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Research Article

The Effect of Glycinebetaine on the Heat Stability of Photosynthetic Reactions in Thylakoid Membranes

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Abstract: Heat inactivation of various photosynthetic electron transport reactions were investigated in the presence/absence of glycinebetaine (betaine) in unstacked thylakoid membranes from spinach. The activity of Photosystem II (PS II) is more sensitive to heat than that of Cytochrome bf (cyt.bf) and Photosytem I (PS I) complexes. The data obtained clearly demonstrated the protection of PS II and PS I electron transfer by betaine under high temperatures. The phenomena observed are probably related to the stabilization of the higher–order structures of PS II and PS I by betaine.

Key Words: Electron transport, heat stress, glycinebetaine.

Tilakoyit Zarlarda Fotosentetik Reaksiyonların Sıcağa Kararlığına Glisinbetain Etkisi

Özet: Ispanak menşeli tilakoyit zarlarda çeşitli fotosentetik elektron taşıma reaksiyonlarının sıcak nedeniyle inaktivasyonu araştırılmıştır. Fotosistem II–nin aktivitesi sıcağa sitokrom bf ve fotosistem I komplekslerininkinden daha duyarlıdır. Bulunan neticelerden glisinbetain'in, fotosistem II ve fotosistem I'yi yüksek sıcaklıklarda savunma etkisine sahip olduğu anlaşılmaktadır. Gözlenen hadiseler glisinbetainin, yüksek sıcaklık sitresi altında fotosistem II ve fotosistem I, 2 in düzenli kuruluşlarını kararlı hale getirdiğini göstermektedir.

Anahtar Sözcükler: Elektron taşıma, sıcaklık sitresi, glisinebetain.

Introduction

Photosynthesis is a very complex process which involves the cooperative functions of three major multiprotein–complexes in thylakoid membranes: photosystem II, the cytochrome bf complex and photosystem I to yield a proton gradient across the membrane and strong reductants on the stromal side. The fourth protein complex (ATP synthase) uses the proton motive force to synthesize ATP.

It is known that photosynthetic systems in plants are most sensitive to high–temperature treatments. The effect of high temperature on photosynthetic membranes results in the loss of grana stacking, due to the dissociation of the peripheral antenna complex of PSII from its core complex (Armond, Bjorkman & Staehelin, 1980; Gounaris, 1984). The PS II protein complex is generally recognized as being one of the most thermally labile components of the photosynthetic apparatus (Srivastava et al., 1997; Al-Khatib & Paulsen, 1989). It has been suggested that the heat–induced loss of PS II activity is the result of the inactivation of the components required for evolution of oxygen (Berry & Bjorkman, 1980; Tompson et al., 1989), while other studies suggest an alteration in Qa to Qb electron transfer at the acceptor site.

It was demonstrated that betaine, an amphipathic compound, which is accumulated in the cells of a number of halotolerant plants and microorganisms, protects PS II complex against the high salt–induced dissociation of the extrinsic proteins and the manganese cluster (Nash, Miyao & Murata, 1985; Papageorgiou, Fujimura & Murata, 1991; Murata et al., 1992). Wyn Jones et al.

(1981) have suggested that betaine is confined largely to the cytoplasm and to the chloroplast in higher plants. Its concentration in spinach chloroplasts reaches 0.3 M when the plant is grown in saline environments. A similar sype of protective effect of betaine has been observed in the oxygen evolution by cyanobacterial thylakoid membrane and PS II submembrane preparations against heat (Mohanty et al., 1993; Mamedov, Hayashi & Murata, 1993; Mamedov et al., 1991), suggesting that betaine prevents the loss of extrinsic polypeptides associated with oxygen evolution and it also stabilizes the coordination of the Mn cluster to the proten cleft.

The data recently obtained suggest that not only the oxygen evolving activity but also the partial electron transfer reactions that are more intimate to the PSII reaction center complex are stabilized by betaine (Allakhverdiev et al., 1996).

In the present study we examined systematically the effects of betaine on the heat stability of various photosynthetic reactions in unstacked thylakoids from spinach.

