

Effect of Membrane Fluidity on Photoinhibition of Isolated Thylakoids Membranes at Room and Low Temperature

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Z. Naturforsch. **56c**, 369–374 (2001); received January 3/February 1, 2001

Membrane Fluidity, Photoinhibition, Thylakoid Membranes

The relationship between thylakoid membrane fluidity and the process of photoinhibition at room and low (4 °C) temperature was investigated. Two different membrane perturbing agents – cholesterol and benzylalcohol were applied to manipulate the fluidity of isolated pea thylakoids. The photochemical activity of photosystem I (PSI) and photosystem II (PSII), polarographically determined, were measured at high light intensity for different time of illumination at both temperatures. The exposure of cholesterol- and benzylalcohol-treated thylakoid membranes to high light intensities resulted in inhibition of both studied photochemical activities, being more pronounced for PSII compared to PSI. Time dependencies of inhibition of PSI and PSII electron transport rates for untreated and membranes with altered fluidity were determined at 20 °C and 4 °C. The effect is more pronounced for PSII activity during low-temperature photoinhibition. The data are discussed in terms of the determining role of physico-chemical properties of thylakoid membranes for the response of photosynthetic apparatus to light stress.

Introduction

It is well known that exposure of plants to high light intensities leads to inactivation of the photosynthetic process (Powles, 1984). Although the detailed mechanisms of photoinhibition are not really known, it is widely accepted that the main target of photoinactivating damage is the photosystem II complex, which undergoes inhibition of electron transport activity followed by degradation and removal of the D1 protein (known as secondary quinone [QB]-binding protein and 32 kD protein) (Krause, 1988; Aro *et al.*, 1993).

In general it has been assumed that PSI, is not or only little affected by excess irradiation *in vivo* (Somersalo and Krause, 1990). In recent years there is growing evidence that PSI may be also inhibited under highlight stress particularly at chilling temperatures. The photoinhibition of PSI

has been observed in cold-sensitive cucumber (Sonoike and Terashima, 1994; Sonoike, 1996; Terashima *et al.*, 1994), potato leaves (Havaux and Davaud, 1994) and barley (Tjus *et al.*, 1999). Recently, Barth and Krause (1999) reported about the different extent of photoinhibition of PSI activity in chilling tolerant and chilling sensitive plants. With isolated thylakoid membranes PSI has been previously shown to be as vulnerable to photoinhibition as PSII (Sato, 1970).

The physical state of cell membranes could be regarded as a very sensitive indicator of changes in environmental factors like temperature or light. It is well documented that the degree of unsaturation of fatty acyl chains of membrane lipids determines the chilling sensitivity of plants (Nishida and Murata, 1996). The correlation between the physical properties of thylakoid membranes and especially the degree of unsaturation of fatty acids are intensively studied with respect to low temperature adaptation and low temperature photoinhibition. At present it is not clear how the unsaturation of fatty acyl chains of membrane lipids affect the low-temperature photoinhibition, but recently it has been shown that the unsaturation of membrane lipids of chloroplasts stabilises the photo-

Abbreviations: PSI (II), photosystem I (II); Chl, chlorophyll; DCMU, 3-(3',4'-dichlorophenyl)-1,1'-dimethylurea; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPIP, 2,6-dichlorophenolindophenol; MV, methylviologen; p-BQ, *p*-benzoquinone; Tricine, N-(tris[hydroxymethyl]methylglycine; N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-glycine).

synthetic machinery against low-temperature photoinhibition *in vivo* in transgenic tobacco plants. (Moon *et al.*, 1995).

The low level of fatty acids unsaturation drastically impairs D1-protein turnover at lowered temperatures (Kanervo *et al.*, 1997). Using the mutants of *Synechocystis* Gombos *et al.* (1994) showed that rather the recovery of PSII is accelerated by unsaturation whilst its inactivation itself is not affected.

