Plant Physiol. (1981) 67, 182-187
0032-0889/81/67/0182/06/$00.50/0

Chilling Susceptibility of the Blue-green Alga *Anacystis nidulans*

II. STIMULATION OF THE PASSIVE PERMEABILITY OF CYTOPLASMIC MEMBRANE AT CHILLING TEMPERATURES[1]

Received for publication May 5, 1980 and in revised form July 23, 1980

TAKA-AKI ONO[2] AND NORIO MURATA[3]
Department of Biology, University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan

ABSTRACT

Potassium ions and amino acids were found to leak from the cytoplasm to the outer medium when the blue-green alga, *Anacystis nidulans*, was exposed to the chilling temperatures. The leakage was marked below the critical temperature regions, the midpoint values for which were around 5 and 14 C in cells grown at 28 and 38 C, respectively. These temperature regions coincided with those critical for the susceptibility of the photosynthetic activities and the carotenoid absorption spectrum previously studied (Ono TA, N Murata 1981 Plant Physiol 67: 176-181).

Potassium and magnesium ions in the cell suspension medium protected the algal cells from the chilling-induced damage of the Hill reaction with 1,4-benzoquinone. The activity of the Hill reaction which had been diminished by the first chilling treatment in a low salt medium was restored by the second chilling treatment of a high salt medium. The chilling susceptibility of the Hill reaction could be attributed to the leakage of cations from the cytoplasm due to increased permeability of the cytoplasmic membrane at the chilling temperatures.

A mechanism is proposed to interpret the chilling susceptibility of *A. nidulans*: (*a*) at chilling temperatures, the bilayer lipids of the cytoplasmic membrane are in the phase separation state; (*b*) ions and solutes having low molecular weights leak from the cytoplasm to the outer medium when the lipids of the cytoplasmic membrane are in the phase separation state; (*c*) decreases in the intracellular concentrations of ions and solutes degrade the physiological activities of the cells.

It is shown in the preceding paper (22) that *Anacystis nidulans* loses the activities of photosynthetic O_2 evolution and the Hill reaction when it is exposed to chilling temperatures. The temperature region critical for chilling susceptibility depends on growth temperature. The midpoint values for the critical regions are 4 and 12 C in cells grown at 28 and 38 C, respectively. These temperatures are much lower than those of the onset of phase separation of thylakoid membrane lipids (15, 16, 19, 21, 22).

In some heterotrophic bacteria, the intracellular solutes, such as permease-accumulated substances (10, 23) and K^+ (7), are released when the temperature is lowered below a critical value. The chilling susceptibility of these bacteria can be attributed to the stimulation of passive permeability of the cytoplasmic membrane at the low temperatures. In *A. nidulans*, some amino acids and pteridines are known to leak from the cells at chilling temperatures (4, 9).

The study presented here was conducted to elucidate the relationship between the leakage of the intracellular substances and the chilling susceptibility of *A. nidulans*. We suggest that the temperature-induced phase change of lipids of the cytoplasmic membrane, but not of the thylakoid membrane, is involved in the chilling susceptibility of this alga.

MATERIALS AND METHODS

A. nidulans was photoautotrophically grown at 28 and 38 C as described previously (22). The cells at the late logarithmic phase were harvested by centrifugation at 5000*g* for 5 min at the growth temperature and were suspended in an appropriate medium, the temperature of which had been adjusted to the growth temperature. The cell density was in a range of 100 to 150 μg Chl/ml for measurements of the releases of K^+, ninhydrin-reacting substances, and pteridines and in a range of 5 to 10 μg Chl/ml for measurements of photosynthesis and the Hill reaction. Chilling treatments of the cells were performed as described previously (22).

To investigate the effect of chilling treatment on the release of K^+, the harvested cells were washed three times by resuspension and recentrifugation with 40 mM Na-phosphate buffer (pH 7.0) at the growth temperature. The resultant pellet was resuspended in the same buffer solution. In experiments for the release of ninhydrin-reacting substances and pteridines. NaH_2PO_4 in the buffer was replaced by KH_2PO_4. After an appropriate period of chilling treatment, the cell suspension was filtrated through a well-washed glass fiber filter (Wattman GF/F; pore size, 0.6 μm). The filtration was completed within 15 s at the temperature of treatment. All of these procedures were done in the dark.