Materials and Methods

Unstacked thylakoids were isolated from spinach leaves *(Spinacia oleracea* L.) as described previously (Jahns et al., 1991) and kept in liquid nitrogen until use. Thylakoid membranes were prepared and stored in solutions that contained 0.5 M betaine. The thylakoid membranes were incubated for 10 min at designated temperatures in darkness in a medium that contained 25 mM HEPES –NaOH (pH 7.5), 0.4 M sucrose and 10 mM NaCl in the absence and in the presence of 0.5 M betaine. The suspension was then cooled to 20°C and after the addition of various electron donors and acceptors the rate of the below–specified photosynthetic reactions were measured.

The transport of electrons from H_2O to PBQ (phenyl–1, 4–benzoquinone), from H_2O to MV (methylviologen), from DQH_2 (duroquinol) to MV and from reduced DAD (1, 4–diamino–2, 3, 5, 6–tetramethylbenzene) to MV were quantitated by polarographic monitoring of the concentration of oxygen in the assay mixture with a Clark–type electrode.

The transport of electrons from H_2O to DCIP (2, 6–dichlorophenol indophenol) in the absence and in the presence of DPC (diphenylcarbazide) were quantitated by measuring photoreduction of DCIP with a single–beam spectrophotometer (Spekol 221, Zeiss) by observing the absorbance change at 600 nm. The chlorophyll (Chl) content of samples was determined according to the method described by Arnon (1949). Thylakoid membranes were added to a final concentration that corresponded to 8–10 µg of Chl/ml.

Results and Discussion

The effect of high temperature on the transport of electrons from H_2O to PBQ in the unstacked thylakoid membranes is shown in Figure 1. When the thylakoid membranes were heated without betaine for 10min at various temperatures the extent of inactivation was considerable at temperatures above 35°c. In the presence of betaine, however, the rate of oxygen evolving in thylakoid membranes increased up to 45°C and then declined only very slowly in samples treated at more elevated temperatures. The protective effect of the betaine is shown further after calculation of the temperature that causes 50% inactivation (Table 1). $T_{1/2}$ was found to be about 39°C in samples in the absence of betaine and 51°C in the presence of betaine. This observation is consistent with previous data derived from thylakoid membranes from *Synechocystis sp.* PCC6803 (Mamedov, Hayashi & Murata, 1993).

The dependence on temperature of the heat inactivation of the electron transport reaction from H_2O to MV was similar to that from H_2O to PBQ (data not shown).

Figure 2 shows the temperature dependence of the heat inactivation of the reaction related to the

Table 1. Temperature for 50% inactivation ($T_{1/2}$) by heat of specific reactions in thylakoid membranes from spinach.

Reactions	$T_{1/2}$ (°C) without betaine	with betaine
$H_2O \rightarrow PBQ$	39	51
$H_2O \rightarrow DCIP$	44	53
$H_2O \rightarrow DPC \rightarrow DCIP$	48	55
$DQH_2 \rightarrow MV$	55	63
$DADH_2 \rightarrow MV$	60	72
$H_2O \rightarrow MV$	44	55

