Thermal Stability of Electron Transport in PS II Membranes and Particles from the Thermophilic Cyanobacteria

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Abstract. Thermal stability of the ferricyanide (FC) and dichlorophenolindophenol (DCIP) reducing reactions was investigated in isolated membrane preparations and PS II particles with active water splitting system from the cyanobacterium Synechococcus elongatus. In a hypotonic medium, the thermal stability was seen to be much higher for the DCIP than for the FC reduction reaction. After the addition of high concentrations of polyethylene glycol (M_wt = 4000) or sodium citrate to the medium, the FC reduction reaction appeared to be more temperature resistant. Data on the effects of temperature, DCMU and detergents on the electron transfer rate in PS II provide evidence suggesting that the different thermal stabilities of the two reactions are due to different physico-chemical properties of the electron donor sites to FC and DCIP. The data suggest that regions of contact between individual macromolecular complexes of the electron transport chain are the most labile sites of the photosynthetic apparatus. The role of the composition and properties of the intracellular medium on thermostability are emphasized.

Key words: Thermal stability — Electron transport — Membrane fragments — Photosynthetic reactions

Introduction

The thermophilic cyanobacteria, in which photosynthetic reactions occur at ambient temperatures as high as 70—75°C, represent a unique example of the adaptive potentials of plant cells. The structure of the photosynthetic apparatus of cyanobacteria and higher plants is known to have much in common (Hladik
and Sofrova 1983); based on this it may be expected that the understanding of the mechanism that make cyanobacteria resistant to high temperatures would permit the formulation of general principles of thermostabilisation of complex photosynthetic lipoprotein structures. The electron transport chain of PS I has been studied most extensively in terms of thermostability (Koike and Katoh 1979, 1980; Koike et al. 1982). This component of the photosynthetic apparatus of cyanobacteria was shown to be most resistant to high temperatures. Thermostability of light-dependent processes in PS II has been much less studied: among others, it remains unknown whether individual links of the electron transport in PS II are homogeneously thermostable.

The objective of the present work was to study the thermostability and temperature dependences of the individual reactions of electron transport in PS II membranes and particles isolated from cells of the thermophilic cyanobacteria *Synechococcus elongatus*.

### Materials and Methods

A *Synechococcus elongatus* cell culture (No. 120, Museum of the K. A. Timiryazev Institute of Plant Physiology) was grown on Kratz-Meiers medium (Kratz and Myers 1955) at 55 °C. The incubation mixture was bubbled with air plus 0.2 % CO₂. The culture was illuminated with white light of luminescent lamps in a changing lighting pattern, starting with 1000—1500 lux at the initial stage of the logarithmic phase of cell growth and gradually reaching 10,000 lux at the end of this stage. The gradual increase in the illumination intensity created more favourable lighting conditions for cell growth in the culture.

Membrane fragments and PS II particles with an active O₂-producing system were obtained as described elsewhere (Kaurov et al. 1986).

**Buffer B** containing 10 mmol/l Hepes/NaOH, 5 mmol/l K, Na-phosphate buffer, and 10 mmol/l MgCl₂ (pH 7.4) was used as the incubation medium for membrane and PS II particle preparations. Chlorophyll A (CHA) was determined by the method of Arnon et al. (1974).

The oxygen production rate by samples in the presence of FC (2 × 10⁻³ mol/l) was measured amperometrically using a Clark-type oxygen electrode.

The DCIP reduction rate was estimated spectrophotometrically (ε₉₀₀ = 21 mmol⁻¹.1.cm⁻¹) (Izawa 1980). The preparations were illuminated with white light of a halogen lamp. The flux of the light energy was 50 mW/cm², and a 50 mm thick water filter was used. The CHA concentration when measuring the electron transport rate was 5—10 μg/ml.

In estimating the thermostability of the PS II sites studied, a concentrated sample aliquot was introduced into the incubation medium maintained at the experimental temperature, kept there for 5 min, and then cooled down on ice to 25 °C to measure the electron transport rate. If not indicated otherwise the concentration of PEG 4000 in the PS II membrane and in particle preparations was 20 %.