The fluidity of thylakoid membranes may play an important role in controlling the light reactions of photosynthesis (Quinn and Williams, 1978). It has been reported that artificial perturbation of the fluidity of thylakoid membranes by cholesterol and benzylalcohol affects the rate of whole chain electron transport, proton uptake, parameters of cation-induced fluorescence and so on (Yamamoto *et al.*, 1981; Pedersen and Cox, 1984; Ford and Barber, 1983).

Modification of the fluidity of thylakoid membranes by cholesterol and benzylalcohol alters the response of photosynthetic apparatus to short-time heat stress, kinetics of fluorescence decay and energy distribution between both photosystems (Dobrikova *et al.*, 1997; Busheva *et al.*, 1998; Zaharieva *et al.*, 1998a; Zaharieva *et al.*, 1998b). Recently it has been reported that there is a close correlation between thylakoid fluidity levels and the threshold temperature required for maximal activation of all of the heat shock-inducible genes and the threshold temperature for damage of photosynthesis (Horvath *et al.*, 1998).

Although the possible role of changes in the fluidity is less understandable with respect to light than to the temperature, there are data indicating that the optimisation of the photosynthetic process in response to the light conditions includes increase of the relative content of the most unsaturated fatty acids in various unicellular algae (Klyachko-Gurvich *et al.*, 1999).

The effect of high light intensity and temperature on photosynthesis *in vivo* is probably the result of modifications of many catalytic and regulatory reactions. In the present study the influence of alteration of thylakoid membrane fluidity by incorporation of cholesterol and benzylalcohol on photoinhibition *in vitro* of PSI and PSII was investigated. The rates of PSI- and PSII-mediated elec-

tron transport were determined in dependence on illumination time at 4 °C and 22 °C.

Materials and Methods

Isolation of thylakoid membranes

Thylakoid membranes from 14 day-old pea leaves (*Pisum sativum* L., Ran 1) were isolated following the procedure described by Goetze and Carpentier (1990). The final pellet was resuspended in a medium containing 0.33 M sucrose, 10 mM Tricine (pH 8.0), 5 mM MgCl₂ and 10 mM NaCl. Chlorophyll (Chl) concentration was determined according to Lichtenthaler (1987).

Perturbation of membrane fluidity by cholesterol and benzylalcohol

Treatment of isolated thylakoid membranes with cholesterol and benzylalcohol was performed as described in Dobrikova *et al.* (1997) with cholesterol concentration of 300 µM (cholesterol:chlorophyll ratio 2.32) and benzylalcohol concentration of 50 mM (benzylalcohol:chlorophyll ratio 380).

Photoinhibitory treatment

The temperature-controlled vessel containing the thylakoid membranes was illuminated with a halogen lamp through a heat filter with circulating cold water. Light intensity at the surface of the vessel was 1000 µmol. m⁻².s⁻¹. Photoinhibitory treatment was carried out at 22 °C and 4 °C with 150 µg Chl/ml and continuous stirring. The samples were taken after different illumination periods and analysed.

Photochemical activity of PSI and PSII

Both photochemical activities were polarographically determined by a Clark-type electrode (Hansatech DW1) in a temperature-controlled cuvette under saturating light intensity. The assay medium contained 20 mM Tricine (pH 7.5), 0.33 M sucrose, 5 mM MgCl₂, 10 mM NaCl and thylakoid membranes equivalent to 25 µg Chl/ml. The electron transport through PSII was determined by the rate of oxygen evolution, with 0.5 mM *p*-benzoquinone as an electron acceptor. PSI-mediated electron transport was determined in the presence of 10 µM DCMU, 5 mM Na₂S₂O₃, 10 mM NH₄Cl, 1 mM

Na ascorbate, 100 μM DPIP and 150 μM MV. Temperature during measurements was 22 °C.