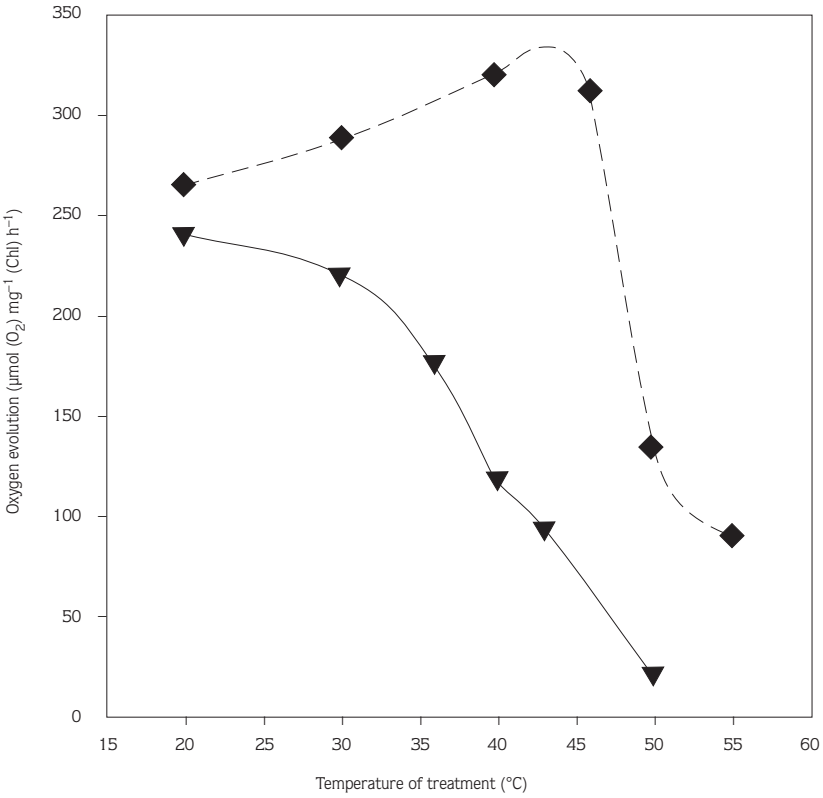

Figure 1. Dependence on incubation temperature of the rate of oxygen evolution in thylakoid membranes. Thylakoid membranes were incubated for 10 min at designated temperatures in 25 mM HEPES–NaOH (pH 7.5), 10 mM NaCl, 0.4 M sucrose and then cooled to 20°C. After the addition of 0.2 mM PBQ the electron–transport activity was measured by monitoring of oxygen evolving ▼–no betaine added to both the preparation and incubation mixtures. ◆–0.5 M betaine was added to these mixtures. Each point represents the mean of 3 experiments.

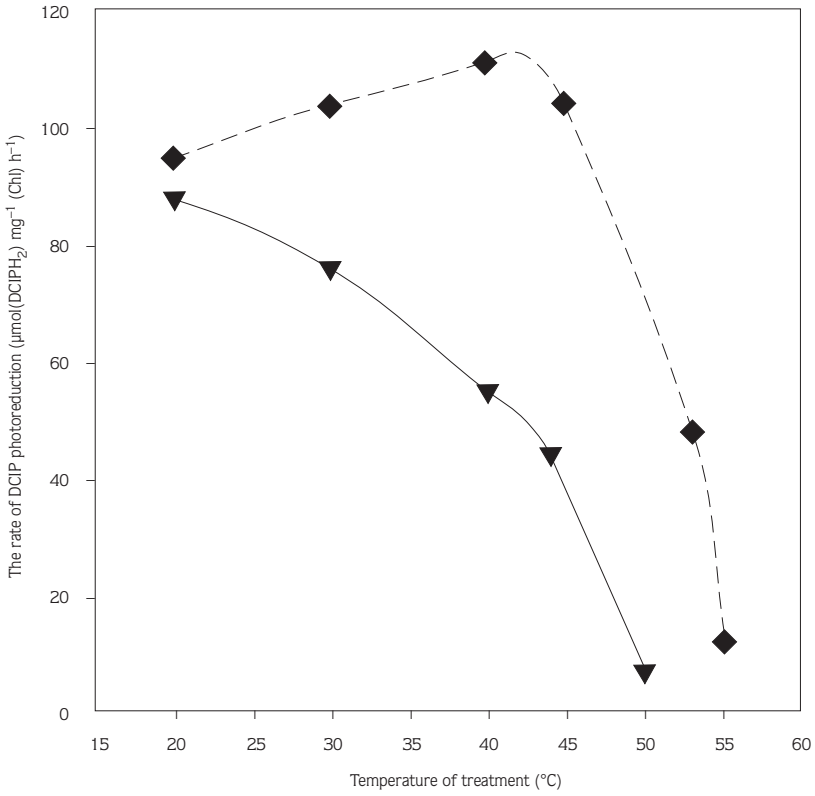

Figure 2. Dependence on incubation temperature of the rate of electron transport from H_2O to DCIP in thylakoid membranes. Thylakoid membranes were incubated for 10min at designated temperatures in 25 mM HEPES–NaOH (pH 7), 10 mM NaCl, 0.4 M sucrose and then cooled to 20° C. After the addition of 0.05 mM DCIP the electron–transport activity was measured by monitoring the photoreduction of DCIP. ▼–no betaine added to both the preparation and incubation mixtures. ◆–0.5 M betaine was added to these mixtures. Each point represents the mean of 3 experiments.

photoreduction of DCIP by H_2O. However, the latter behaves comparably with the reaction of $H_2O \rightarrow PBQ$. For example, betaine changes the temperature for 50% inactivation of the photoreduction of DCIP from 44°C to 53°C. In addition, the rate of DCIP reduction at 40°C was 17% higher than of the rate at 20°C.

