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TEMPERATURE DEPENDENCE OF THE PHOTOSYNTHETIC ACTIVITIES IN THE THYLAKOID MEMBRANES FROM THE BLUE-GREEN ALGA *ANACYSTIS NIDULANS*

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Summary

The photosynthetic electron transport and phosphorylation reactions were measured in the room temperature region in the thylakoid membranes prepared from the blue-green alga, *Anacystis nidulans*. The Arrhenius plot of the Hill reaction with 2,6-dichlorophenolindophenol showed a distinct break of straight lines at 21°C in the membranes from cells grown at 38°C, and at 12°C in those from cells grown at 28°C. The Arrhenius plot of the Hill reaction with ferricyanide showed a break at 13°C in the membranes from cells grown at 38°C, and at 7°C in those from cells grown at 28°C. On the other hand, the Arrhenius plot of the System I reaction with methylviologen as an electron acceptor and 2,6-dichlorophenolindophenol and ascorbate as an electron donor system was composed of a straight line in the membranes from cells grown at 28°C as well as at 38°C. The Arrhenius plot of the System II reaction measured by the ferricyanide reduction mediated by silicotungstate in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea also showed a break at 11°C in the membranes from cells grown at 38°C.

The Arrhenius plot of the phosphorylation mediated by *N*-methylphenazonium methylsulfate showed a break at 21°C in the membranes from cells grown at 38°C and at 12°C in those from cells grown at 28°C. The Arrhenius plot of the phosphorylation mediated by the System I reaction showed a break at 24°C in the membranes from cells grown at 38°C.

The characteristic features in the Arrhenius plots of the photosynthetic electron transport and phosphorylation reactions are discussed in terms of the transition of physical phase of the thylakoid membrane lipids.

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Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

Introduction

Temperature has great influences on the physiological properties and activities of biological membranes. The temperature-dependent phenomena of membranes are partly interpreted by the physical state of membrane lipids. Most drastic changes in the membrane properties occur at the thermotropic transition of physical phase of membrane lipids. Arrhenius plots of activities of some membranous enzymes show discontinuities and/or changes in inclination of straight lines at the temperature of phase transition [1—4].

In the photosynthetic membranes, the relationship between the physical phase of membrane lipids and the temperature dependences of physiological activities has been studied in some chilling-sensitive higher plants and algae [5—11]. In the blue-green alga *Anacystis nidulans*, studies on the spin labeling and the chlorophyll *a* fluorescence have revealed that the phase transition of the thylakoid membrane lipids between the liquid crystalline and the phase separation states occurs around 24°C in cells grown at 38°C and around 13°C in cells grown at 28°C [7,8]. The phase transitions are accompanied by the characteristic changes in various photosynthetic activities of intact cells such as the photosynthetic electron transport reaction [7], the intensity of delayed fluorescence of chlorophyll *a* [11], the pigment state 1-state 2 shift and O_2 evolution [7].

The breaks of the Arrhenius plots of the photosynthetic electron transport reactions have been observed also in the chloroplasts isolated from higher plants [5,12,13]. They are discussed in relation to the chilling sensitivity of plants [5,14]. Nolan and Smillie, on the other hand, reported that the breaks appeared in the Arrhenius plot of the Hill reaction with DCIP in the chloroplasts of chilling-resistant plants and inferred that there would be no relatioship between the breaks and the phase transition of membrane lipids [12,13].

In a previous paper [15], we described a method to prepare the thylakoid membranes from *A. nidulans*. The membrane preparation was highly active in the cyclic and non-cyclic photophosphorylation as well as the electron transport reactions such as the Hill reactions with DCIP and ferricyanide, the System I reaction and the Mehler reaction mediated by methylviologen. In the present study, we measured the temperature dependences of the photosynthetic electron transport and phosphorylation reactions in the thylakoid membranes of *A. nidulans*, and investigated the effects of lipid phase transition on the membrane activities. The results are discussed in terms of the physical phase transition of the thylakoid membrane lipids.

