Plant Cell Physiol. 33(7): 933–941 (1992)

Modes of Fatty-Acid Desaturation in Cyanobacteria

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The mode of desaturation of fatty acids in the membrane lipids of cyanobacteria was studied by analyzing the composition of fatty acids, the distribution of fatty acids at the *sn* position of the glycerol moiety, and the position of double bonds in the fatty acids. Cyanobacterial strains can be classified into four groups on the basis of the mode of desaturation of fatty acids. Cyanobacteria in Group 1 can introduce only one double bond at the $\Delta 9$ position of fatty acids at the *sn*-1 position. Cyanobacteria in Groups 2, 3 and 4 are characterized by a unique positional distribution of fatty acids; the C18 and C16 fatty acids are esterified, respectively, to the *sn*-1 and *sn*-2 positions of the glycerol moiety. In Group 2, the C18 acid is desaturated at the $\Delta 9$, $\Delta 12$, and $\Delta 15$ positions and the C16 acid is desaturated at the $\Delta 9$ and $\Delta 12$ positions; in Group 3, the C18 acid is desaturated at the $\Delta 6$, $\Delta 9$, and $\Delta 12$ positions; and in Group 4, the C18 acid is desaturated at the $\Delta 6$, $\Delta 9$, $\Delta 12$, and $\Delta 15$ positions. The C16 acid is not desaturated in Groups 3 and 4. Both unicellular and filamentous strains are distributed among all four groups.

Key words: Classification — Cyanobacteria — Desaturation — Fatty acids — Membrane lipids.

Kenyon (1972) and Kenyon et al. (1972) classified the cyanobacteria into four groups by reference to their fatty-acid components. Strains in the first group contain only saturated and monounsaturated fatty acids, such as 16:1 and 18:1, whereas strains in the other groups contain polyunsaturated fatty acids in addition to saturated and monounsaturated fatty acids. The second group is characterized by the presence of 18:3α, the third group by 18:3γ, and the fourth group by 18:4.

It has been demonstrated that cyanobacteria incorporate only saturated fatty acids during the biosynthesis of polar lipids, and that the desaturation of fatty acids takes place when the fatty acids are already in lipid-bound form (Lem and Stumpf 1984, Sato and Murata 1982, Stapleton and Jaworski 1984). This situation is different from that in higher plants where the first double bond is introduced while the fatty acid is bound to acyl-carrier protein (McKeon and Stumpf 1982, Stumpf 1981). Now that the biosynthesis of fatty acids in cyanobacteria has been well characterized (Murata and Nishida 1987), it is possible to examine the modes of fatty-acid desaturation in these microorganisms and to reevaluate the classification of cyanobacteria proposed by Kenyon (1972) and Kenyon et al. (1972).

In the present study, we examined the fatty acids of cyanobacteria with special emphasis on the fatty-acid compositions of individual lipid classes and the positional distributions of fatty acids at the *sn*-1 and the *sn*-2 positions of the glycerol moiety. The classification of cyanobacteria according to the unsaturation of fatty acids is reevaluated in terms of the mode of fatty-acid desaturation.

Abbreviations: 16:0, hexadecanoic acid (palmitic acid); 16:1, $\Delta 9$-hexadecenoic acid (palmitoleic acid); 16:2, $\Delta 9,12$-hexadecadienoic acid; 18:0, octadecanoic acid (stearic acid); 18:1, $\Delta 9$-octadecenoic acid (oleic acid); 18:2, $\Delta 9,12$-octadecadienoic acid (linoleic acid); 18:3α, $\Delta 9,12,15$-octadecatrienoic acid (α-linolenic acid); 18:3γ, $\Delta 6,9,12$-octadecatrienoic acid (γ-linolenic acid); 18:4, $\Delta 6,9,12,15$-octadecatetraenoic acid; ACP, acyl-carrier protein; DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PG, phosphatidylglycerol; SQDG, sulfoquinovosyl diacylglycerol.

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Materials and Methods

Organisms and culture conditions—The culture media were the MDM medium of Watanabe (1960) for *Nostoc*

muscorum and *Plectonema boryanum* and the BG-11 medium of Stanier et al. (1971), supplemented with 20 mM HEPES-NaOH (pH 7.5), for the other strains. Cells were grown photoautotrophically under continuous illumination from incandescent lamps at 70 μE m^{-2} s^{-1} with aeration by sterile air that contained 1% CO_2 (Ono and Murata 1981).

