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THE EFFECT ON PHOTOSYNTHETIC ELECTRON TRANSPORT OF TEMPERATURE-DEPENDENT CHANGES IN THE FLUIDITY OF THE THYLAKOID MEMBRANE IN A THERMOPHILIC BLUE-GREEN ALGA

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Summary

Various electron transport reactions in cell or isolated thylakoid membranes of the thermophilic blue-green alga, *Synechococcus* sp. were measured at a different temperatures between 72 and 3°C. They are classified into two groups with respect to their temperature dependency. The first group involves cytochrome 553 photooxidation, methyl viologen photoreduction with reduced 2,6-dichlorophenolindophenol as electron donor and 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea-resistant ferricyanide photoreduction determined in the presence or absence of silicomolybdate. The Arrhenius plot of these reactions showed a single straight line with the activation energy of about 10 kcal/mol throughout wide temperature ranges studied.

Methyl viologen photoreduction with water as electron donor, reduction of flash-oxidized cytochrome 553, ferricyanide photoreduction and photosynthetic O_2 evolution form the second group. Their Arrhenius plots are characterized by discontinuities or breaks at about 30 and 10°C, which respectively correspond to the upper and lower boundaries of the lateral phase separation of the membrane lipids.

The first group reactions represent short spans of electron transport which are mediated either by Photosystem I or Photosystem II alone and not related to plastoquinone, whereas all the reactions of the second group involve plastoquinone. It is concluded therefore that the membrane fluidity affect electron transport specifically at the region of plastoquinone.

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid .

It is proposed that the reaction center chlorophyll-protein complexes of both Photosystems I and II are closely associated with related electron carrier proteins to form functional supramolecular assemblies so that electron transfer within such a cluster of proteins proceeds independently of the phase changes in the membrane lipids. On the other hand, the role of plastoquinone as a mobile electron carrier mediating electron transfer from the protein assembly of Photosystem II to that of Photosystem I through the fluid hydrophobic matrix of the membranes is highly sensitive to the physical state of the membrane lipids.

Introduction

Activities of biological membranes or associated enzymes depend upon the fluidity of the membranes which varies as a function of the ambient temperature. The Arrhenius plot of a membrane activity often shows a break or dicontinuity, i.e., an abrupt change in the apparent activation energy, which is related to the phase transition or phase separation of the membrane lipids [1—6].

Photosynthetic electron transport in higher plants or algae is located in the thylakoid membranes. The relationship between the activity and the fluidity of the thylakoid membranes is, however, difficult to be assessed, because the phase transitions of the membranes lipids, which are particularly abundant in polyunsaturated linolenic acid [7—9], would occur only at subzero temperatures. In this respect, a group of blue green algae (cyanobacteria) which can grow at high temperatures provide an unique advantage that the phase changes of the membrane lipids occur at temperatures above 0°C. Photosynthetic activities of the blue-green algae have been studied at different temperatures to relate them to the physical state of the membrane lipids [5,10—12].

In the present work, temperature dependency of various segments of photosynthetic electron transport, which are mediated by either Photosystem I or Photosystem II alone, or both, as well as that of photosynthetic O_2 evolution, in the thermophilic blue-green alga, *Synechococcus* sp., were studied to examine how the membrane fluidity affects the electron transport. Previous works have shown that growth and photosynthesis of the alga are maximal at a high temperature close to 60°C [13] and that the thermotropic phase transition of lipids of the algal thylakoid occurs at suboptimal temperatures above 0°C [12]. The results indicate that non-cyclic electron transport is affected by the membrane fluidity specifically at the plastoquinone site, whereas electron transfer among protein carriers including the reaction center complexes is independent of the changes in the physical state of the membrane lipids. Implication of the results will be discussed in terms of supramolecular organization of electron transport in the thylakoid membranes.

