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TEMPERATURE DEPENDENCE OF THE DELAYED FLUORESCENCE OF CHLOROPHYLL *a* IN BLUE-GREEN ALGAE

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SUMMARY

1. The delayed fluorescence of chlorophyll *a* was measured with a phosphoroscope by changing the temperature in a range of room temperatures in intact cells of blue-green algae, *Anacystis nidulans*, two strains of *Anabaena variabilis* and *Plectonema boryanum*, and other kinds of algae, *Cyanidium caldarium* and *Chlorella pyrenoidosa*. The induction of delayed fluorescence remarkably depended on the temperature of measurement. Nevertheless, the induction pattern was characterized by three levels of intensity; the initial rise level at the onset of excitation light, the maximum level after a period of excitation and the steady-state level after 10 min of excitation.

2. In *A. nidulans* and a strain of *A. variabilis* grown at various temperatures, a close relationship was found between the phase transition of membrane lipids and the initial rise and the steady-state levels of delayed fluorescence. The initial rise level showed the maximum at the temperature of phase transition between the liquid crystalline and the mixed solid-liquid crystalline states. The steady-state level showed a remarkable change from a high in the liquid crystalline state to a low level in the mixed solid-liquid crystalline state.

3. The millisecond decay kinetics of the delayed fluorescence measured at the steady-state level in *A. nidulans* grown at 38 °C consisted of two components with different decay rates. The half-decay time of the fast component was about 0.17 ms and was constant throughout the temperature range of measurement. The half decay time of slow component ranged from 0.6 to 1.5 ms, depending on the temperature of measurement.

INTRODUCTION

In recent years, the role of the physical phase of lipids in the biological membranes has been recognized in some of the characteristic temperature dependencies of

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the membraneous activities [1, 2]. Discontinuity in the physiological activities is often observed at the temperature of transition of the lipid phase [1, 2].

In the photosynthetic membranes, the transition of physical phase of lipids was found in a blue-green alga, *Anacystis nidulans* [3]. The yield of chlorophyll *a* fluorescence in the thylakoid membrane showed characteristic temperature dependence having a maximum at the temperature of phase transition between the liquid crystalline and the mixed solid-liquid crystalline states [4]. Some photosynthetic activities revealed discontinuity points in the Arrhenius plot at the phase transition temperature [3]. The phase transition of membrane lipids and the related characteristic change in the electron transport reaction were also reported in the chloroplasts of chilling-sensitive plants [5, 6].

The delayed fluorescence of chlorophyll *a* in the thylakoid membrane of photosynthetic organisms is in close relation to the primary photochemical reaction of photosynthesis. It originates from a reverse process of the primary photochemical reaction at the reaction center 2 (refs. 7 and 8). The high energy state of phosphorylation [9], the pH gradient across the thylakoid membrane [10], the membrane potential [11] or possibly the pH inside the thylakoid [12] stimulates this process.

In the present study the temperature dependence of the delayed fluorescence of chlorophyll *a* was investigated in the blue-green and other kinds of algae, in order to elucidate the relationship between the physical phase of membrane lipids and the delayed fluorescence.

METHODS

A. nidulans and *Anabaena variabilis* strain M-3 were grown at 38, 28 and 20 °C in Kratz and Myers' C medium [13]. *Plectonema boryanum* and *A. variabilis* strain M-2 were grown in the same medium but only at 28 °C. *Chlorella pyrenoidosa* was grown in the medium of Kuhl [14] at 28 °C. *Cyanidium caldarium* was grown at 38 °C in the medium of Watanabe [15]. The strains of these algae were obtained from the Algal Collection of the Institute of Applied Microbiology, the University of Tokyo. The cultures were bubbled with air enriched with 3 % CO_2. The cultures at the logarithmic phase were used for the measurement of delayed fluorescence after a suitable dilution with the culture media to give a chlorophyll concentration of 3 μg/ml. In the case of *A. variabilis* and *P. boryanum*, 0.5–1.0 % methyl cellulose was added to the cell suspension in order to prevent the precipitation of the cells during the measurement. This concentration of methyl cellulose did not alter any characteristics of the delayed fluorescence.