Results and Discussion

Membrane fluidity manipulations

Our previous investigations have shown that incorporation of cholesterol leads to a decrease of thylakoid membrane fluidity at room temperature determined by the degree of polarisation of fluorescence of a DPH probe, while the treatment with benzylalcohol increases the membrane fluidity (Ford and Barber, 1980; Dobrikova *et al.*, 1997). Changes of the steady-state fluorescence polarisation *P* of DPH do not depend linearly on the concentrations of the cholesterol and benzylalcohol used for treatment. Fig. 1 presents the relationship between concentrations of both membrane-perturbing agents and the measured values for *P*. The most considerable change of *P* was observed at low concentrations of the applied agents. The concentrations of cholesterol and benzylalcohol applied in this study were 300 μM and 50 mM, respectively. After these treatments the measured values for *P* were found as 0.354 and 0.222, respectively,

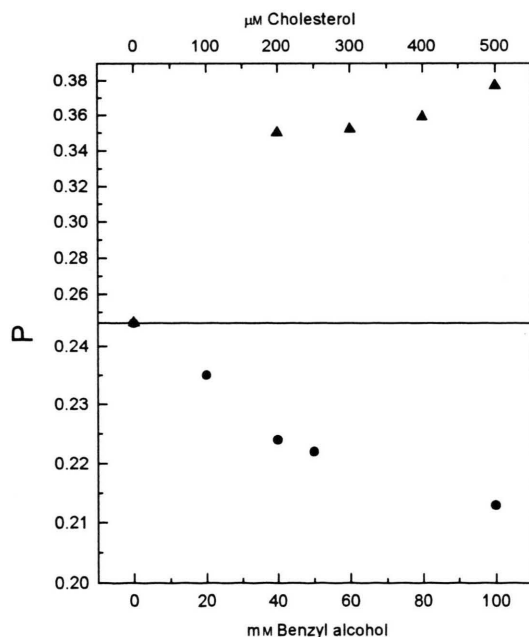


Fig. 1. Values for *P* (fluorescence polarization of DPH, incorporated into thylakoid membranes) for different concentrations of cholesterol and benzylalcohol. ● – benzylalcohol; ▲ – cholesterol.

and for control untreated sample 0.249. The rigidification of the thylakoid membranes is more pronounced than that for fluidization, the latter achieved by benzylalcohol.

Both treatments affect the PSI and PSII electron transport rates. Cholesterol gave a more pronounced effect on PSI – up to 50% decreased activity, while the decrease induced by benzylalcohol treatment is less with 10–15%. Benzylalcohol affects to the same extent PSII- and PSI-mediated electron transport rate. The cholesterol effect on PSII was less pronounced – yielding up to 30% decrease of photochemical activity (Busheva *et al.*, 1998; Zaharieva *et al.*, 1998a).

The aim of the present study is to determine to what extent the fluidity of the lipid phase of the thylakoid membranes affects the process of the photoinhibition of photochemical activity of both photosystems at room and low (4 °C) temperature.

Photoinhibition of PSI- and PSII-mediated electron transport at room temperature

The dependence of the rates of PSI electron transport on the time of high light treatment at 22 °C is presented by Fig. 2A. With increasing of illumination time the PS I activity decreased, more pronounced for cholesterol – treated samples. The activity of control remained higher during the whole period of light treatment. The cholesterol-treated samples are more susceptible to photoinhibition at this temperature. The extent of photoinhibition of PSI activity was found lower than this for PSII (see Fig. 2B). After a 90 min. illumination approx. half of the activity was still present: 55% in benzylalcohol – treated samples and 40% in cholesterol – treated samples. So, the PS I activity, measured as the rate of electron transport from DPIP_H to MV seems to be more rapidly inhibited in thylakoids with decreased membrane fluidity.