The amount of K^+ in the filtrate was determined in the flame-photometer with an atomic absorption spectrophotometer (Hitachi 508 A). The amount of ninhydrin-reacting substances was determined in the method of Yemm and Cocking (25). To identify the amino acids released from the cells, the filtrate was applied to the two-dimensional TLC on silica gel with the developing solvents 1-butanol-acetic acid H_2O (4:1:1, by volume) and H_2O-saturated phenol (18). Spots were detected with ninhydrin. The amount of pteridines in the filtrate was photometrically determined. After the complete oxidation of pteridines by exposing the filtrate to air at 4 C for 12 h (4), the *A* at 410 nm was measured.

To investigate the effect of salts and sucrose in the suspension medium on the chilling susceptibility of the Hill reaction with 1,4-benzoquinone and photosynthesis, the cells grown at 38 C were twice washed with 15 mM Tes-NaOH buffer (pH 7.0) at the growth temperature. The resultant pellet was resuspended in the same buffer solution at 38 C containing various concentrations of either

[1] This work was supported by Grant-in-Aid 448012 for Scientific Research (to N. M) from the Japanese Ministry of Education, Science, and Culture and was taken from a dissertation submitted by T.-A. O. to the University of Tokyo in parital fulfillment of the requirements for the Doctor of Science degree.

[2] Present address: Laboratory of Plant Physiology, Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan.

[3] To whom reprint requests should be addressed.

KCl, NaCl, or $MgCl_2$ in the salt effects, and 300 mM sucrose in the sucrose effects. After being kept at 38 C for 20 min, the cells were treated at 0 C or 60 min. They were warmed to 38 C and, after being kept at the temperature for 10 min, were subjected to the measurements. In experiments of the two-step treatment, the cells were washed with and resuspended in 15 mM Tes-NaOH buffer (pH 7.0) at 38 C. They were treated at 0 C for 60 min (the first treatment), warmed up to 38 C, and kept at the temperature for 10 min. 10 mM $MgCl_2$ was added to the cell suspension, and the cells were treated at 0 or 38 C for 30 min (the second treatment). After being rewarmed to 38 C and being kept at temperature for 10 min, the cells were subjected to the measurements. All of these procedures were done in the dark.

Activities of the Hill reaction with 1,4-benzoquinone and the photosynthesis were measured as described previously (22), Chl *a* concentration was determined in 80% aqueous acetone according to the method of MacKinney (11).

RESULTS

EFFECT OF CHILLING TREATMENT

On Release of Potassium Ions. When the algal cells grown at 38 C were exposed to 0 C, the leakage of K^+ from the cells to the suspension medium was greatly stimulated. Figure 1 shows time courses of K^+ release at 0 and 38 C. The K^+ release was small and slow at 38 C, *i.e.* the growth temperature, whereas it was marked and rapid at 0 C. The amount of leaked K^+ attained almost to the final level within 10 min at the low temperature.

There was a small, but distinct, amount of K^+ release at zero time even at 38 C. A non-zero value of K^+ release on changing the suspension medium has been observed in *E. coli* (7). This type of K^+ release might occur in *A. nidulans*.

An intracellular concentration of K^+ was estimated from the amount of released ions, on the assumption that all of K^+ leaked and that a cell had a cylindrical shape 3.0 μm in length and 0.5 μm in diameter (20). The estimated concentration was approximately 0.2 M.

Figure 2 shows the temperature dependence of the K^+ release. In cells grown at 38 C, the extent of K^+ released in 10 min was high below 10 C and very low above 17 C. A midpoint value for the temperature region critical for the K^+ release was around 14 C. In cells grown at 28 C, a similar feature of temperature dependence was found. A midpoint value for the critical temperature was around 4 C, although it could not be precisely determined. We note that the critical temperature region depended on the growth temperature.

On Release of Ninhydrin-reacting Substances. Jansz and MacLean (9) discovered that some amino acids were released from the cells of *A. nidulans* when they were exposed to the chilling temperatures. We re-examined this phenomenon using the cells grown at 38 C. Figure 3 shows that the ninhydrin-reacting substances leaked to the outer medium at 0 and 10 C. Most of the leakage was completed within 10 min. A small and slow release seemed to begin after 30 min of chilling treatment at 0 C. TLC on silica gel revealed that a major component of the released ninhydrin-reacting substances was glutamic acid. Two minor ninhydrin-positive spots were found on the thin layer plate but were not identified.

Figure 4 shows the temperature dependence of the release of ninhydrin-reacting substances. The temperature regions critical for the release appeared with midpoints around 5 and 13 C in cells grown at 28 and 38 C, respectively. The critical temperature regions coincided with those observed in the K^+ release. These findings indicate that a common mechanism underlies the chilling-induced releases of K^+ and the ninhydrin-reacting substances.