Analysis of lipids—Lipids were extracted from 100-ml cultures by the method of Bligh and Dyer (1959). Lipids were analyzed by the method of Sato and Murata (1988). The lipid classes were separated by thin-layer chromatography on precoated silica-gel plates (5721; Merck, Darmstadt, F.R.G.) with a mixture of $CHCl_3$, CH_3OH and H_2O that contained 28% NH_4OH (65 : 35 : 5, by volume) as the mobile phase. The separated lipids were subjected to methanolysis in 5% HCl in methanol at 85°C for 2.5 h. The resultant methyl esters were analyzed with a gas-liquid chromatography system (GC-7A; Shimadzu, Kyoto) equipped with a capillary column coated with cyanopropylmethyl silicone of 0.25 μm thickness (50 m × 0.25 mm internal diameter; CPS-1; Quadrex, New Haven, CT, U.S.A.) and a hydrogen flame ionization detector. The relative amounts of fatty acid methyl esters were calculated by comparing the areas under the chromatographic peaks with a data processor (C-R 2AX; Shimadzu). The distribution of fatty acids at the *sn* positions of the glycerol moiety of the glycerolipids was analyzed, using the position-specific lipase from *Rhizopus delemar*, by the method of Sato and Murata (1988).

The numbers of carbon atoms and double bonds of fatty acid methyl esters were determined with a gas chromatograph-mass spectrometer (JMS-DX300; JEOL, Tokyo) equipped with a mass-analysis system (BJMS-3100; JEOL). The capillary column was identical to that used for GLC analysis. The positions of double bonds in fatty acids were determined by the procedure of Andersson et al. (1975) on a column (1.0 m × 2.5 mm, internal diameter) packed with 3% OV-1 (Gasukuro Kogyo, Tokyo).

Results

Fatty-acid composition—Table 1 shows the fatty-acid compositions of the total lipids from twelve strains of cyanobacteria; six strains were filamentous and the other six were unicellular (Rippka et al. 1979). As suggested by Kenyon (1972) and Kenyon et al. (1972), strains were classified into four groups according to fatty-acid unsaturation. Table 1 also shows that, although the growth temperature affected the relative proportions of individual fatty acids, the presence of unsaturated fatty acids characteristic of the individual groups was unaltered.

Group 1 included a filamentous strain, *Mastigocladus laminosus,* and three unicellular strains, *Synechococcus* PCC7942, *Synechococcus* PCC6301 and *Synechococcus lividus*. These strains contained saturated and monounsaturated fatty acids but no polyunsaturated fatty acids. At a low growth temperature, as compared with a high growth temperature, not only the relative proportions of unsaturated fatty acids, but also the relative proportions of C18 fatty acids increased at the expenses of the C16 and C14 fatty acids particularly in the unicellular strains. The change in the number of carbon atoms in fatty acids with growth temperature was characteristic of this group.

Group 2 included three filamentous strains, *Plectonema boryanum, Nostoc muscorum*, and *Anabaena variabilis*, and a unicellular strain *Synechococcus* PCC7002. These strains contained 16:1, 18:2 and 18:3α, but no 18:3γ or 18:4. The occurrence in 16:2 in *A. variabilis* is not unique because this fatty acid is found in *Plectonema terebrans, Oscillatoria williamssii* (Parker et al. 1967), *Anabaena cylindrica* and *Anabaena flos-aquae* (Nichols and Wood 1967) which are classified in this group. Although growth temperature affected the relative proportions of unsaturated fatty acids in *A. variabilis* and *Synechococcus* PCC7002, the various fatty acids that were characteristic of this group were present in cells grown at all temperatures tested.

Group 3 included a filamentous strain, *Spirulina platensis*, and a unicellular strain, *Synechocystis* PCC6714. These strains contained 18:1, 18:2, and 18:3γ, but neither 18:3α nor 18:4. The level of 16:1 was very low. Even when the growth temperature was decreased, no 18:3α or 18:4 was detected.

Group 4 included a filamentous strain, *Tolypothrix tenuis*, and a unicellular strain, *Synechocystis* PCC6803. These strains contained 18:1, 18:2, 18:3α, 18:3γ and 18:4. The level of 16:1 was very low in both strains. Although the growth temperature affected the relative proportions of fatty acids in this group also, the fatty acids, such as 18:3α, 18:3γ and 18:4, that characterize this group were present irrespective of growth temperature.

These data in Table 1 are compatible with the previously published ones (Fork et al. 1979, Kenyons 1972, Kenyons et al. 1972, Nichols and Wood 1967, Parker et al. 1967, Sato et al. 1979, Wada and Murata 1989). All these results confirm the validity of the classification of cyanobacteria by fatty-acid composition, as proposed by Kenyon (1972) and Kenyon et al. (1972). A new finding in the present study, which was not known to Kenyon (1972) or to Kenyon et al. (1972), is that each of the groups includes both filamentous and unicellular strains. The mode of fatty-acid desaturation in individual groups was studied as follows.

