Relationships between the Transition of the Physical Phase of Membrane Lipids and Photosynthetic Parameters in *Anacystis nidulans* and Lettuce and Spinach Chloroplasts

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Norio Murata

Faculty of Science, Department of Biophysics and Biochemistry, University of Tokyo, Hongo-Tokyo, Japan

John H. Troughton

Physics and Engineering Laboratory, Department of Scientific and Industrial Research, Lower Hutt, New Zealand

David C. Forre

Department of Plant Biology, Carnegie Institution of Washington, Stanford, California 94305

**ABSTRACT**

The transition of the physical phase of lipids in membrane fragments of a blue-green alga *Anacystis nidulans* was studied by a spin labeling technique. The maximum hyperfine splitting of the electron spin resonance spectrum of the N-oxy-1',4',4'-dimethyloxazolidine derivative of 5-ketostearic acid plotted against the reciprocal of the absolute temperature gave a discontinuity point that was characteristic of a transition of the physical phase of the hydrocarbon region of membrane lipids. The phase transition appeared at approximately 13 or 24 C in the organisms grown at 28 or 35 C, respectively.

The temperature dependence curve of chlorophyll a fluorescence in intact cells, membrane fragments, and extracted lipids of *Anacystis* cells suspended in a buffer solution showed that the fluorescence yield became maximum near the phase transition temperatures. These findings suggest that chlorophyll a in the thylakoid membrane works as a native fluorescence probe for the detection of phase transition.

The temperature dependence of photosynthetic electron transport reactions was studied by measuring the oxidoreductive reactions of P700 and by measuring O2 evolution. Each of the Arrhenius plots of the reaction rates was composed of two straight lines with a break near the phase transition temperatures. The activation energy was always lower above than below the transition temperatures. It is proposed to explain these phenomena that a reaction involving plastoquinone is influenced by the physical state of membrane lipids.

The shift between the pigment state 1 and state 2 measured by fluorescence transients also showed a characteristic break in the Arrhenius plots near the phase transition temperatures; below the transition temperatures the shift almost disappeared. This suggests that the configurational change of the thylakoid membrane related to the state 1 and state 2 shift is dependent on the physical state of membrane lipids. In the chloroplasts of lettuce and spinach, on the other hand, there was no break in the Arrhenius plot of the electron transport reactions or of Mg2+-induced changes of chlorophyll a fluorescence.

It is suggested that the transitions of the hyperfine splitting of the ESR signal, electron transport, and the configurational change, as well as the appearance of the maximum of chlorophyll a fluorescence, in the thylakoid membranes of *Anacystis nidulans* are all related to the transition of the physical phase of membrane lipids between the liquid crystalline state and the mixed liquid crystal-solid state.

It has been well demonstrated that the physical phase of lipids of biological membranes plays an important role in the physiological function of membranes. The proper functioning of biological membranes requires the presence of the smectic liquid crystalline state in which rotational and translational movements of lipid and protein molecules in the membrane are possible. It has been established in model membranes that the temperature of phase transition between the liquid crystalline and the solid states depends on the lipid species as well as the fatty acid composition; the higher the degree of unsaturation of fatty acids, the lower the phase transition temperature. The same relationship between the phase transition temperature and fatty acid composition also appears in the biological membranes of *Escherichia coli* (44, 50, 59), *Mycoplasma laidlawii* (14, 35, 55, 56), mitochondria (30, 32, 47), and yeast (13). Drastic changes of physiological activities of the biological membranes are observed at the phase transition temperatures. This may be changes in growth, transport, and respiration in *E. coli* (44, 59) and in phosphorylation, respiration, and conformational changes in mitochondria (24, 31, 32, 48).

The phase transition between the solid and liquid crystalline states appears in a rather narrow temperature range in the model membrane systems composed of a single species of phospholipid with the same fatty acid composition. In a mixture of lipids, the transition occurs over a wide range of temperatures (10, 46). In
the spin labeling study of Shimshick and McConnell (52), two discontinuity points appear at the start and at the end of the phase transition between the solid and liquid crystalline states. Between these two transition temperatures, the membrane is composed of regions having both the solid and the liquid crystalline states that are separated laterally from each other (52).

Lipid species present in biological membranes are in most cases heterogeneous. This predicts a wide range of temperature for the lateral phase separation. This is actually seen in the spin labeling studies of mitochondria (49) and E. coli (6, 29). The respiratory activities of mammalian and plant mitochondria (49, 60) and the glucoside and galactoside transport in E. coli (29) showed two discontinuity points in the Arrhenius plots. These two points corresponded to the start and end points of the transition of the physical phase and to the two discontinuity points seen in the spin labeling study.