On Release of Pteridines. When the cells grown at 38 C were exposed to chilling temperatures, a yellow-colored and fluorescent substance with absorption peaks at 410 and 270 nm leaked from the cells. Forrest *et al.* (4) identified it as isosepiapterin. The pteridine began to leak only after 30 min of treatment at 0 C and 50 min at 10 C (Fig. 5). We note that the pteridine release was much slower than the releases of K^+ and ninhydrin-reacting

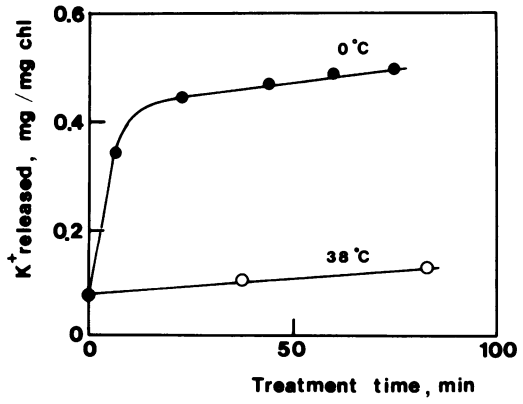

FIG. 1. Effect of chilling treatment on the K^+ release in cells grown at 38 C. The cells were suspended in 40 mM Na-phosphate buffer (pH 7.0) and treated at 38 C (○) or 0 C (●).

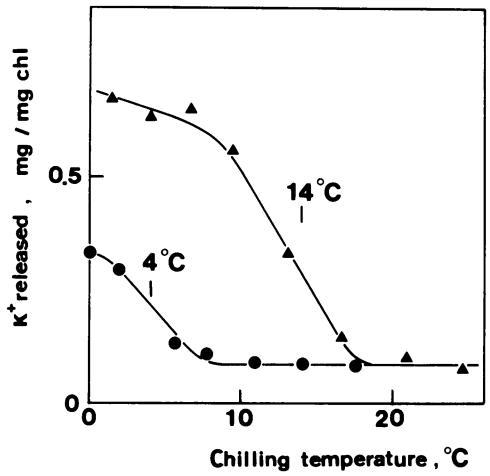

FIG. 2. Dependence of the K^+ release on chilling temperatures in cells grown at 28 C (●) and 38 C (▲). The cells were suspended in 40 mM Na-phosphate buffer (pH 7.0) and treated for 10 min at chilling temperatures.

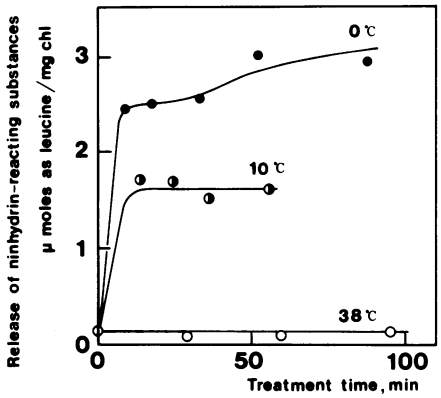

FIG. 3. Effect of chilling treatment on the release of ninhydrin-reacting substances in cells grown at 38 C. The cells were suspended in 40 mM Na/K-phosphate buffer (pH 7.0) and treated at 38 C (○), 10 C (◑), or 0 C (●).

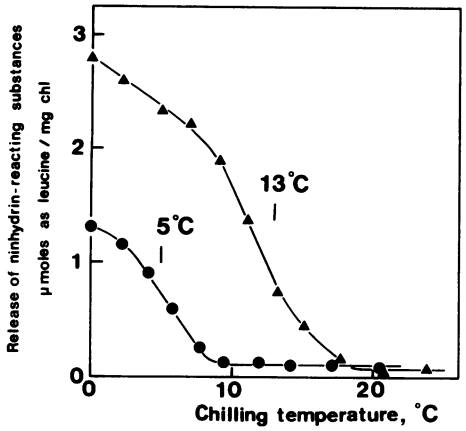

FIG. 4. Dependence of the release of ninhydrin-reacting substances on the chilling temperatures in cells grown at 28 C (●) and 38 C (▲). The cells were suspended in 40 mM Na/K-phosphate buffer (pH 7.0) and treated for 10 min at chilling temperatures. The amount of ninhydrin-reacting substances was calculated in terms of leucine.