Group 1—The composition and positional distribution of fatty acids of the individual lipid classes of the strains in Group 1 were examined. Table 2 shows the fatty-acid compositions of individual lipid classes of *M. laminosus* and *Synechococcus* PCC7942. It is notable

Table 1 Fatty-acid composition of the total lipids from various cyanobacterial strains

Organism	Growth temp. (°C)	Fatty acid (mole %)										
		14:0	14:1	16:0	16:1	16:2	18:0	18:1	18:2	18:3α	18:3γ	18:4
Group 1												
Mastigocladus laminosus (F)	34	1	0	34	31	0	5	29	0	0	0	0
	28	1	0	19	50	0	1	27	0	0	0	0
Synechococcus PCC7942 (U)	34	1	0	49	36	0	4	10[a]	0	0	0	0
Synechococcus PCC6301 (U)	38	1	1	48	38	0	4	7[a]	0	0	0	0
	28	1	3	46	46	0	1	3[a]	0	0	0	0
Synechococcus lividus (U)	55	0	0	54	10	0	22	14	0	0	0	0
	38	0	0	42	36	0	1	20	0	0	0	0
Group 2												
Plectonema boryanum (F)	28	t	0	36	22	0	1	3[a]	10	29	0	0
Nostoc muscorum (F)	28	t	0	41	14	0	1	2	7	35	0	0
Anabaena variabilis (F)	38	0	0	31	22	t	1	24	22	t	0	0
	22	0	0	29	22	3	t	7	15	24	0	0
Synechococcus PCC7002 (U)	34	t	0	40	16	0	1	27	14	t	0	0
	22	1	0	35	19	0	t	10	25	10	0	0
Group 3												
Spirulina platensis (F)	32	t	t	53	3	0	1	1	13	0	29	0
Synechocystis PCC6714 (U)	34	t	t	59	2	0	t	9	16	0	12	0
	22	t	t	52	3	0	1	2	19	0	23	0
Group 4												
Tolypothrix tenuis (F)	30	0	0	55	3	0	1	2	5	6	11	17
Synechocystis PCC6803 (U)	34	1	0	58	3	0	1	7	12	t	17	t
	30	1	0	54	3	0	1	3	9	3	24	2
	22	t	0	51	3	0	t	2	6	8	21	8

t: Trace (less than 0.5%).

[a] Mixture of Δ9-octadecenoic acid (oleic acid) and Δ11-octadecenoic acid (*cis*-vaccenic acid). F and U in parentheses correspond to filamentous and unicellular strains, respectively.

that, in *M. laminosus*, the fatty-acid compositions of MGDG and DGDG were similar to one another. The fatty-acid compositions of SQDG and PG, which were also similar to one another, were very different from those of MGDG and DGDG. 16:1 was very abundant in MGDG and DGDG, but it was a very minor component of SQDG and PG. However, in *Synechococcus* PCC7942, such a discrete difference in fatty-acid composition was not apparent among the lipid classes.

Table 3 shows the positional distribution of fatty acids in the individual lipid classes from *M. laminosus*. In all the lipid classes, the *sn*-1 and *sn*-2 positions were esterified predominantly by C18 and C16 fatty acids, respectively. Moreover, half of the C16 fatty acids at the *sn*-2 position of MGDG and DGDG were monounsaturated, while the hardly any of the C16 fatty acids at the *sn*-2 position of SQDG and PG were unsaturated. The *sn*-1 positions of MGDG and DGDG were exclusively esterified with monounsaturated fatty acids, such as 16:1 and 18:1. In clear contrast, the predominant fatty acids esterified at the *sn*-1 position were 16:0 and 18:1 in SQDG, and 18:0 and 18:1 in PG. It is estimated from the positional distribution of fatty acids (Table 3) that the major molecular species of MGDG and DGDG are of the *sn*-1-(16:1 or 18:1)-*sn*-2-(16:0 or 16:1) type, and that those of SQDG and PG are of the *sn*-1-(16:0 or 18:1)-*sn*-2-(16:0) and *sn*-1-(18:0 or 18:1)-*sn*-2-(16:0) types, respectively.

The positional analysis was repeated for the individual lipid classes from *Synechococcus* PCC7942. A uniform characteristic of this strain was that only 16:0 was esterified

Table 2 Fatty-acid composition of lipid classes of *Mastigocladus laminosus* and *Synechococcus* PCC7942 (Group 1) grown at 34°C

Lipid class	Fatty acid (mole %)					
	14:0	16:0	16:1	18:0	18:1	ΣC16
M. laminosus						
MGDG	1	22	49	1	28	71
DGDG	1	23	50	1	25	73
SQDG	t	59	2	6	33	61
PG	t	46	3	19	32	49
Synechococcus PCC7942						
MGDG	1	47	36	5	6	83
DGDG	2	53	32	4	5	85
SQDG	1	64	28	2	4	90
PG	1	53	31	2	8	84

t: Trace (less than 0.5%).

at the *sn*-2 position. The *sn*-1 position was esterified mainly by unsaturated fatty acids such as 16:1 and 18:1 (data not shown). A similar distribution of fatty acids at the *sn* positions was reported some time ago for *Synechococcus* 6301, previously known as *Anacystis nidulans* (Sato et al. 1979).

