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# Role of lipids in the organization and function of Photosystem II studied by homogeneous catalytic hydrogenation of thylakoid membranes in situ

Gábor Horváth [a], Anastasios Melis*, Éva Hideg [a], Magdolna Droppa [a] and László Vigh [b]

[a] *Institute of Plant Physiology and* [b] *Institute of Biochemistry, Biological Research Center, Szeged (Hungary)*

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Key words: Chloroplast lipid; Homogeneous catalytic hydrogenation; Fluorescence induction; Photosystem II heterogeneity; Thylakoid membrane; Membrane hydrogenation

**The effects of homogeneous catalytic hydrogenation on the organization and function of Photosystem II was investigated by fluorescence induction kinetic measurements with isolated chloroplasts. The results showed that saturation of the double bonds of fatty acyl constituents of membrane lipids had distinct effects on PS II. We found that progressive hydrogenation resulted in a gradual decrease of PS II electron transport with a concomitant increase of the initial fluorescence rise from $F_0$, the non-variable level, to $F_{p1}$, the intermediate plateau level, indicating that an optimal saturation level of fatty acids of membrane lipids is important in maintaining the efficient electron transfer from $Q_A^-$ to plastoquinone pool. In addition, the fluorescence induction kinetics of DCMU-poisoned chloroplasts showed that the proportion of PS $II_\beta$ in the membrane was increased by hydrogenation, suggesting that the saturation level of fatty acids may play an important role in regulating the association of the peripheral chlorophyll *a/b* light-harvesting complex II (LHC II-peripheral) with PS $II_\beta$, thus determining the PS II heterogeneity in PS $II_\alpha$ and PS $II_\beta$.**

## Introduction

The concept of PS II heterogeneity was introduced in order to explain the biphasic nature of the fluorescence induction kinetics measured in DCMU-poisoned chloroplasts [1–3]. It has been proposed that there are two structurally and functionally independent forms of PS II and they were designated as PS $II_\alpha$ and PS $II_\beta$ centers [1–3]. During this decade the PS II heterogeneity has been extensively studied and several aspects of the differences between the two types of centers have become well characterized.

It is well established now that PS $II_\alpha$ is localized in the grana partition region and PS $II_\beta$ is exclusively found in stroma-exposed thylakoids [4]. Furthermore, PS $II_\alpha$ participates in the linear electron transport and non-cyclic phosphorylation, while PS $II_\beta$ does not show a fast interaction with the PQ pool, probably because the intermediate PQ pool is absent from the thylakoid membrane in which PS $II_\beta$ is localized [4–6]. The two types of PS II unit differ in the light-harvesting antenna size as well. PS $II_\alpha$ units contain both the intrinsic and peripheral Chl *a/b* LHC II

* Permanent address: Division of Molecular Plant Biology, University of California, Berkeley, CA, U.S.A.

Abbreviations: Chl, chlorophyll; F, fluorescence; LHC, light-harvesting complex; MGDG, monogalactosyldiacylglycerol; $Pd(QS)_2$, Pd(II) sulfonated alizarine complex; PQ, plastoquinone; PS, Photosystem; $Q_A$ and $Q_B$, the 'primary' and 'secondary' acceptors of PS II.

Correspondence: G. Horváth, Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, Szeged, Hungary, H-6701.

complex [5,7]. On the other hand, PS $II_\beta$ units contain only the tighly bound intrinsic LHC II [5,6,8]. It was recently proposed that a developmental relationship between PS $II_\alpha$ and PS $II_\beta$ exists [6]. According to this model, during chloroplast development PS $II_\beta$ is formed firstly. As a second step, PQ and the peripheral LHC II associate with the preformed PS $II_\beta$ centers, resulting in the formation of PS $II_\alpha$ centers. However, the regulatory mechanism required for the association of reaction centers (RC) and LHC is as yet not known.

