Response of isolated thylakoid membranes with altered fluidity to short term heat stress

Maya Velitchkova, Dessislava Lazarova and Antoaneta Popova

Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev str. bl. 21, 1113 Sofia, Bulgaria

ABSTRACT

The effect of alterations of lipid phase order of thylakoid membranes on the thermosensitivity of photosystem I (PS I) and photosystem II (PS II) was studied. Plant sterols stigmasterol and cholesterol were applied to decrease the fluidity in isolated membranes. After sterol treatment, a decrease of the temperature of 50 % inhibition of PSII activity was observed. Heat stress-induced stimulation of PSI-mediated electron transport rate was registered for control, but not for sterol-treated membranes. Effect of altered lipid order on oxygen evolving complex was evaluated by means of flash oxygen yields revealing changes in the stoichiometry of PSIIα and PSIIβ centers. The effect of sterol incorporation on the changes in the thermotropic behavior of the main pigment-protein complexes was studied by differential scanning calorimetry (DSC). DSC traces of control thylakoids in the temperature range 20-98 °C exhibited several irreversible endothermic transitions. Incorporation of cholesterol and stigmasterol results in superimposition of the transitions and only two main bands could be resolved. While high temperature band peaks at the same temperature after treatment with both sterols, the band that combines low temperature transitions shows different melting temperature (Tm) : 70 °C for stigmasterol- and 65 °C for cholesterol-treated membranes. The data presented here emphasise the crucial role of lipid order for the response of thylakoids to high temperatures, mediated not only by changes in the fluidity of bulk lipid phase as result of sterol incorporation but also by changes in the thermotropic properties of pigment-protein complexes. [Physiol. Mol. Biol. Plants 2009; 15(1) : 43-52] E-mail : mayav@bio21.bas.bg

Key words : Cholesterol, Fluidity, Heat stress, Oxygen flash yields, Thylakoid membrane, Stigmasterol

INTRODUCTION

The fluidity and permeability of membrane lipid phase play an important role in controlling light reactions of photosynthesis, for effective electron and energy transfer (Siegenthaler and Tremolieres, 1998). The lateral separation of the main pigment-protein complexes in thylakoid membranes, participation of mobile electron carriers in the electron transport reactions and regulation of energy distribution between PSI and PSII through physical movement of the light-harvesting chlorophyll a/b complex emphasize the role of lipid matrix and in particular, of its fluidity for the effectiveness of the photosynthetic process – linear electron transport, capture and transmitting of light energy. Several studies on this topic, including artificially manipulated thylakoids (incorporation of cholesterol or cholesteryl hemisuccinate, catalytic hydrogenation) or by using membranes from lipid mutants (genetically modified membrane fluidity) discuss the effects of altered properties of lipid matrix on functional characteristics of photosynthetic apparatus (Siegenthaler and Tremolieres, 1998; Williams, 1998). The importance of membrane fluidity is clearly evident in respect to plant response to changes of environmental conditions. Changes of lipid saturation level inevitably reflect membrane fluidity, which is well-characterized phenomenon of plant and algal acclimation to temperature and light conditions (Raison et al., 1982; Klyachko-Gurvich et al., 1999). To date, a number of studies have been reported on the influence of the degree of fatty acid unsaturation on the extent of low temperature photoinhibition of some cyanobacteria (Kanavero et al., 1997) and tobacco transgenic plants (Moon et al., 1995).