Group 2—Table 4 shows the fatty-acid composition of lipid classes of *N. muscorum, P. boryanum*, and *Synechococcus* PCC7002. A unique characteristic common to all these strains was that the sum of C16 acids was about 50% in MGDG, DGDG and PG, but far greater than 50% in SQDG, and that the C16 acids were mixtures of 16:0 and 16:1 in MGDG and DGDG but were mostly 16:0 in SQDG and PG. The mode of unsaturation of C18 acids was essentially the same among the various lipid classes in all the strains.

The positional analysis of fatty acids in *Synechococcus* PCC7002 indicated that the *sn*-1 position was esterified predominantly by C18 acids in MGDG, DGDG and PG, but by a mixture of C18 acids and 16:0 in SQDG. The *sn*-2 position was esterified exclusively by 16:0 and 16:1 in MGDG and DGDG, and by 16:0 in SQDG and PG. These observations suggest that the major molecules in Group 2 are of the *sn*-1-(unsaturated C18)-*sn*-2-(16:0 or 16:1) type in MGDG and DGDG, and of the *sn*-1-(unsaturated C18)-*sn*-2-(16:0) type in SQDG and PG. In addition, the *sn*-1-(16:0)-*sn*-2-(16:0) species is also present in SQDG.

Group 3—Table 5 shows the fatty-acid composition of lipid classes of *S. platensis* and *Synechocystis* PCC6714. The 16:0 acid accounted for about 50% of total lipids in MGDG, DGDG and PG, but far more than 50% in SQDG. Although the mode of unsaturation of the C16 acids and of the C18 acids was different from that in Group 2, the positional distribution (data not shown) indicates that the C18 acids and 16:0 are distributed to the *sn*-1 and *sn*-2 posi-

Table 3 Positional distribution of fatty acids in lipid classes of *Mastigocladus laminosus* grown at 34°C

Lipid class	Position	Fatty acid (mole %)					
		14:0	16:0	16:1	18:0	18:1	ΣC16
MGDG	*sn*-1	t	t	25	t	25	25
	sn-2	1	22	21	1	5	43
DGDG	*sn*-1	t	t	28	1	22	28
	sn-2	2	24	16	1	6	40
SQDG	*sn*-1	t	14	1	4	32	15
	sn-2	t	45	1	2	1	46
PG	*sn*-1	t	5	2	14	29	7
	sn-2	t	45	1	3	1	46

t: Trace (less than 0.5%).

The values represent the averages calculated from two independent measurements. The deviation of the values was within ±1.2%.

Table 4 Fatty-acid composition of lipid classes of *Nostoc muscorum, Plectonema boryanum*, and *Synechococcus* PCC7002 (Group 2) grown at 34°C

Lipid class	Fatty acid (mole %)								
	14:0	16:0	16:1	18:0	18:1	18:1(11c)	18:2	18:3α	ΣC16
N. muscorum									
MGDG	t	37	15	t	2	t	8	39	52
DGDG	t	37	17	1	2	t	7	35	54
SQDG	t	60	3	2	7	1	7	20	63
PG	1	55	3	1	5	t	9	26	58
P. boryanum									
MGDG	t	27	27	t	2	1	10	33	54
DGDG	t	24	31	t	1	1	9	34	55
SQDG	t	68	2	2	4	1	9	15	70
PG	t	56	2	1	3	1	12	24	58
Synechococcus PCC7002									
MGDG	t	35	20	t	24	t	20	1	55
DGDG	t	30	26	1	14	t	27	2	56
SQDG	1	65	7	1	21	t	5	t	72
PG	t	54	3	1	27	t	15	t	57

t: Trace (less than 0.5%).

tions, respectively, in MGDG, DGDG and PG. A unique feature of this group was that the major C18 acid at the *sn*-1 position was 18:2 in SQDG and PG but 18:3γ in MGDG and DGDG. This result suggests that unsaturation at the Δ6 position is present in MGDG and DGDG, but not in SQDG and PG. These observations further suggest that the major molecules of these lipid classes are of the *sn*-1-(unsaturated C18)-*sn*-2-(16:0) type. SQDG additionally includes a molecule of the *sn*-1-(16:0)-*sn*-2-(16:0) type.