In the photosynthetic systems, i.e. chloroplast or thylakoid membranes, there has been no good demonstration of a relationship between the transition of the physical phase of membrane lipids and photosynthetic activities. In chloroplasts of higher plants and most algae, the content of linolenic acid is very high (8). This predicts that the phase transition would appear far below room temperature. Shipley et al. (53) have shown that the phase transition occurs at -30 C in monogalactosyl and -50 C in digalactosyl diglyceride of a higher plant, Pelargonium. Holton et al. (20), Hirayama (19), and Nichols, et al. (42), however showed that in the blue-green alga, Anacystis nidulans, there was no linolenic acid and that the major fatty acids were palmitic and hexadecenoic acids. These two fatty acids account for 85 to 90% of the total fatty acids. This suggests that the transition of the phase of the lipids may occur at room temperature. The former authors also reported that the fatty acid content changed depending upon the growth temperature; the higher the growth temperature, the higher the content of saturated fatty acids (20).

We used this alga grown at different temperatures as a test material for a study of the transition of the physical phase of membrane lipids in relation to photosynthetic activities. The chloroplasts of lettuce and spinach were also used as examples of higher plants.

**MATERIALS AND METHODS**

Anacystis nidulans was grown at 28 and 38 C in Kratz and Myers’ C medium (26) bubbled with air enriched with 3% CO2. The culture was filtered through filter paper to remove precipitates and aggregated cells, and then in most cases diluted with the same culture medium. The concentration of cells was adjusted to give approximately 5 µg Chl/ml in the measurement of light-induced absorbance changes and 1 µg Chl/ml in the measurement of fluorescence. In order to prepare the membrane fragments of Anacystis, the cells were collected by centrifugation at 2,000g for 5 min, suspended in a medium of 0.4 M sucrose, 0.01 M NaCl, and 0.2 M MES buffer, pH 6.6, recentrifuged, and resuspended in the same medium. The suspension was passed twice through the French pressure cell at 6,000 psi. The homogenate was centrifuged at 2,000g for 10 min to remove unbroken cells and cell walls. After centrifuging the supernatant at 105,000g for 45 min, the membrane fragments were obtained as a precipitate that was resuspended in the same medium to serve as the test material.

Lipids and Chl a were extracted from the membrane fragments with 80% acetone. After centrifugation at 500g for 10 min, the acetone solution of the extract was added to 50 volumes of 0.05 M phosphate buffer, pH 7.3, and shaken to form micelles or lamellar structures of lipids in H2O. These preparations were used for the measurement of the temperature dependence of Chl a fluorescence in lipids suspended in H2O.

Romaine lettuce (Lactuca sp. var. Romaine, purchased at Germain’s Seed Co., Los Angeles) was cultivated in a growth cabinet with a supply of nutrient solution. The temperature in the cabinet was controlled at 15 or 25 C. Light and dark periods were 14 and 10 hr, respectively. Leaves were harvested in the light period. Spinach leaves were purchased at a local market. The leaves were ruptured with a Waring Blender in a medium of 0.4 M sucrose, 0.01 M NaCl, and 0.05 M phosphate buffer, pH 7.4. After centrifugation at 200g for 1 min to remove cell walls, nuclei, and unbroken leaf pieces, the chloroplasts were collected by centrifugation at 800g for 5 min. For the measurement of the MgCl2 effect on Chl a fluorescence, the chloroplasts were suspended in a medium of 0.4 M sucrose, 0.01 M NaCl, and 0.01 M HEPES buffer, pH 7.4. The suspension was centrifuged at 800g for 5 min, and the chloroplasts obtained as a precipitate were resuspended in the same medium. The suspension thus obtained was centrifuged at 200g for 1 min, and the supernatant was diluted with the same medium to give 1 µg Chl/ml in the chloroplast preparation. For measurements of light-induced absorbance changes and the Hill reaction with DCIP, the same procedure of chloroplast preparation was employed but using a medium containing 0.05 M phosphate buffer, pH 7.4, instead of HEPES buffer, and the concentration of chloroplasts was adjusted to 5 µg Chl/ml.

In the measurement of the ESR spectrum of nitroxide-free radical, a Varian (Model V4502) ESR spectrometer was used. A spin labeling reagent 5-SAL, purchased from Syva, Palo Alto, Calif., was added to a suspension of highly concentrated membrane fragments of Anacystis. To improve the signal to noise ratio, the ESR spectrum was obtained as an average of 32 traces by means of a signal averager.