The obtained value for the rate of DCIP reduction in the presence of DPC as the artificial electron donor (Figure 2) indicates that PSII complexes in the thylakoid membranes retain almost all of the reaction centers active for oxygen evolution. At the same time, as shown in Figure 3 betaine was capable of affecting RC PSII activity at high temperatures when the oxygen evolving complex was inactivated.

Thus, all the results obtained clearly suggest that betaine enhanced and protected the electron transport reactions of PSII complex against heat stress.

Figure 4 shows the temperature dependence of the heat inactivation of the electron transport from DQH_2 to MV, which involves the activities of PSI and cyt. bf complexes. The rate of electron transport observed, in contrast to the whole–chain and PSII electron transport, was more tolerant to heat treatments. Temperatures for 50% heat inactivation were 55°C and 63°C in the absence and in the presence of betaine, respectively.

The temperature dependence of inactivation of the PSI activity measured as oxygen uptake with reduced DAD as an electron donor, and MV as an acceptor, in the presence of DCMU (dichlorophenyl–dimethylurea), inhibitor of electron transfer between the primary and secondary plastoquinone molecules of PSII is shown in Figure 5. PSI was also claimed to be destroyed by heat treatments, but the stability of PSI is much higher than that of PSII (5). The temperature for 50% inactivation in the absence of betaine was about 60°C, while in the presence of betaine the rate of oxygen uptake by PSI increased along with temperature until a maximum was reached at about 50°C and $T_{1/2}$ was 72°C.

The findings that there are no marked differences in the stability to heating of the $DQH_2 \rightarrow MV$ and $DADH_2 \rightarrow MV$ reactions suggest that the protection and stimulation effects of betaine on the electron transport in the thylakoid membranes are due to its interaction with PSI rather than cyt bf complexes.