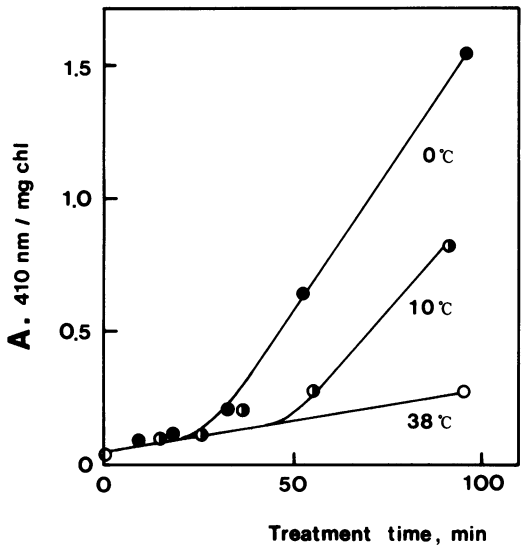

FIG. 5. Effect of chilling treatment on the release of pteridines in cells grown at 38 C. The cells were suspended in 40 mM Na/K-phosphate buffer (pH 7.0) and treated at 38 C (○), 10 C (◑), or 0 C (●). *A* at 410 nm due to pteridines was measured after pteridine solution was kept at 4 C for 12 h.

substances.

Effects of Salts and Sucrose on Chilling Susceptibility of Hill Reaction. KCl and $MgCl_2$ in the suspension medium were found to protect the cells against the chilling-induced damage of the Hill reaction with 1,4-benzoquinone. In Figures 6 and 7, the Hill reaction was measured after the cells grown at 38 C were treated at 0 or 38 C in the presence of various concentrations of KCl or $MgCl_2$. On treatment at 0 C (Fig. 6), the activity of the Hill reaction increased from 250 μmol O_2 evolved/mg Chl·h without KCl to 500 μmol O_2 evolved/mg Chl·h with 25 mM KCl. Further additions of KCl slightly suppressed the activity. On treatment at 38 C (Fig. 6), the activity decreased from 700 μmol O_2 evolved/mg Chl·h without KCl to 550 μmol O_2 evolved/mg Chl·h with 80 mM KCl. In increasing the KCl concentration, the relative activity (the activity of the 0 C-treated cells divided by that of 38 C-treated cells) augmented from 40% without KCl to 75% with 30 to 80 mM KCl. These findings indicate that KCl significantly protects the algal cells from the chilling-induced damage of the

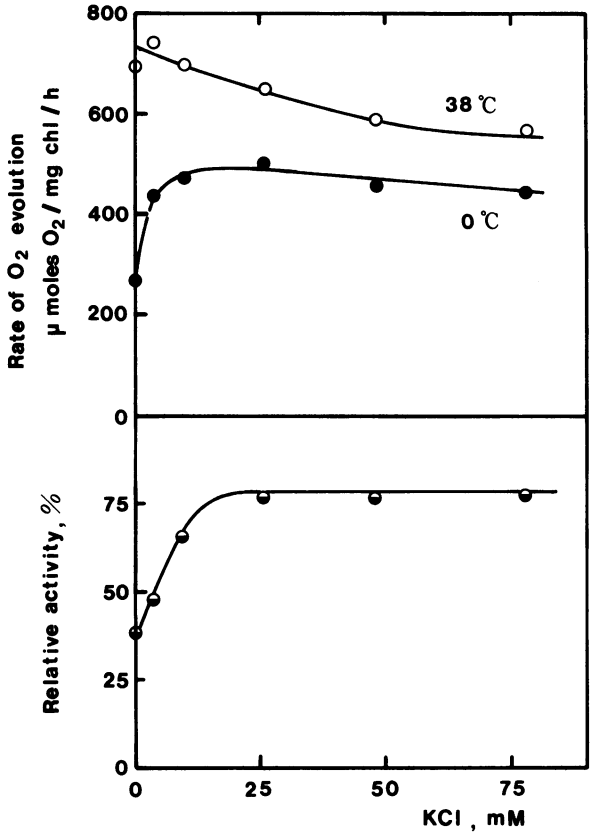

FIG. 6. Effect of the KCl concentration in the suspension medium on chilling susceptibility of the Hill reaction with 1,4-benzoquinone. Cells grown at 38 C were suspended in 15 mM Tes-NaOH buffer (pH 7.0) containing various concentrations of KCl and treated for 60 min at 38 C (○) or 0 C (●). Relative activity (◒) stands for the activity in the chilled cells divided by that in the unchilled cells.

Hill reaction. A KCl concentration required for the half-maximal effect of protection was 7.5 mM. NaCl at concentrations lower than 40 mM had the same effects as KCl did, although NaCl at concentrations higher than 50 mM strongly inhibited the Hill reaction at both 0 and 38 C.