Materials and Methods

Growth conditions of the thermophilic blue-green alga *Synechococcus* sp. were as described in a previous paper [13] except that the inorganic medium of

Dyer and Gafford [14] were enriched in iron and copper salts [15]. The alga grows more rapidly and steadily in the modified medium mainly due to the increased level of iron. After 24 h of growth, cells were harvested by centrifugation, suspended in the fresh medium containing 25 mM Hepes-NaOH (pH 7.5) to give a final chlorophyll concentration of 8 to 12 μg/ml and kept at 25°C under illumination with white light of 1000 lux prior to the measurement. Chlorophyll was determined by the method of Mackinney [16].

The procedure for preparation of the thylakoid membranes was described previously [13] but, after lysozyme-treatment of cells, protoplasts were suspended in a medium containing 1 M sucrose and 50 mM Hepes-NaOH (pH 7.0) and passed through a French pressure cell. In contrast to the membranes prepared in the medium containing poly(ethylene glycol) [13], the membranes thus prepared lost most of phycobilins but were still highly active in the O_2 evolution with ferricyanide as electron acceptor.

Photosythetic O_2 evolution and methyl viologen photoreduction either with water or reduced DCIP as electron donor were measured with a Clark-type oxygen electrode which had been calibrated to the oxygen concentration of distilled water in equilibrium with air at each measuring temperature. Cells were illuminated with white light from a 650 W Ushio halogen lamp through a water filter of 6 cm thickness and a Hoya HA-50 infrared absorbing filter. The light intensity was $6.5 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. Additions made were 5 mM $NaHCO_3$ for photosynthetic O_2 evolution; 2 mM methyl viologen and 1 mM NaN_3 for methyl viologen photoreduction with water as electron donor; and 2 mM methyl viologen, 50 μM DCIP, 1 mM ascorbate, 10 μM DCMU and 1 mM KCN for methyl viologen photoreduction mediated by Photosystem I alone.

Reduction kinetics of flash-oxidized cytochrome 553 was determined at 553 nm with a single beam spectrophotometer as described previously [15]. The duration of a xenon flash was 20 μs at its half maximum height. Cytochrome photooxidation was measured with an Union Giken spectrophotometer with a shorter xenon flash (half duration, 5 μs). Flashes passed through Toshiba VR-66 and VR-67 filters and the photomultiplier was protected against the atinic light with two Corning 4-96 filters. Both flashes were saturating. Flash-excitation was repeated at 1 to 0.2 Hz and 256 or 500 signals were averaged to improve the signal/noise ratio.

Photoreduction of ferricyanide in the thylakoid membranes was determined by measuring absorbance changes at 420 nm with a Hitachi 356 spectrophotometer in the split-beam mode [13]. The actinic light was provided from a Nikon microscope illuminator with a 100 W halogen lamp and passed through a water layer of 6 cm thickness, a Hoya HA-50 filter and a Toshiba VR-60 cut-off filter ($1.0 \cdot 10^6$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}). A photomultiplier was guarded with a Corning 4-96 and a Hoya B-390 band pass filters. The membranes were suspended at a final chlorophyll concentration of 5 μg/ml in the medium containing 1 M sucrose, 50 mM Mes-NaOH (pH 6.5), 10 mM $MgCl_2$, 20 mM NaCl and 1 mM ferricyanide.

Silicomolybdate-mediated photoreduction of ferricyanide was determined with the same set-up but an actinic light of $3.2 \cdot 10^6$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1} obtained from a 500 W xenon lamp was used. The reaction medium contained 1 M sucrose, 50 mM Mes-NaOH (pH 5.5), 10 mM $MgCl_2$, 20 mM NaCl, 10 μM

DCMU and indicated amounts of silicomolybdate. The final chlorophyll concentration was 5 μg/ml.

Temperature of the reaction medium was controlled by circulating thermostated water through a water jacket of the sample cuvette and monitored with a calibrated thermocouple. The sample was preincubated at indicated temperature for 5 min and renewed at each measuring temperature. The temperature dependence of reactions was determined with three to five different cell cultures or thylakoid preparations. Although absolute rates of photochemical reactions varied to some extent with cultures or membrane preparations, characteristics features of their Arrhenius plots such as slopes and breaks of plots were satisfactorily reproducible.

