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MINI-REVIEW

Low-Temperature Effects on Cyanobacterial Membranes

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Abstract

The effect of change in ambient temperature on fatty acid unsaturation has been studied in the cyanobacterium *Anabaena variabilis*. When cells isothermally grown at 22°C are compared with those grown at 38°C, the relative content of oleic acid decreases and that of linolenic acid increases in all of the lipid classes. After a temperature shift from 38 to 22°C, palmitic acid is rapidly desaturated in monogalactocyldiacylglycerol, but in no other lipids, and oleic acid is slowly desaturated in most lipid classes. When cells of *Anacystis nidulans* are exposed to low temperature such as 0°C, they lose physiological activities and finally die. This low-temperature damage is initiated by the phase transition of lipids in the plasma membrane. The phase transition of thylakoid membrane that occurs at intermediate temperature produces loss of activity related to photosynthesis. This is, however, recovered when the cells are rewarmed to growth temperature. A model for the mechanism of the low-temperature damage in the cyanobacterial cells is proposed.

Key Words: Cyanobacterium; fatty acid desaturation; lipid phase transition; low-temperature damage; temperature effect; *Anabaena variabilis*; *Anacystis nidulans*.

Introduction

The cyanobacteria are classified as Gram-negative bacteria, and their cell envelope is composed of the outer membrane and plasma (inner) membrane separated by a peptidoglycan layer. In addition, they have intracellular photosynthetic membranes, i.e., the thylakoid membrane. The structure of the membrane is similar to that of the chloroplast, which contains an outer

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and inner envelope membrane, and an intrachloroplast membrane, i.e, the thylakoid membrane.

The thylakoid membranes of cyanobacteria contain chlorophyll *a* and *β*-carotene (Omata and Murata, 1983, 1984a), and attach phycobilisomes to their outer surface (Gantt and Conti, 1969). They are the site of both photosynthetic and respiratory electron transport (Omata and Murata, 1984b, 1985). The plasma membrane and outer membrane, on the other hand, contain xanthophylls, but no or little chlorophyll *a* or *β*-carotene (Murata *et al.*, 1981; Omata and Murata, 1983, 1984a; Resch and Gibson, 1983; Jürgens and Weckesser, 1985).

The thylakoid and plasma membranes contain glyceroglycolipids and phosphatidylglycerol, whereas the outer membrane contains lipopolysaccharides and hydrocarbons in addition to the glycerolipids (Murata and Nishida, 1987). The biosynthetic pathway of glyceroglycolipids in the cyanobacteria differs from that in higher plants and green algae (Sato and Murata, 1982a–c; Murata and Nishida, 1987).

Changes in the lipid composition, membrane fluidity, and physiological activities induced by ambient low temperature have been studied in limited strains of cyanobacteria, such as *Anacystis nidulans, Anabaena variabilis, Synechococcus lividus* and *Synechocystis* PCC 6803. Therefore, this mini-review deals with these four strains of cyanobacteria.

Glycerolipids and Fatty Acids

The cyanobacteria contain four major glycerolipids—monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG), and phosphatidylglycerol (PG)—and a minor component, monoglucosyl diacylglycerol (GlcDG) (reviewed by Murata and Nishida, 1987). MGDG represents 50–60% of the total glycerolipid content, and DGDG, SQDG, and PG each amount to 10–20% (Sato *et al.*, 1979), whereas GlcDG amounts to only ~1% (Sato and Murata, 1982a).

The glycerolipid compositions of the thylakoid membranes, plasma membrane, and outer membrane was compared in *A. nidulans* (Omata and Murata 1983; Murata *et al.*, 1981), and no significant difference between the three types of membranes was observed. However, the glycerolipid content relative to the total dry weight was found to be 19%, 57%, and 3% in the thylakoid, plasma, and outer membrane, respectively (Murata *et al.*, 1981; Omata and Murate, 1983).