The time dependencies of oxygen evolution at 22 °C in the presence of p-BQ as electron donor for control, cholesterol- and benzylalcohol-treated samples are presented by Fig. 2B. At room temperature the PSII activity of control thylakoids decreased gradually with increasing time of illumination. Treatments with cholesterol and benzylalcohol did not change considerably the shape of the time dependence and finally approx. the same degrees of PSII photoinhibition were ob-

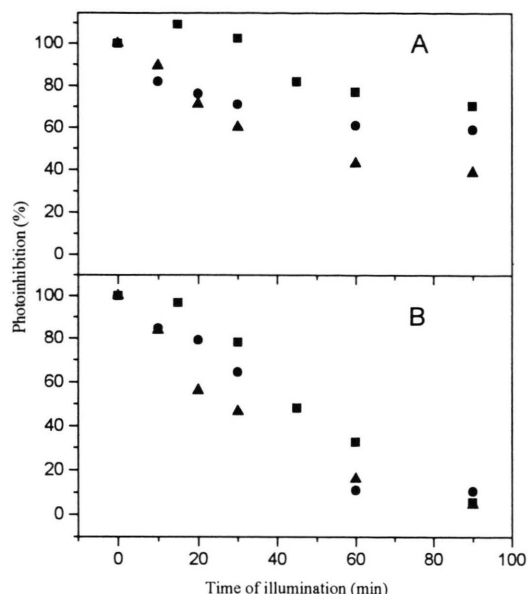


Fig. 2. Photoinhibition of PSI and PSII-mediated electron transport during photoinhibition at room temperature. A – Photosystem I – electron transport rate; B – Photosystem II – electron transport rate. ■ – control thylakoid membranes; ● – benzylalcohol treated membranes; ▲ – cholesterol-treated membranes. Light intensity – $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. 100% correspond to $694.96 \pm 51.2 \mu\text{mol O}_2\cdot\text{mg Chl}^{-1}\cdot\text{h}^{-1}$ for control, $601.17 \pm 60.3 \mu\text{mol O}_2\cdot\text{mg Chl}^{-1}\cdot\text{h}^{-1}$ for benzylalcohol-treated and $416.49 \pm 30.2 \mu\text{mol O}_2\cdot\text{mg Chl}^{-1}\cdot\text{h}^{-1}$ for cholesterol-treated membranes for PSI electron transport rate. For PSII electron transport 100% correspond to $118.6 \pm 3.8 \mu\text{mol O}_2\cdot\text{mg Chl}^{-1}\cdot\text{h}^{-1}$, $92.61 \pm 6.2 \mu\text{mol O}_2\cdot\text{mg Chl}^{-1}\cdot\text{h}^{-1}$ and $78.2 \pm 4.7 \mu\text{mol O}_2\cdot\text{mg Chl}^{-1}\cdot\text{h}^{-1}$ respectively.

served. In the first minutes of treatment the inhibition of cholesterol treated samples proceeded more rapidly. PS II photochemical activity was inhibited to a greater extent than PSI and after 90 min of illumination only 10–15% of the initial activity could be detected.

At room temperature the greater extent of photoinhibition of PSI-mediated electron transport at cholesterol-treated samples could be explained in terms of involvement of mobile electron carriers as well as the decreased ability of thylakoid membranes to undergo structural changes after rigidification. With benzylalcohol-treated samples the inhibition of PSI activity proceeds more slowly than in the controls, but finally the values for both samples are higher than those for cholesterol-treated samples. The slower inhibition of electron trans-

port through PSI in benzylalcohol treated samples could be regarded as a result of greater possibility of more fluid membrane to compensate for more time the damaging effect of high light. Both treatments do not change significantly the response of the PSII machinery to photoinhibition at room temperature, at least determined by oxygen evolution in presence of *p*-benzoquinone.

Photoinhibition of the rates of PSI- and PSII-mediated electron transport at low temperature

Fig. 3A presents the time dependencies of PSI-mediated electron transport for three samples. Photoinhibition is observed in all samples measured, but in benzylalcohol-treated thylakoid membranes photoinhibition proceeded slowly. After 90 min. of illumination benzylalcohol-treated samples showed approx. 70% of their initial activity, while for control thylakoids the remaining activity was about 35%. Apparently, the treatment with benzylalcohol protects to some extent the photoinhibition of PSI, whilst cholesterol-treated