Materials and Methods

A. nidulans was obtained from the Algal Collection of the Institute of Applied Microbiology, University of Tokyo. The cells were grown at 28 and 38°C in the previously described method [15]. The thylakoid membranes were prepared in a way essentially the same as previously described [15]. However, the conditions for the lysozyme treatment were modified. The cells grown at 38°C were treated at 38°C for 3 h with lysozyme in the presence of EDTA and were disrupted at 30°C by a short period of sonic oscillation, while the cells

grown at 28°C were treated at 28°C for 4 h and disrupted at 20°C. The thylakoid membranes thus prepared were stored overnight at 0°C and then used for the experiments [15].

The Hill reaction with DCIP and ferricyanide and the System II reaction mediated by silicotungstate were measured spectrophotometrically, and the System I reaction was measured with a Clark type oxygen electrode as previously described [15]. Sensitivity of the oxygen electrode was corrected for temperature. The basal reaction mixture for the electron transport reactions contained 600 mM sucrose, 10 mM NaCl, 15 mM *N*-tris(hydroxymethyl)-methyl-2-aminoethane sulfonic acid/NaOH buffer, pH 7.0. 0.08 mM DCIP was added for the Hill reaction with DCIP. 1 mM potassium ferricyanide and 1 mM potassium ferrocyanide were added for the Hill reaction with ferricyanide. 1 mM potassium ferricyanide, 1 mM potassium ferrocyanide, 0.025 mM silicotungstate and 0.005 mM DCMU were added for the System II reaction. For the System I reaction, 2 mM sodium ascorbate and 0.1 mM DCIP were used as an electron donor system and 0.2 mM methylviologen as an electron acceptor.

The activities of photosynthetic phosphorylations were measured by using radioactive isotope of phosphorus according to the method of Avron [16]. The basal reaction mixture contained 600 mM sucrose/10 mM NaCl/5 mM $MgCl_2$/5 mM K_2HPO_4/3 mM ADP/15 mM *N*-tris(hydroxymethyl)methyl glycine-NaOH buffer, pH 7.5. 0.03 mM *N*-methylphenazonium methylsulfate was used as a cofactor for the cyclic phosphorylation. The concentrations of electron carriers for the non-cyclic phosphorylation were the same as in the assay for the electron transport reaction of System I. The details of assays were previously described [15].

For the measurement of activities, 0.03 ml of the suspension of thylakoid membranes at 0°C was added to 3.5 ml of the reaction mixture at the designated temperature. Thus, the mixture was transferred to a four-side transparent cuvette in a cell container with water jacket, through which water at the designated temperature was circulated. After standing for 5 min in the dark to obtain constant temperature, the activities were assayed. Temperature during the measurements was monitored with a calibrated copper-constantan thermocouple or a thermister (Taiyokagaku). The change in temperature during the measurement did not exceed 0.5°C.

The System II reaction was measured by the reduction of ferricyanide mediated by silicotungstate. The thylakoid membranes were preincubated with the medium containing 600 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$, 0.05 mM silicotungstate and 15 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid/NaOH buffer, pH 7.0, in the dark at 30°C for 10 min. 0.25 ml of this mixture was added to 3.0 ml of the reaction mixture which had been set at the designated temperature. After standing for 5 min the activity was assayed. Without this pretreatment the reaction rate progressively increased during the measurement, and the exact values for the activity could not be determined.

The concentration of chlorophyll *a* was determined according to the method of MacKinney [17].

Results

Fig. 1 shows the Arrhenius plots for the Hill reaction with DCIP of the thylakoid membranes prepared from the cells of *A. nidulans* grown at 38 and 28°C. The plots were composed of two straight lines with a break at 21°C in the membranes from cells grown at 38°C, and at 12°C in the membranes from cells grown at 28°C. It is noted that the temperature for the break point depended on the growth temperature and that the apparent activation energies above and below the break points were almost the same in the two samples.

The result in Fig. 1 was obtained without adding $MgCl_2$ to the reaction mixture. The temperature dependence of the Hill reaction with DCIP was measured also in the presence of 20 mM $MgCl_2$ in the temperature region from 9 to 35°C in the membranes from cells grown at 38°C (data not shown). $MgCl_2$ did not alter the essential features of the temperature dependence. A break in the Arrhenius plot was found at 22°C and activation energy was 10.4 kcal/mol above and 15.0 kcal/mol below the break point.

