

BRIEF COMMUNICATION

Influence of saccharides and glycine betaine on freezing of photosystem 2-enriched particles: a chlorophyll fluorescence study

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Abstract

Freeze-thaw treatment of photosystem 2 (PS2) enriched subchloroplast membranes induced changes in the relative proportion and antenna sizes of PS2 α and PS2 β . The addition of disaccharides (trehalose or saccharose) and glycine betaine before subjecting to freezing prevented these changes.

Additional key words: cryoprotection; disaccharides; freeze-thaw treatment; photosystem 2 heterogeneity; *Pisum sativum*; saccharose; subchloroplast membranes; trehalose.

Plant cell membranes are extremely frost sensitive (Heber *et al.* 1981). Investigations on isolated thylakoid membranes have shown that the decrease in the activity of photosynthetic electron transport chain after freeze-thaw treatment is primarily due to the freeze-sensitivity of PS2 (Santarius 1990). The protective efficiency of different saccharides and low concentration of glycine betaine (100 mM) on PS2 has been demonstrated after freezing of chloroplasts and PS2 subchloroplast membranes (Coughlan and Heber 1982, Busheva and Apostolova 1989, Hinch 1989, 1990). Glycine betaine protects the PS2 complex also against heat inactivation, salt-induced dissociation of extrinsic proteins and against inactivation of the oxygen-evolving system of PS2 (Papageorgiou *et al.* 1991, Mamedov *et al.* 1993). In this study we further analysed the influence of the freeze-thaw treatment upon the PS2 complex. We estimated changes in the chlorophyll (Chl) antenna size and in the amounts of PS2 α and PS2 β after subjection to freeze-thaw treatment of PS2-enriched subchloroplast membranes from pea in the presence and absence of cryoprotectors.

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For isolation of PS2-enriched subchloroplast membranes from pea leaves (*Pisum sativum* L. cv. Ran-1) we used mild digitonin and Triton X-100 treatment, following the procedure of Yamamoto *et al.* (1982). The low temperature (77 K) Chl fluorescence ratio F_{735}/F_{685} of the isolated PS2 particles was 0.5 ± 0.2 . The value was similar to that reported by Dunahay *et al.* (1984) and indicated a contamination with some amounts of PS1 complex. Suspensions of PS2-enriched membranes, containing $450 \text{ g(Chl) m}^{-3}$, 100 mM NaCl, and 300 mM disaccharides (saccharose or trehalose) or 100 mM glycine betaine, were frozen down to -27°C . The rate of freezing was $1.5^\circ\text{C per min}$. After 20 h at -27°C the samples were thawed ($3.3^\circ\text{C per min}$) in a water bath at 20°C . The fast Chl fluorescence induction kinetics were measured as described in Maslenkova *et al.* (1993). The subchloroplast particles were resuspended in 0.3 M saccharose, 4 mM MgCl_2 , 10 mM MES (pH 6.5), and 33 mM DCMU. The Chl concentration was 10 g(Chl) m^{-3} . The samples were dark adapted for 5 min. The actinic radiation was provided from a 150 W *Tungsram* lamp with the 430 nm *Corning* filter. The Chl fluorescence signals were recorded in a time span from 0 to 2 s with a data accusation rate of 0.22 ms. The kinetic analysis of Chl fluorescence induction curves of PS2-enriched subchloroplast membranes in the presence of DCMU was done according to Melis and Homann (1976).

Table 1. Effect of freeze-thaw treatment on chlorophyll fluorescence induction curve parameters of PS2-enriched subchloroplast membranes. Mean values \pm S.E. were calculated from 5 independent experiments.