For the measurement of the delayed fluorescence, the quartz cuvette (20 mm × 20 mm wide and 2 mm thick) containing the algal cell suspension was immersed in water of designed temperature in a Dewar vessel having quartz windows. The temperature of the sample was monitored with a copper-constantan thermocouple. After the cuvette containing the sample was put in the Dewar vessel, the sample was kept in the dark for 9 min before turning on the excitation light. The change in temperature during the measurement of delayed fluorescence of 10 min did not exceed 0.5 °C.

The delayed fluorescence was measured with a modified Bequerel type phosphoroscope as described previously [16]. A cycle of excitation and measurement was

2.5 ms; 0–0.85 ms for excitation, 0.85–2.5 ms for darkness. The delayed fluorescence during 0.05–1.75 ms after the cessation of excitation flash was measured. The excitation light was obtained from a 500 watt xenon arc lamp and through optical filters, Hoya HA50, Toshiba V-Y50 and V-R60. The light intensity measured by a thermopile (Kipp and Zonen) was 54 000 ergs/cm^2 per s on the surface of the cuvette. The delayed fluorescence was detected by a photomultiplier (Hamamatsu TV R374). In the measurement of long term induction of delayed fluorescence the signal from the photomultiplier was smoothed by a combination of resistor and capacitor with a time constant of 50 ms, amplified, and recorded on a strip chart servo recorder (Riken Denshi SPH-4). In the measurement of millisecond decay kinetics, the signal was directly fed to a synchroscope (Hitachi V-018) with a time constant of 0.03 ms.

RESULTS

Fig. 1 shows the induction of the millisecond delayed fluorescence of chlorophyll *a* induced by the excitation with repetitive flashes in *A. nidulans* and *P. boryanum* both grown at 28 °C. The induction pattern varied depending on the temperature of measurement. It was noted, however, that the time course was composed of a rapid increase to the initial rise level, and a slow increase to the maximum level followed by a gradual decline to the steady-state level. This was similar to the typical induction pattern of millisecond delayed fluorescence (200 μs decay component) of the spinach chloroplasts [9, 16]. At temperatures below 20 °C in *A. nidulans* and 10 °C in *P. boryanum*, a trough between the initial rise and the maximum levels was marked, while at higher temperatures it disappeared because of a rapid development of the maximum level.

It is noted that the same shapes of the induction pattern appeared at lower temperatures in *P. boryanum* than in *A. nidulans*. For example, a trace at 7 °C in *P. boryanum* was similar to that at 20 °C in *A. nidulans*. This shift in temperature for the induction pattern of delayed fluorescence is possibly related to a difference in fluidity of membrane lipids. The phase transition of membrane lipids occurs at 13 °C in *A. nidulans* [3], and around 0 °C in *P. boryanum* as will be seen later. These findings suggest that the lipid phase of thylakoid membrane is more fluid in *P. boryanum* than in *A. nidulans*, and thus that the same fluidity should appear at a lower temperature in *P. boryanum* than in *A. nidulans*.

The intensities of delayed fluorescence measured at the initial rise, the steady-state and the maximum levels were plotted against temperature in *A. nidulans* grown at 28 and 38 °C (Fig. 2). Broken lines in the figure (see ref. 3), indicate the temperature dependence of chlorophyll *a* fluorescence in the presence of 3-(3′, 4′-dichlorophenyl)-1,1-dimethylurea in the same organism grown at the same temperatures. In the cells grown at 28 °C (Fig. 2a) the initial rise level showed a maximum at 13 °C. The steady-state level showed a marked intensity change from the high to the low level with a midpoint at 14 °C. The temperatures for the maximum in the initial rise level and for the midpoint in the steady-state level corresponded to the temperature of lipid phase transition (about 13 °C) detected by the spin label and the chlorophyll *a* fluorescence [3]. However, no clear correlation was found between the phase transition and the maximum level.