Fig. 2 shows the Arrhenius plots for the Hill reaction with ferricyanide in the thylakoid membranes in the presence of 20 mM $MgCl_2$. A break was found at 13°C in the membranes from cells grown at 38°C, and at 7°C in the membranes from cells grown at 28°C. It can be noted that the activation energies above and below the breaks were almost the same irrespective of the growth temperature.

The temperature dependence of the Hill reaction with ferricyanide was also measured in the absence of $MgCl_2$. In this case, however, the Arrhenius plots were not approximated by two straight lines with one break point (data not shown).

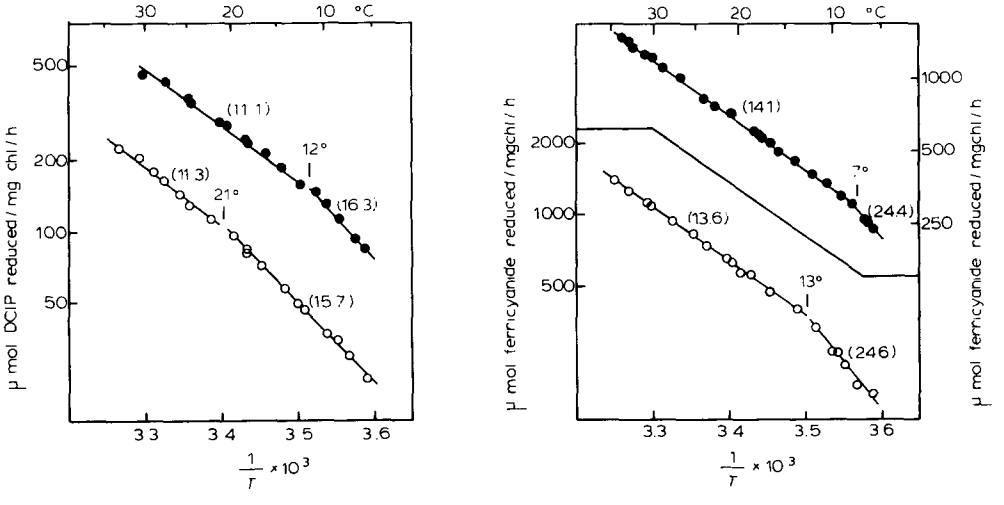

Fig. 1. Arrhenius plots of the Hill reaction with DCIP. The numbers in parentheses are the activation energies in kcal/mol. Thylakoid membranes were prepared from cells grown at 38°C (○) and 28°C (●).

Fig. 2. Arrhenius plots of the Hill reaction with ferricyanide in the presence of 20 mM $MgCl_2$. The numbers in parentheses are the activation energies in kcal/mol. Thylakoid membranes were prepared from cells grown at 38°C (○) and 28°C (●).