In order to measure the light-induced absorbance changes and the light-induced and the magnesium ion-induced fluorescence changes at various temperatures, a cuvette was made in which the temperature was controlled over a range of 0 to 40 C. The cuvette was round shaped and horizontally placed. It contained 30 to 40 ml of sample medium. The measuring, excitation, and actinic beams all came from above the cuvette and the changes in absorbance and in fluorescence yield were detected by a photomultiplier placed underneath the cuvette. Temperature control was achieved without disturbing the light beams by means of a coil of stainless steel tubing through which H2O or H2O-alcohol mixture of appropriate temperature was circulated. In order to accelerate heat exchange, the sample medium was stirred by a small propeller turning at 120 rpm. It was removed during the measurements of absorbance and fluorescence changes. During measurements of light-induced absorbance changes a signal averager (Nicolet Model 1010) was used occasionally to improve the signal to noise ratio; 8 or 16 measurements were averaged to obtain one trace. The temperature dependence of Chl a fluorescence was automatically measured by using a thermocouple that was connected to the x axis of an x-y recorder. In this case the propeller was turning during the measurement.

The Hill reaction with DCIP was measured spectrophotometrically by following the absorbance change at 590 nm. A molecular extinction coefficient of 19 m&sup2; cm&sup-1 was used for the calculation of the absolute rate of the reaction (5). Oxygen exchange was measured by using a Clark-type oxygen electrode (Rank Brothers). A correction for the change of electrode sensitivity with temperature was made for each experiment.

**RESULTS**

Spin Labeling of Membrane Fragments of Anacystis. Spin labeling of biological membranes with 5-SAL has been used to detect the fluidity of membrane lipids (22, 50, 51). Figure 1 shows
The absolute point grown cystis nitroxide ture 13 lines straight line and phase of probes ESR Anacystis For this unbound lines of suspension Concentrations the molar ratio of Chl a to 5-SAL being 10. The distance between the vertical lines in each spectrum indicates the maximum hyperfine splitting 2Tm.

ESR spectra of 5-SAL bound to the membrane fragments of Anacystis nidulans at various temperatures. There is no sign of unbound nitroxide radical, which, if present, should show a characteristic sharp signal overlapping the broad membrane-bound one. The ESR spectrum changed depending upon the temperature (Fig. 1).

The hyperfine splitting 2Tm (the distance between the two vertical lines in Fig. 1) can be taken as an indicator of mobility of the nitroxide group in the hydrophobic region of the membrane (50). In Figure 2 the 2Tm was plotted against the reciprocal of the absolute temperature. In the membrane fragments of Anacystis grown at 28 or 38°C, there appeared a discontinuity point around 13 or 24°C, respectively. This break between the two straight lines is related to the transition of the physical phase of the membrane lipids (50).

Although only one transition point appeared in the curve for ESR hyperfine splitting versus temperature in each sample, this point will correspond to the transition between the liquid crystalline and the mixed solid-liquid crystalline states.

Chl a Fluorescence as Indicator of Phase Transition. Fluorescence probes are used to indicate the transition of the physical phase of lipids in model and biological membranes (45, 50, 57). For this purpose we tried to use 8-anilinonaphthalene-1-sulfonate and N-phenyl-1-naphthylamine (45, 50, 57, 58). However, in the intact cells as well as the membrane fragments of Anacystis, the fluorescence yield of membrane bound probes was very low and the characteristic fluorescence spectra due to the membrane-bound probes were not observed. A very efficient excitation transfer from the probes to Chl a and/or carotenoids in the thylakoid membrane is likely to be the cause of this effect. Colbow (11) studied the fluorescence yield of Chl a dissolved in phospholipid liposomes to show that the pigment works as a fluorescent probe to detect the phase transition. This idea was applied to the thylakoid membrane of Anacystis in the present study.

Figure 3 shows the temperature of the fluorescence yield of Chl a in intact cells, membrane fragments, and in a mixture of lipids and Chl a suspended in a buffer solution. In the

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**Fig. 1.** ESR spectra of 5-SAL at various temperatures in a dense suspension of membrane fragments of Anacystis nidulans grown at 28°C. Concentrations of Chl a and 5-SAL were 650 and 20 μg/ml, respectively, the molar ratio of Chl a to 5-SAL being 10. The distance between the vertical lines in each spectrum indicates the maximum hyperfine splitting 2Tm.