Photosynthetic reactions exhibit different heat sensitivity. Exposure of isolated chloroplast or leaves of higher plants to elevated temperatures leads to considerable changes in structural organization of thylakoid membranes and their photosynthetic activities.
(Bukhov and Mohanty, 1999). PSII-catalyzed water oxidation is especially sensitive to heat, while PSI demonstrates much higher heat tolerance. Heat-induced changes of thylakoid stacking and rearrangement of lipids and of pigment-protein complexes as a result of heat stress are well documented (Gounaris et al., 1983; Sundby and Andersson, 1985). The heterogeneity of PSII centers is also influenced by heat treatment (Bukhov and Carpentier, 2000). Alterations of grana stacking, partial dissociation of LHCII from the core complex of PSII and other heat-induced changes of mutual organization of thylakoid membranes and their constituents are dependent on the properties and composition of the lipid phase. It has been shown that the thermal stability of thylakoid membrane proteins in the process of development of thylakoid membrane during greening is related to the concomitant change of composition of the lipid phase. It has been shown that the thermal stability of thylakoid membrane proteins in the process of development of thylakoid membrane during greening is related to the concomitant change of composition, dynamics and overall fluidity of the lipid component in greening seedlings (Kota et al., 2002). All reported data indicate that the changes in the properties of lipid matrix affect the effectiveness of the photosynthetic process, but the mechanisms underlying are very complex and are far from completely understood.

The aim of the present study was to investigate the effect of changes in lipid phase properties on the response of isolated thylakoid membranes to high temperature stress. Artificial manipulation of membrane fluidity was achieved by treatment with cholesterol or stigmasterol. Although sterols are mainly found in plasma membranes of animals and higher plants and only in very low concentration in intracellular membranes, they could be used for artificial manipulation of thylakoid membrane fluidity (Ford and Barber, 1983). It is shown that incorporation of sterol molecules results in changes in the response of thylakoid membranes to short term heat stress and alters the thermotropic behavior of the main pigment-protein complexes.

**MATERIALS AND METHODS**

**Isolation of thylakoid membranes**

Thylakoid membranes from 14 day-old pea leaves (*Pisum sativum* L., Ran 1) were isolated by the procedure described by Popova et al. (2007). The final pellet was resuspended in a medium containing 0.33 M sucrose, 10 mM N-(2-hydroxy-1,1-bis(hydroxymethyl) ethyl) glycine (TRICINE) (pH 8.0), 5 mM MgCl₂ and 10 mM NaCl. Chlorophyll concentration was determined according to the procedure described in Lichtenthaler (1987).

**Incorporation of sterols**

For cholesterol and stigmasterol incorporation, a procedure described in Popova et al. (2007) was followed. Sterols were added to thylakoid membranes from a stock solution in ethanol and incubated in the dark at room temperature while stirring gently for 10 min. Ethanol concentration in the samples was kept as low as not to affect the measurements proven by control samples treated only with ethanol. After incubation, the membranes were washed two times in order to remove the non-incorporated sterol and resuspended in a medium appropriate for further measurements. Alternatively, the method of Yamamoto et al. (1981) to incorporate cholesterol by the use of polyvinyl pyrrolidone as a mediator was checked. Fluorescence polarization (P) of 1,6-diphenyl-1,3,5-hexatriene (DPH) indicates that with both methods, the level of cholesterol incorporation is approximately the same. The level of cholesterol incorporation depends on the concentration of sterol during incubation (Yamamoto et al., 1981; Ford and Barber, 1983). Application of the first method - direct treatment with cholesterol (from ethanol solution) could be complicated by the presence of cholesterol micelles, which at high concentration form aggregates with thylakoid membranes. These complications were observed at cholesterol:chlorophyll ratio higher than 5 during incubation (Ford et al., 1981). In our experiments, we used a lower incubation ratio of cholesterol:chlorophyll – 2.32, thus avoiding aggregation. At this incubation ratio, the cholesterol:chlorophyll value in the final pellet was about 0.26 (Ford et al., 1981).

**Estimation of membrane fluidity**

The fluidity of isolated membranes was estimated by measuring the steady-state fluorescence polarization of DPH at room temperature, as described previously (Dobrikova et al., 1997). Fluorescent probe is often used to determine the fluidity of the hydrophobic interior of biological membranes and especially for thylakoid membranes, as it tends to distribute reasonably evenly between all lipid domains and no energy transfer occurred between DPH and photosynthetic pigments (Ford and Barber, 1980). A P value of about 0.23 indicates a relatively fluid membrane, around zero for an isotropic solution and is higher - 0.49, for a totally rigid system (Kinosita et al., 1981).

**High temperature treatment**

Short term heat treatment was performed in a water thermostat. Thylakoid membranes were incubated at