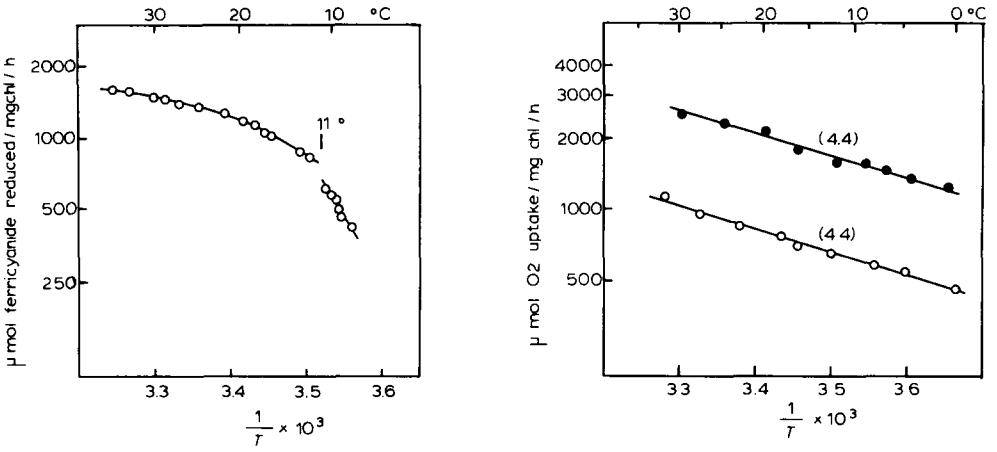

Fig. 3. Arrhenius plot of the System II reaction. 0.005 mM DCMU and 0.01 mM gramicidin J were present in the reaction mixture. Thylakoid membranes were prepared from cells grown at 38°C. The experimental details are described in Materials and Methods.

Fig. 4. Arrhenius plots of the System I reaction. The numbers in parentheses are the activation energies in kcal/mol. Thylakoid membranes were prepared from cells grown at 38°C (○) and 28°C (●).

It has been elucidated that silicotungstate and silicomolybdate accept electrons from the primary electron acceptor of Photoreaction II and transfer them to ferricyanide [18,19]. Fig. 3 shows the Arrhenius plot of the reduction of ferricyanide mediated by silicotungstate in the presence of DCMU and gramicidin J [20] in the thylakoid membranes from cells grown at 38°C. A discontinuity of lines appeared at 11°C. Although the line above the discontinuity was not straight, it was impossible to mark another break point.

Fig. 4 shows the Arrhenius plots of the System I reaction with methylviologen as an electron acceptor and DCIP and ascorbate as an electron donor system. The Arrhenius plots were composed of straight lines with no breaks. The activation energies were 4.4 kcal/mol in the two samples. This value was much lower than those for the activation energies of the Hill reactions and the phosphorylation reactions.

Fig. 5 shows the Arrhenius plots of the phosphorylation reaction mediated by *N*-methylphenazonium methylsulfate in the presence of DCMU. Although the number of data points was not enough to obtain exact temperatures of breaks, it seemed reasonable to point a break of lines somewhere around 21°C in the membranes from cells grown at 38°C and a discontinuity somewhere around 12°C in the membranes from cells grown at 28°C.

It is not determined in this phosphorylation reaction whether the characteristic break points originated from the electron transport mediated by *N*-methylphenazonium methylsulfate or the phosphorylation reaction itself. Thus, we investigated the temperature dependence of the phosphorylation coupled to the System I reaction, which did not reveal any break point in the Arrhenius plots (Fig. 4). It can be inferred that if a break point appears in the Arrhenius plot of the phosphorylation reaction, the phosphorylation reaction but not the

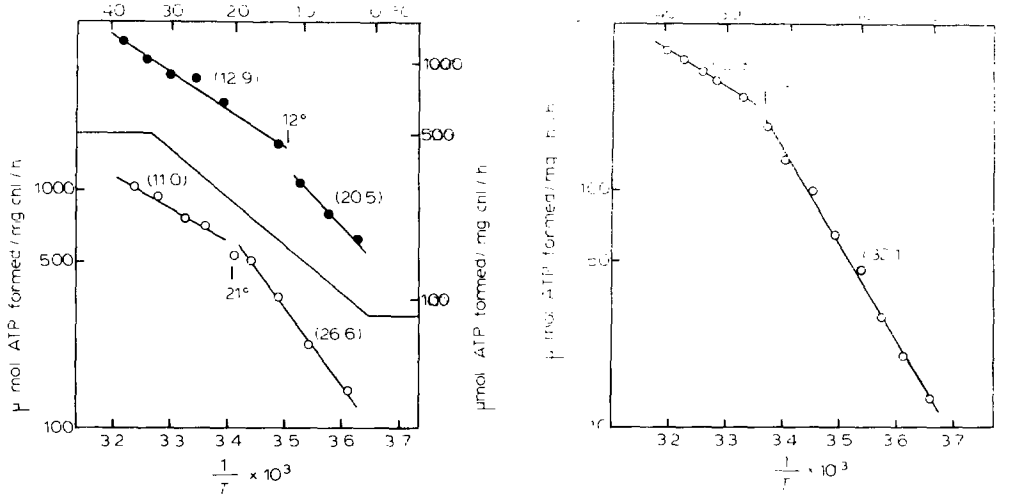

Fig. 5. Arrhenius plots of the phosphorylation mediated by *N*-methylphenazonium methylsulfate in the presence of DCMU. The numbers in parentheses are the activation energies in kcal/mol. Concentrations of *N*-methylphenazonium methylsulfate and DCMU were 0.03 mM and 0.005 mM, respectively. Thylakoid membranes were prepared from cells grown at 38°C (○) and 28°C (●).