**Fig. 2.** Maximum hyperfine splitting of ESR spectrum, 2Tm', plotted against the reciprocal of absolute temperature. 5-SAL was added to the suspension of membrane fragments of Anacystis grown at 28 and 38°C. Concentration of 5-SAL was 21 μg/ml. Concentration of Chl a was 650 and 335 μg/ml in the samples grown at 28 and 38°C, respectively.

**Fig. 3.** Temperature dependence of Chl a fluorescence in intact cells and membrane fragments of Anacystis and in a mixture of extracted lipids and Chl a suspended in buffer solution. Excitation light, 430 nm, 35 nm half-bandwidth, 4,000 ergs/cm²-sec. Chl a fluorescence was measured at 684 nm. Ten μM DCMU was added in intact cells and membrane fragments. Temperature was changed from the low to the high at a rate of 2°C/min. Solid line obtained from the low to the high at a rate of 2°C/min. Solid line obtained with cells grown at 28°C; dashed line obtained with cells grown at 38°C.
sample obtained from cells grown at 28 C, the maximum fluorescence yield appeared at 13 and 11 C in intact cells and membrane fragments, respectively. These temperatures for the maximum yield of fluorescence corresponded well to the transition temperatures seen in the ESR spectra in the spin labeling study. In the sample grown at 38 C, the maxima appeared at 21 and 19 C in the intact cells and the membrane fragments. Also in this case, a good correspondence between the transition temperature of the ESR signal and the positions of the fluorescence maxima were seen.

These results were obtained when the electron transport reaction was completely inhibited by the presence of DCMU. It can be concluded, therefore, that this characteristic change of fluorescence yield should reflect the physical environment of Chl a in the thylakoid membrane.

In model membranes and in biological (but not photosynthetic) membranes, the artificial fluorescent probes show a maximum fluorescence yield at the transition between the liquid crystalline and the mixed solid-liquid crystalline states and the minimum fluorescence yield at the transition between the solid and the mixed solid-liquid crystalline states (45, 50). Chl a in the phospholipid model membrane behaves in a similar way (11). On the assumption that Chl a in the thylakoid membrane of Anacystis reflects the transition of the physical phase of membrane lipids as do the artificial fluorescent probes, the occurrence of the maximum fluorescence yield of Chl a in Anacystis indicates that the transition of the ESR signal is due to the phase transition between the liquid crystalline and the mixed solid-liquid crystalline states.

Considerable effort was made, but without success, to determine the temperature of the minimum fluorescence yield of Chl a that would correspond to the transition between the solid and the mixed solid-liquid crystalline states. The absence of a minimum in the fluorescence yield above 0 C suggests that, depending upon the temperature, the lipids of the thylakoid membrane of Anacystis at room temperatures exist either in the liquid crystalline or the mixed solid-liquid crystalline states but not in the solid state.

This characteristic feature of the curve for temperature versus fluorescence was observed in membrane-bound Chl. When Chl a and membrane lipids were extracted from membrane fragments of Anacystis with 80% acetone, the curve for temperature versus fluorescence in this solution did not show a maximum or minimum but only a gradual and monotonic decrease with increase in temperature. By analogy to the phospholipid model membrane (11), it may be assumed that when a small amount of the 80% acetone extract containing most of galactolipids and Chl a of the thylakoid membrane is added to a large volume of buffer solution, the galactolipids and Chl a form lamellar or vesicular structures. The mixture of lipids and Chl a suspended in buffer solution produced a temperature curve almost the same as that seen for membrane fragments (Fig. 3). The maximum of the fluorescence yield appeared at 10 and 18 C in the two cultures grown at 28 and 38 C, respectively. These findings suggest that the occurrence of the fluorescence maximum is produced by the interaction of Chl a and membrane lipids and is not due to the nature of Chl a itself.

The results in Figure 3 were obtained by changing the temperature from low to high. When the temperature was changed from high to low, the maximum fluorescence yield appeared at lower temperatures than in Figure 3 especially in the intact cells (Murata and Fork, unpublished data). In Anacystis grown at 28 C, the maximum appeared at 11 instead of 13 C, and in the cells grown at 38 C it appeared at 16 C instead of 21 C. This hysteresis effect has been studied in the artificial fluorescent probes in the model and biological membranes, and it is a characteristic nature of the fluorescent probes (45, 50). In the membrane fragments and in the mixture of lipids and Chl a suspended in the buffer solution, this hysteresis effect of the maximum fluorescence yield was less significant being about 1 C.