The fatty acids thus far known to be present in cyanobacteria are palmitic acid (16 : 0), palmitoleic acid (16 : 1), hexadecadienoic acid (double bond positions undetermined) (16 : 2), stearic acid (18 : 0), oleic acid (18 : 1),

Table I. Fatty Acid Composition of the Total Lipids of Cyanobacteria

Strain	Growth temp. (°C)	Fatty acid										
		14:0	16:0	18:0	14:1	16:1	18:1	16:2	18:2	α-18:3	γ-18:3	18:4
Anacystis nidulans[a]	38	1	48	4	1	39	7[b]	0	0	0	0	0
	22	2	43	tr	9	45	tr	0	0	0	0	0
Synechococcus lividus[c]	55	0	54	22	0	10	14	0	0	0	0	0
	38	0	42	1	0	36	20	0	0	0	0	0
Anabaena variabilis[a]	38	0	31	1	0	22	24	tr	22	tr	0	0
	22	0	29	tr	0	22	7	3	15	24	0	0
Synechocystis PCC 6803[d]	34	tr	54	tr	0	4	7	0	14	0	20	tr
	22	tr	51	tr	0	3	2	0	6	8	21	8

[a]Sato *et al.* (1979), [b]Mixture of ω9 and ω7, [c]Fork *et al.* (1979) and [d]Wada and Murata (unpublished).

linoleic acid (18 : 2), α-linolenic acid (α-18 : 3), γ-linolenic acid (γ-18 : 3), and ω3,6,9,12-octadecatetraenoic acid (18 : 4) (reviewed by Murata and Nishida, 1987). *Anacystis nidulans* contains, in addition, vaccenic acid (Sato *et al.*, 1979). Table I shows the fatty acid composition of the total lipids in the four strains of cyanobacteria. It is noted that *A. nidulans* and *S. lividus* contain saturated and monounsaturated fatty acids. *Anabaena variabilis* contains, in addition, 18 : 2 and α-18 : 3. *Synechocystis* PCC 6803 contains unique fatty acids γ-18 : 3 and 18 : 4, in addition.

The fatty acid composition of MGDG is similar to that of DGDG, and the fatty acid composition of SQDG is similar to that of PG in all four strains. Distribution of specific fatty acids between the C-1 and C-2 positions of the *sn*-glycerol moiety can be categorized into two types. In *A. nidulans*, most of the monounsaturated fatty acids, 14 : 1, 16 : 1, and 18 : 1, are esterified at the C-1 position and most of 16 : 0 is at the C-2 position in all lipid classes (Sato *et al.*, 1979). In *A. variabilis* (Sato *et al.*, 1979) and *Synechocystis* PCC 6803 (Wada and Murata, unpublished), the C_{18} acids are esterified to the C-1 position, and the C_{16} acids to the C-2 position in all lipid classes. Figure 1

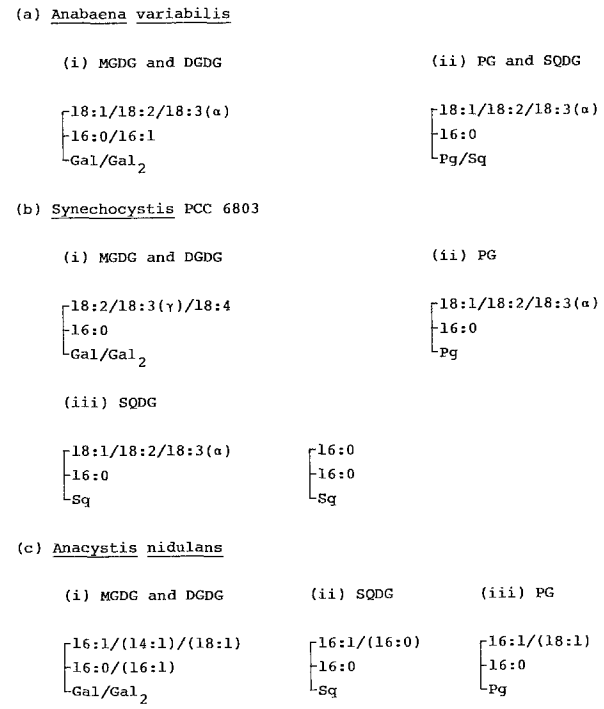

Fig. 1. Fatty acid distribution to the *sn*-glycerol of glycerolipids in cyanobacteria. Minor fatty acids are neglected in this scheme. Glc, glucose; Gal, galactose; Pg, phosphoglycerol; Sq, sulfoquinovose; 18 : 3 (α), α-linolenic acid (α-18 : 3); and 18 : 3 (γ), γ-linolenic acid (γ-18 : 3).

shows the positional distribution of fatty acids and major molecular species of individual lipids in *A. variabilis*, *Synechocystis* PCC 6803, and *A. nidulans*.

