The Unsaturation of Membrane Lipids Stabilizes Photosynthesis against Heat Stress

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Glycerolipids of thylakoid membranes not only serve as a major constituent of the membrane-forming bilayers, but they also provide hydrophobic ligands to membranous proteins (Doyle and Yu, 1985). It has been suggested that four abundant glycerolipids of thylakoid membranes in the chloroplasts of higher plants and in the cells of cyanobacteria play important roles in maintaining the photosynthetic electron-transport machinery. It has been reported that sulfoquinovosyl diacylglycerol is associated with the ATP synthase (Pick et al., 1987) and that MGDG is bound to the reaction center of PSII (Murata et al., 1990).

The degree of unsaturation of acyl residues of glycerolipids determines the physical characteristics of membranes (Chapman, 1975; Quinn, 1988; Quinn et al., 1989) and, consequently, the molecular motions of these lipids in the membranes. Therefore, one can postulate that fatty-acid unsaturation should affect various functions of membrane-bound proteins. It is of interest to examine the way in which the fatty-acid unsaturation of the glycerolipids of thylakoid membranes is related to the thermal tolerance of photosynthesis.

Altrations in fatty-acid unsaturation of glycerolipids in thylakoid membranes can be achieved by changing the growth temperatures of photosynthetic organisms. Pearcy (1978) and Raison et al. (1982) observed that increases in growth temperature increase the level of saturated fatty acids in membrane lipids and enhance the heat stability of photosynthesis. These results led them to conclude that the saturation of fatty acids increases heat stability. However, such studies fail to correlate definitively the degree of saturation of fatty acids with heat stability, because a change in growth temperature affects not only the fatty-acid saturation but also various other metabolic factors.

Santrarius and Müller (1979) observed that, in spinach, the increase in heat tolerance of photosynthesis during the acclimation to high temperature is not associated with changes in the level of saturation of membrane lipids. McCourt et al. (1987) observed that a decrease in the level of sn-1-18:3/sn-2-16:3-MGDG and a corresponding increase in the level of sn-1-18:2/sn-2-16:2-MGDG in a mutant strain of Arabidopsis did not affect the heat stability of photosynthesis. Hugly et al. (1989) observed only insignificant differences in the thermal stability of the photosynthetic electron transports from H$_2$O to methyl viologen and H$_2$O to dichlorophenol indophenol in thylakoid membranes from the fadC mutant of Arabidopsis, which contain a reduced level of sn-1-18:3/sn-2-16:3-MGDG and an enhanced level of sn-1-18:1/sn-2-16:1-MGDG (Browse et al., 1989) compared with those from wild-type leaves. When the thermal stability of the electron transport of thylakoid membranes from the wild-type Arabidopsis was compared with that from the fadB mutant, which contained a reduced level of sn-1-18:3/sn-2-16:3-MGDG and an enhanced level of sn-1-18:3/sn-2-16:0-MGDG, there was no distinct difference (Kunst et al., 1989a, 1989b). In a previous study (Gombos et al., 1991) we also demonstrated that the complete elimination of trienoic fatty acids by mutation of Synechocystis PCC6803 had no effect on the thermal stability of photosynthesis. All these studies tend to suggest that heat stability is not associated with the level of saturation of membrane lipids.

The oxygen-evolving activity is more sensitive to heat than other photosynthetic activities (Berry and Bjorkman, 1980; Mamedov et al., 1993). Nash et al. (1985) demonstrated that

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Abbreviations: Kmr, kanamycin-resistance gene; MGDG, monogalactosyl diacylglycerol; X:Y, fatty acid containing X carbon atoms with Y double bonds in the cis configuration.
a direct result of heat inactivation is the release of functional manganese ions from the PSII complex.

Recently we developed a novel system in which the desaturation of fatty acids can be eliminated in a stepwise manner (Wada and Murata, 1989; Wada et al., 1992). In the present study we addressed the question of whether fatty-acid unsaturation plays a key role in the heat stability of photosynthesis using this cyanobacterial system. We demonstrated that, in contrast to the previous hypothesis, unsaturation of membrane lipids stabilizes, to a small but distinct extent, photosynthesis against heat inactivation.

MATERIALS AND METHODS

Organisms and Culture Conditions

Wild-type and Fad6 strains of Synechocystis PCC6803 were obtained as described by Wada and Murata (1989). The transformant of Synechocystis PCC6803, Fad6/desA::Kmr, was obtained as described previously (Wada et al., 1990, 1992). These strains were grown photoautotrophically under illumination from incandescent lamps at an intensity of 0.07 mE m$^{-2}$ s$^{-1}$ in BG-11 medium (Stanier et al., 1971) supplemented with 20 mM Hepes-NaOH (pH 7.5), with aeration by sterile transformant of Synechocystis PCC6803, Fad6/desA::Kmr, was obtained as described by Wada and Murata (1989). The obtained as described previously (Wada et al., 1990, 1992).