It has been shown previously that isolated PS II contains a relatively high proportion of lipids [9,10]. The functional significance of these lipids has been discussed in the literature. It was demonstrated that phosphatidylglycerol (PG) and in particular its constituent, the $\Delta^3$-*trans*-hexadecenoic acid (*trans*-C16 : 1), played role in the formation of the oligomeric form of LHC, as well as in the association of LHC to the RC [11–14]. From fluorescence measurements with isolated chloroplasts, it has been found that the amount and composition of monogalactosyldiacylglycerol (MGDG) regulated the supramolecular structure of PS II complexes [15]. Especially, those molecular species of MGDG, which contained highly unsaturated fatty acids, were effective in restoring the energy transfer between LHC and RC [15]. It is evident from these publications that the lipid constituents of PS II as well as their saturation level play important role in the functional organization of the PS II complex. These lipids might be one of the factors that regulates the association of LHC II and RC, thus determining the differentiation of PS $II_\alpha$ and PS $II_\beta$.

The recently introduced homogeneous catalytic hydrogenation [16,17] is a suitable technique for studying the role of lipids in the association of LHC II and RC. By the application of this technique, one can modulate the lipid composition of the membrane by saturating in situ the fatty acyl double bonds of the membrane lipids [17]. The technique is highly selective for fatty acids only [18] and it does not affect the existing architecture of chloroplasts [16,19].

In the present report we describe the effect of homogeneous catalytic hydrogenation of membrane lipids on the PS II organization and function. We have demonstrated that progressive hydrogenation gradually decreased the activity of PS II. The lower PS II activity correlated well with the proportional increase of the slow $\beta$ phase in the fluorescence induction kinetics. The elevated proportion of $\beta$ phase in the fluorescence kinetics indicated that following hydrogenation, the peripheral LHC II and PQ became functionally disconnected from the PS II core in the treated membrane. A model is presented to explain the dissociation of LHC and PQ from PS II.

## Materials and Methods

Chloroplasts were isolated from pea seedling (*Pisum sativum* L.) grown in greenhouse conditions for 18–22 days as described by Reeves and Hall [20] except the concentration of $MgCl_2$ was 2 mM in the suspension medium [17].

Hydrogenation was carried out according to the procedure described in Ref. 17. The catalyst was $Pd(QS)_2$ (QS, sulfonated alizarine: $C_{14}H_6O_7NaS$); Chl/catalyst ratio, 0.125; Chl concentration, 10 $\mu$g/ml; temperature, +4°C; $H_2$ pressure 0.3 MPa and the reaction time up to 30 min. As a control, chloroplasts were kept under 0.3 MPa $N_2$ pressure at +4°C for 30 min in the presence of catalyst. The $N_2$ treatment resulted in less than 20% decrease in photosynthetic activity [17]. Extraction of lipids and the analysis of fatty acids were performed as in Ref. 21. PS II electron transport was measured by using 0.25 mM *p*-benzoquinone in a Clark-type electrode under saturating white light [22]. Fluorescence induction measurement was carried out as described by Demeter et al. [23]. Actinic excitation was provided by green light filtered from the light of a projector lamp by a combination of CS 4-96 and CS 3-69 Corning glass filters. The fluorescence signal was transmitted through a red filter (Corning CS 2-64) and detected by an EMI 9558B photomultiplier, amplified by a home made differential amplifier system and stored in a multichannel analyzer (KFKI ICA 70). The kinetic analysis of the chloroplast fluorescence were done as described in Refs. 1 and 24.

For heat treatment the chloroplast suspension was incubated in a water bath at +40°C for 1 min [25]. The heat treatment was terminated by

returning the thylakoid suspension to 0°C followed by measurement of fluorescence induction.

## Results

The time-course of hydrogenation of thylakoid membranes at +4°C is shown in Fig. 1. The results suggest efficient hydrogenation of lipids at +4°C. This temperature was selected in order to minimize heat damage to the chloroplasts and to allow fluorescence measurements at various levels of hydrogenation. Under these conditions, about 35% of all double bonds were saturated within 30 min. The most intensive hydrogenation occurred within the first 20 min.