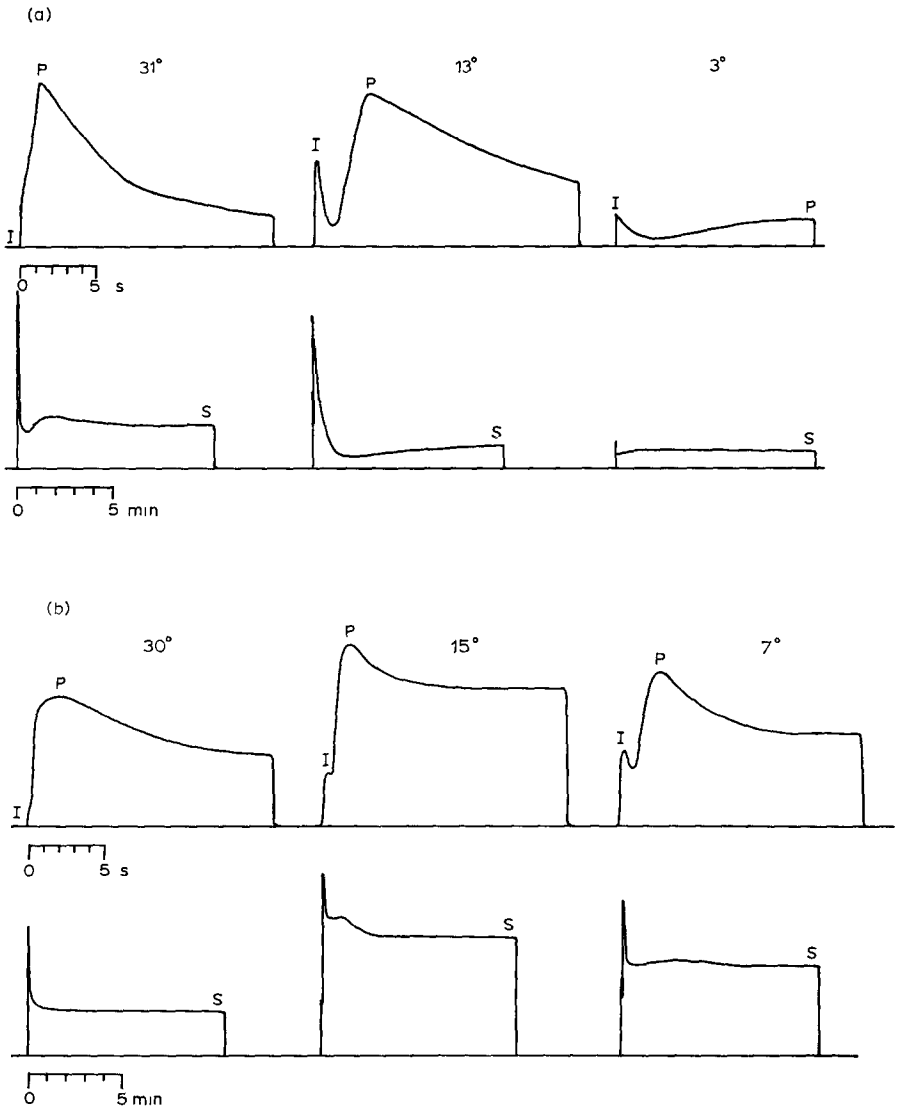

Fig 1. Induction of delayed fluorescence of chlorophyll *a* in *A. nidulans* (a) and *P. boryanum* (b) both grown at 28 °C. I, S and P represent the initial rise, the steady-state and the maximum levels, respectively.

The temperature dependence of the delayed fluorescence of *A. nidulans* grown at 38 °C is shown in Fig. 2b. The initial rise, the steady-state and the maximum levels responded to the temperature in a way similar to the cells grown at 28 °C, but the characteristic changes appeared at higher temperatures. The initial rise level showed a maximum at 21 °C. The steady-state level showed a steep decline on decreasing temperature with a midpoint at 22 °C. These temperatures correspond to that of the phase transition of lipids of thylakoid membrane of the alga grown at 38 °C [3].