Temperature-Dependent Changes in Glycerolipids

Isothermal Growth

When cells of *A. variabilis* grown at 22°C are compared with those grown at 38°C, the relative contents of 18 : 1 and 18 : 2 are lower and the relative content of α-18 : 3 is higher in the total lipids (Table I). Similar changes are observed at the C-1 position of all the major lipid classes, while the levels of C_{16} acids remain nearly constant except for a minor decrease in 16 : 1 and increase in 16 : 2 at the C-2 positions of MGDG and DGDG (Sato *et al.*, 1979; Sato and Murata, 1980b). The temperature dependence of the molecular species composition in *A. variabilis* indicates that a decrease in growth temperature from 38°C to 22°C decreases 18 : 1/16 : 0 and 18 : 2/16 : 0 species and increases α-18 : 3/16 : 1 in all the major lipid classes, and decreases 18 : 1/16 : 1 and increases α-18 : 3/16 : 1 and α-18 : 3/16 : 2 in MGDG and DGDG (Sato and Murata, 1980a).

When cells of *Synechocystis* PCC 6803 grown at 22°C were compared with those grown at 34°C, the relative contents of 18 : 1 and 18 : 2 are lower and those of α-18 : 3 and 18 : 4 are higher in the total lipids (Table I). Changes in the fatty acids are characteristic in the individual lipids. In the expense of 18 : 1 and 18 : 2, 18 : 4 increases in MGDG and DGDG, and α-18 : 3 increases in PG. All of these changes occur at the C-1 position of *sn*-glycerol moiety. SQDG responds to the temperature differently; with decrease in growth temperature from 34°C to 22°C, the levels of 16 : 0 and 18 : 0 decrease and those of 18 : 2 and α-18 : 3 increase at the C-1 position (Wada and Murata, unpublished). The temperature dependence of the molecular species in *Synechocystis* PCC 6803 indicates that a decrease in growth temperature induces decreases in 18 : 1/16 : 0 and 18 : 2/16 : 0 in MGDG, DGDG, and PG and increases in 18 : 4/16 : 0 in MGDG and in α-18 : 3/16 : 0 in PG. SQDG responds to the temperature change by decreasing 16 : 0/16 : 0 and increasing 18 : 2/16 : 0 and α-18 : 3/16 : 0.

In contrast to *A. variabilis* and *Synechocystis* PCC 6803, *A. nidulans* and *S. lividus*, which contain only saturated and monounsaturated fatty acids, respond to change in growth temperature differently. A decrease in growth temperature decreases 16 : 0 and 18 : 0 and increases 16 : 1 and 18 : 1 in the total lipids (Table I). Sato *et al.* (1979) and Murata *et al.* (1979) have found that, in *A. nidulans*, the mode of changes with growth temperature in the fatty acid composition of all of the major lipid classes is similar to that of the total lipids. They have also demonstrated that, with decrease in growth

temperature, the average chain length of monounsaturated acids is reduced at the C-1 positions of all the major lipid classes, whereas the relative content of 16 : 1 increases and 16 : 0 concomitantly decreases at the C-1 positions of MGDG and DGDG. Although the molecular species composition in lipids from *A. nidulans* have not been well documented, it can be estimated from the composition and positional distribution of fatty acids. When cells grown at 28°C are compared with those grown at 38°C, the 16 : 0/16 : 0 and 18 : 1/16 : 0 species decrease and the 16 : 1/16 : 0 and 14 : 1/16 : 0 species increase in all the lipid classes.

The increase in unsaturation and decrease in the chain length of esterified fatty acids are known to lower the phase transition temperature of membrane lipids (Chapman *et al.*, 1974). The changes in fatty acid composition with growth temperature can be regarded as an adaptive response to changes in the ambient temperature (Ono and Murata, 1982; Murata *et al.*, 1984).