Fig. 2 shows the effect of saturation of fatty acyl double bonds on the fluorescence kinetics of isolated chloroplasts. The dark-adapted control chloroplasts exhibit a typical fluorescence induction curve. The small initial rise of fluorescence from the non-variable level ($F_0$) to the intermediate plateau level ($F_{pl}$) is followed by a slower but more pronounced fluorescence increase to $F_{max}$ level. It was recently demonstrated that the initial rise from $F_0$ to $F_{pl}$ was due to the prompt photoreduction of the primary electron acceptor $Q_A$ of PS $II_\beta$ [6], whereas the lag and subsequent rise of fluorescence from $F_{pl}$ to $F_{max}$ is indicative of the PQ pool photoreduction [6]. The electron accumulation between the two photosystems is caused by the slow rate of electron flow on the acceptor side of PS I [5]. Such a limitation, however, is largely alleviated by the addition of methyl viologen to the sample, which removes electrons efficiently from the FeS centers of PS I, thus resulting in lower $F_{max}$. Under these conditions, the initial

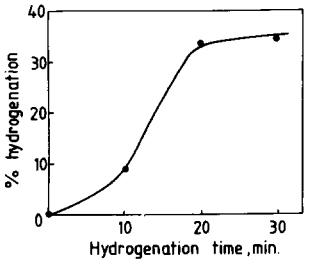


Fig. 1. The time-course of hydrogenation of spinach thylakoid lipids carried out at +4°C. Other experimental conditions as given in Materials and Methods.

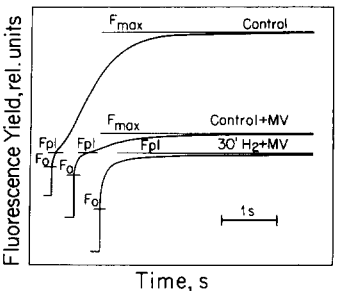


Fig. 2. The fluorescence induction kinetics of isolated spinach thylakoids in the absence (control) and in the presence of 200 $\mu$M methyl viologen (+MV). The lower curve shows the induction kinetics of thylakoid membranes hydrogenated for 30 min and measured in the presence of 200 $\mu$M methyl viologen.

fluorescence from $F_0$ to $F_{pl}$, reflecting the activity of PS $II_\beta$ in the sample [6], is more easily resolved (cf. Fig. 2, middle curve). The lower curve of Fig. 2 shows that after 30 min of hydrogenation, the $F_0$ level was practically unchanged in the methyl viologen-containing sample, but the level of $F_{pl}$ increased significantly.

The change of the $F_{pl}$ level in relation to the progress of hydrogenation is shown in Fig. 3. It is quite interesting that even a 10% saturation of double bond of fatty acids (corresponding to 10

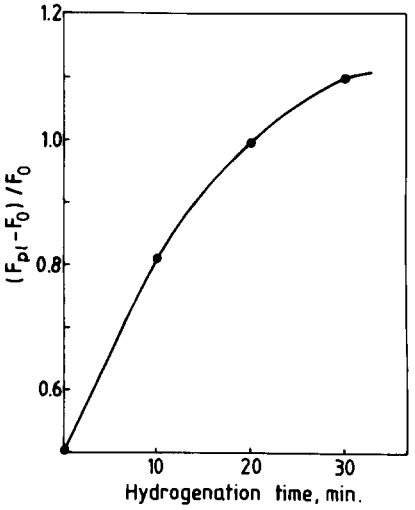


Fig. 3. The change of the initial rise of the fluorescence induction curve ($F_0$ to $F_{pl}$) as a function of hydrogenation time. The $(F_{pl} - F_0)/F_0$ values were calculated from fluorescence induction traces obtained in the presence of 200 $\mu$M methyl viologen.