Results

The blue green alga employed in the present work shows high growth rate only at high temperatures between 50 and 60°C [13]. The optimal functioning of photosynthesis also requires high temperatures [12,13]. Fig. 1 shows the Arrhenius plot of photosynthetic O_2 evolution in the algal cells. The activity was maximal at about 58°C and decreased sharply at temperatures above 60°C, where the thermal inactivation of photosynthesis occurs [13]. On the lower temperature side of the optimum, the plot shows a linear decrease from 55 to 29°C, then the second linear decrease with an increased slope as temperature is lowered to 11°C, followed by the third linear decrease with a much steeper slope below 11°C. The apparent activation energies calculated from the slopes

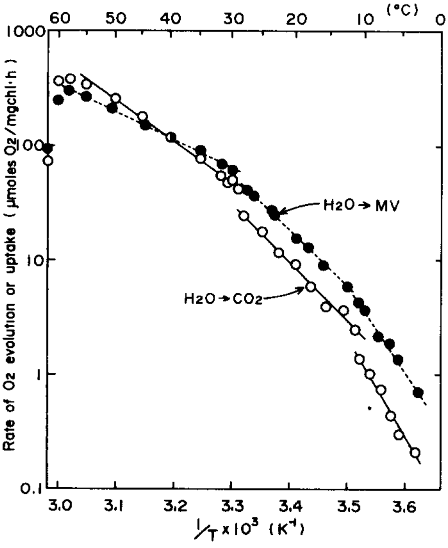


Fig. 1. Arrhenius plots of photosynthetic O_2-evolution and methyl viologen photoreduction. O_2 evolution was determined with a Clark-type oxygen electrode. For the assay of photosynthetic O_2-evolution, cells were suspended in the growth medium supplimented with 25 mM Hepes-NaOH (pH 7.5) and 5 mM $NaHCO_3$ and illuminated with white light of $6.5 \cdot 10^5$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}. For the assay of O_2-uptake by methyl viologen photoreduction, $NaHCO_3$ was replaced by 2 mM methyl viologen and 1 mM NaN_3.

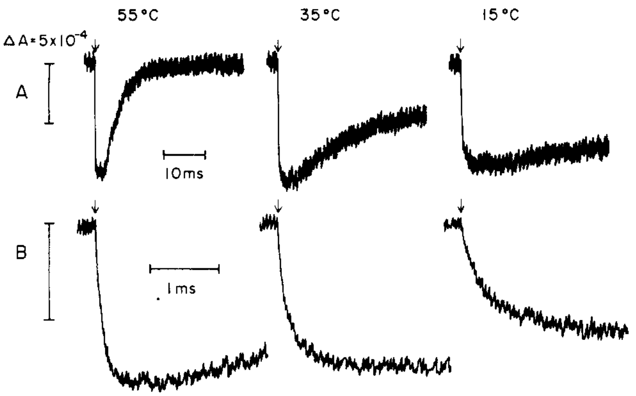


Fig. 2. Time courses of flash-induced changes of cytochrome 553 at different temperatures. Cells were suspended in the growth medium supplimented with 25 mM Hepes-NaOH (pH 7.5). (A) Reduction kinetics. Flash of 20 μs duration at its half maximum height was fired when indicated by arrows. Repetition rates were 1 Hz at 55 and 35°C and 0.2 Hz at 15°C. 256 signals were averaged. (B) Photooxidation kinetics. A shorter flash (5 μs) was used and 500 signals were averaged. At temperatures at and below 15°C, 1 mM ascorbate and 50 μM DCIP were added.

of the three straight lines are 17, 24 and 46 kcal/mol in the order of decreasing temperature.

Fig. 1 also shows the Arrhenius plot of methyl viologen photoreduction which represents electron transport involving both Photosystems I and II. The maximum rate was again attained at 58°C. Between 58 and 3°C, the plot is also composed of three straight lines with activation energies of 11, 23 and 34 kcal/mol.