Temperature Shift

When the growth temperature is suddenly shifted downward, the fatty acid composition of *A. variabilis* is rapidly altered (Sato and Murata, 1980a, b). For 10 h after the temperature shift from 38 to 22°C, the total amount of lipids stays at a constant level (Sato and Murata, 1980a), but a decrease in 16 : 0 and a concomitant increase in 16 : 1 take place at the C-2 position of MGDG. Then, as lipid synthesis resumes, the ratio of 16 : 0 to 16 : 1 is slowly restored to that seen prior to the temperature shift. This type of transient desaturation of 16 : 0 to 16 : 1 is not observed in the other major lipid classes such as DGDG (Murata and Nishida, 1987). The rapid and transient introduction of a *cis*-double bond into 16 : 0 that takes place at the C-2 position in MGDG is regarded as an emergency acclimation to compensate for the decrease in membrane fluidity due to the decrease in temperature (Sato and Murata, 1980a, b). The rapid change in levels of unsaturation of the C_{16} acids after the temperature shift does not require *de novo* synthesis of fatty acids (Sato and Murata, 1981). The mass spectrometric analysis of ^{13}C-enriched MGDG indicates that, during the change in unsaturation, 16 : 0 is desaturated in the lipid-bound form and not replaced by previously synthesized 16 : 1 (Sato *et al.*, 1986). Molecular oxygen, but not light, is necessary for the desaturation (Sato and Murata, 1981). The findings that desaturation is greatly suppressed by chloramphenicol, an inhibitor of protein synthesis, and rifampicin, an inhibitor of RNA synthesis, suggests that a fatty acid desaturase, specific to 16 : 0 at the C-2 position of MGDG, is transiently synthesized after the downward shift in temperature (Sato and Murata, 1981).

Decreases in the relative contents of 18 : 1 and 18 : 2 and an increase in α-18 : 3 take place at the C-1 position of MGDG, SQDG, and PG, but these occur more slowly than the changes in C_{16} acids of MGDG. DGDG most slowly responds to the temperature shift. The desaturation of 18 : 1 and 18 : 2 after the temperature shift is also abolished by inhibitors of protein synthesis and RNA synthesis (Sato and Murata, 1981).

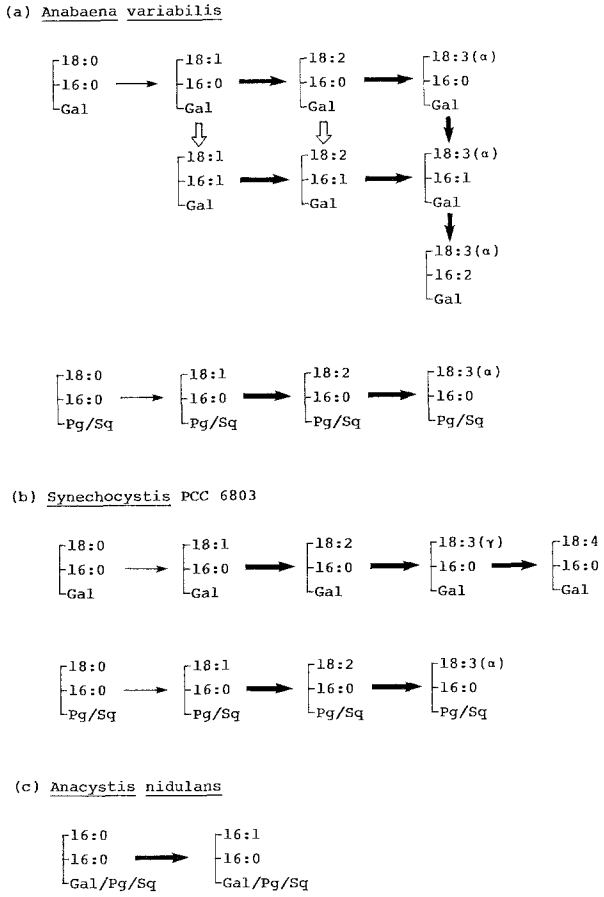

Fig. 2. A scheme for the biosynthesis of glycerolipid molecular species and the effect of downward temperature shift on the fatty acid desaturation: (closed broad arrows) desaturation stimulated after the downward temperature shift, (broad open arrows) desaturation stimulated only transiently after the downward temperature shift, and (thin arrows) desaturation that is highly active regardless of the temperature change. (a) *Anabaena variabilis*; temperature change from 38°C to 22°C. (b) *Synechocystis* PCC 6803; temperature change from 34°C to 22°C. (c) *Anacystis nidulans*; temperature change from 38°C to 28°C.

The study on biosynthesis of lipid molecular species in *A. variabilis* was reviewed by Murata and Nishida (1987). The cyanobacterium lacks the ability to desaturate fatty acids in the thioester form (Lem and Stumpf, 1984; Stapleton and Jowarski, 1984). Only saturated fatty acids are esterified to the *sn*-glycerol, and then desaturated to unsaturated fatty acids in the lipid-bound form (Sato and Murata 1982b, c; Sato *et al.*, 1986). Figure 2 shows the biosynthetic pathway of lipid molecular species in *A. variabilis* and the steps that are stimulated after a downward temperature shift.