Lysozyme and Hepes/NaOH (Serva, West Germany) and lauryl dimethyl aminoxide (LDAO; Onyx Chemical Co., USA) were used as reagents; all other reagents were of inland origin and were of at least analytical grade.
Fig. 1. Thermostability of electron transport in membrane fragments obtained from the cells of the thermophilic cyanobacteria *Synechococcus elongatus* under different conditions. 1 — membranes obtained in the presence of PEG-4000; 2 — membrane obtained in medium without PEG-4000; 3 — membranes obtained without stabilizer and transferred into medium with PEG-4000; 4 — membranes obtained without stabilizer and transferred into medium with sodium citrate (0.74 mol/l). Electron transport rate (V) was estimated by oxygen release. Electron acceptor FC. Rates of oxygen release by membranes in various buffer systems measured at 25 °C were used as control values 100%.

Results

A review of literature concerning the physico-chemical characteristics of the photosynthetic apparatus of thermophilic cyanobacteria reveals significant differences in estimates of thermostability of isolated membrane fragments. The membranes of the filiform cyanobacteria *Phormidium luridium* (Binder et al. 1976) and *Phormidium laminosum* (Stewart and Bendall 1980) lack thermostable oxygen release reactions. At the same time, membranes obtained from the cyanobacteria *Synechococcus sp.* (Yamaoka et al. 1978) have retained the initial thermostability of photosynthetic reaction. A comparison of the results of the
Fig. 2. Thermostability of FC and DCIP reduction membrane with fragments from *S. elongatus* cell. 1 — reduction of FC with membranes in incubation medium; 2 — reduction of DCIP with membranes in incubation medium; 3 — reduction of FC with membranes in medium with PEG-4000; 4 — reduction of DCIP with membranes in medium with PEG-4000.

Relative reduction rate (\(V\)) of FC was estimated as described in legend to Fig. 1; \(V\) for DCIP was determined as described in Materials and Methods.

reports mentioned above suggests that these discrepancies are due to specific features of the membrane-extraction procedures. The reported high stability of membranes from *Synechococcus sp.* cells (Yamaoka et al. 1978) was apparently due to the fact that PEG-4000 was used in the isolation procedure. Indeed, it is evident from our results (Fig. 1, curve 1) that membranes isolated from *Synechococcus elongatus* cells in a medium containing 30% PEG-4000 show high thermostability of the FC reducing reaction with \(T_{50\%}=63^\circ C\). In conventional buffer systems (including the hypotonic ones) employed by Binder et al. (1976) and Yamaoka et al. (1978) for *P. luridium* and *P. laminosum* cells the thermostability of the reaction studied is lost, and \(T_{50\%}\) decreases by 17°C to equal only 46°C (Fig. 1, curve 2). This change in thermostability is not caused by the loss of some component of the photosynthetic apparatus, since the transfer of the same membranes into a medium containing PEG-4000 virtually restores the thermostability (Fig. 1, curve 3). A similar effect is also observed when the
membranes are suspended in a buffer with a high concentration of sodium citrate (Fig. 1, curve 4).

The results illustrated in Fig. 1, show an interesting phenomenon, namely the effect of thermostabilizers heated to experimental temperature on activated electron transport reactions in membrane preparations. This was particularly evident in preparations with sodium citrate. As a rule, after the samples were kept at 25 °C for 15—20 min the activation effect disappeared. Quite likely, the observed phenomenon was due to reversible conformational changes in the photosynthetic apparatus that slowly restored.

PS II is known to be the most thermolabile elements in the complex of structures that conduct light-dependent processes in cyanobacterial cells (Koike and Katoh 1979, 1980; Koike et al. 1982). Owing to the considerable structural and functional heterogeneity of PS II one may expect that the thermostability of the components of the cyanobacteria electron transport system would be heterogenous. To shed light on the issue, we studied the thermostability of electron transport from water to artificial acceptors of various classes in PS II membranes and particles isolated from Synechococcus elongatus.

FC (class I acceptor) and DCIP (class II acceptor) were used as electron acceptors. It is evident from Fig. 2 (curves 1 and 2) that the thermostability of DCIP and FC reduction is significantly changed in the absence of thermostabilizers: $T_{50\%} = 59^\circ C$ for DCIP, while being only $46^\circ C$ for FC. Obviously the connection between the photosystems of isolated cyanobacteria membranes is impaired (Yamaoka et al. 1978; Hirano et al. 1981). In our tests, acceptors were reduced at the PS II level only. In addition to different thermostability of FC and DCIP reducing reactions, the two substances also differ in their sensitivities to diurone (Table 1). The release of oxygen by membranes in the presence of FC is inhibited by diurone to 92 %, while DCIP is reduced only to 22 %. These differences are apparently due to the fact that FC and DCIP are reduced at different sites of the electron transport chain.