Group 4—Table 6 shows the fatty-acid composition of lipid classes of *T. tenuis* and *Synechocystis* PCC6803. 16:0 accounted for about 50% of fatty acids in all the lipid classes except for SQDG from *Synechocystis* PCC6803. The positional analysis suggested that the C18 acids were esterified to the *sn*-1 position with a minor contribution of 16:0 and that 16:0 was esterified exclusively to the *sn*-2 position. 18:3γ and 18:4 were abundant components of MGDG and DGDG, while they were at a very low level in SQDG and PG. This result indicates that the Δ6-unsaturated acids are abundant in MGDG and DGDG but negligible in

Table 5 Fatty-acid composition of lipid classes of *Synechocystis* PCC6714 and *Spirulina platensis* (Group 3) grown at 34°C

Lipid class	Fatty acid (mole %)							
	14:0	14:1	16:0	16:1	18:1	18:2	18:3γ	ΣC16
S. platensis								
MGDG	t	t	52	3	1	1	42	55
DGDG	t	t	51	5	2	3	38	56
SQDG	1	t	60	2	7	26	1	62
PG	1	t	55	1	5	35	1	56
Synechocystis PCC6714								
MGDG	t	t	53	2	5	16	23	55
DGDG	t	t	55	2	4	13	25	57
SQDG	t	t	64	3	13	17	1	67
PG	1	t	53	1	8	35	1	54

t: Trace (less than 0.5%).

Table 6 Fatty-acid composition of lipid classes of *Tolypothrix tenuis* and *Synechocystis* PCC6803 (Group 4) grown at 30°C

Lipid class	Fatty acid (mole %)									
	14:0	16:0	16:1	18:0	18:1	18:2	18:3α	18:3γ	18:4	ΣC16
T. tenuis										
MGDG	0	56	3	t	1	2	2	14	21	59
DGDG	0	58	3	t	2	3	2	13	19	61
SQDG	0	60	1	t	19	11	4	t	t	61
PG	0	60	1	t	4	10	23	t	t	61
Synechocystis PCC6803										
MGDG	1	51	3	t	2	7	t	32	3	54
DGDG	1	51	3	1	2	7	t	32	4	54
SQDG	1	68	5	1	6	13	4	1	t	73
PG	3	51	2	1	5	17	20	1	t	53

t: Trace (less than 0.5%).

SQDG and PG. It is estimated that the predominant molecules present in Group 4 are of the *sn*-1-(unsaturated C18)-*sn*-2-(16:0) type in all the lipid classes. In addition, the *sn*-1-(16:0)-*sn*-2-(16:0) species is also present in SQDG.

Lipid composition—Table 7 shows the composition of lipid classes for eight strains from the four groups. The level of MGDG ranged from 44% to 63% and the levels of each of the other three lipid classes ranged mainly from 8% to 23% of the total. These results suggest that MGDG can account for about a half of the total glycerolipids and that DGDG, SQDG and PG account for the other half, making approximately similar contributions.

Discussion

The present analysis of fatty acids in various cyanobacterial strains indicates that cyanobacteria can be classified into four groups according to the degree of unsaturation and positional distribution of fatty acids. This result is consistent with the proposal by Kenyon (1972) and Kenyon et al. (1972) with respect to the existence of the four groups, but it is not consistent with it with respect to the distribu-

Table 7 Composition of lipid classes from cyanobacterial strains

Strain	Growth temp.	Lipid class (mole %)			
		MGDG	DGDG	SQDG	PG
Group 1					
Mastigocladus laminosus	34°C	51	14	15	20
Synechococcus PCC7942	34°C	57	13	9	21
Group 2					
Plectonema boryanum	28°C	51	21	13	15
Nostoc muscorum	28°C	63	18	8	11
Synechococcus PCC7002	34°C	58	12	19	13
	22°C	57	9	11	23
Group 3					
Spirulina platensis	32°C	47	16	17	20
Synechococcus PCC6714	34°C	44	23	23	10
Group 4					
Tolypothrix tenuis	30°C	60	16	9	15
Synechocystis PCC6803	34°C	59	17	16	8
	22°C	54	18	15	13

tion of unicellular and filamentous strains among the four groups. Kenyon et al. (1972) inferred that the unicellular strains belonged to Groups 1 and 3 whereas the filamentous strains were distributed in Groups 2, 3 and 4. We found, in the present study, that both unicellular and filamentous strains belong to each of the four groups.

In the present study, we analyzed fatty acids from all the known transformable strains of cyanobacteria, namely, *Synechococcus* PCC7942, *Synechococcus* PCC7002, *Synechocystis* PCC6714 and *Synechocystis* PCC6803 (Golden et al. 1987). These strains differ in terms of the mode of fatty-acid desaturation because they are members of the four different groups, even though they share the common characteristic of transformability. As demonstrated by Wada et al. (1990), the transformability of these strains is extremely useful and holds the promise of the possible manipulation of genes that are related to the desaturation of fatty acids.