When the growth temperature of *Synechocystis* PCC 6803 is shifted from 34°C to 22°C, the total amount and composition of lipids stay constant for 10 h (Wada and Murata, unpublished). During this period, however, 18:2 and γ-18:3 are desaturated to 18:4 at the C-1 position of MGDG, and 18:1 and 18:2 are desaturated to α-18:3 at the C-1 position of PG and SQDG, while the fatty acids of DGDG are unchanged. In contrast to the C_{18} acids, the 16:0 at the C-2 position is not desaturated in any of the lipid classes. These changes in fatty acid unsaturation take place only in the light and are inhibited by DCMU, showing a contrast to the case in *A. variabilis*. Figure 2 shows a hypothetical pathway of lipid synthesis in *Synechocystis* PCC 6803 and the steps that are stimulated after the downward temperature shift.

In *A. nidulans*, a downward temperature shift from 38 to 28°C produces both rapid and slow changes in the fatty acid composition (Murata *et al.*, 1979). In contrast to *A. variabilis* and *Synechocystis* PCC 6803, 16:0 at the C-1 positions of MGDG, SQDG, and PG is converted to 16:1 within 10 h after the temperature shift. Decrease in chain lengths of the monounsaturated fatty acids at the C-1 position also takes place in all the lipid classes. Desaturation of 16:0 to 16:1 at the C-2 position of MGDG and DGDG occurs only slowly. The rapid desaturation of 16:0 to 16:1 at the C-1 position of the lipids, i.e., the conversion of 16:0/16:0 species into 16:1/16:0 in MGDG, PG, and SQDG plays an important role in the acclimation of *A. nidulans* to the lower temperature.

Lipid Phase of Membranes

Anacystis nidulans

Temperature-dependent changes in the physical phases of membrane lipids in *Anacystis nidulans* have been studied by several techniques such as differential scanning calorimetry, X-ray diffraction, spin probe electron paramagnetic resonance spectrometry, freeze-fracture electron microscopy, carotenoid absorption, and chlorophyll fluorescence. Some of these

techniques can be applied to the plasma membranes, and others to the thylakoid membranes.

Freeze-fracture electron microscopy was used to observe the phase-separation state of the plasma membranes of *A. nidulans* (Ono and Murata, 1982). Temperatures for the onset of phase separation in the plasma membranes determined by this method were 5°C and 16°C in cells grown at 28°C and 38°C, respectively (Table II). However, the method cannot be applied to determine the temperature for the onset of phase separation in the thylakoid membrane, since this membrane is fractured only when a large proportion of the membrane area is in the gel state (Ono and Murata, 1982).

Yamamoto and Bangham (1978) observed that zeaxanthin incorporated into phosphatidylcholine liposomes reveals a characteristic absorption change when the liposome membrane goes from a liquid-crystalline into a phase-separated state. A very similar absorption change can be measured in intact cells, membrane fragments, and extracted lipids of *A. nidulans* when they are exposed to low temperature (Brand, 1977, 1979; Ono and Murata,

Table II. Temperatures for the Onset of Phase Separation of Plasma and Thylakoid Membranes, and for the Characteristic Changes in Physiological Phenomena, in *Anacystis nidulans* Grown at 28°C and 38°C

Method and phenomenon	Sample	28°C-cells		38°C-cells	
		PM[a]	TM[b]	PM	TM
Freeze-fracture electron microscopy[c]	C	5	—	16	—
Carotenoid absorption[d]	C	5	—	15	—
Chlorophyll *a* fluorescence[e]	C	—	13	—	25
Spin probe[f]	M	5	14	13	23
X-ray diffraction[g]	M	—	16	—	26
Critical for irreversible damage					
Photosynthesis[d,h]	C	5		16	
Hill reaction[d]	C	5		15	
Leakage					
Electrolytes[h]	C	8		14	
Potassium ion[i]	C	7		17	
Animo acids[i]	C	7		17	
Break in Arrhenius plot					
Photosynthesis[h,j]	C	14		22	
Phosphorylation[k]	M	—		24	

C and M stand for intact cells and isolated membranes, respectively. [a]Plasma membrane; [b]Thylakoid membranes; [c]Ono and Murata (1982); [d]Ono and Murata (1981a) and Vigh *et al.* (1985); [e]Murata and Fork (1975), Murata and Ono (1981), and Vigh and Joó (1983); [f]Wada *et al.* (1984) and Murata *et al.* (1975); [g]Tsukamoto *et al.* (1980); [h]Murata *et al.* (1984); [i]Ono and Murata (1981b); [j]Murata *et al.* (1983); [k]Ono and Murata (1979).