### Table 1. Diurone inhibited FC and DCIP reactions with PS II membranes and particles from Synechococcus elongatus cells

<table>
<thead>
<tr>
<th>Acceptor Incubation medium</th>
<th>DCIP Membranes</th>
<th>PS II</th>
<th>FC (O$_2$-evolving) Membranes</th>
<th>PS II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without stabilizer</td>
<td>22</td>
<td>57</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>PEG-4000</td>
<td>0</td>
<td>43</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>

1) In all tests, diurone concentration was 5 μmol/l
2) Control values of reaction rates for membranes: see Figs. 2. and 3.
Table 2. Effects of PEG-4000 and LDAO on reduction rates (V) of FC and DCIP with membrane fragments from *Synechococcus elongatus* cells

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Incubation medium</th>
<th>FC Without stabilizer</th>
<th>PEG-4000</th>
<th>DCIP Without stabilizer</th>
<th>PEG-4000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without LDAO</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>LDAO</td>
<td>70</td>
<td>75</td>
<td>250</td>
<td>135</td>
</tr>
</tbody>
</table>

LDAO: CHA ratio = 1:1

<sup>a</sup> control

![Graph showing thermostability of FC and reduction with O₂-evolving particles from *S. elongatus* cells.](image)

Fig. 3. Thermostability of FC and reduction with O₂-evolving particles from *S. elongatus* cells. 1 — reduction of FC with PS II particles in incubation medium; 2 — reduction of DCIP with PS II particles in incubation medium; 3 — reduction of FC with PS II particles in medium with PEG-4000; 4 — reduction of DCIP with PS II particle in medium with PEG-4000. Relative reduction rate acceptor was estimated as described in legend to Fig. 2.
Introduction into the membrane incubation medium of PEG-4000 (20 %) substantially reduced the differences in thermostability of the reactions studied. In the absence of stabilizers the difference in $T_{50\%}$ was 13°C, and it decreased to 4°C in the presence of PEG-4000 (Fig. 2, curves 3 and 4). Obviously PEG-4000 decreases the total electron transport rate in PS II. At 25°C, the reduction rate of FC was 50 %, and that of DCIP 70 %. Moreover, PEG-4000 reduced the sensitivity of both reaction to diuron (Table 1). These effects are apparently due to a reduced structural and functional mobility of elements of the electron transport chain and to a changed accessibility to the acceptors and inhibitors of the corresponding donor and herbicide-binding sites. The fact that the detergent lauryl dimethyl aminoxide (LDAO) reduces the inhibitory effect
Fig. 5. Temperature dependence of DCIP reduction by PS II membrane fragments and O₂-evolving particles. 1 — PS II membrane fragments without PEG-4000; 2 — PS II particles in medium without PEG-4000; 3 — PS II membrane fragments with PEG-4000; 4 — PS II particle in medium with PEG-4000.

of PEG-4000 (Table 2) can be considered as supporting the latter assumption. LDAO activates most effectively the reduction of the hydrophobic acceptor DCIP.