In increasing the $MgCl_2$ concentration (Fig. 7), the activity remained almost constant at 38 C, whereas it augmented from a low to high level at 0 C. The relative activity increased from 35 to 75%. A $MgCl_2$ concentration for the half-maximum effect was 1.5 mM. $MgCl_2$ was 5 times as effective as KCl in protecting the cells from the chilling-induced damage.

Table I shows the effect of $MgCl_2$ on the chilling susceptibility of the Hill reaction in cells grown at 38 C. The activity of the Hill reaction which had decreased to 35% of control after the treatment at 0 C in the absence of $MgCl_2$ was restored to 74% of control when the cells were retreated at 0 C in the presence of 10 mM $MgCl_2$. This recovered activity was approximately the same as the activity in the cells which were directly treated at 0 C in the presence of $MgCl_2$. The Hill reaction was partially restored on the second treatment with $MgCl_2$ even at 38 C, although the recovered activity was lower than that at 0 C. It can be inferred, therefore, that the chilling is not necessary in recovering the declined activity in the second treatment.

To investigate the effect of osmotic pressure on the chilling susceptibility of the Hill reaction, the cells grown at 38 C were treated at 0 or 38 C in the presence of 300 mM sucrose (Table II). Sucrose decreased the activity to about two-thirds, irrespective of the presence or absence of $MgCl_2$ and the temperature of treatment. As a result, the chilling treatment in the presence of sucrose

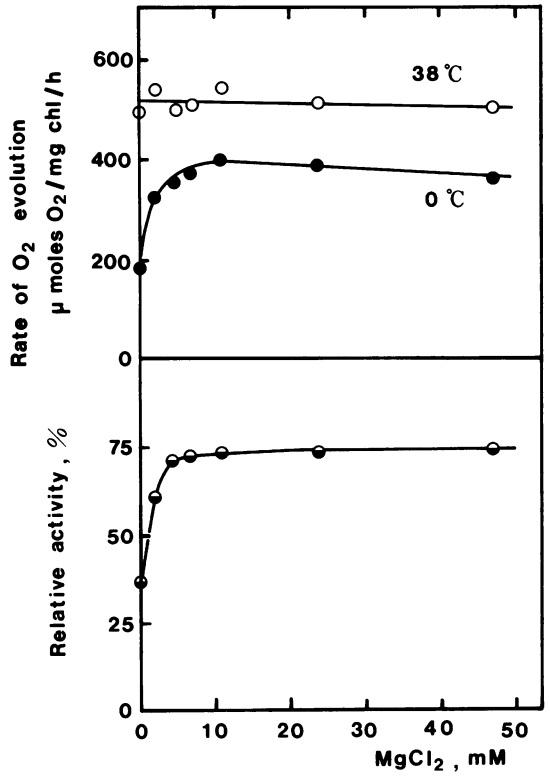

FIG. 7. Effect of the $MgCl_2$ concentration in the suspension medium on chilling susceptibility of the Hill reaction with 1,4-benzoquinone. Cells grown at 38 C were suspended in 15 mM Tes-NaOH buffer (pH 7.0) containing various concentrations of $MgCl_2$ and treated for 60 min at 38 C (○) or 0 C (●). Relative activity (◒) stands for the activity in the chilled cells divided by that in the unchilled cells.

Table I. *Activity of Hill Reaction with 1,4-Benzoquinone after Two-step Treatment in Presence and Absence of $MgCl_2$.*

Cells grown at 38 C were suspended in 15 mM Tes-NaOH buffer (pH 7.0), with or without 10 mM $MgCl_2$ as indicated, and were treated at 0 or 38 C for 60 min in the first treatment and for 30 min in the second treatment.

First Treatment		Second treatment		O_2 Evolved
Temperature	$MgCl_2$	Temperature	$MgCl_2$	
C		*C*		*μmol/mg Chl·h*
38	−			524
0	−			186
0	+			416
0	−	0	−	200
0	−	0	+	391
0	−	38	−	147
0	−	38	+	323

diminished the activity of the Hill reaction to 28% in the absence and 78% in the presence of 10 mM $MgCl_2$. We conclude that the osmotic pressure does not affect the protective effect of $MgCl_2$.

Effect of Salt on Chilling Susceptibility of Photosynthesis. The chilling susceptibility of photosynthesis O_2 evolution, on the other hand, was not influenced by $MgCl_2$. Figure 8 shows the effect of the $MgCl_2$ concentration on the photosynthesis when the cells grown at 38 C were treated at 0 or 38 C. At 38 C, $MgCl_2$ stimulated the photosynthetic activity at high concentrations. At 0 C, the activity was markedly suppressed throughout the $MgCl_2$ concentrations from 0 to 50 mM. The relative activity remained at 25% at all the $MgCl_2$ concentrations examined.