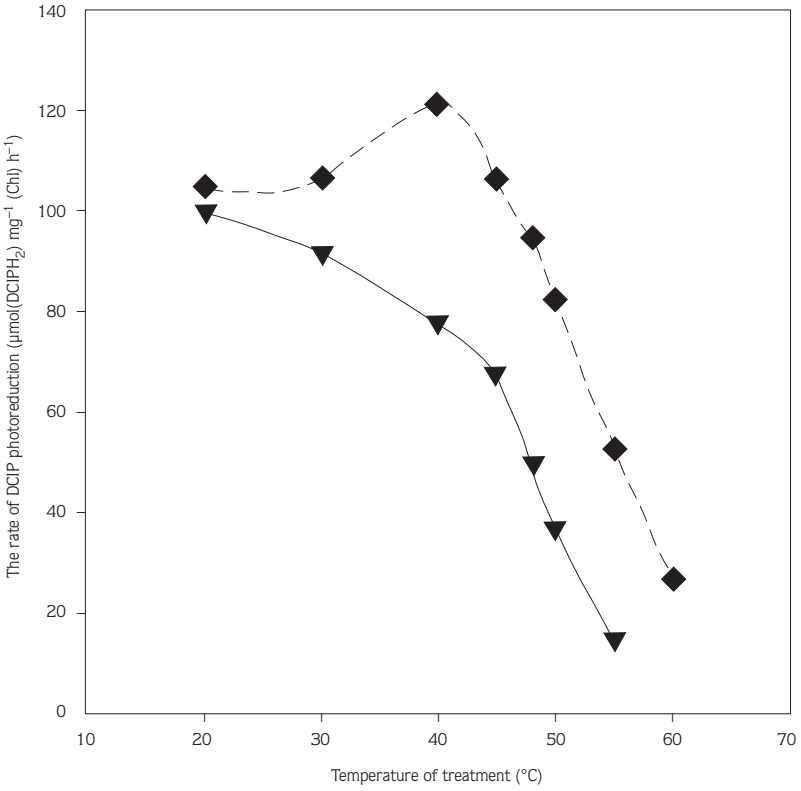

Figure 3. Dependence on incubation temperature of the rate of electron transport from H_2O+DPC to DCIP in thylakoid membranes. Thylakoid membranes were incubated for 10 miN at designated temperatures in 25 mM HEPES–NaOH (pH 7), 10 mM NaCl, 0.4 M sucrose and then cooled to 20°C. After the addition of 0.05 mM HCIP, 0.5 mM DPC the electron–transport activity was measured by monitoring the photoreduction of DCIP. ▼–no betaine added to both the preparation and incubation mixtures. ◆–0.5 M betaine was added to these mixtures. Each point represents the mean of 3 experiments.

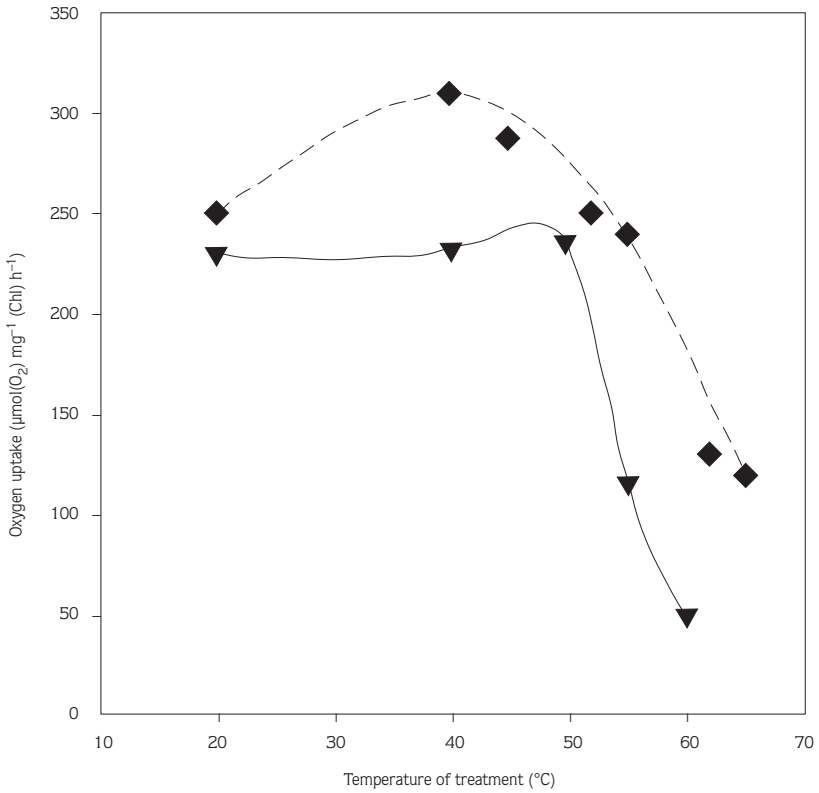

Figure 4. Dependence on incubation temperature of the rate of electron transport from DQH_2 to MV in thylakoid membranes. Thylakoid membranes were incubated for 10 min at designated temperatures in 25 mM HEPES–NaOH (pH 7.5), 10 mM NaCl, 0.4 M sucrose and then cooled to 20°C. After the addition of 0.01 mM DCMU, 0.5 mM DQH_2 0.2 mM MV, 75 units/ml SOD the electron–transport activity was measured by monitoring of oxygen uptake. ▼–no betaine added to both the preparation and incubation mixtures. ◆–0.5 M betaine was added to these mixtures. Each point represents the mean of 3 experiments.