Analysis of Lipids and Fatty Acids

The lipids were extracted from the intact cells by the method of Bligh and Dyer (1959). Analyses of lipids and fatty acids were performed as described by Wada and Murata (1989).

Measurement of Photosynthetic Activities

Photosynthetic evolution of oxygen by intact cells was measured with a Clark-type oxygen electrode (Gombos et al., 1991), either with no exogenously added electron acceptor and donor, or with 1 mM 1,4-benzoquinone and 1 mM K$_3$Fe(CN)$_6$ as electron acceptors. Light was provided from an incandescent lamp, after passage through a red optical filter (R62; Hoya Glass Co., Tokyo, Japan), at an intensity of 3.5 mE m$^{-2}$ s$^{-1}$. The light treatment of cells was carried out as described by Gombos et al. (1992). The Chl concentration of cells was adjusted to about 10 $\mu$g Chl mL$^{-1}$, as determined by the method of Arnon et al. (1974).

Flash dependence of the evolution of oxygen was measured with an unmodulated bare-platinum oxygen electrode (Vass et al., 1990). A suspension of cells at a concentration that corresponded to 50 $\mu$g Chl mL$^{-1}$ was preilluminated with a train of 50 short flashes, which was followed by 5 min of dark adaption. The evolution of oxygen was induced by a series of flashes of 3 ms duration and a frequency of 1 Hz, which were provided by a xenon flash lamp (1539-A xenon flash; GenRad, Concord, MA). Signals from the electrode were detected with a custom-built amplifier and monitored with a multichannel analyzer set at 2.5 to 10 ms/point (ICA KFKI, Budapest, Hungary).

RESULTS

The temperature profiles of the heat inactivation of the photosynthetic activities of intact wild-type, Fad6, and Fad6/desA::Kmr' cells were measured in terms of net photosynthesis and the photosynthetic evolution of oxygen, which was monitored with 1,4-benzoquinone as an artificial electron acceptor (Fig. 1). Essentially the same inactivation profiles were observed with the wild type and the Fad6 mutant. The Fad6/desA::Kmr' mutant, by contrast, revealed a small but distinct decrease in the heat stability of photosynthesis and oxygen-evolving activity (Fig. 1). The temperatures for 50% inactivation of oxygen-evolving activity were 49.0 ± 0.2°C, 49.2 ± 0.2°C, and 47.8 ± 0.2°C in the wild type, the Fad6 mutant, and the Fad6/desA::Kmr' mutant, respectively. These results are compatible with those reported by Mamedov et al. (1993).

The effect of light on the heat stability was studied to examine the possibility of cooperation of heat with light. Figure 2 shows that temperatures for 50% inactivation of the
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Figure 2. The effect of light intensity on the temperature for 50% inactivation of photosynthetic oxygen-evolving activity. Wild-type, Fad6, and Fad6/desA::Km' cells grown at 34°C were incubated at various temperatures under light of designated intensity for 40 min. Cells were suspended in BG-11 medium at a concentration of Chl that corresponded to 4 µg mL⁻¹. The photosynthetic evolution of oxygen with 1,4-benzoquinone as the acceptor of electrons was measured at 34°C. The values were obtained from results of three independent experiments. •, Wild type; □, Fad6; ○, Fad6/desA::Km'.

Figure 3. Changes in the pattern of oxygen yield for a sequence of flashes. Wild-type and Fad6/desA::Km' cells grown at 34°C were incubated in BG-11 medium at 36°C (A), 40°C (B), 44°C (C), and 46°C (D) in darkness for 40 min. The Chl concentration during the heat treatment and during the measurement of oxygen yield was 50 µg mL⁻¹. •, Wild type; ○, Fad6/desA::Km'.

Figure 4. Estimated composition in terms of molecular species of membrane lipids in Synechocystis PCC6803. Values were calculated on the basis of the fatty-acid composition of total lipids (Wada et al., 1992). For calculations, we assumed that the sn-2 position of the glycerol moiety of Synechocystis PCC6803 is esterified exclusively by 16:0, it is possible to calculate the number of unsaturated bonds in lipid molecules (Murata et al., 1992).

evolution of oxygen fell with increases in light intensity. Nevertheless, the extent of the effect of light on the heat inactivation appeared to be the same for the wild type, the Fad6 mutant, and the Fad6/desA::Km' mutant. These observations suggest that the differences in terms of the heat stability of photosynthesis among the three strains were not affected by light.