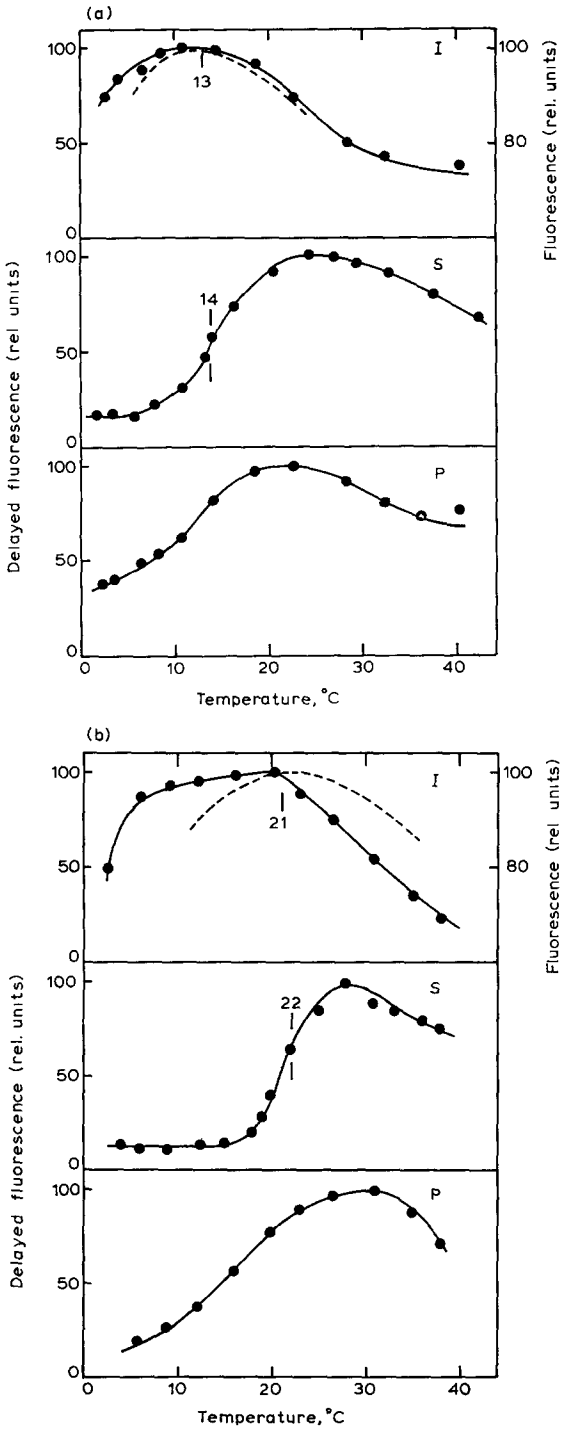

Fig. 2. Temperature dependence of delayed fluorescence at the initial rise (I), the steady-state (S) and the maximum (P) levels in *A. nidulans*. Temperature dependence of chlorophyll *a* fluorescence (dashed lines) is reproduced from ref. 3. (a) Cells grown at 28 °C. (b) Cells grown at 38 °C.

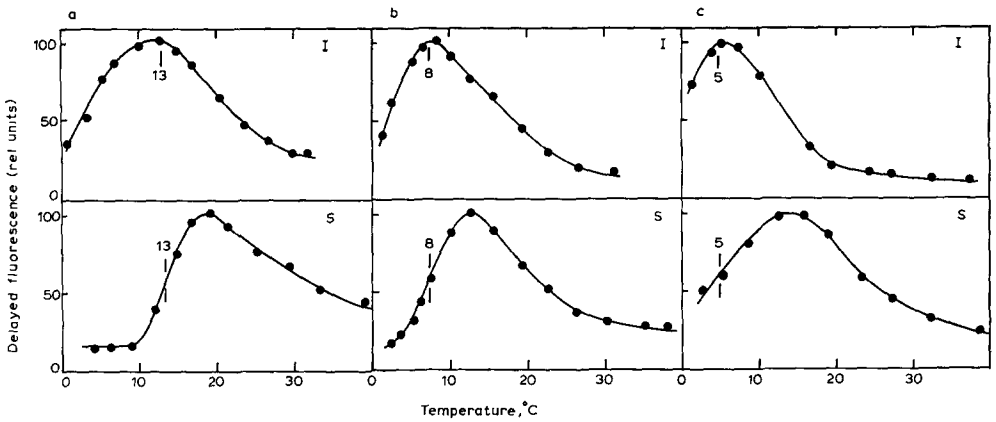

Fig. 3. Temperature dependence of delayed fluorescence in *A. variabilis* strain M-3 at the initial rise and steady-state levels. Growth temperature was 38 °C (a), 28 °C (b) and 20 °C (c). 0.5 % methyl cellulose was added to prevent the precipitation of cells during the measurement.