Note that the two Arrhenius plots show clear discontinuous points or breaks at about 29 and 11°C. Similar discontinuity or break of the Arrhenius plot has been reported with various activities of biological membranes and ascribed to the phase changes of the membrane lipids [1—6]. The fluidity of the thylakoid membrane isolated from the thermophilic blue green alga has been studied by a spin labelling technique at various temperatures [12]. The plot of the membrane fluidity against the reciprocal of absolute temperature showed a discontinuity point between 35 and 30°C and a break at about 10°C. The results were interpreted to indicate the phase separation of the membrane lipids. Thus, the upper and lower discontinuous points or breaks of the Arrhenius plot of photosynthesis and methyl viologen photoreduction corresponding to these two temperatures where abrupt changes in the temperature dependency of the membrane fluidity occurred indicate that the two reactions are sensitive to the phase separation of the membrane lipids.

The results also suggest that the temperature dependency of photosynthesis is determined by that of electron transport. Note, however, that the activation energy of photosynthesis is significantly higher than that of methyl viologen photoreduction between 55 and 29°C. This indicates that photosynthesis is not limited by electron transport at the physiological temperature of the alga.

Fig. 2A illustrates time courses of flash-induced redox changes of a cytochrome showing the α-band peak at 553 nm at different temperatures. The cytochrome could be either cytochrome *c*-553 [17,18] or cytochrome *f*-553

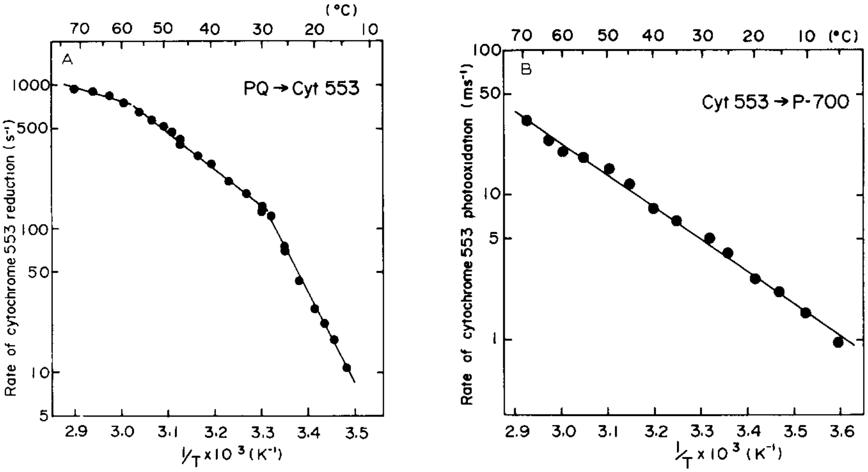


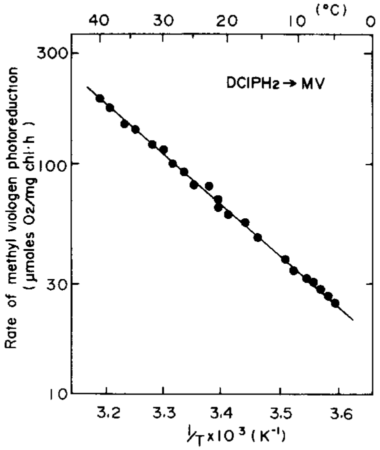
Fig. 3. Arrhenius plots of dark reduction (A) and photooxidation (B) of cytochrome 553.

[19], or both. Cytochrome 553 was rapidly oxidized by flash and after showing a lag of a few ms returned to the dark level. A previous work has demonstrated that reduction rate of flash-oxidized cytochrome 553 depends upon the redox state of plastoquinone which serves as electron donor for the cytochrome [15]. Plastoquinone was, therefore, kept in the reduced state by illuminating cells prior to the measurement and also by adding fructose which serves as a good electron donor for plastoquinone [15] to the reaction medium. The dark reduction, which can be approximated by the first order of kinetics, was slowed down with decreasing temperature. The Arrhenius plot of the reduction rates showed a break at 29°C (Fig. 3A). The two straight lines above and below 29°C gave the activation energies of 12 and 28 kcal/mol which are comparable to those of the methyl viologen photoreduction at corresponding temperature ranges. This is compatible with the view that electron transport from plastoquinone to the cytochrome is a rate limiting step of the photosynthetic electron transport [20,21].