Validity of the classification of cyanobacteria by fatty-acid unsaturation—The temperature of growth affects the unsaturation of fatty acids in a number of cyanobacterial strains that have been examined (Sato and Murata 1980, Sato et al. 1979, Wada and Murata 1990). The extent of unsaturation is enhanced at low temperature within the range of physiological growth conditions. This phenomenon has raised the question of the validity of the classification of cyanobacteria according to the unsaturation of fatty acids. The present study clearly demonstrates that, although the growth temperature effectively changes the relative proportions of the constituent fatty acids, at no temperature of growth is any specific fatty acids eliminated that is characteristic of each particular group (Table 1).

Mode of desaturation of fatty acids—Table 8 summarizes the distribution of major fatty acids at the *sn*-1 and *sn*-2 positions of lipid classes in the four groups of cyanobacteria. Group 1 is unique in the absence of polyunsaturated fatty acids. In this group, 16:1 is found at the *sn*-2 position in MGDG and DGDG but not in SQDG and PG. Group 2 is characterized by the unsaturated C16 acids at the *sn*-2 position of MGDG and DGDG and by the presence of 18:3*α*. Strains in Group 3 contain 18:3*γ* in MGDG and DGDG. Group 4 is characterized by 18:4 in MGDG and DGDG.

The desaturation of fatty acids in plants and cyanobacteria is characteristic in that double bonds are introduced into fatty acids in the lipid-bound form (Harwood 1988, Jaworski 1987, Sato and Murata 1982, Sato et al. 1986). This type of desaturation can be termed acyl-lipid desaturation by analogy to acyl-CoA desaturation in animals (Holloway 1983). The desaturation process in cyanobacteria is particularly unique, in that all the desaturation reactions take place with fatty acids in the lipid-bound form (Sato and Murata 1982). In eukaryotic plants, by contrast, the first double bond is introduced into 18:0 when it is in the form of 18:0-(acyl-carrier protein), and further desaturation is of the type of acyl-lipid desaturation (Harwood 1988, Jaworski 1987).

On the basis of these considerations and the data in Table 8, it is possible to consider the mode of desaturation of fatty acids in cyanobacteria as depicted in Table 9. In Group 1, both 16:0 and 18:0 at the *sn*-1 position are desaturated at the *Δ*9 position of the fatty acids. At the *sn*-2 position, 16:0 is desaturated at its *Δ*9 position in MGDG and DGDG of *M. laminosus*. In Group 2, 18:0 at the *sn*-1 posi-

Table 8 Major fatty acids at the *sn*-1 and *sn*-2 positions of the glycerol moiety of glycerolipids of the four groups of cyanobacteria

Group	Major molecular species in		
	MGDG and DGDG	SQDG	PG
1	16:1/18:1 16:0/16:1 Gal/Gal_2	16:0/18:1 16:0 Sq	18:0/18:1 16:0 Pg
2	18:1/18:2/18:3*α* 16:0/16:1/16:2 Gal/Gal_2	16:0/18:1/18:2/18:3*α* 16:0 Sq	18:1/18:2/18:3*α* 16:0 Pg
3	18:1/18:2/18:3*γ* 16:0 Gal/Gal_2	16:0/18:1/18:2 16:0 Sq	18:1/18:2 16:0 Pg
4	18:1/18:2/18:3*γ*/18:4 16:0 Gal/Gal_2	16:0/18:1/18:2/18:3*α* 16:0 Sq	18:1/18:2/18:3*α* 16:0 Pg

Gal, galactosyl; Gal_2, digalactosyl; Sq, sulfoquinovosyl; Pg, glycerylphosphatidyl.

Table 9 The Δ position of fatty-acid desaturation

Classification	Δ Position of desaturation			
	MGDG and DGDG		SQDG and PG	
	sn-1 (C18)	*sn*-2 (C16)	*sn*-1 (C18)	*sn*-2 (C16)
Group 1	9[a]	N[b] or 9	9[a]	N
Group 2	9, 12, 15	9, 12	9, 12, 15	N
Group 3	6, 9, 12	N	9, 12	N
Group 4	6, 9, 12, 15	N	9, 12, 15	N

[a] Mixture of C18 and C16.