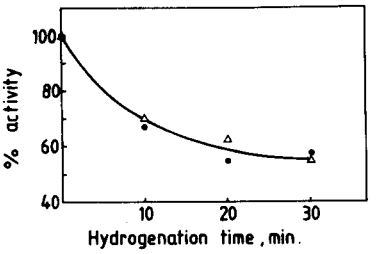


Fig. 4. The effect of hydrogenation on the activity of PS II electron flow measured from $H_2O$ to *p*-benzoquinone (●) and the percentage of active PS II centers (△). The fraction of active PS II centers was estimated by the method described in Refs. 26 and 27.

min of hydrogenation) increased the $F_{pl}$ level by 60% compared to that of the control. In samples hydrogenated for 30 min (corresponding to 35% saturation of fatty acyl double bond) the $F_{pl}$ level was higher by about 120%.

In earlier work, Joliot and Joliot [26] and Melis and Schreiber [27] presented an empirical relationship between the photoreduction of Q and the variable fluorescence yield, showing the fraction of the PS II centers that were closed during the fluorescence rise. Using this relationship we estimated the proportion of active PS II centers during hydrogenation. Fig. 4 shows that the decrease of active PS II centers and the change in PS II electron flow (measured from $H_2O$ to *p*-benzoquinone), expressed as % of control were identical during progressive hydrogenation. This means that the previously described decrease of PS II activity [19] caused by hydrogenation is a result of the decreased number of active centers in the photosynthetic lamellae. The inactivation of PS II by hydrogenation is probably because of the inability of PQ to interact with $Q_A^-$ at the PQ-binding site [6].

The next step in our experimentation was to determine whether hydrogenation is accompanied by the formation of PS $II_\beta$ centers via uncoupling of the LHC II-peripheral antenna of PS $II_\alpha$. To determine the proportion of PS $II_\alpha$ and PS $II_\beta$ units as a function of hydrogenation, we measured the fluorescence induction kinetics of DCMU-poisoned chloroplasts. In DCMU-poisoned chloroplasts the oxidation of $Q_A^-$ is fully inhibited and upon illumination a prompt fluorescence rise from $F_0$ to $F_{max}$ is observed, as is shown in Fig. 5. The

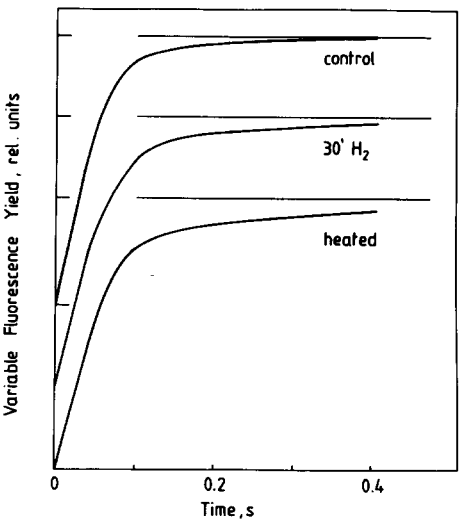


Fig. 5. Chloroplast fluorescence induction kinetics measured in the presence of 5 μM DCMU. Upper trace: control thylakoids middle trace: thylakoids subjected to 30 min of hydrogenation; lower trace: thylakoids heat-treated at +45°C for 1 min.

fluorescence induction kinetics in the presence of DCMU are biphasic, however. The fast non-exponential α component reflects the photoactivity of PS $II_\alpha$ and the slower exponential β component reflects the photoactivity of PS $II_\beta$ [1–8]. Comparing the fluorescence curve of control and hydrogenated chloroplasts, it is observed that the amplitude of the slow β phase is higher in hydrogenated than in control samples (Fig. 5, upper and middle trace). This transition is similar to that observed with heated thylakoids (Fig. 5 lower curve). It was recently shown that incubation of