Redox changes of cytochrome 553 were observed at temperature as high as 72°C, where methyl viologen photoreduction was rapidly inactivated. The Arrhenius plot of the cytochrome reduction at the high temperature range shows a straight line with a break at 58°C. From the slop of the line, the apparent activation energy was calculated as 4 kcal/mol.

On the other hand, whether or not there is a break or discontinuity at about 10°C could not be determined, because cytochrome 553 photooxidation was diminished as temperature was lowered below 15°C and virtually disappeared at about 10°C. This reflects a situation that the reduction rate becomes too slow to accumulate a sufficient amount of reduced cytochrome during the flash intervals at such low temperatures. In fact, cytochrome photooxidation fully reappeared at and below 10°C when DCIP and ascorbate were added as reductant of the cytochrome.

Fig, 2B illustrates time courses of cytochrome photooxidation at different temperatures. At temperatures at and below 15°C, kinetics of cytochrome changes were determined in the presence of the DCIP and ascorbate couple.



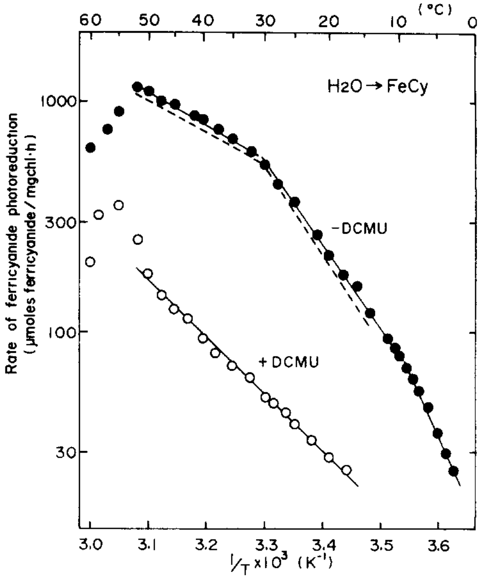


Fig. 4. Arrhenius plot of methyl viologen photoreduction with reduced DCIP as electron donor. Experimental conditions were as described in Fig. 1 except that $NaHCO_3$ was omitted and 2 mM methyl viologen, 50 μM DCIP, 1 mM ascorbate, 1 mM KCN and 10 μM DCMU were added.

Fig. 5. Arrhenius plots of ferricyanide photoreduction in the absence and presence of DCMU. The thylakoid membranes were suspended in a medium containing 1 M sucrose, 50 mM Mes-NaOH (pH 6.5), 10 mM $MgCl_2$, 20 mM NaCl and 1 mM ferricyanide and illuminated with a red light of $1.0 \cdot 10^6$ ergs · cm^{-2} · s^{-1}. Closed circles, no addition. Open circles, 10 μM DCMU. Dashed line indicates the plot for the DCMU-sensitive part of ferricyanide photoreduction calculated by subtracting reduction rates determined in the presence of DCMU from those determined in the absence of DCMU at respective temperatures.

The time needed to oxidize 50% of the cytochrome was 50 μs at 55°C but markedly lengthened at lower temperatures. In contrast to the reactions determined above, the Arrhenius plot of photooxidation rates (reciplocals of the half oxidation times) gives a single straight line with a constant slope throughout a wide temperature range covering 65°C difference (Fig. 3B). The activation energy was 10 kcal/mol. Obviously, the reaction is insensitive to the transitions of the physical state of the membrane lipids.

Another Photosystem I reaction, methyl viologen photoreduction with the DCIP and ascorbate couple as electron donor shows a similar linear Arrhenius plot between 40 and 5°C (Fig. 4). The activity could not be determined accurately at temperatures above 40°C because of rapid autooxidation of the reductants. Activation energy of the reaction is 10 kcal/mol in good agreement with that of the cytochrome photooxidation. Ono and Murata have also shown a linear Arrhenius plot for the reaction in the membrane isolated from *Anacystis nidulans* [22]. Thus, a short span of electron transport associated with Photosystem I is not sensitive to temperature induced changes in the fluidity of the thylakoid membranes.