[b] N stands for no desaturation at the *sn*-2 position.

tion is desaturated at the Δ9, Δ12 and Δ15 positions in all lipid classes and 16:0 at the *sn*-2 position is desaturated at the Δ9 position of MGDG and DGDG. In Group 3, 18:0 at the *sn*-1 position is desaturated at the Δ6, Δ9 and Δ12 positions of MGDG and DGDG and at the Δ9 and Δ12 positions of SQDG and PG. In Group 4, 18:0 at the *sn*-1 position is desaturated at the Δ6, Δ9, Δ12 and Δ15 positions of MGDG and DGDG and at the Δ9, Δ12 and Δ15 positions of SQDG and PG (see also Ref. Wada and Murata 1989). It is unlikely, however, that the fatty acids are desaturated in DGDG (Sato and Murata 1980). Our previous study in which we used radiolabeled tracers to monitor fatty-acid synthesis in *A. variabilis* suggested that molecules of DGDG characterized by the constituent fatty acids are synthesized by galactosylation of the corresponding molecules of MGDG.

The effect of growth temperature on the fatty-acid desaturation can be reevaluated in reference to the mode of fatty-acid desaturation in Table 9. When cells grown at low temperature are compared with cells grown at high temperature, following changes are observed. In Group 1, the Δ9 desaturation at the sn-1 and sn-2 positions is accelerated. In Group 2, the Δ15 and Δ12 desaturations are induced at the sn-1 and sn-2 positions, respectively. In Group 3, the Δ6 desaturation at the sn-1 position of MGDG is accelerated. In Group 4, the Δ15 desaturation at the sn-2 position is induced in all lipid classes, and the Δ6 desaturation at the sn-1 position of MGDG is accelerated.

Transfer of acyl moieties—In addition to the mode of fatty-acid desaturation, it is important to mention the specificity of the transfer of acyl moieties at the early steps of the biosynthesis of lipids in cyanobacteria. As summarized in Table 8, the specific and characteristic distribution of fatty acids to the *sn* positions according to their chain lengths is apparent. In all the groups, the *sn*-2 position is esterified exclusively by the C16 fatty acids. This observation suggests that transfer of acyl moieties to this position is specific for 16:0. The same type of specificity for 16:0 is found in the transfer of acyl moieties from acyl-ACP to the *sn*-2 position of 1-acylglycerol-3-phosphate in eukaryotic chloroplasts (Frentzen et al. 1983).

With respect to the transfer of acyl moieties to the *sn*-1 position in cyanobacteria, there is diversity among the groups. The strains of Group 1 transfer both 18:0 and 16:0 to the *sn*-1 position, whereas the strains of Groups 2, 3 and 4 esterify 18:0 at the *sn*-1 position. However, this specificity toward 18:0 at the *sn*-1 position is not as strict as that toward 16:0 at the *sn*-2 position. This type of flexibility of transfer of acyl moieties at the *sn*-1 position is also observed in the transfer of acyl groups from acyl-ACP to glycerol-3-phosphate in chloroplasts of higher plants (Frentzen et al. 1983, 1987). The enzyme from temperate plants that is responsible for this reaction, namely, acyl-ACP: glycerol-3-phosphate acyltransferase, is specific for 18:1-ACP, whereas the enzymes from tropical plants fail to discriminate effectively between 18:1-ACP and 16:0-ACP (Frentzen et al. 1987).

It is of interest that the two low temperature-sensitive strains of cyanobacteria, namely, *Synechococcus* PCC7942 and *Synechococcus* PCC6301, are members of Group 1 in which the specificity of this transfer of acyl moieties is rather flexible, reflecting the situation in tropical plants which exhibit flexible specificity of the acyltransfer reaction.

The authors are grateful to Professors Yoshihiko Fujita and Kaori Ohki, National Institute for Basic Biology, for their generous gift of several strains of cyanobacteria.

References

Andersson, B.A., Christie, W.W. and Holman, R.T. (1975) Mass spectrometric determination of positions of double bonds in polyunsaturated fatty acid pyrrolidides. *Lipids* 10: 215–129.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid

extraction and purification. *Can. J. Biochem. Physiol.* 37: 911–917.

Fork, D.C., Murata, N. and Sato, N. (1979) Effect of growth temperature on the lipid and fatty acid composition, and the dependence on temperature of light-induced redox reactions of cytochrome *f* and of light energy distribution in the thermophilic blue-green alga, *Synechococcus lividus*. *Plant Physiol.* 63: 524–530.

Frentzen, M., Heinz, E., Mckeon, T.A. and Stumpf, P.K. (1983) Specificities and selectivities of glycerol-3-phosphate acyltransferase and monoacylglycerol-3-phosphate acyltransferase from pea and spinach chloroplasts. *Eur. J. Biochem.* 129: 629–636.

Frentzen, M., Nishida, I. and Murata, N. (1987) Properties of the plastidial acyl-(acyl-carrier protein): glycerol-3-phosphate acyltransferase from the chilling-sensitive plant squash (*Cucurbita moschata*). *Plant Cell Physiol.* 28: 1195–1201.