**Photonsynthetic Electron Transport Measured by P700 Reduction in Anacystis.** In the photosynthetic electron transport system of Anacystis, the electron carriers, P700, Cyt f, and plastoquinone, are oxidized by illumination of pigment system 1 and reduced by illumination of pigment system 2 (2, 3, 40). In order to study the effect of the transition of the phase of membrane lipids on the electron transport reactions, the oxidation-reduction reactions of P700 were measured at various temperatures. In this experiment a suspension of algal cells was illuminated for 15 sec by intense actinic light having wavelengths longer than 620 nm that were absorbed by both pigment systems. After turning off this actinic light, the reduction of P700 and Cyt f in the dark was measured by following the absorbance changes in the blue light region.

Figure 4 shows the light minus dark difference spectra measured at 12 C and 30 C. The two difference spectra were very similar in shape and magnitude. P700 with the peak at 435 nm and Cyt f with the peak at 423 nm were two major components that were oxidized in the light and reduced in the dark. The P700 reduction in the dark measured at 435 nm is contaminated little by the Cyt f change, because the latter component has very little absorbance change upon oxidation or reduction at this wavelength. We assumed that the rate of absorbance change at 435 nm was attributed exclusively to the reaction of P700. Figure 5 shows the rate of P700 reduction in the dark represented as the reciprocal of the half-time of dark decay after 15 sec illumination versus the reciprocal of absolute temperature. The rather fast reduction of P700 in the dark indicates the presence of an electron pool, possibly plastoquinone (2), which is reduced in the light and functions as an electron donor to P700.

The Arrhenius plot of the rate of P700 reduction in the dark in Figure 5 shows two straight lines with a break at 10 C for the cells grown at 28 C, and at 20 C for the cells grown at 38 C. The activation energies above the transition temperatures were 14 and 13 kcal/mole in the cells grown at 28 and 38 C, respectively, and below the transition temperatures were 21 and 26 kcal/mole in the cells grown at 28 and 38 C, respectively. It can be noted that although the transition of P700 reduction appeared at different temperatures, the activation energy in the liquid crystalline state was about the same for the two cultures.

From the values for the half-time of P700 reduction after turning off the actinic illumination, the rate of photosynthetic electron transfer would be: $...$
Fig. 5. Arrhenius plot of P700 reduction after turning off the 15-sec actinic illumination in intact cells of *Anacystis* grown at 28 and 38°C. The rate of P700 reduction was plotted as the reciprocal of the half decay time of the absorbance change at 435 nm. The experimental procedures were the same as in Fig. 4. The rate of electron transport presented on the right side of the figure was calculated from the half decay time of P700 reduction using the assumption that P700 reduction is a monomolecular reaction and that the molar ratio of Chl a to P700 is 100 (23). The temperature of the sample was changed from high to the low.

The results shown in Figure 5 were obtained by decreasing the temperature of the sample. An experiment was also done to see whether a different result would be obtained by using increasing temperatures. The results are given in Figure 6, where one sample was used throughout the experiment in which the sample was first cooled from the growth temperature to 6°C and then heated up to 33°C. With increasing temperatures, the transition of the activation energy appeared at the same temperature as with decreasing temperatures. The lines below the break point followed each other with about the same activation energy. Above the break point, however, the activation energy was lower with increasing than with decreasing temperatures. The finding of lowered electron transport reactions above the transition temperature suggests that the chilling treatment below the transition temperature induced some damaging effects on the thylakoid membrane that suppressed electron transport at temperatures above the transition temperature. This hysteresis effect may be related to the cold shock of *Anacystis* cells as demonstrated by Forrest et al. (16).

In the presence of DCMU that inhibits the electron transport from photoreaction 2 to plastoquinone (2), the dark reduction of P700 is significantly retarded. Since under this condition the electron pool, plastoquinone, is not reduced in the light, P700 must be reduced by unknown endogenous electron donor(s). This P700 reduction in the presence of DCMU was measured at different temperatures. Figure 7 shows that the algal cells grown at 28°C gave a discontinuity in the Arrhenius plot at 12°C and those grown at 38°C at 20°C. It can be noted in these cases also that the break points appeared near the transition temperatures of the lipid phase in the two cultures.

