Biochimica et Biophysica Acta, 979 (1989) 361–364
Elsevier

BBA 74344

Saturation of membrane lipids by hydrogenation induces thermal stability in chloroplast inhibiting the heat-dependent stimulation of Photosystem I-mediated electron transport

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(Received 5 July 1988)

Key words: Photosystem I; Electron transport; Catalytic hydrogenation; Lipid saturation; Thermal stability

Mild hydrogenation (up to 20%) of *cis* double bonds of acyl lipids within thylakoid membranes results in a marked increase in the threshold temperature at which heat stress-induced stimulation of $DCPIPH_2$-supported PS I-mediated electron transport is initiated. Lipid saturation above 35% totally prevents the appearance of any increase in electron flow to PS I upon heat pretreatment. Experiments conducted on uncoupled chloroplast show that photosynthetic control of electron transport is not involved in the protective effect associated with lipid saturation. Homogeneous catalytic hydrogenation of unsaturated fatty acids within chloroplast membranes proved to be a powerful technique in verifying that heat stability of chloroplasts is coupled to the level of fatty acid unsaturation.

Exposure of higher plant cells to heat stress results in irreversible damage to photosynthesis prior to impairment of other cell functions [1,2]. Photosystem II-mediated electron transport rates and photophosphorylation are particularly susceptible to high temperature [2–5]. It was suggested that elevated temperatures result in the blockage of PS II reaction centers and then cause dissociation of the peripheral light-harvesting apparatus of PS II [2,4]. The loss of activity was accompanied by a sharp increase in chlorophyll *a* fluorescence from PS I [6].

The PS I-mediated electron transport, however, was apparently stimulated rather than inhibited at the threshold temperature at which the damage to PS II occurred. Recent studies of Thomas et al. [7] have provided new data concerning the origin of the changes in PS I activity observed in the heat-stressed chloroplast. The stimulation of PS I-mediated electron transport from reduced 2,6-dichlorophenolindophenol ($DCPIPH_2$) to methyl viologen (MV) can not be accounted for by either thermal uncoupling or grana destacking as was suggested previously [3,4]. Instead, the observed increase in PS I activity can mainly be attributed to the thermal stress-induced formation of new acceptor sites for $DCPIPH_2$ within the cytochrome f/b_6 complex. Newly formed electron donation sites were located at the reducing site of the Rieske Fe-S center tested by using EDAC as an inhibitor of the electron transport chain.

It was also suggested that the breakdown of membrane organisation coupled with the apparent stimulation of PS I may be related to the phase separation of non-bilayer forming lipids in heat-stressed membranes [3,7]. Evidence that the appearance of non-bilayer lipid phase under heat stress might be related to the reduced thermal stability of chloroplast has been described recently [8].

Employing the technique of homogeneous catalytic hydrogenation of the biomembranes [9–11] we were able to demonstrate that the selective saturation of *cis* double bonds of lipid alkyl chains within the intact thylakoids resulted in a marked increase in the threshold temperatures at which both the thermal damage of PS II-mediated electron transport and formation of non-bilayer lipid phase occurred [8]. Heat-induced stimulation of PS I may also be controlled by the level of unsaturated lipids in the thylakoid membranes.

Data presented in this paper indicate that the reduction of the double bond content of fatty acids by

Abbreviations: DCPIP, 2,6-dichlorophenolindophenol; $DCPIPH_2$, reduced DCPIP; MV, methyl viologen; PS, Photosystem; EDAC, ethyldimethylaminopropylcarbodiimide; $Pd(QS)_2$, palladium-di(sodium alizarine monosulphonate); DCMU, 3-(3,4-dichlorophenyl)-1,1'-dimethylurea; LHC, light-harvesting complex.

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catalytic hydrogenation in thylakoid membranes prevents thermally induced changes in $DCPIPH_2$-supported electron transport through PS I.

The catalyst, $Pd(QS)_2$, is a product of Molecular Probes, Eugene, OR. DCPIP, MV and EDAC were purchased from Sigma Co. All other chemicals were of commercial grade.

Chloroplasts were isolated from leaf tissues of 3-week-old pea seedlings (*Pisum sativum* L. var BR-52, Orosháza) by the method of Stokes and Walker [12] and suspended in isolation medium consisting of 0.33 M sorbitol, 5 mM $MgCl_2$, 2 mM EDTA, 10 mM NaCl, 1 mM $MnCl_2$, 30 mM phosphate buffer (pH 6.5). To yield class D chloroplasts (naked thylakoids) they were then lysed by resuspension in a medium consisting of 2 mM EDTA, 10 mM NaCl, 2 mM $MgCl_2$, 15 mM phosphate buffer (pH 6.5).