1981a). The spectral change is observed predominantly in the plasma membranes in which zeaxanthin amounts to 70% of the total carotenoids, but little change is observed in the thylakoid membranes in which β-carotene is the major carotenoid component (Omata and Murata, 1983). These findings suggest that the carotenoid absorption change is an indicator for the lipid phase of the plasma membrane, but not that of the thylakoid membranes. The temperature for the onset of phase separation of the plasma membrane determined by carotenoid absorption is presented in Table II. Gombos and Vigh (1986) reached the same conclusion by using the nitrate-starved cells of *A. nidulans*, which retain a normal plasma membrane, but contain degraded thylakoid membranes.

In model membrane systems composed of synthetic lipids and chlorophyll *a*, the chlorophyll *a* fluorescence shows characteristic changes at phase transition temperature of the membranes (Lee, 1975; Knoll *et al.*, 1980). Since chlorophyll *a* is localized in the thylakoid membranes, but not in the plasma or outer membranes (Omata and Murata, 1983, 1984), the fluorescence can be regarded as a tool to detect the lipid phase of the thylakoid membranes. The temperature dependence of fluorescence yield in intact cells shows a characteristic peak at temperatures (Table II) that correspond to those for the onset of phase separation (Murata and Fork, 1975; Murata and Ono, 1981; Vigh and Joó, 1983).

The spin probe method can be applied to both plasma and thylakoid membranes prepared from *A. nidulans* (Wada *et al.*, 1984; Murata *et al.*, 1975). Clear breaks in the Arrhenius plot of the rotational correlation time of a fatty acid spin probe are observed (Wada *et al.*, 1984). This indicates an abrupt change occurring in the fluidity of the membrane lipids, i.e., the phase transition (Table II).

The X-ray diffraction of lipid bilayers gives a sharp reflection line corresponding to a Bragg spacing of 4.2 A when the membrane is in the gel state, and a diffuse line corresponding to a Bragg spacing of 4.5 A when they are in the liquid-crystalline state (Rivas and Luzzati, 1969). Tsukamoto *et al.* (1980) studied the temperature dependence of the 4.2-A and 4.5-A reflections in thylakoid membranes, and suggested that the membranes from *A. nidulans* cells grown at 28°C are in the phase-separated state between 16°C and − 5°C, while those from cells grown at 38°C are in the phase-separated state between 26°C and 0°C (Table II).

The temperatures critical for the onset of phase separation of the plasma membrane and thylakoid membranes studied by various methods are summarized in Table II. The methods of freeze-fracture electron microscopy, carotenoid absorption, and spin labeling provide nearly the same values for the temperature for the onset of phase separation in the plasma membranes from cells grown at 28°C and 38°C. The three methods—chlorophyll fluorescence,

spin labeling, and X-ray diffraction—provide about the same values for the temperature of the onset of phase separation in the thylakoid membranes from cells grown at 28°C or 38°C. According to the data summarized in Table II, the following facts are suggested with respect to the lipid phase of the membranes in *A. nidulans*: (a) At growth temperatures, both types of membranes are in the liquid-crystalline state. (b) With decrease in temperature to below those that permit growth, the thylakoid membranes first enter a phase-separated state. (c) At ~ 10°C below the onset of phase separation in the thylakoid membranes, the plasma membrane enters a phase-separated state. (d) The temperature for the onset of phase separation of both types of membranes depends on the growth temperature.

Irrespective of different features of the phase transition of the plasma and thylakoid membranes, there is no discernible difference between the plasma membranes and thylakoid membranes with respect to their polar lipid and fatty acid composition (Omata and Murata, 1983). The higher phase transition temperature of the thylakoid membrane with respect to the plasma membrane may be due to the high protein content of the thylakoid membranes, 70% of the dry weight, compared with the corresponding value of 40% found in plasma membranes (Omata and Murata, 1983). The shift in phase transition temperature due to the growth temperature corresponds to the growth temperature-dependent alteration in the fatty acid composition (Sato *et al.*, 1979).