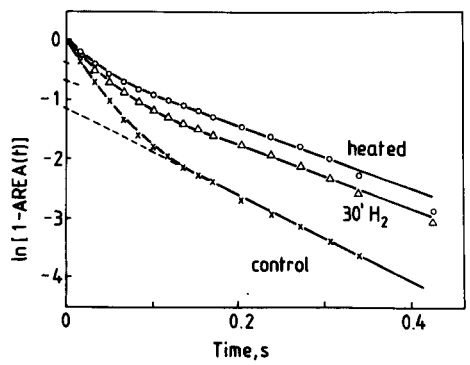


Fig. 6. First-order kinetic analysis of the area over the variable part of the fluorescence induction traces shown in Fig. 5.

chloroplasts at +45°C for 1 min resulted in a substantial conversion of PS $II_\alpha$ into PS $II_\beta$ [25] by dissociation of the LHC II-peripheral from PS $II_\alpha$.

Quantitation of this phenomenon was provided by a first-order kinetic analysis to the area over the variable part of the fluorescence induction curves [1–3]. The semilogarithmic plots of such traces with chloroplasts exposed to hydrogenation and heat-treatment are shown in Fig. 6. From the intercepts of the slow linear phases with the ordinate at zero time we calculated the relative amount of PS $II_\beta$. Fig. 6 (crosses) shows that in control chloroplasts, PS $II_\beta$ accounts for about 30% of all PS II present. This proportion of PS $II_\beta$ is in agreement with estimates published previously [4,6]. By heat treatment (circles), about 65% of all PS II was accounted for as PS $II_\beta$, in agreement with results described in Ref. 25. After 30 min of hydrogenation the proportion of PS $II_\beta$ was equal to 50% of the total. The time-course of the formation of PS $II_\beta$ concentration increase during hydrogenation is shown in Fig. 7. It is obvious from this result that the amount of PS $II_\beta$ obtained after heat-treatment at various levels of hydrogenation was constant. The amounts of PS $II_\beta$, however, converted by the saturation of the double bonds of fatty acids increased progressively with the level of hydrogenation. This indicates that saturation of double bonds of fatty acyl constituents of membrane lipids may play an important role in controlling the association of LHC II-peripheral with PS $II_\beta$.

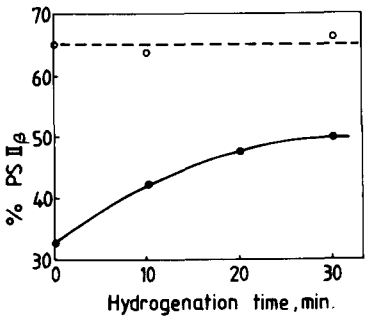


Fig. 7. The effect of hydrogenation (●) and of subsequent heat-treatment of hydrogenated samples (○) on the proportion of PS $II_\beta$ centers.

## Discussion

The interplay between membrane lipid hydrogenation and the structural and functional organization of PS II is demonstrated by the results in this work. It was previously shown that lipids had a functional significance in modulating both energy and electron transfer processes within PS II in the thylakoid membrane [9,11–15]. In particular, MGDG and PG were found to be effective. Homogeneous catalytic hydrogenation, introduced by Chapman and Quinn [28], and first applied to thylakoid membranes by Restall et al. [16], makes possible the in situ removal of double bonds of fatty acids in membrane lipdis [16,28]. However, some unsatisfactory features of the initial procedure (long incubation time, water insolubility of the catalyst) limited the comprehensive application of this process. The recently developed new homogeneous hydrogenation technique, with $Pd(QS)_2$ catalyst eliminates the above-mentioned shortcomings [17–19,29].

