaves of the *dgd1* mutant of *Arabidopsis thaliana* deficient in the galactosyldiacylglycerol. *Biochemistry* 36: 11769–11776
- Řezanka T, Doucha I, Mareš P, Podojil M (1987) Effect of cultivation temperature and light intensity on fatty acid production in the red alga *Porphyridium cruentum*. *J Basic Microbiol* 23: 275–278
- Sato N, Murata N (1981) Studies on the temperature shift induced desaturation of fatty acids in monogalactosyldiacylglycerol in the blue-green alga (cyanobacterium), *Anabaena variabilis*. *Plant Cell Physiol* 22: 1043–1050
- Semenenko VE, Abdullaev AA (1980) Parametric control of β -carotene biosynthesis in the cells of intensive culture of *Dunaliella salina*. *Sov Plant Physiol* 27: 22–30
- Sud'ina EG, Lozovaya GI (1982) *Fundamentals of the Evolutionary Biochemistry of Plants*. Naukova Dumka, Kiev, p 358
- Trémolières A, Jacques R, Mazliak P (1973) Régulation par la lumière de l'accumulation de l'acide linoléique dans la jeune feuille de pois. *Physiol Veg* 11: 239–251
- Tsoglin LN, Klyachko-Gurvich GL (1980) Changes in functional activity of the chloroplast in the *Chlorella* cell cycle. *Sov Plant Physiol* 27: 871–877
- Vladimirova MG, Semenenko VE (1962) Intensive culture of unicellular algae. In: Nichiporovich AA (ed) *Izd Akad Nauk SSSR, Moscow*, 60 p
- Vladimirova MG, Markelova AG (1983) The cultures of unicellular algae. In: Gromov BV (ed) *The Cultures of Algal Collection Strains. Izd Akad Nauk SSSR, Leningrad*, pp 57–74
- Wada H, Schmidt H, Heinz E, Murata N (1993) In vitro ferredoxin-dependent desaturation of fatty acids in cyanobacterial thylakoid membranes. *J Bacteriol* 175: 544–547