Table II. *Effect of Sucrose on Chilling Susceptibility of Hill Reaction with 1,4-Benzoquinone in Presence and Absence of $MgCl_2$.*

Cells grown at 38 C were suspended in 15 mM Tes-NaOH buffer (pH 7.0) containing 0 or 300 mM sucrose and 0 or 10 mM $MgCl_2$ as indicated and were treated at 0 or 38 C for 60 min.

Temperature	Additions		O_2 Evolved
	Sucrose	$MgCl_2$	
C			*μmol/mg Chl·h*
38	−	−	661
38	−	+	721
0	−	−	168
0	−	+	499
38	+	−	426
38	+	+	500
0	+	−	120
0	+	+	336

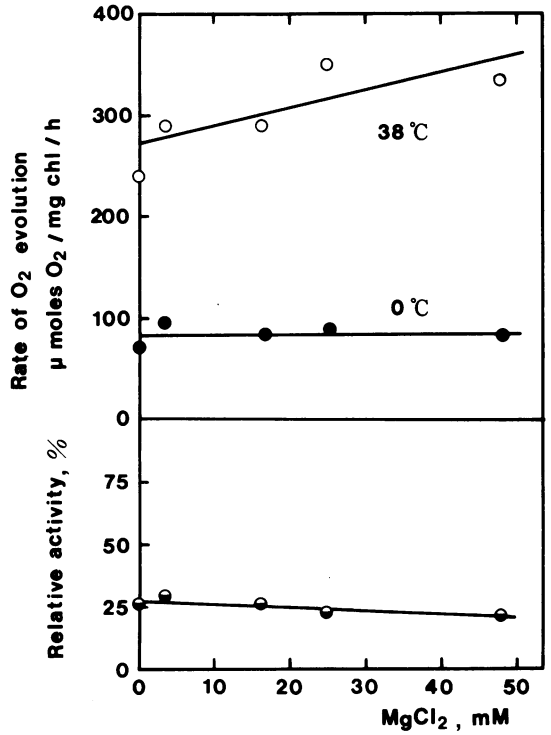

FIG. 8. Effect of the $MgCl_2$ concentration in the suspension medium on chilling susceptibility of photosynthetic O_2 evolution. Cells grown at 38 C were suspended in 15 mM Tes-NaOH buffer (pH 7.0) containing various concentrations of $MgCl_2$ and treated for 60 min at 38 C (○ or 0 C (●). Relative activity (◒) stands for the activity in the chilled cells divided by that in the unchilled cells.

DISCUSSION

We found that the critical temperature regions for the release of K^+ and amino acids were the same as for the susceptibility of the photosynthetic activities and the carotenoid absorption spectrum (22). Similar time courses were found in the release of the intracellular ions and solutes here and the responses of the photosyn-

thetic activities and the absorption spectrum in the preceding paper (22). We also note that the critical temperature regions depended on growth temperature in similar ways. The findings suggest that a common mechanism underlies chilling susceptibility and the release of intracellular ions and solutes.

Forrest *et al.* (4) inferred that the release of pteridines from the cells at chilling temperatures would be related to chilling susceptibility. As shown here, however, it is much slower than the inactivation of the photosynthesis and the Hill reaction and the leakage of K^+ and amino acids. These findings may indicate that the leakage of pteridines is not directly related to chilling susceptibility but, rather, is the result of chilling injury.

Studies on the lipid phase of thylakoid membrane in *A. nidulans* (15, 16, 19, 21) have shown that the onset of phase separation occurs around 15 and 25 C in cells grown at 28 and 38 C, respectively. These temperatures, however, are about 10 C higher than the critical temperature regions of chilling susceptibility and ion leakage. It is likely that a membrane system other than the thylakoid membrane is responsible for the chilling susceptibility.

Studies on the freeze-fracture electron microscopy (1, 2, 5, 24) have provided information on the lipid phase of cytoplasmic membrane of *A. nidulans*. Armond and Staehelin (1) have shown that the particle-free area where the lipids are probably in the gel state is much smaller in the cytoplasmic than in the thylakoid membrane at low temperatures. This fact seems to indicate that the onset of phase separation occurs at a lower temperature in the cytoplasmic than in the thylakoid membrane. Furtado *et al.* (5) have shown that the particle-free area is found in the cytoplasmic membrane at 5 C in cells grown at 28 C and at 15 C in cells grown at 38 C.