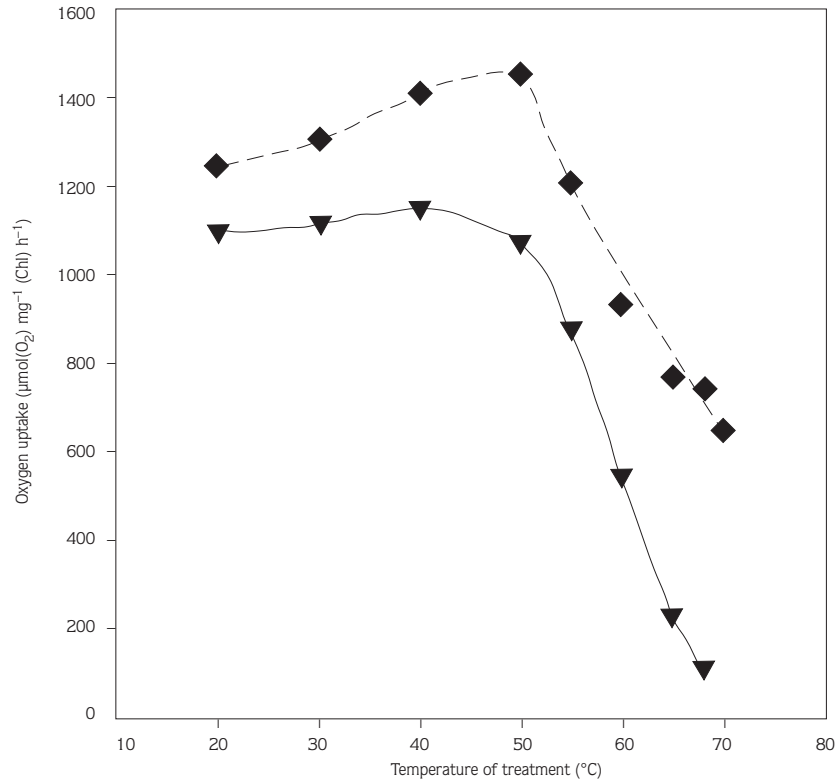

Figure 5. Dependence on incubation temperature of the rate of electron transport from DAD to MV in thylakoid membranes. Thylakoid membranes were incubated for 10 min at designated temperatures in 25 mM HEPES–NaOH (pH 7.5), 10 mM NaCl, 0.4 M sucrose and then cooled to 20°C. After the addition of 0.01 mM DCMU, 0.5 mM DAD, 1 mM Na–ascorbate, 0.2 mM MV, 75 units/ml SOD the electron–transport activity was measured by monitoring of oxygen uptake. ▼–no betaine added to both the preparation and incubation mixtures. ◆–0.5 M betaine was added to these mixtures. Each point represents the mean of 3 experiments.

The main target for the inactivation of photosynthetic electron transport activity by heat has been generally considered to be the PSII complex. The photosystem II is unique with respect to other photosynthetic multiprotein complexes in that it not only performs light–induced charge separation, but also can oxidize the water to molecular oxygen. Thermal inactivation of oxygen evolution has been correlated with the release of functional Mn from PSII together with the loss of three extrinsic polypeptides of 33, 24 and 18 kDa associated with oxygen evolution (Nash, Miyao & Murata, 1985).

Regarding the mechanism of action of betaine, it was initially suggested that it prevents the release of Mn ions from the oxygen–evolving complex (Mohanty et al., 1993). Almost the same proposition was declared based on the data derived from *Synechocystis* sp. PCC6803 thylakoid membranes (Mamedov, Hayashi & Murata, 1993; Mamedov et al., 1991). The results recently obtained on Mn–depleted PSII preparations (Allakhverdiev et al., 1996) showed that not only oxygen evolving activity but also the PSII reaction center complex was affected by betaine. The stabilization effect is understood in terms of the minimization of protein–water interactions as proposed by the theory of Arakawa and Timasheff. Therefore, the data concerning the effect of betaine on the rate of oxygen uptake by PSI complexes in thylakoid membranes (Figure 5) are reasonable.

Thus, the present study clearly demonstrates that betaine protects and stimulates the electron transport in unstacked thylakoid membranes from spinach within PSII and PSI complexes. We propose that betaine is probably effective in stabilizing the higher–order structure of pigment–protein complexes. This is the first report of the effect of betaine on PSI although the examination of the stability to heating of various electron transport reactions of photosynthesis in thylakoid membranes isolated from *Synechocystis* sp. PCC6803 showed that betaine was effective in protecting the evolution of oxygen, but none of the other reactions (Mamedov et al., 1991).

In order to confirm the relationship between the protection and stimulation of photosynthetic activities in thylakoids and to examine the mechanistic basis of the effect of betaine on photosystems, detailed in vitro studies are still necessary.

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