Golden, S.S., Brusslan, J. and Haselkorn, R. (1987) Genetic engineering of the cyanobacterial chromosome. *Methods Enzymol.* 153: 215–231.

Harwood, J.L. (1988) Fatty acid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39: 101–138.

Holloway, P.W. (1983) Fatty acid desaturation. *In* The Enzymes, Vol. 16. Edited by Boyer, P.D. pp. 63–83. Academic Press, Orlando, Florida.

Jaworski, J.G. (1987) Biosynthesis of monoenoic and polyenoic fatty acids. *In* The Biochemistry of Plants, Vol. 9. Edited by Stumpf, P.K. pp. 159–174. Academic Press, Orlando, Florida.

Kenyons, C.N. (1972) Fatty acid composition of unicellular strains of blue-green algae. *J. Bacteriol.* 109: 827–834.

Kenyons, C.N., Rippka, R. and Stanier, R.Y. (1972) Fatty acid composition and physiological properties of some filamentous blue-green algae. *Arch. Microbiol.* 83: 216–236.

Lem, N.W. and Stumpf, P.K. (1984) In vitro fatty acid synthesis and complex lipid metabolism in the cyanobacterium *Anabaena variabilis*. I. Some characteristics of fatty acid synthesis. *Plant Physiol.* 74: 134–138.

McKeon, T.A. and Stumpf, P.K. (1982) Purification and characterization of the stearoyl-acyl carrier protein desaturase and the acyl-acyl carrier protein thioesterase from maturing seeds of safflower. *J. Biol. Chem.* 257: 12141–12147.

Murata, N. and Nishida, I. (1987) Lipids of blue-green algae (cyanobacteria). *In* The Biochemistry of Plants, Vol. 9. Edited by Stumpf, P.K. pp. 315–347. Academic Press, Orlando, Florida.

Nichols, B.W. and Wood, B.J.B. (1967) The occurrence and biosynthesis of gamma-linolenic acid in the blue-green alga, *Spirulina platensis*. *Lipids* 3: 46–50.

Ono, T.-A. and Murata, N. (1981) Chilling susceptibility of the blue-green alga *Anacystis nidulans*. I. Effect of growth temperature. *Plant Physiol.* 67: 176–181.

Parker, P.L., Van Baalen, C. and Maurer, L. (1967) Fatty acids in eleven species of blue-green algae: geochemical significance. *Science* 155: 707–708.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111: 1–61.

Sato, N. and Murata, N. (1980) Temperature shift-induced responses in lipids in the blue-green alga *Anabaena variabilis*: The central role of diacylmonogalactosylglycerol in thermoadaptation. *Biochim. Biophys. Acta* 619: 353–366.

Sato, N. and Murata, N. (1982) Lipid biosynthesis in the blue-green alga, *Anabaena variabilis*. II. Fatty acids and lipid molecular species. *Biochim. Biophys. Acta* 710: 279–289.

Sato, N. and Murata, N. (1988) Membrane lipids. *Methods Enzymol.* 167: 245–251.

Sato, N., Murata, N., Miura, Y. and Ueta, N. (1979) Effect of growth temperature on lipid and fatty acid compositions in the blue-green algae, *Anabaena variabilis* and *Anacystis nidulans*. *Biochim. Biophys. Acta* 572: 19–28.

Sato, N., Seyama, Y. and Murata, N. (1986) Lipid-linked desaturation of palmitic acid in monogalactosyl diacylglycerol in the blue-green alga (cyanobacterium) *Anabaena variabilis* studied in vivo. *Plant Cell Physiol.* 27: 819–35.

Stanier, R.Y., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. (1971) Purification and properties of unicellular blue-green algae (order *Chroococcales*). *Bacteriol. Rev.* 35: 171–205.

Stapleton, S.R. and Jaworski, J.G. (1984) Characterization of fatty acid biosynthesis in the cyanobacterium *Anabaena variabilis*. *Biochim. Biophys. Acta* 794: 249–255.

Stumpf, P.K. (1981) Plants, fatty acids, compartments. *Trends Biochem. Sci.* 6: 173–176.

Wada, H. and Murata, N. (1989) *Synechocystis* PCC6803 mutants defective in desaturation of fatty acids. *Plant Cell Physiol.* 30: 971–978.

Wada, H. and Murata, N. (1990) Temperature-induced changes in the fatty acid composition of the cyanobacterium, *Synechocystis* PCC6803. *Plant Physiol.* 92: 1062–1069.

Wada, H., Gombos, Z. and Murata, N. (1990) Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. *Nature* 347: 200–203.

Watanabe, A. (1960) List of algal strains in collection at the Institute of Applied Microbiology, University of Tokyo. *J. Gen. Appl. Microbiol.* 6: 283–292.

(Received June 3, 1992; Accepted July 22, 1992)