We have also studied the lipid phase of cytoplasmic membrane by means of the freeze-fracture electron microscopy (unpublished data). The result indicates that the onset of phase separation of the cytoplasmic membrane occurs around 14 C in cells grown at 38 C and 4 C in cells grown at 28 C. The membrane lipids are in the liquid crystalline state above, and in the phase separation state below, these temperatures. These temperature regions coincide with those critical for chilling susceptibility and ion leakage. This fact suggests that the lipid phase of cytoplasmic membrane is closely related to these temperature-dependent phenomena in *A. nidulans*.

It has been shown in the artificial membranes that the passive permeability for ions and solutes of low mol wt drastically alters according to the physical phase of lipid hydrocarbon core (6). The permeability is very high when the membrane lipids are in the phase separation state (6). The ions and solutes leak probably through the disordered structure at the interface between liquid crystalline and gel phases. A similar situation might be involved in the cytoplasmic membrane of *A. nidulans*.

The structure of cell envelope of *A. nidulans* is similar to the gram-negative bacteria (3). It is likely that the diffusion barrier for the intracellular solutes of low mol wt is the inner cytoplasmic membrane. The leakage of K^+ and amino acids from the cytoplasm upon chilling treatment suggests that the inner cytoplasmic membrane is the site of chilling susceptibility.

The effect of salts in protecting the cells from the chilling-induced damage of the Hill reaction can be rationalized by the stimulated passive permeability of cytoplasmic membrane. In the experiments of two-step treatment, the reduced activity of the Hill reaction after chilling treatment in a low salt medium was restored on treating the cells in a high salt medium. This fact strongly indicates that the cytoplasmic membrane becomes passively permeable and that the intracellular ions regulate the activity of the Hill reaction.

Also in the two-step treatment (Table I), the salt was found to restore partly the Hill reaction in the second treatment even at 38 C. This suggests that the cytoplasmic membrane was irreversibly damaged at the first chilling treatment and was still permeable after the cells were rewarmed to 38 C. The same conclusion was reached by Brand *et al.* (2) in a study on the freeze-fracture electron microscopy.

$MgCl_2$ is 5 times as effective as KCl and NaCl in protecting the cells from chilling-induced damage of the Hill reaction. This is an indication that cations, but not anions, are essential to the protective effect of salts. This type of valency-dependent effectiveness of cations is seen in the neutralization of surface potential of thylakoid membrane (8), the stacking of grana thylakoid (12) and the regulation of light energy distribution (13, 14).

The change in osmotic pressure due to 300 mM sucrose had no influence on the $MgCl_2$ effect in protecting the cells from the chilling-induced damage of the Hill reaction. It can be inferred, therefore, that the osmotic pressure by KCl and $MgCl_2$ did not afford the protective effect of salts on the Hill reaction.

The chilling-induced damage of photosynthetic O_2 evolution cannot be explained solely by the changes in the intracellular concentrations of cations because the high-salt medium does not have any protective effect on the activity. Releases of solutes other than cations may be also involved in the chilling susceptibility of photosynthesis.

It has been found by Nakajima *et al.* (17) that the intracellular concentration of K^+ is about 0.25 M in *E. coli* and this concentration is required for the growth of the bacterium. The intracellular concentration of K^+ (about 0.2 M) in *A. nidulans* estimated from the amount of released ions on chilling treatment is almost equal to that of *E. coli*.

We propose a mechanism for the chilling susceptibility of *A. nidulans* on the basis of the findings disclosed here and in the preceding paper (22): (*a*) at chilling temperatures, the bilayer lipids of the cytoplasmic membrane are in the phase separation state in which the passive permeability of the membrane is high; (*b*) ions and solutes with low mol wt, such as K^+ and amino acids, leak from the cytoplasm to the outer medium; (*c*) decreases in the intracellular concentrations of ions and solutes inactivate photosynthesis and other physiological activities.

Acknowledgments—The authors thank Dr. S. Okamura of Department of Chemistry. Faculty of Science, Toho University for the use of the atomic absorption spectrophotometer.