Similar thermostability data were obtained from PS II particles with oxygen-evolving system (Fig. 3, Table 1). Compared with the membrane fragments, the PS II particles showed a slightly lower thermostability in the absence of PEG-4000 in the buffer, and a higher sensitivity of electron transport activity to DCMU, perhaps due to the effect of the femnant LDAO in the preparation. After heating in the presence of PEG-4000, the electron transport activity was activated in PS II particles, and the difference in the thermostability between the
two reactions persisted. If the DCIP and FC are reduced at different sites, as suggested above, it is reasonable to expect that the state of the lipid phase influences the two reactions to different extent. One way to elucidate the effect of lipid fluidity is to investigate the effect of thermotropic phase transitions on the electron transfer rate. In experiments using ferricyanide as the electron acceptor, the presence of a thermostabilizer is necessary because high temperatures (above 25°C) inactivate PS II-driven electron transport (Figs. 2, 3). Fig. 4 shows Arrhenius plots of temperature dependences of oxygen evolution rates for membrane fragments (curve 1) and PS II particles (curve 2) in the presence of FC and PEG-4000. The inflexion points at approx. 37°C may be due to lipid phase transitions, since in thermophilic microorganisms structural alterations in the proteins occur at elevated temperatures (Koike and Katoh 1979, 1980; Koike et al. 1982; Brock 1967; Singleton and Amelunxen 1973; Berezin and Mozhaev 1985; Kutuzova et al. 1982). Thus, the influence of the state of the lipid phase on FC reduction in our preparations is obvious. Intermediates that determine the pattern of this dependence are presumably secondary quinones. It must be pointed out that there is a marked difference between curve 1 (Fig. 4) and a similar dependence for Synechococcus sp. membranes in the presence of 1 mol/l saccharose I (Hirano et al. 1981). In the latter, the inflexion point was at approx. 30°C and the transition was accompanied by a decrease of the FC reduction activation energy, while in our experiment the activation energy increased. Two phenomena may presumably be responsible for the discrepancy, namely, the influence of PEG-4000 and saccharose on electron transport and properties of the membranes themselves, which can change considerably with growth conditions. Hirano et al. (1981) reported the disappearance of the lipid-dependent inflexion in membrane fragments of Synechococcus sp. grown in an iron-deficient medium.

A general behaviour pattern of the temperature dependence of electron transport activity was only seen in membrane fragments when DCIP was reduced by water in the absence of thermostabilizers (curve 1, Fig. 5). It should be noted that at high temperatures thermal inactivation of the membranes may contribute to the slope of curve 1 (Fig. 5), since thermostability decreases with increasing temperature (Fig. 2, curve 2). In the absence of thermostabilizers in the PS II particles (curve 2) and in the presence of PEG-4000 in both the membrane fragments and PS II particles, the temperature dependence of DCIP reduction showed no inflexion (curves 3 and 4, respectively). This suggests that DCIP may be reduced by the secondary quinone pool in the membrane fragments in the absence of PEF-4000 only. The linearity of the Arrhenius plot of the temperature dependence for PS II particles in buffer B may presumably be accounted for by the exposure of much of the hydrophobic donor moiety of the PS II core to DCIP owing to the detergent present in the preparation. As seen
from Fig. 5, the addition of PEG-4000 had no effect on the pattern of the temperature dependence for PS II particles (curves 2 and 4). In the membrane fragments, the curve straightened in the presence of PEG-4000, presumably as a consequence of pronounced structural alterations in the membrane portion of the electron transport chain. An indication of this is the specific behavior of the Arrhenius plot of the temperature dependence of the oxygen production rate for membrane fragments in the presence of PEG-4000 (Fig. 4). In view of the above, the effect of temperature on electron transport of PS II appears to be of a complicated nature. It depends on the class of the acceptor used, on properties of the incubation medium and on the sample under study. An important inference from the data of Figs. 4 and 5 is the different nature of the sites donating electron to FC and DCIP.

Discussion

The data obtained here suggest that in the absence of thermostabilizers, the PS II-driven electron transport chain of the thermophilic cyanobacterium *S. elongatus*, is heterogenous as for the thermostability of its different paths (Figs. 2—4). Of the two reactions investigated, the FC reducing reaction is thermally much more labile than the other one. This suggests that FC and DCIP obviously accept electrons at different sites of PS II. The reduction of DCIP occurs at a thermally stable site before the site at which FC is reduced. It is known that in chloroplasts of higher plants the electron donating sites located on the acceptor side of PS II have similar affinities to both hydrophilic and hydrophobic acceptors (Trebst and Reimer 1973a, b) and that even acceptors of a common class can be reduced at different sites and have different sensitivities to DCMU (Gould and Izawa 1973). A similar situation presumably occurred in our experiments. Results shown in Table 1 and Figs. 2 and 3 suggest that DCIP reduction by membrane fragments occurs at the site of the primary acceptor Qₐ. The apoenzyme Qₐ is firmly attached to the hydrophobic region of the PS II core. Under normal conditions with neutral pH, it is little exposed to hydrophilic acceptors such as ferricyanide (Itoh and Nishimura 1977; Itoh 1978). Ferricyanide, which is most sensitive to DCMU under our experimental conditions, is presumably reduced at the site of the secondary acceptor Qₐ. The oxidation-reduction of the secondary quinones, by virtue of their high mobility involve such steps as binding to apoenzyme Qₐ, disintegration of the complex and the release of reduced plastoquinones into the lipid matrix of the membrane (Kyle 1985; Millner and Barber 1984). The high mobility and complicated pattern of intermolecular interactions of the secondary quinones are likely to contribute much to the thermal lability of this part of the electron transport chain of PS II.
Consistent with this assumption are observations of the effect of temperature on electron transport in membrane fragments and PS II particles (Figs. 4, 5). The data also provide evidence for the different sites donating electrons to DCIP and FC.