The temperature dependence of the initial rise and the steady-state levels of delayed fluorescence in *A. variabilis* strain M-3 grown at 38, 28 and 20 °C is shown in Fig. 3. The delayed fluorescence responded to the temperature in a way similar to that of *A. nidulans*. The maximum of the initial rise level and the midpoint of charac-

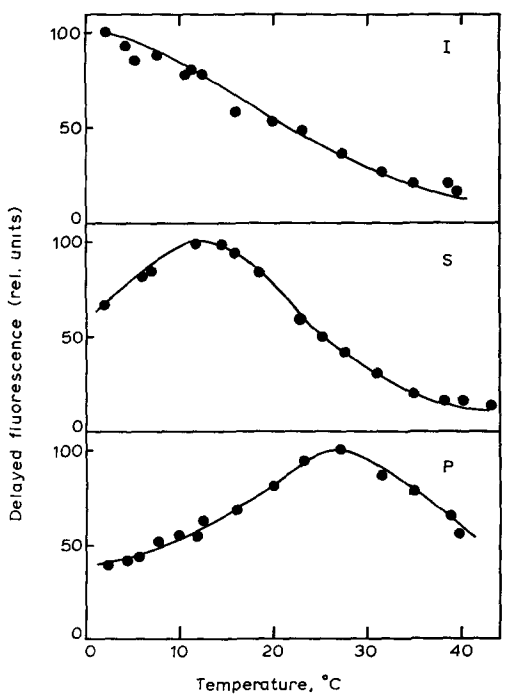

Fig. 4. Temperature dependence of delayed fluorescence at the initial rise (I), the steady-state (S) and the maximum (P) levels in *P. boryanum* grown at 28 °C. 0.5% methyl cellulose was added during the measurement.

teristic changes in the steady-state level coincided with each other in all the cultures of different growth temperatures; 13 °C in the 38 °C-grown cells, 8 °C in the 28 °C-grown cells and 5 °C in the 20 °C-grown cells. This temperature dependence of the delayed fluorescence corresponds to the findings that the phase transition of membrane lipids detected by the fluorescence measurement in this alga appears to be approx. 10 °C (unpublished data).

Fig. 4 shows the temperature dependence of the initial rise, the steady-state and the maximum levels of the delayed fluorescence in *P. boryanum*. Only a monotonous increase without maximum was observed in the initial rise level. The decrease in increment near 0 °C, however, may indicate the maximum would occur near 0 °C. The steady-state level seemed to give a midpoint of the characteristic change near 0 °C, although the low level was not attained in the region of room temperature. These findings may suggest that the physical phase transition would occur near 0 °C in this alga.

A. variabilis strain M-2 did not grow above 30 °C, exhibiting a contrast to the strain M-3 of the same species that grew even at 40 °C. The temperature dependence of the delayed fluorescence of the strain M-2 grown at 28 °C was approximately the same as that of *P. boryanum*. This suggests that the phase transition of membrane lipids would occur near 0 °C in this strain.