LITERATURE CITED

1. ARMOND, PA, LA STAEHELIN 1979 Lateral and vertical displacement of integral membrane proteins during lipid phase transition in *Anacystis nidulans*. Proc Natl Acad Sci USA 76: 1901–1905
2. BRAND JJ, SJ KIRCHANSKI, R RAMIREZ-MITCHELL 1979 Chill-induced morphological alterations in *Anacystis nidulans* as a function of growth temperature. Planta 145: 63–68
3. DREWS G, H MEYER 1964 Untersuchungen zum chemischen Aufbauder Zellwände von *Anacystis nidulans* und *Chlorogloea fritschii*. Arch Microbiol 48: 259–267
4. FORREST HS, C VAN BAALEN, J MYERS 1957 Occurrence of pteridines in a blue-green alga. Science 125: 699–700
5. FURTADO D, WP WILLIAMS, APR BRAIN, PJ QUINN 1979 Phase separation in membrane of *Anacystis nidulans* grown at different temperatures. Biochim Biophys Acta 555: 352–357
6. DE GIER J, MC BLOK, PWM VAN DIJICK, C MOMBERS, AJ VERKLEIJ, ECM VAN DER NEUT-KOK, LLM VAN DEENEN 1978 Relations between liposomes and biomembranes. Ann N Y Acad Sci 308: 85–100
7. HAEST CWM, J DE GIER, GA VANES, AJ VERKLEIJ, LLM VAN DEENEN 1972 Fragility of the permeability barrier of *Escherichia coli*. Biochim Biophys Acta 288: 43–53
8. ITOH S 1978 Electrostatic state of the membrane surface and the reactivity between ferricyanide and electron transport components inside chloroplast membranes. Plant Cell Physiol 19: 149–166
9. JANSZ ER, FI MACLEAN 1973 The effect of cold shock on the blue-green alga *Anacystis nidulans*. Can J Microbiol 19: 381–387
10. LEDER IG 1972 Interrelated effects of cold shock and osmotic pressure on the permeability of the *Escherichia coli* membrane to permease accumulated substances. J Bacteriol 111: 211–219
11. MACKINNEY G 1941 Absorption of light by chlorophyll solutions. J Biol Chem 140: 315–322

12. IZAWA S, NE GOOD 1966 Effect of salts and electron transport on the conformation of isolated chloroplast. I. Light-scattering and volume changes. Plant Physiol 41: 533–543
13. MURATA N, H TASHIRO, A TAKAMIYA 1970 Effect of divalent metal ions on chlorophyll *a* fluorescence in isolated spinach chloroplasts. Biochim Biophys Acta 197: 250–256
14. MURATA N 1971 Effect of monovalent cations on light energy distribution between two pigment systems of photosynthesis in isolated spinach chloroplasts. Biochim Biophys Acta 226: 422–432
15. MURATA N, DC FORK 1975 Temperature dependence of chlorophyl *a* fluorescence in relation to the physical phase of membrane lipids in algae and higher plants. Plant Physiol 56: 791–796
16. MURATA N, JH TROUGHTON, DC FORK 1975 The relationship between transition of physical phase of membrane lipids and photosynthetic parameters in *Anacystis nidulans* and lettuce and spinach chloroplasts. Plant Physiol 56: 508–517
17. NAKAJIMA H, I YAMATO, Y ANRAKU 1979 Quantitative analysis of potassium ion pool in *Escherichia coli* K-12. J Biochem 85: 303–310
18. NIEDERWISSER A 1972 Thin-layer chromatography of amino acids and derivatives. Methods Enzymol 25: 60–99
19. ONO T-A, N MURATA 1977 Temperature dependence of the delayed fluorescence of chlorophyll *a* in blue-green algae. Biochim Biophys Acta 460: 220–229
20. ONO T-A, N MURATA 1978 Photosynthetic electron transport and phosphorylation reactions in thylakoid membranes prepared from the blue-green alga *Anacystis nidulans*. Biochim Biophys Acta 502: 47–485
21. ONO T-A, N MURATA 1979 Temperature dependence of the photosynthetic activities in the thylakoid membranes from the blue-green alga *Anacystis nidulans*. Biochim Biophys Acta 545: 69–76
22. ONO T-A, N MURATA 1981 Chilling susceptibility of the blue-green alga *Anacystis nidulans*. I. Effect of growth temperature. Plant Physiol 67: 176–181
23. RING K 1965 Der Einfluss der Adaptationstemperatur auf die Kältestabilität der Zellmembran von *Streptomyces hydrogenans*. Biochim Biophys Acta 94: 598–600
24. VERWER W, PHJY VERVERGAERT, J LEUNISSEN-BIJVELT, AJ VERKLEIJ 1978 Particle aggregation in photosynthetic membranes of the blue-green alga *Anacystis nidulans*. Biochim Biophys Acta 504: 231–234
25. YEMM EW, EC COCKING 1955 Determination of amino acids with ninhydrin. Analyst 80: 209–213