Photosynthesis (*O₂ Evolution*) in *Anacystis*. The temperature dependence of photosynthesis was also studied in *Anacystis*. Figure 8 shows the Arrhenius plot of the rates of *O₂* evolution in the two cultures grown at 28 and 38°C. The transition of the activation energy appeared at 13 and 24°C; again near the transition temperatures of lipid phase. The activation energy above the transition temperatures was 19 kcal/mole in both the cultures and below the transition temperature was 37 and 34 Kcal/mole in the cells grown at 28 and 38°C, respectively. The activation energy of the reaction is constant in the liquid crystalline state irrespective of the growth temperature.

Pigment State 1 and State 2 Shift in *Anacystis*. A kind of light adaptation of algal photosynthesis to the color of light is called the pigment state 1 and state 2 shift (9, 36, 38). When algal cells are illuminated for several sec with light absorbed preferentially by pigment system 2, a special kind of configurational alteration...
of the thylakoid membrane takes place resulting in a change in light quantum distribution between the two pigment systems that provides more light quanta to pigment system 1. This stimulates the reaction of pigment system 1 that is the rate-limiting step of the over-all electron transport reaction. This state is called pigment state 2. When the cells are illuminated by light absorbed preferentially by pigment system 1, the reverse change of light quantum distribution happens. This state is called pigment state 1. This flexibility of quantum redistribution is advantageous for plants and allows them to utilize light energy efficiently for photosynthesis. This phenomenon is now understood by analogy to cation-induced control of light quantum distribution in isolated chloroplasts of higher plants (37, 39, 41) as the preferential illumination of one of the pigment systems of algal cells produces a kind of configurational change of thylakoid membrane that leads to an alteration of excitation transfer between the two pigment systems and results in more light quanta distributed in the other pigment system. It was of interest to investigate whether such a configurational change would be influenced by the physical state of membrane lipids. The state 1 and state 2 shift was measured at various temperatures in Anacystis cells grown at 28 and 38°C.

Figure 9 shows time courses of fluorescence transients at 33 and 17°C in Anacystis cells grown at 38°C. In this experiment, light preferentially absorbed by pigment system 2 at an intensity of 800 ergs cm⁻² sec⁻¹ was always furnished for the excitation of Chl a fluorescence. Under this condition the cells were in state 2. A beam of light absorbed by pigment system 1 at the intensity of 5,000 ergs cm⁻² sec⁻¹ was turned on and given for 1 min in addition to the excitation light absorbed by system 2. At 33°C the fluorescence yield after turning off the system 1 light increased from an initial level (I) to a peak (P), then slowly decreased to a steady state level (S), the same as that seen before the illumination of pigment system 1. The appearance of the peak suggests a shift from state 2 to state 1 during the illumination with the overlapped system 1 light, and the slow decrease from the P to the S level indicates a shift from state 1 back to state 2 again. The results at 17°C show that the state 1 and state 2 shift is almost lacking at this temperature.

In order to present a quantitative representation of the state 1 to state 2 shift, a quantity for the rate of shift from state 1 to state 2, R, was introduced. R is equal to the maximum rate of fluorescence yield change after the peak $\frac{d(\Delta F)}{dt}\max$ divided by the difference of fluorescence yields at P and S ($\Delta F$);

$$ R = \frac{d(\Delta F)/dt\max}{\Delta F} $$

Figure 10 shows an Arrhenius plot of the rate of state 1 to state 2 shift represented by $R$ in Anacystis cells grown at 28 and 38°C.
then after reduction was a Far Calflex C. methylviologen.

that the dark with absorbance decrease from a C. The chloroplasts were illuminated in the presence of methyl viologen with far red light for 500 msec, during which time P700 and Cyt f and possibly plastoquinone were oxidized. After 30 msec of darkness, during which time these components remained oxidized, a 3-μsec flash of red light was given. The differences in absorbance induced by the flash (illustrated as ΔA in the figure) were plotted against wavelength. This difference spectrum shows a positive peak at 430 nm and negative peaks at 450 and 400 nm. This suggests that P700, but not Cyt f, was reduced by the short flash of red light. Thus, the temperature dependence of P700 reduction could be measured as the rate of the absorbance change at 430 nm after the red flash.

Figure 12 shows the rate of P700 reduction induced by the flash measured as illustrated in Figure 11, as well as the rate of the Hill reaction in strong continuous light with DCIP as the electron acceptor. The Arrhenius plots of these reactions were composed of straight lines with no break points. This corresponds to the observations of Murata and Fork (unpublished data) showing that a maximum in the curve for temperature versus fluorescence does not occur at room temperature in lettuce chloroplasts.