The temperature dependence of the delayed fluorescence was investigated also in a thermophilic alga *C. caldarium* grown at 38 °C (Fig. 5). The initial rise level

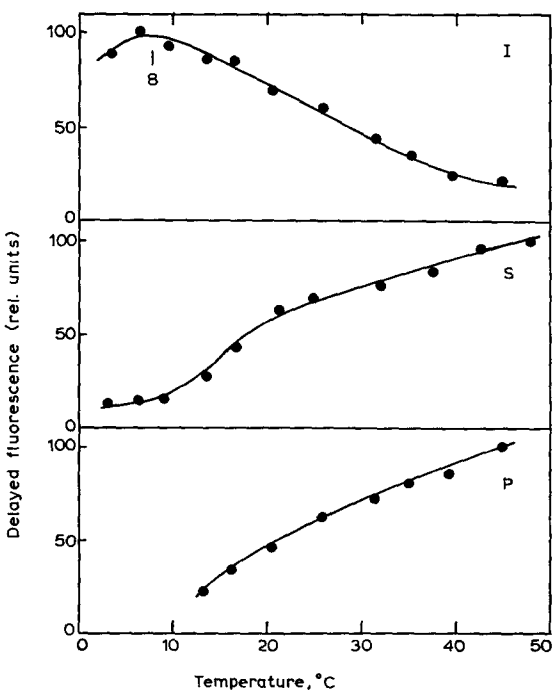

Fig. 5. Temperature dependence of delayed fluorescence at the initial rise (I) the steady-state (S) and the maximum (P) levels in *C. caldarium* grown at 38 °C.

showed a maximum at 8 °C, while the steady-state level did not show the corresponding remarkable intensity changes as seen in *A. nidulans* and *A. variabilis* strain M-3. A recent study of *C. caldarium* on the chlorophyll *a* fluorescence and the Arrhenius plot of the dark reduction of cytochrome *f* and the dark recovery of carotenoid shift [17] revealed that the transition of lipid phase from the liquid crystalline to the mixed solid-liquid crystalline states occurs at 7–9 °C in the thylakoid membranes of this organism. It can be inferred, therefore, that the initial rise level responds to the phase transition, but the steady-state level does not.

The temperature dependence of delayed fluorescence was also measured in the initial rise and the steady-state levels in a green alga *C. pyrenoidosa* grown at 28 °C (Fig. 6). While a remarkable decrease of the steady-state level was observed near 0 °C, the maximum of the initial rise level was not. The chlorophyll *a* fluorescence versus temperature curve (dashed line in Fig. 6) suggests that the phase transition of lipids of thylakoid membrane would occur below 0 °C. The change in the steady-state level at 3 °C may not respond to the phase transition in this organism. It was noted that the steady-state level gradually increased by increasing the temperature above 35 °C. This would result from the inhibition of the oxygen evolving system by exposing the cells to high temperatures, that may enhance the delayed fluorescence [12].

The millisecond decay kinetics of the delayed fluorescence was measured at various temperatures at the steady-state level in *A. nidulans* grown at 38 °C. The decay curve consisted of two exponentially-decaying components with different decay rates. Fig. 7 shows the temperature dependence of half-decay time of the two components. The half-decay time of the fast component was 0.15–0.19 ms and seemed constant throughout the temperature range of 3 and 39 °C. The half-decay time of the slow component ranging from 0.5 to 1.4 ms depended on the temperature of measurement. However, no remarkable change in the decay rates was found at the temperature of phase transition of membrane lipids around 20 °C.

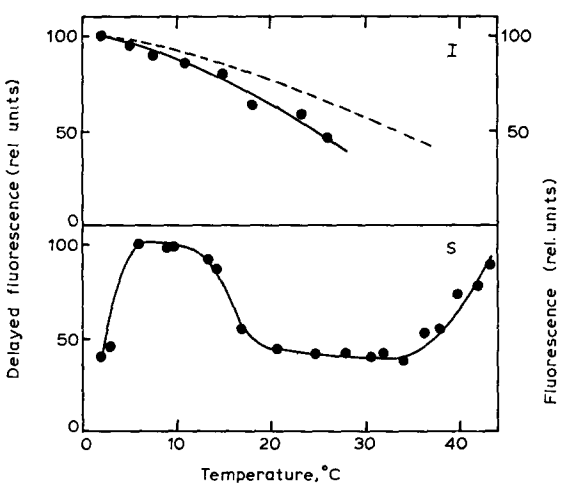

Fig. 6. Temperature dependence of delayed fluorescence at the initial rise (I) and the steady-state (S) levels in *C. pyrenoidosa* grown at 28 °C. Temperature dependence of chlorophyll *a* fluorescence (dashed line), presented for comparison, is reproduced from ref. 4.