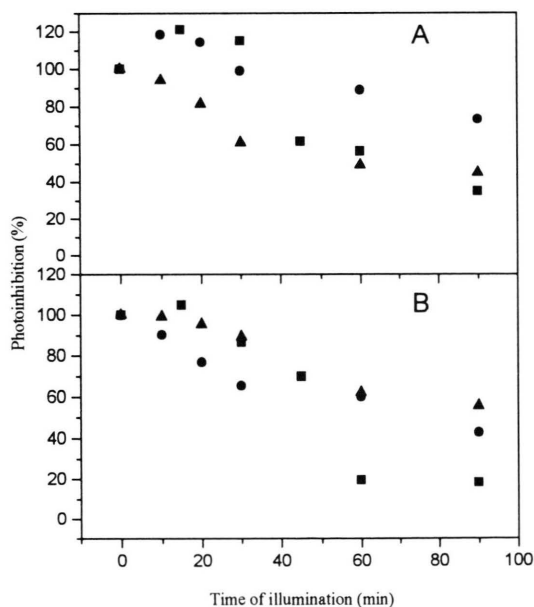


Fig. 3. Photoinhibition of PSI and PSII-mediated electron transport during photoinhibition at 4 °C. A – Photosystem I – electron transport rate; B – Photosystem II – electron transport rate. ■ – control thylakoid membranes; ● – benzylalcohol treated membranes; ▲ – cholesterol-treated membranes.

membranes are inhibited approx. to the same extent as at room temperature.

The difference between control and treated membranes was more pronounced when measuring the photoinhibition of PS II activity. Although the photoinhibition of PSII electron transport at 4 °C was less than that at room temperature, in control thylakoids after 90 min of illumination the rate of electron transport from H₂O to p-BQ was suppressed by about 75% (Fig. 3B). Surprisingly, the inhibition of PSII mediated electron transport was less in cholesterol- and benzylalcohol – treated thylakoid membranes – 45% and 60%, respectively. It should be noted that although at the beginning of illumination the inhibition of control thylakoids proceeded more slowly, after a 1 hour of photoinhibitory treatment the extent of photoinhibition of PSII activity in cholesterol- and benzylalcohol-treated samples was lower than in the control.

At 4 °C the more pronounced differences of time dependence of PSII photoinhibition between control and treated samples are observed. The changes of membrane fluidity decrease the extent of PSII activity inhibition and it seems that that PSII is more resistant to photoinhibition after incorporation of cholesterol and benzylalcohol. The similar protecting effect at 4 °C on PSII activity of both used agents is due to the fact that cholesterol causes fluidisation of the solid states (below the temperature of phase transition) of phospholipid bilayers (Demel *et al.*, 1976). Cholesterol concentration used in our study seems to be in the region that such kind of action could be expected (Koyanova *et al.*) and the temperatures of phase transi-

tion of intact thylakoid membranes from chilling-sensitive plants are between 9 °C and 19 °C (Fork *et al.*, 1981). It is reasonable to expect that at 4 °C (below to the temperature of phase transition) the incorporation of cholesterol could increase the thylakoid membrane fluidity as do the treatment with benzylalcohol.

Data presented showed that cholesterol expresses a different action on both photosystem complexes. Most probably it is due to the fact that cholesterol distributes heterogeneously in the plane of the model membrane with different lipid composition and express differences in interactions with various lipid species. (De Kruijff *et al.*, 1974). Taking in mind the distribution of the pigment-protein complexes of PSI and PSII in stroma and grana regions, as well as the lateral heterogeneity of lipid classes of thylakoid membranes, it is reasonable to expect that cholesterol will affect to different manner the lipid environment of pigment protein complexes of PSI and PSII.

It must be noted also that the effect of changes of thylakoid membrane fluidity is more pronounced after a 30–40 min light treatment. This means that with increasing of illumination time a mechanism of photodamages is included in the photoinhibitory process that is more related with the fluidity properties of thylakoid membranes especially at low temperature.

Acknowledgements

This work was supported by the Bulgarian National Fund for Scientific Investigations under project K-807/1998.

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