The activation energy was 28 kcal/mole for P700 reduction induced by a red flash and 10 kcal/mole for the Hill reaction. This difference in the activation energy may be due to different reactions of the rate-determining step in the electron transport system. In the Hill reaction with DCIP the site of oxidation of plastoquinone is likely to be the rate-limiting step of the over-all reaction. In the experiment of P700 reduction by the flash, the electron carriers between photoreaction 1 and 2 including plastoquinone are all oxidized after illumination with far red light, and then only one electron is released by flash excitation of photoreaction 2. The electron passes quickly through the electron transport chain until it reaches P700. In this case it is difficult to pinpoint the rate-determining site.

Mg2+-induced Fluorescence Yield Change in Lettuce and Spinach Chloroplasts. An addition of divalent cations at concentrations about 5 mM to the chloroplast suspension of low salt medium produces an increase in the fluorescence yield of Chl a (21, 37, 39, 41). This effect is apparently caused by the cation-dependent distribution of light quanta between the two pigment systems of photosynthesis (37). Cations induce a configurational change of the thylakoid membrane that is reflected by a change in the efficiency of excitation transfer between the two pigment systems (39). Figure 13 shows the time courses of fluorescence changes upon the addition of 7 mM MgCl2 measured at 6 and 33 C in the chloroplasts of lettuce leaves grown at 25 C. It can be noted that the fluorescence change is extremely temperature-dependent. In Figure 14 the rate of fluorescence change upon the addition of MgCl2 was plotted against the reciprocal of absolute temperature in lettuce and spinach chloroplasts. There were no transitions found in the Arrhenius plots in these chloroplasts. In the case of chloroplasts of lettuce leaves grown at 15 and 25 C, the points followed the same line. This suggests that the fluidity of the membrane lipids in lettuce chloroplasts is independent of the growth temperature. This is in contrast to the case of Anacystis nidulans.
DISCUSSION

This study establishes that the transition of the physical phase of membrane lipids produces characteristic influences on the photosynthetic parameters of Anacystis nidulans. This is reflected by the appearance of the maximum yield of Chl a fluorescence and by a change in activation energy of the electron transport reactions and a configurational change of the thylakoid membrane. Figure 15 summarizes the temperatures for the transition and the characteristic changes of the photosynthetic parameters.

In the organism grown at 28°C, the characteristic transition temperatures appeared between 10 and 13°C, and in organisms grown at 38°C, they appeared between 18 and 24°C. It can be noted in the cells grown at 28°C that the characteristic transition temperatures appeared at 13°C (the higher limit) in the spin label mobility, the Chl a fluorescence in intact cells, O₂-evolution and the state 1 to state 2 shift, while they appeared at 10°C (the lower limit) in the measurements of the fluorescence in lipid-H₂O mixture and the electron transport in the absence of DCMU. In the other parameters studied the transitions appeared at intermediate temperatures. A similar pattern of distribution of the characteristic transition temperatures (but with more scattering) is also seen in the cells grown at 38°C. It can be concluded in this respect that these photosynthetic parameters respond to the transition of the phase of membrane lipids in a slightly different manner.

Another feature observed in the transition from the liquid crystalline to the mixed solid-liquid crystalline state in Anacystis is that the transition temperature depended upon the growth temperature. As noted above, the transition appeared around 10°C in cells grown at 28°C and around 20°C in cells grown at 38°C. This is due to the growth temperature-dependent alteration of the fatty acid composition of the membrane lipids of Anacystis (20). Such a tendency of the fatty acid composition and of the fluidity of membrane lipids to change with the growing temperature has been well studied in bacterial membranes (12, 33, 34) and Tetrahymena (43). This is in contrast to the finding in lettuce chloroplasts that the Arrhenius plots of MgCl₂-induced fluorescence change followed the same line irrespective of the growth temperature. The fluidity of lettuce chloroplast membranes does not seem to be influenced by the growth temperature.

It is worthwhile to estimate the site of electron transport that is influenced by the transition of the lipid phase of the thylakoid membrane. In the measurement of P700 reduction in the absence of DCMU, the rather long illumination of Anacystis cells with red light absorbed by the two pigment systems caused P700 and Cyt f to be oxidized and plastoquinone (1, 2, 18) to be reduced. It has been well studied in Porphyridium cruentum (4) and spinach chloroplasts (17) that the site of plastoquinone oxidation is the rate-determining step of the over-all electron transport reactions. This may also be the case for Anacystis. In this condition, the rate of P700 reduction after turning off the actinic light is controlled by the rate of plastoquinone oxidation. The appearance of a change in the rate of P700 reduction corresponding to a transition in the phase of membrane lipids leads to the conclusion that this part of electron transport including plastoquinone is influenced by the phase transition of the lipids. The fact that the plastoquinone is the most lipophilic component in the electron transport system also suggests that plastoquinone is most likely to be influenced by the fluidity of membrane lipids. It has been seen in the respiratory electron transport of mitochondria (48) and bacterial membranes (15) that the oxidation-reduction reactions including ubiquinone show a transition in the activation energy that is related to the transition of phase of membrane lipids.