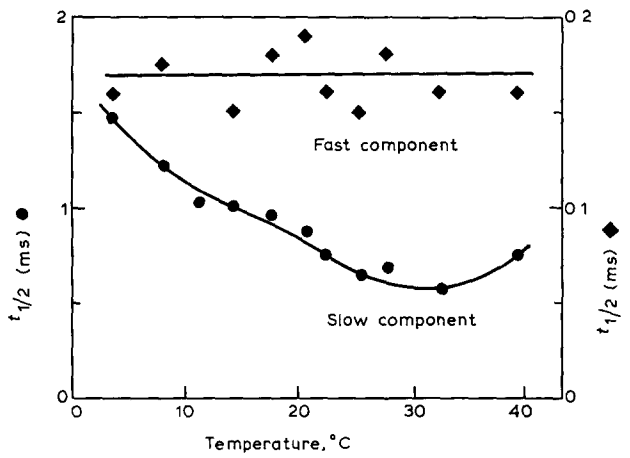

Fig. 7. Temperature dependence of half-decay time of the fast (◆) and the slow (●) components in the dark decay kinetics of delayed kinetics in *A. nidulans* grown at 38 °C. The decay kinetics was measured at the steady-state level after 10 min of excitation with repetitive flashes.

A relative proportion of the fast to the slow decay component did not significantly change throughout the temperature of measurement.

DISCUSSION

It has been found in the present study that the delayed fluorescence of chlorophyll *a* in the blue-green algae shows characteristic responses to the temperature of measurement. At the transition of physical phase of lipids of thylakoid membrane between the liquid crystalline and the mixed solid-liquid crystalline states, the initial rise level shows the maximum and the steady-state level shows the remarkable change. It is also noted that the temperature of phase transition depends on the growth temperature not only in *A. nidulans* but also in *A. variabilis* strain M-3. These findings probably correspond to the effects of growth temperature on the fatty acid composition of the membrane lipids (ref. 18 and Sato, N. and Murata, N., unpublished).

A conclusive explanation, however, cannot be presented at present for the mechanism that is involved in the characteristic responses of delayed fluorescence to the phase transition of membrane lipids in the blue-green algae.

The yield of chlorophyll *a* fluorescence becomes maximum at the phase transition between the liquid crystalline and the mixed solid-liquid crystalline states in the biological and the artificial membranes [3, 19, 20]. The initial rise level of delayed fluorescence in the blue-green algae responds to the phase transition in a manner similar to the chlorophyll *a* fluorescence. The same mechanism may operate in the temperature dependence of chlorophyll *a* fluorescence and the initial rise level of delayed fluorescence.

The steady-state level of delayed fluorescence shows a remarkable change from a high level in the liquid crystalline state to a low level in the mixed solid-liquid crystalline state. The steady-state level measured in the phosphoroscope is influenced

by some factors, such as the oxidation-reduction level of the primary electron acceptor and donor of the reaction center 2 (ref. 7) and the pH inside the thylakoid membrane [12]. It is quite possible to assume that the thylakoid membrane, when in the mixed solid-liquid crystalline state, is permeable to hydrogen ions. This suppresses the formation of low pH inside the thylakoid, and thus reduces the delayed fluorescence. It is also possible that the electron transport is suppressed especially at the site of plastoquinone in the mixed solid-liquid crystalline state [3] that leads to a reduction of the primary electron acceptor of reaction center 2. Since the delayed fluorescence is produced by the reaction center which is open before the excitation flash comes [7], the delayed fluorescence becomes suppressed with the reduced state of the primary electron acceptor of reaction center 2.

Although the delayed fluorescence at the steady-state level responded to the physical phase transition of membrane lipids in the blue-green algae investigated in the present study, such a clear relationship was not found in other kinds of algae, *C. caldarium* and *C. pyrenoidosa*. The reason why this relationship exists only in the blue-green algae is so far obscure.

ACKNOWLEDGEMENT

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