To obtain more direct information about the effect of lipid unsaturation on the heat stability of the evolution of oxygen, we measured the oxygen yield after repeated flashes of light in heat-treated cells (Fig. 3). Heat treatment at 44°C decreased the oxygen yield in both wild-type and Fad6/desA::Km' cells, but there were no changes in the patterns of oscillation of the yield. This result suggests that the heat treatment entirely destroyed part of the oxygen-evolving manganese complex but left the remaining part fully operative in both wild-type and Fad6/desA::Km' cells. However, the results in Figure 3 indicate that the wild-type cells were more resistant to heat than the Fad6/desA::Km' cells; after treatment at 44°C for 40 min, the wild-type cells retained 50% of their oxygen-evolving activity, whereas the Fad6/desA::Km' cells lost 75% of their activity.

DISCUSSION

In the present study, our aim was to determine, using genetically engineered strains of Synechocystis PCC6803, whether the heat stability of photosynthesis is correlated with unsaturation of membrane lipids. Since the sn-2 position of the glycerol moiety of Synechocystis PCC6803 is esterified exclusively by 16:0, it is possible to calculate the number of unsaturated bonds in lipid molecules (Murata et al., 1992). Figure 4 shows the major molecular species estimated in this way. In the wild-type cells, sn-1-18:3/sn-2-16:0 and sn-1-18:2/sn-2-16:0 account for 35 and 24%, respectively, whereas the Fad6 mutant contains sn-1-18:2/sn-2-16:0 at a level equivalent to 50% of the total molecular species but no sn-1-18:3/sn-2-16:0. In the Fad6/desA::Km' mutant, sn-1-18:1/sn-2-16:0 accounts for 85% of the total molecular species, and there are no polyunsaturated species. In all three
strains, the saturated molecule 16:0/16:0 remained at a constant level of about 15%.

The heat stability of the photosynthetic machinery in the Fad6 and the wild-type cells cannot be distinguished (Fig. 1). The photosynthetic machinery in the Fad6/desA::km' mutant, which does not contain any polyunsaturated lipid molecules, exhibited a small but distinct decrease in heat tolerance compared with that in the wild-type and the Fad6 mutant cells. The present study demonstrates that unsaturation of lipid molecules does indeed stabilize photosynthesis.

This conclusion stands in contrast to the previous hypothesis, based on physiological studies (Raison et al., 1982), that the saturation of lipid molecules stabilizes the photosynthetic evolution of oxygen against heat inactivation. Since this contradiction might be the result of different experimental conditions, such as the presence or absence of light, we examined the effect of lipid unsaturation on the heat stability of photosynthesis under light of various intensities. With increases in light intensity, the inactivation of photosynthesis was accelerated (Fig. 2). Nevertheless, the effect of the unsaturation on the inactivation of photosynthesis was not altered in the light.

In contrast to the results of Santarius and Müller (1979) and our observations, Kunst et al. (1989a) and Hugly et al. (1989) inferred that the thermal tolerance of photosynthesis was enhanced by mutation of chloroplastic desaturation of membrane lipids in Arabidopsis. However, changes in the level of unsaturation of membrane lipids in their mutants were only partial because the cytoplasmic pathway for the supply of lipids to chloroplasts was fully active (Browse et al., 1989; Kunst et al., 1989b). Moreover, changes in the thermal tolerance were insignificant. Therefore, the relationship between the unsaturation of membrane lipids and the thermal tolerance was not clear in their reports. Using the Pd-catalyzed hydrogenation of membrane lipids of pea thylakoids, Thomas et al. (1986) observed that the thermal stability of PSII was enhanced after hydrogenation of up to 90% of the total double bonds of lipids in membranes in which the fully saturated lipid molecules were present at a substantial level. After hydrogenation of up to 40% of the total double bonds, which may correspond to changes within physiological conditions, the thermal stability was not altered at all.

However, it has been observed in higher plants (Schreiber and Berry, 1977; Santarius and Müller, 1979; Havaux, 1992) and cyanobacteria (Nishiyama et al., 1993) that heat stability of photosynthesis increases with changes in environmental factors such as temperature, water stress, and light. The extent of increases in heat stability due to growth temperature ranges from 3°C to 6°C and is much larger than that caused by the modification of the unsaturation of membrane lipids in the present study. We previously demonstrated (Nishiyama et al., 1993) that the thylakoid membranes isolated from cyanobacterial cells that had been acclimated to non-lethal high temperature retained heat stability of photosynthetic electron transport. These observations suggest that unknown biochemical factors associated with the thylakoid membranes are responsible for the enhanced thermal stability of photosynthesis in cells grown at high temperature.

LITERATURE CITED


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