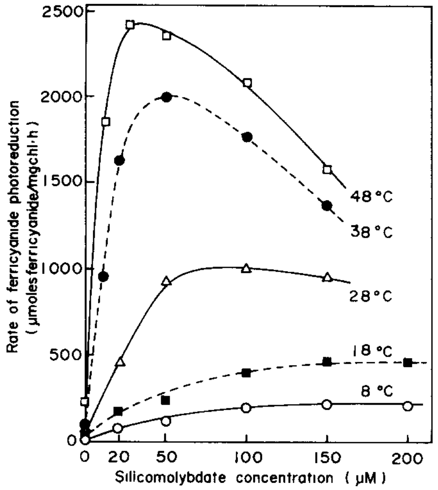
In order to examine the temperature dependency of Photosystem II reaction, ferricyanide photoreduction in the thylakoid membrane isolated from the alga was determined at different temperatures. The membranes are largely devoid of cytochrome *c*-553 so that electron transport is interrupted between

Photosystems I and II [12,13]. Thus the ferricyanide photoreduction is mediated by Photosystem II alone. The activity was determined at pH 6.5, which had been found to be the optimum in the membranes prepared in the present work and its Arrhenius plot is presented in Fig. 5. As described by Yamaoka et al. [13], the maximum reduction rate was obtained at about 50° C reflecting an increased heat-sensitivity of the membrane after isolation. At the low temperature side of the optimum, the plot consists of three straight lines with breaks at 30 and 9° C. The activation energies were 7, 17 and 26 kcal/mol in the order of decreasing temperature. Thus, the temperature dependency of the ferricyanide photoreduction is similar to that of methyl viologen photoreduction mediated by the two photosystems.

The results are, however, in variance with the previous observation of Yamaoka et al. [12] that the plot showed no break at about 30° C. The discrepancy between the previous and present works may be related to difference in the accessibility of the primary electron acceptor of Photosystem II to ferricyanide in the thylakoid preparations employed. In the thylakoid preparation employed in the present work, 90% inhibition of ferricyanide photoreduction was observed in the presence of 10 to 50 μM DCMU, an inhibitor which blocks electron transport from the primary electron acceptor of Photosystem II to plastoquinone. This indicates that ferricyanide accepts electrons mostly via plastoquinone. In contrast, about a half of the activity survived in the presence of 10 μM DCMU in the previous work [12]. Obviously, the shield covering the primary electron acceptor of Photosystem II in the membranes which had been isolated from cells grown under an iron-deficient condition for 4 to 5 days was much more leaky. Since the DCMU-resistant part of the ferricyanide photoreduction showed no break at about 30° C in its Arrhenius plot (see below and Fig. 5), the break at 30° C must have been obscured by a large flux of electrons from the primary Photosystem II acceptor to ferricyanide in the previous work. (The activation energies given in Fig. 5 of Ref. 12 were miscalculated and should be divided by a factor of 2.3.)

Fig. 5 shows that the Arrhenius plot of the DCMU-resistant part of the activity is markedly different from that of ferricyanide photoreduction determined in the absence of the poison. The plot upwardly deviated from a linearity at temperature about 50° C, presumably reflecting an increase in the accessibility of the primary electron acceptor of Photosystem II to ferricyanide at high temperatures where an irreversible thermal inactivation of the control activity occurs. Below 15° C, reduction rates became too slow to be determinated accurately. Between 50 and 15° C, however, the plot was linear showing no break or discontinuity around 30° C and giving a constant activation energy of 11 kcal/mol. The results strongly suggest that electron transport from the water splitting enzyme and the primary electron acceptor of Photosystem II is not affected by the membrane fluidity.