Also in the Hill reaction with DCIP in lettuce chloroplasts, the rate of fluorescence change divided by the fluorescence level before MgCl₂ addition was taken as the rate in the plot. The experimental conditions were the same as in Fig. 13. Lettuce grown at 25°C (○—○); lettuce grown at 15°C (●—●); market spinach (△—△).

![Fluorescence change](image)

**Fig. 13.** Changes in the fluorescence yield upon addition of MgCl₂ at 6 and 38°C in the chloroplast of lettuce grown at 25°C. Ten μM DCMU was added. The final concentration of MgCl₂ added was 7 mM. Excitation light, 435 nm, 30 nm half-bandwidth, 4,000 ergs/cm²/sec.

**Fig. 14.** Arrhenius plot of MgCl₂-induced fluorescence changes in the chloroplasts of lettuce and spinach leaves. The initial rate of fluorescence increase divided by the fluorescence level before MgCl₂ addition was taken as the rate in the plot. The experimental conditions were the same as in Fig. 13. Lettuce grown at 25°C (○—○); lettuce grown at 15°C (●—●); market spinach (△—△).

**Fig. 15.** Summary of the transition temperatures of photosynthetic parameters in Anacystis nidulans.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grown at 28°C</th>
<th>Grown at 38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spin label</td>
<td></td>
<td>○</td>
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<tr>
<td>Fluorescence</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Cells</td>
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</tr>
<tr>
<td>Membrane</td>
<td>●</td>
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</tr>
<tr>
<td>Lipid-water</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>P700 reduction-DCMU</td>
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<td>○</td>
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<tr>
<td>+DCMU</td>
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</tr>
<tr>
<td>O₂-evolution</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>State 1→2 shift</td>
<td>●</td>
<td>○</td>
</tr>
</tbody>
</table>

Temperature, °C

10 15 20 25
rate-determining step is likely to be the site of oxidation of plastoquine. Lien and Bannister (28) suggested that DCIP was reduced by photoreaction 1. Kok et al. (25) in their kinetic analysis of the oxidation-reduction reaction of DCIP estimated the half-time of the reaction between the reductant of photoreaction 1 and 0.1 mm DCIP to be 40 μs. The calculated rate of DCIP reduction based on this value is about 10^4 to 10^5 times higher than that measured in the Hill reaction with DCIP. Therefore in this case, the rate-determining step of the Hill reaction is also estimated to exist between photoreaction 1 and 2. If this situation, which is considered to be the case for Anacystis, is also true for lettuce chloroplasts, then the site of plastoquinone oxidation is likely to be the rate-determining step of the over-all reaction. The absence of a discontinuity point in the Arhenius plot of the Hill reaction with DCIP in lettuce chloroplasts suggests that the transition of the lipid phase, which in turn would affect the activity of plastoquinone, does not take place at room temperature.

By measuring partial reactions of photosynthetic electron transport Shneyour et al. (54) inferred that the transition occurring at about 10°C in the Arhenius plots of electron transport reactions including the ferredoxin-NADP oxidoreductase in sweet potato and tomato chloroplasts is due to the transitions of membrane lipids. As indicated in the fluorescence study (Murata and Fork, unpublished data), and also pointed out by the above mentioned authors, the membrane lipids of tomato chloroplasts do not show the transition of the physical phase of lipids in the room temperature region. Possibly, the transition of activation energy of the reaction of the flavin enzyme was induced by an as yet unknown factor other than the transition of the physical phase of membrane lipids.

It was found that the state 1 to state 2 shift of light quantum distribution between the two pigment systems was highly dependent upon the fluidity of membrane lipids. It almost disappeared when the membrane lipids were in the mixed solid-liquid crystalline state. The concentration-dependent of Chl a fluorescence in lettuce and spinach chloroplasts showed rather high activation energy, and thus was quite sensitive to the temperature, even when the membrane lipids were in the liquid crystalline state.

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LITERATURE CITED