In PS II particles of *S. elongatus*, the thermal stability of the secondary donor is in line with the general thermal stability of the path from water to DCIP (Figs. 2, 3). It is probable that each individual patch of the PS II electron transport chain in thermophilic cyanobacteria has high thermal stability due to the structural arrangement itself of the pigment-protein associations. In support of this idea is the ability to perform light-induced charge separation at temperature as high as 70 °C by isolated pigment-protein complexes from *Synechococcus sp.* that retain PS II activity (Nakayama et al. 1979), and also the resistivity of the PS I reaction center of this culture to 5 min heating to 99 °C (Koike et al. 1982). From what has been said it is suggested that regions of contact between individual macromolecular complexes of the PS II electron transport chain are thermally most labile, determining the lower limit of thermal stability of the path. The regions of low thermal stability may include the electron transfer path between the primary and secondary quinone, the coupling site of the light-harvesting complex and reaction center, the polypeptides of the water-splitting complex. The two latter regions are thermally very labile. It is known that phycobilisomes (Yamaoka et al. 1978; Katoh and Gannt 1979) and proteins of the water-splitting complex (Koike and Inoue 1985; England and Evans 1985) can easily be removed while washing out membranes of the cyanobacteria.

PEG-4000 is very likely to exert its thermostabilizing effect on regions like those mentioned above (Figs. 1—4). The mechanism of its effect on thermostability is presumably increase of the dehydration-induced efficiency of intramolecular and intermolecular interactions of the components of the photosynthetic apparatus. An illustration of the structuring effect of PEG-4000 is the prevention of phycobilisome detachment in the presence of PEG-4000 in isolated membrane vesicles from cyanobacteria (Yamaoka et al. 1978; Fujita and Suzuki 1979). There are other compounds which improve the thermostability of the photosynthetic membranes by making their structure more stable. Membranes of the *Synechococcus* stock exhibit a high thermostability in the presence of high concentrations of sucrose (1 mmol/l) (Hirano et al. 1981) or Na-citrate in the medium (Fig. 1). Na-citrate was seen to increase the thermostability of spinach chloroplasts (Stewart 1982). Thus, the thermal stability of the electron transfer reaction in the presence of artificial acceptors largely depends on the ambient medium. The influence of the medium may presumably account for the contradicting estimates of the temperature sensitivity of isolated membranes (Binder et al. 1976; Stewart and Bendall 1980; Yamaoka et al. 1978), native

Certainly, there are much more compounds beyond those considered here that are capable of increasing the stability of cyanobacteria membranes, both structurally and functionally. They are in fact potent thermostabilizers. Many polysaccharides, salt ions of lyotropic class obviously belong to such compounds. The stabilizing effects of these compounds on macromolecules (proteins, nucleic acids) have been well studied (Berezin and Mozhaev 1985; Kutuzova et al. 1982; Hippel and Schleich 1973). Studies of the mechanisms of stabilization of macromolecular ensembles are only at their beginnings. However, the experimental material available to date makes us believe that in thermophilic bacteria the composition and properties of the intracellular medium are important in maintaining the in vivo thermostability of structure and function of both individual macromolecules and membranes.

**Abbreviations**

FC — ferricyanide  
DCIP — 2,6 dichlorophenol-indophenol  
CHA — chlorophyll A  
PEG-4000 — polyethylene glycol (Mw = 4000)  
LDAO — lauryl dimethyl amine-N-oxide  
T_{50} — temperature at which a 50% reduction of reaction occurs  
DCMU — 3-3 (3, 4-dichlorophenyl)-1,1-dimethyl-urea

**References**


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