Mannock *et al.* (1985a, b) studied the phase behavior of MGDG, DGDG, SQDG, and PG isolated from *A. nidulans* by differential scanning calorimetry and X-ray diffraction. In a cooling scan of aqueous dispersions of MGDG, DGDG, SQDG, and PG from cells growtn at 38°C, the phase separation begins to appear at 17°C, 11°C, 15°C, and 13°C, respectively.

A number of studied have demonstrated that *A. nidulans* is susceptible to low temperature, since Forrest *et al.* (1957) first observed the release of pteridines and glutamic acid from intact cells, and a corresponding loss of photosynthetic activity at 4°C. When the algal cells are exposed to temperatures near 0°C, viability declines (Rao *et al.*, 1977), and activities of overall photosynthesis, photosynthetic electron transport, and phosphorylation all diminish (Forrest *et al.*, 1957; Jansz and MacLean, 1973; Rao *et al.*, 1977; Ono and Murata, 1981a; Vigh and Joó, 1983; Vigh *et al.*, 1985). Also, potassium ions are released from the cells (Forrest *et al.*, 1957; Jansz and MacLean 1973; Ono and Murata, 1981b; Vigh *et al.*, 1985), and morphological alterations of the plasma and the thylakoid membranes are detectable (Brand *et al.*, 1979). These low temperature-dependent phenomena are irreversible and, even when the cells are again warmed to growth temperature, only partial recoveries or none at all are observed. The critical temperatures for irreversible damage of the cells depend on growth temperature

(Rao *et al.*, 1977; Ono and Murata, 1981a, b; Vigh *et al.*, 1985), suggesting that the temperature-dependent alteration of membrane lipids (Sato *et al.*, 1979) may be involved in the susceptibility to low temperature.

Temperatures critical for irreversible damage and ion leakage, and for breaks in the Arrhenius plots, are also listed in Table II. The critical temperatures for the irreversible damage of photosynthesis and the Hill reaction are 5°C and 15°C in cells grown at 28°C and 38°C, respectively, and correspond to the onset of phase separation in the plasma membranes. At about the same temperatures, potassium ions, free amino acids (Ono and Murata, 1981b) and electrolytes (Murata *et al.*, 1984) begin to leak from the cells to the surrounding medium. In contrast, breaks in the Arrhenius plots of photosynthesis, phosphorylation, and state-1–state-2 transition, and the declines of delayed fluorescence appear at ~ 15°C and ~ 25°C in cells grown at 28°C and 38°C, respectively (Murata *et al.*, 1975, 1983; Ono and Murata, 1977, 1979), which correspond to the onset of phase separation in the thylakoid membranes.

Based on these observations, a mechanism as shown in Fig. 3 can be proposed for low-temperature-induced phenomena in *A. nidulans*. The plasma membrane and thylakoid membranes are barriers for ions and small molecules, whereas the outer membrane of the cell envelope is permeable to them. At the growth temperature, the plasma membrane and thylakoid membranes are both in the liquid-crystalline state and are impermeable to ions and small molecules. With decrease in temperature, the thylakoid membranes go into a phase-separated state and become permeable to ions and small molecules. Under these conditions, physiological activities such as photosynthesis and photosynthetic ATP formation are reversibly diminished as revealed by a break in the Arrenhius plot (Murata *et al.*, 1975, 1983; Ono and Murata, 1979). With a further decrease in temperature, the plasma membrane enters a phase-separated state and becomes permeable. Under these conditions, ions and small molecules in the cytoplasm leak out, and those in the surrounding medium leak in. This diminishes cellular metabolism, leading to the death of the cell. Even when the temperature is raised to that which supports growth in unchilled cells, the concentrations of ions and small molecules are not recovered, thus resulting in irreversible damage of all physiological activities of the cells.

The suggestion that the irreversible damage at low temperature in *A. nidulans* is induced by the phase transition in the plasma membrane, but not in the thylakoid membrane, has been verified by Vigh *et al.* (1985), who selectively hydrogenated most of the unsaturated fatty acids in the plasma membrane, but not those of the thylakoid membrane of *A. nidulans*. They observed parallel shifts in the degree of fatty acid saturation, the temperature