Fig. 6. Arrhenius plot of the phosphorylation mediated by DCIP and ascorbate as an electron donor system and methylviologen as an electron acceptor. The numbers in parentheses are the activation energies in kcal/mol. Thylakoid membranes were prepared from cells grown at 38°C.

electron transport produces the characteristic break point. Fig. 6 shows the Arrhenius plot of the phosphorylation reaction coupled to the System I reaction in the membranes from cells grown at 38°C. A clear break of the line appeared around 24°C.

Discussion

In the present study, the temperature dependence of photosynthetic electron transport and phosphorylation reactions were investigated in the thylakoid membranes isolated from the blue-green alga, *A. nidulans*. The Arrhenius plots of these reactions revealed break or discontinuity points except for the electron transport reaction of System I.

In the Hill reaction with DCIP and the phosphorylation reactions, the characteristic points appeared at 21—24°C in the membranes prepared from cells grown at 38°C, and around 13°C in the membranes from cells grown at 28°C. The previous studies on spin-labeling and chlorophyll *a* fluorescence [7,8] have indicated that the temperature-dependent transition of the physical phase of membrane lipids between the liquid crystalline and phase separation states occurs in the same temperature regions; around 24°C in cells grown at 38°C and around 13°C in cells grown at 28°C. This is confirmed in our recent studies on X-ray diffraction and differential thermal analyses (unpublished data). Thus, we suggest that the above-mentioned characteristic break points in the Arrhenius plot are related to the phase transition of thylakoid membrane lipids.

Since the thylakoid membranes prepared in the present study retained the electron transport system from H_2O to Photoreaction I (ref. 15), it is likely that DCIP accepts electrons from the reducing side of Photoreaction I. Thus, the rate-determining step of the Hill reaction with DCIP must be located between the two photoreactions, and this step is affected by the phase transition of membrane lipids. It is noted that the break point appeared in the Arrhenius plot of the phosphorylation reaction coupled to the electron transport of the System I reaction, which did not show any break in the Arrhenius plot. Therefore, it is concluded that a process somewhere between the ATP formation and the coupling to electron transport is affected by the physical phase of membrane lipids as seen in various ATPase systems [2,21,22]. It is likely that activities of CF_1, the enzyme associated with photosynthetic phosphorylation, and/or formation of the ion gradient across the thylakoid membrane are affected by the physical phase of the membrane lipids.

The breaks in the Arrhenius plots were seen in lower temperature regions in the Hill reaction with ferricyanide and the System II reaction mediated by silicotungstate; They appeared at 11—13°C in the membranes prepared from cells grown at 38°C and around 7°C in those from cells grown at 28°C. However, the characteristic points appearing in this temperature region are observed in intact cells of this alga in the chilling-sensitivity of photosynthesis (Ono, T. and Murata, N., unpublished) and the inhibition of excitation transfer from phycobilins to chlorophyll *a* (Schreiber, U. and Murata, N., unpublished).

In the System II reaction silicotungstate is reduced directly by the primary electron acceptor of Photoreaction II [18]. In the Hill reaction with ferricyanide, the ferricyanide reduction probably takes place at the reducing side of Photoreaction II, especially in the presence of divalent cations such as Mg^{2+} [23]. These findings suggest that the characteristic break points in the lower temperature regions are produced by the reaction between the oxidants and the reducing side of Photoreaction II. Since the characteristic points in the lower temperature regions depended on the growth temperature, it is likely that they are also related to the lipid phase, although details of the mechanism are not yet clarified.

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