	PS2 α [%]	PS2 β [%]	k_α [s $^{-1}$]	k_β [s $^{-1}$]	F_v/F_0	F_v/F_m
unfrozen control	65	35	11.2	4.6	1.44 ± 0.09	0.68 ± 0.04
100 mM NaCl	33	67	8.8	5.9	0.70 ± 0.09	0.45 ± 0.07
100 mM NaCl+300 mM saccharose	48	52	8.3	3.8	1.13 ± 0.10	0.56 ± 0.05
100 mM NaCl+300 mM trehalose	53	47	10.9	4.4	0.97 ± 0.10	0.59 ± 0.07
100 mM NaCl+100 mM glycine betaine	59	41	10.8	4.5	0.95 ± 0.15	0.55 ± 0.04

The time course of Chl fluorescence rise of PS2-enriched subchloroplast membranes could be resolved into two kinetically different phases. The slow β phase increased exponentially with a rate constant k_β , and the faster α phase was sigmoidal in shape with a rate constant k_α . The area above the induction curve and below its asymptote was used to measure the relative proportion of PS2 α and PS2 β centres. The differences in the rates of fluorescence increase (k_α and k_β) were interpreted as characteristics for the different absorption cross-section of light-harvesting antenna pigments for both pools of PS2 (PS2 α and PS2 β) (Karukstis 1992).

The Chl fluorescence induction curves of PS2-enriched subchloroplast membranes in the presence of DCMU both for frozen and unfrozen (control) samples were biphasic (values not shown). The control particles revealed 65 % of PS2 α and 35 % of PS2 β centres. Particles frozen in the presence of 100 mM NaCl showed an increase of the amount of PS2 β by 32 % (Table 1). The rate constant of radiation

absorption k_{β} after freezing was higher than in the control particles. The observed changes in k_{β} suggested that the antenna size of the PS2 β after freezing was not the same as that of the PS2 β in the control particles. Sundby *et al.* (1986) have shown a similar heat-induced conversion of PS2 α to PS2 β of thylakoid membranes, but in their case the antenna size of the newly formed PS2 β is the same as that of the original PS2 β .

The relative proportion of PS2 α and PS2 β in the samples frozen in the presence of disaccharides (trehalose or saccharose) or glycine betaine was close to that in the unfrozen particles (Table 1). In the presence of trehalose and glycine betaine the values for k_{β} were in the range of freshly isolated PS2 particles (Table 1), while in the presence of saccharose the k_{β} was different from that of the control particles.

Jensen *et al.* (1981) show an alteration in the membrane surface charge and suppression of Coulombic interactions between membrane components after freezing without cryoprotectors. The ability of saccharides to form hydrogen bonds has long been viewed as an important aspect of the membrane stabilising effect of saccharides (Santarius and Bauer 1983). However, in addition to the number of hydroxyl groups available for hydrogen bonding, structural characteristics of the saccharides also play a role in membrane protection (Hinch 1990). However, the stabilizing effect of glycine betaine may be a result of an interaction between the positive quaternary ammonium cation with the membrane surface (Coughlan and Heber 1982). Hence the protection of PS2 heterogeneity by disaccharides and glycine betaine, which are chemically very different substances, could be a result of an unspecific interaction with the membrane surface.

The ratios of variable Chl fluorescence (F_v) to maximal Chl fluorescence (F_m) and variable fluorescence to initial fluorescence (F_0) were used as a measure of the efficiency of PS2 photochemistry (Krause and Weis 1991). A decrease in the ratios F_v/F_m and F_v/F_0 of the particles frozen in 100 mM NaCl showed inhibition of PS2 activity. The changes in these parameters were smaller in the presence of trehalose, saccharose, and glycine betaine, which indicated that the PS2 activity was protected to a great extent. The latter results correlate with the previous observation about the protective effect of these compounds on PS2-mediated electron transport (Coughlan and Heber 1982, Busheva and Apostolova 1989).

Our experiments showed that the decrease in the PS2 activity after freeze-thaw treatment was connected with a decrease in amount of PS2 α and an increase in amount of PS2 β . The cryoprotective effect of disaccharides (trehalose and saccharose) and glycine betaine was associated with prevention of changes in the relative proportion of the two forms of PS2.

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