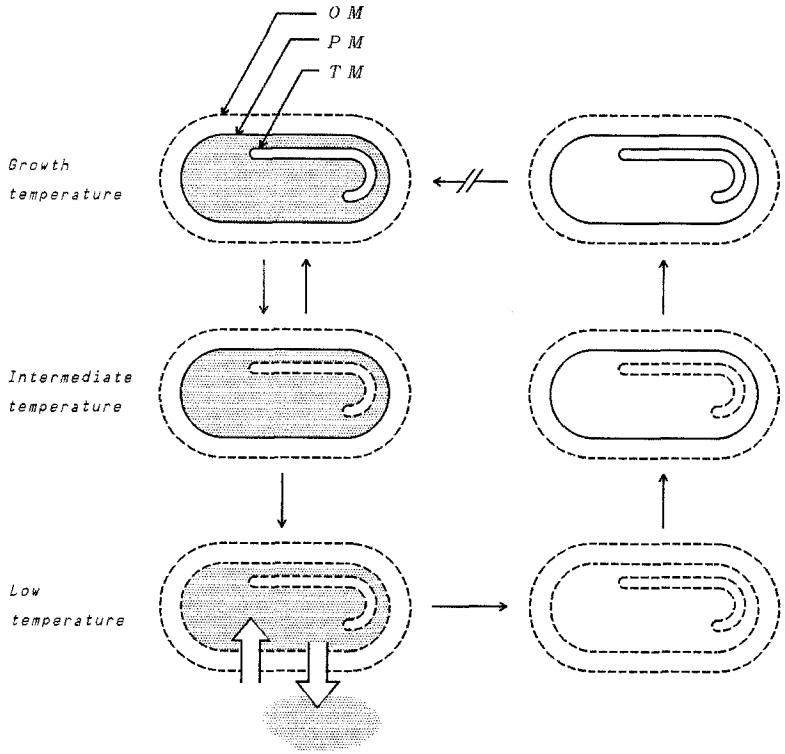

Fig. 3. A scheme for the low-temperature-induced irreversible damage in *Anacystis nidulans*. OM, PM, and TM stand for outer membrane, plasma membrane, and thylakoid membrane, respectively. Bold and dashed lines, respectively, indicate membranes impermeable and leaky to ions and small molecules. At growth temperature, the plasma membrane is in the liquid-crystalline state and impermeable. With decrease in temperature, the thylakoid membrane first enters a phase-separated state. Under these conditions, the cell loses most physiological activities, but only reversibly. With further decrease in temperature, the plasma membrane enters a phase-separated state and therefore becomes leaky. Under these conditions, ions and small molecules in the cytoplasm leak out and those in the outer medium leak in. Upon rewarming the cells to growth temperature, the plasma membrane resumes the liquid-crystalline state and recovers impermeability. Under these conditions, however, the ions and small molecules that have once been leaked out are not restored, leading to the irreversible damage of the cells.

for the onset of phase separation in the plasma membrane, and the temperature critical for K^+ leakage and the irreversible decline of photosynthesis. Gombos and Vigh (1986), using nitrate-starved cells of *A. nidulans*, which contain intact plasma membrane but degraded thylakoid membranes, observed that K^+ leakage occurs at the same temperature as the temperature that induces the onset of phase separation in the plasma membrane.

Anabaena variabilis

Anabaena variabilis is tolerant of low temperatures. Freeze-fracture electron microscopy reveals that the plasma membrane of cells grown at 22°C and 38°C are in the liquid-crystalline state at 0°C (Ono and Murata, 1982). The electron paramagnetic resonance signal of a spin probe suggests that the thylakoid membranes from cells grown at 22°C and 38°C enter a phase-separated state at ~ 5°C and ~ 15°C, respectively (Wada *et al.*, 1984). Again, in this alga, the temperature-dependent phase transition of thylakoid membranes depends on the growth temperature, and is related to the growth temperature-dependent alteration of unsaturation level of their component fatty acids (Sato *et al.*, 1979).

No irreversible damage to photosynthesis or electrolyte leakage occurs at 0°C in cells grown at either 22°C or 38°C (Murata *et al.*, 1984). These results are related to the finding that the plasma membrane is in the liquid-crystalline state > 0°C (Ono and Murata, 1982; Wada *et al.*, 1984). In contrast, the thylakoid membranes enter the phase-separated state > 0°C, and a clear break in the Arrhenius plot of photosynthesis appears at the temperature for the onset of phase separation in the thylakoid membranes (Wada *et al.*, 1984; Murata *et al.*, 1984). Therefore, in *A. variabilis* as in *A. nidulans*, phase transition of the thylakoid membranes does not induce irreversible damage. This is consistent with the mechanism proposed in Fig. 3 for the low-temperature effect on *A. nidulans*, i.e., a phase transition in the plasma membrane is directly related to low-temperature damage.

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