This possibility was further explored using siliçomolybdate which has been shown to bypass the DCMU-inhibition site by accepting electrons direclty from the primary acceptor of photosystem II and transferring them to ferricyanide [23,24]. As described previously with spinach chloroplasts [24], rates of ferricyanide reduction in the presence of silicomolybdate decreased as the reaction proceeded. When the initial reduction rates were determined, ferricyanide



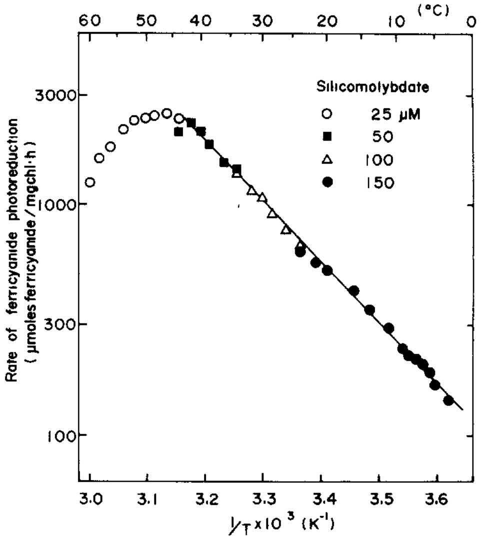


Fig. 6. Dependence of ferricyanide photoreduction on silicomolybdate concentrations at different temperatures. The reaction medium contained 1 M sucrose, 50 mM Mes-NaOH (pH 5.5), 10 mM $MgCl_2$, 20 mM NaCl, 10 μM DCMU, 5 μg chlorophyll/ml, and indicated amounts of silicomolybdate. Light intensity was $3.2 \cdot 10^6$ ergs · cm^{-2} · s^{-1}.

Fig. 7. Arrhenius plot of ferricyanide photoreduction determined in the presence of DCMU and silicomolybdate.

photoreduction was 2- to 3-fold stimulated by the addition of silicomolybdate to the algal membranes. The enhanced activity was largely insensitive to DCMU. Thus a high intensity of the actinic light is needed to saturate the silicomolybdate-supported reaction in the membranes which lost of most of phycobilins. However, even under the saturating light intensity, the Arrhenius plot tended to bend downwardly at hight temperatures when the activity was determined at a fixed concentration (50 μM) of silicomolybdate (data not presented).

It was found that the optimum concentrations of silicomolybdate vary as a function of the assay temperatures (Fig. 6). At and below 18° C, the maximum reduction rate was obtained with 150 μM silicomolybdate, whereas the optimum concentration decreased with elevating temperature to reach 25 μM at 48° C. An excess of silicomolybdate was inhibitory at high temperatures. Results suggest that the optimum concentration thus determined is a composite of the stimulating and inhibitory effects of silicomolybdate and may not reflect the saturating concentration. Then, the Arrhenius plot is expected to deviate downwardly from a linearity. However, this was found not to be the case. The plot of the activity determined at the optimum concentrations of silicomolybdate at each assay temperature is linear between 44 and 3° C with no break or discontinuity at about 30 and 10° C. Its activation energy was comparable with that of DCMU-insensitive part of ferricyanide photoreduction. Thus, the two DCMU-resistant ferricyanide photoreductions determined with and without silicomolybdate are independent of the phase changes in the membrane lipid. It is concluded from these observations that electron transport from water to the primary electron acceptor of photosystem II is not affected by the changes in the fluidity of the thylakoid membrane.

Discussion

Electron transport reactions studied here can be divided into two groups with respect to their temperature dependency. The first group involves cytochrome 553 photooxidation, methyl viologen photoreduction with reduced DCIP as electron donor, DCMU-resistnat part of ferricyanide photoreduction and silicomolybdate-supported ferricyanide photoreduction. These reactions are characterized by a constant activation energy in the wide temperature range studied.

Methyl viologen photoreduction with water as electron donor, ferricyanide photoreduction in the absence of DCMU and reduction of flash-oxidized cytochrome 553 form the second group. All reactions in this group showed abrupt transitions of the activation energy at about 30 and 10°C which, respectively, correspond to the upper and lower boundaries of the lateral phase separation of the membrane lipids detected by the spin labelling experiment [12]. Thus, the second group reactions are influenced by the fluidity of the thylakoid membranes.