Chlorophyll concentrations were estimated by the method of Arnon [13]. Aliquots of thylakoid suspensions (15 μg chlorophyll/ml) were hydrogenated essentially as described earlier [8,14].

At the end of the procedure, chloroplasts were washed twice with fresh isolation medium then transferred to an assay medium containing 40 μM DCPIP, 100 μM MV, 2 mM sodium ascorbate, 0.8 mM NaN_3 and 16 μM DCMU, which was also used for measuring PS I activity (oxygen uptake).

An aliquot (5 ml) of control (where oxygen-free nitrogen in place of hydrogen was used in the presence of catalyst) or partially hydrogenated chloroplasts (adjusted to 10 μg chlorophyll/ml) were suspended in PS I assay medium in the absence or presence of 5 mM NH_4Cl as uncoupler [7] and were incubated at different temperatures by rotating the tubes at 30 rpm for 5 min. The samples were then cooled to 25°C. Electron transport rates through PS I were estimated from rates of O_2 uptake associated with electron flow from $DCPIPH_2$ to methyl viologen, according to Ref. 7. In some cases the effects of incubation temperatures on PS I-mediated oxygen uptake were measured in chloroplasts which had been pretreated with 2 mM EDAC, a blocker of the heat-stimulated electron flow acting before cytochrome *f* [7].

Extraction and analysis of fatty acids were performed as described in Ref. 10. Data on figures are the means of triplicate analyses from a single experiment on a single set of membranes and are in all cases typical of other experiments performed under identical conditions.

Hydrogenation of thylakoid vesicles led to very rapid increases in the level of fatty acid saturation: approx. 70% of the average number of double bonds of the lipids are lost after 25 min of the reaction. Fatty acid composition of lipids at partial saturation has been presented recently [14].

The PS I-mediated electron transport (from $DCPIPH_2$ to MV) was apparently unaffected by the progressive saturation of *cis* double bonds in the chloroplast lipids (Fig. 1). The same phenomenon was observed previously in intact chloroplasts [11].

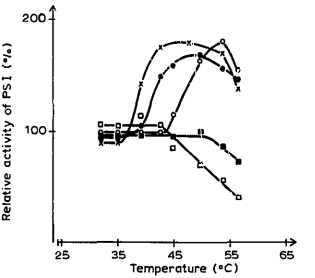

Fig. 1. The relative rates of PS I-mediated electron transport in nonhydrogenated (× ——— ×) and partially hydrogenated (● ——— ●, 10%, ○ ——— ○, 20%; ■ ——— ■, 35%; □ ——— □, 55%) samples, heat-stressed at different temperatures. Assay conditions are described in the text.

In agreement with earlier studies [7,15] exposure of thylakoid vesicles to temperatures above 35°C led to a stimulation of PS I-mediated electron transport rate (Fig. 1). The hydrogenation procedure itself had no effect on the heat-induced changes in PS I since both untreated and control samples that were put through the procedure but exposed to N_2 instead of H_2 showed the same PS I activity. Conversion of acyl-lipids within the intact photosynthetic membranes to their more saturated form, however, resulted in substantial alterations in the effects of heat stress on the PS I-mediated electron transport. In mildly hydrogenated samples (10 and 20% hydrogenation, respectively) the critical temperatures at which PS I stimulation was initiated, shifted gradually upwards (Fig. 1). The protective effect, apparently associated with saturation of the lipids, was more evident in samples saturated to a higher degree (35% and 55%). In these cases there was no sign of heat stress-induced stimulation of PS I, instead, in samples saturated up to 35%, the relative activity of PS I apparently remained constant up to 50°C, and declined thereafter. It was of interest, that while the thermal stress-induced stimulation was also absent, the PS I-mediated electron transport was inhibited at lower temperature (approx. 43°C) in extensively saturated (55%) samples. That inhibition of $DCPIPH_2$-supported electron transport through PS I at temperatures above about 50–55°C was also reported and discussed in other studies [6].

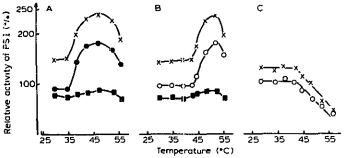

Fig. 2. The effect of incubation temperature on the relative rates of PS I-mediated oxygen uptake in the presence (×——×) or absence (●——●) of NH_4Cl as uncoupler and on PS I activities on chloroplasts pretreated with EDAC (■——■). Comparison was made with nonhydrogenated (Fig. 2A), mildly (20%) hydrogenated (Fig. 2B) and extensively (55%) hydrogenated (Fig. 2C) samples, respectively.