The Arrhenius plot of cytochrome 553 reduction showed the third break at 58°C, the optimum temperature for photosynthesis. The cause of this transition of the activation energy is not known at present. However, the absence of the break from the Arrhenius plot of cytochrome 553 photooxidation suggests that the break also reflects a change in the membrane fluidity.

Methyl viologen photoreduction with water as electron donor was determined without addition of any uncoupler. Thus observed changes in its activation energy may be, at least partly, related to temperature-dependent changes in the pH difference across the thylakoid membranes which is known to regulate electron transport [21]. However, this explanation cannot be applied to other reactions of the second group. Ferricyanide photoreduction was determined in the uncoupled thylakoid membranes [13]. Cytochrome 553 reduction was measured with repetitive flash excitation which could not accumulate a significant pH difference across the thylakoid membranes. We conclude, therefore, that some part of electron transport common among the three reactions of the second group are direclty affected by the transition of physical state of the membrane lipids.

In this regard, of special interest is a fact that, whereas the first group reactions do not involve plastoquinone, the three electron transport reactions of the second group are related to the functional quinone. This strongly suggests that phase change of the membrane lipids affect electron transport specifically at the region of the plastoquinone pool. As compared with other electron carriers, plastoquinone has a higher mobility in the lipid phase of the thylakoid membranes due to its small molecule size as well as its lipophilic property. Thus plastoquinone may shuttle or relay electrons between Photosystem I and Photosystem II through the fluid hydrophobic matrix of the membranes. As such, the electron transferring capacity of plastoquinone strongly depends upon the physical state of the membrane lipids.

Electron transport reactions classified into the first group are mediated by either Photosystem I or Photosystem II alone. The reaction centers of Photosystems I and II are located in chlorophyll-protein complexes which could be isolated with aid of detergents [25—27]. Thus the functioning of the reaction center is independent of the thermotropic behaviour of the membrane matrix. In fact, the primary charge separation and its dark reversal proceed even at the cryogenic temperatures [28,29]. However, Photosystem I reactions studied in the present work cover the span of electron transport from cytochrome 553 to Photosystem I reaction center. DCMU-insensitive ferricyanide photoreductions in the presence and absence of silicomolybdate involve all redox components on the oxidizing side of photosystem II reaction center. Thus, the results indicate that electron transport through proteineous electron carriers is insensitive to temperature-dependent changes in the membrane fluidity.

We suggest, therefore, that the reaction center complexes are present in close association with related electron carrier proteins to form functional supramolecular assemblies. Cytochrome 553, any intermediate carrier between cytochrome 553 and P-700 if occurs , and P-700 chlorophyll protein complex form the assembly of Photosystem I. In this respect, it is noteworthy that cytochrome *f* and *b*-563 have been isolated in a complexed form [30]. Ferredoxin and ferredoxin-NADP oxidoreductase may be a part of the protein cluster, although further experiments are needed to establish this point. On the other hand, chlorophyll protein complex of Photosystem II and redox proteins involved in oxygen evolving reaction form the assembly of Photosystem II. The interaction among all protein components of an assembly may not necessarily be very strong but still sufficient to allow electron transfer within such a cluster of proteins to proceed independently of the phase changes in the membrane lipids.

Thus, non-cyclic electron transport consists of the two protein assemblies connected by plastoquinone. The model is compatible with observations that electron exchanges between electron transport chains occur at the region of plastoquinone [31,32]. A plastoquinone molecule, as a mobile electron carrier, may not necessarily connect a particular pair of Photosystems I and II but, when reduced by a Photosystem II reaction center, would diffuse into the lipid phase of the membrane and donate its electrons to one of neighbouring Photosystem I assemblies the molecule happens to encounter. Extent of the electron exchange would increase if electrons from Photosystem II were relayed by more than one plastoquinone molecules until they reach Photosystem I. The presence of the mobile electron carrier between the two photosystems would promote a smooth and efficient functioning of photosynthesis especially under weak light conditions, because it will aid electron transport from water to $NADP^+$ to complete without a simultaneous excitation of a fixed pair of Photosystems I and II. Finally, the mobile quinone carrier would be important with respect to the chemiosmotic energy conservation of the thylakoid membrane [21].

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