Relief of photosynthetic control by the addition of 5 mM NH_4Cl as uncoupler resulted in a pronounced stimulation in the basal activity of PS I-mediated electron transport in both the untreated and hydrogenated samples (Fig. 2). It was shown in our previous experiments that progressive hydrogenation of lipids in the photosynthetic membranes is paralleled with extensive uncoupling between the non-cyclic phosphorylation and the linear electron transport flow [11]. In contrast, cyclic phosphorylation around PS I proved to be far more resistant to an equivalent level of saturation. Predictibly, the hydrogenated systems remained in a well-coupled state and the addition of uncoupler brought about only a slightly lower level of stimulation in basal activity than that observed when compared with nonhydrogenated control.

Plots showing temperature dependency of the rate of PS I-mediated electron transport, tested in the presence or absence of uncoupler, ran nearly parallel with each other, both in the nonhydrogenated (Fig. 2A), and mildly (20%) (Fig. 2B) or extensively hydrogenated (55%) (Fig. 2C) samples, respectively. Thus, our results further support the proposal, that increased rates in PS I seen following the heat stress of chloroplasts cannot be accounted for by thermal uncoupling. Moreover, it is postulated that photosynthetic control of electron transport is not involved in the protective effect associated with an elevated level of lipid saturation.

The final step in elucidating the nature of thermal protection brought about by mild hydrogenation of lipid was to establish whether electrons from $DCPIPH_2$ entered the electron chain at the same point in the control and hydrogenated samples. Treatment with EDAC, slightly reduced the basal electron flow, but almost completely blocked the heat-induced stimulation of PS I-mediated electron transport, both in control and mildly (20%) hydrogenated chloroplasts (Figs. 2A, 2B). (EDAC was apparently ineffective in the case of 55% saturation.) These results indicated, that mildly hydrogenated systems still maintained the same electron donation site of $DCPIPH_2$ formed by heat damage, but 20% hydrogenation resulted in an upward shift in the temperature, required for the 'opening' of this new acceptor site(s). The higher level of fatty acid saturation obtained at 35% hydrogenation may totally prevent the structural reorganization of the membranes responsible for the heat stimulation of PS I activity.

Several studies indicate that acclimation of plant cells to high temperatures results in the appearance of polar thylakoid lipids with more saturated fatty acids [1,16–18]. Increased lipid saturation observed during thermal acclimation has been considered to be a factor that reduces the possibility of the conversion of lamellar phase of lipids to inverted hexagonal [2,3]. It has also been suggested, that alteration of fatty acid pattern observed during high temperature acclimation would tend to reduce the overall level of membrane fluidity [17]. This assumption predicted that threshold temperatures for irreversible heat damage are related to an upper limit of thylakoid membrane fluidity above which the membrane becomes unstable and disintegrates [2,17]. The availability of the technique of homogeneous catalytic hydrogenation using the sulphonated alizarine derivative of Pd(II) as catalyst [9–11] permitted us to directly test the adaptive value of lipid saturation and to assess the validity of the assumptions described above. Results, presented in this investigation, indicated that even a mild hydrogenation manifests in a marked increase in the threshold temperature for assumed rearrangement of the thylakoid membrane which consequently leads to the stimulation of PS I-mediated electron transport. Extensive saturation of thylakoid lipids (above 35%) totally prevents the appearance of any increase in electron flow to PS I upon heat treatment.

One of the main interests of the above and previous [8] findings is the apparent difference found in the extent of thermal protection afforded by mild hydrogenation to the regions of PS II [8] and to cytochrome f/b_6. A possible explanation could be, that because the major pool of PS II is localized in the appressed membranes of grana [19], the access of catalyst to the PS II core, in contrast to the lipids in the vicinity of the evenly distributed cytochrome f/b_6 [20], is relatively restricted. For similar reason, it is also conceivable that areas of heat-stressed membranes where separation of non-bilayer forming lipids, inspite of mild saturation, still occurred [8], are spatially segregated from those regions, where thermal protection resulting from mild hydrogenation is manifested. The assumption, that membranes can act as a barrier to the catalyst employed in the present study has also been evidenced by previous observations [21]. More study is needed to clarify this issue.

In summary, the method of homogeneous catalytic hydrogenation proved to be a useful experimental ap-

proach to verify that acquired heat tolerance, both of PS II-mediated electron transport [8] and $DCPIPH_2$-supported electron flow through PS I can essentially be mediated by saturation of fatty acyl chains as a common adaptive strategy. Since the molecular mechanism, inducing thermal tolerance of chloroplasts in vivo still remains to be elucidated, further studies are in progress, focussing mainly on the hydrogenation-afforded heat protection within intact algal cells.

Acknowledgements

This study was supported by the Hungarian National Scientific Research Foundation (OTKA No. 543). The authors are grateful to Éva Petrényi for help with isolation and heat treatment of chloroplasts and Béla Dusha for the photos.

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