In the present work, we demonstrated that homogeneous catalytic hydrogenation of the lipid constituents of the photosynthetic membrane, in situ, induces significant changes in PS II activity which strictly correlate with the changes of the fluorescence induction kinetics of these membranes. We have found that progressive hydrogenation resulted in a gradual decrease of PS II electron transport with a concomitant increase of the initial fluorescence rise from $F_0$ to $F_{p1}$ level, in agreement with the previous finding [29]. It was recently demonstrated that the initial rise from the $F_0$ to $F_{p1}$ level was identical to that of $\beta$ component detected in DCMU-poisoned chloroplasts [6]. The initial rise from $F_0$ to $F_{p1}$ is due to the prompt photoreduction of the primary quinone acceptor of PS $II_\beta$ because of the impaired interaction of PS $II_\beta$ with the intermediate PQ pool [6]. Thus, we may conclude that the appropriate saturation level of fatty acids of membrane lipids is important to maintain the efficient electron flow from $Q_A$ to the PQ pool.

Lipids play an additional role in controlling the association of LHC II-peripheral with PS $II_\beta$. Both MGDG and PG as well as their saturation level were found to be important in this respect [12,15,30]. In the present work we measured in-

duction kinetics with DCMU-inhibited chloroplasts, and found that the proportion of $\beta$ centers in the membrane was increased by hydrogenation. It has been shown previously that one of the major differences btween PS $II_\alpha$ and PS $II_\beta$ is their effective light-harvesting antenna size [5–8]. PS $II_\alpha$ has the complete peripheral Chl *a/b* LHC II, while PS $II_\beta$ possesses only the small complements of the intrinsic LHC II. The hydrogenation-induced increase of $\beta$ phase in fluorescence induction traces means that hydrogenation affects not only the interaction between $Q_A$ and $Q_B$ but the organization of the LHC II in the membrane. Siefermann-Harms et al. [15] found that the saturation level of $C_{18}$ fatty acid of MGDG controlled the association of LHC and PS II reaction center [15]. Similarly, it was found that PG and its hexadecenoic acid content played a crucial role in the association of the monomeric forms of LHC II as well as that of the LHC II and RC [11–14,30]. A recent publication [31] provided detailed analysis of the lipid classes that are subject to hydrogenation from the $Pd(QS)_2$ catalyst. It was reported that the fatty acyl residues of all lipid classes were hydrogenated, albeit at various extents. The susceptibility of major lipid classes to hydrogenation (e.g., rate of hydrogenation) occurred in the following order: monogalactosyldiacylglycerol → digalactosyldiacylglycerol → sulfoquinovosyldiacylglycerol → phosphatidylglycerol. The most interesting finding, however, was that the hexadecenoic acid content of phosphatidylglycerol displayed the same high hydrogenation rate as MGDG. The lipid analysis date [31], together with the data presented in this work, are in good agreement with the earlier findings [11–15,30] indicating that lipids, probably MGDG and the hexadecenoic acid constituent of phosphatidylglycerol, through their saturation level, indeed play a very important role in controlling the association of LHC II-peripheral with PS $II_\beta$. It has been proposed recently that PS $II_\alpha$ assembly occurs in a two-step process: the first step is the formation of PS $II_\beta$ via the association of RC and the LHC II-intrinsic, which is followed by the second step by addition of the peripheral Chl *a/b* LHC II complex and of the PQ to the thylakoid membrane of the PS $II_\beta$ centers [6]. The present work suggests a role for thylakoid membrane lipids, i.e., the saturation level of the fatty acyl constituents of the membrane lipids, especially MGDG and phosphatidylglycerol, as one of the factors that regulate the association of the peripheral Chl *a/b* LHC II with PS II.

In summary, we present a model which might help to explain the effect of hydrogenation with respect to PS II organization and function. Fig. 8 is a schematic of the functional organization of PS II showing the reaction center complex (P-680, pheophytin, $Q_A$) with the herbicide-quinone binding site ($Q_B$). Attached to this complex is the core Chl *a* containing antenna (cPa) and the LHC II-intrinsic (LHC* II). Functionally, the above constitute PS $II_\beta$. The association of the LHC II-peripheral (LHC II) with PS $II_\beta$ results in the formation of PS $II_\alpha$. Catalytic hydrogenation of membrane lipids has a multiple effect on PS II. On the one hand, hydrogenation decreases the rate of electron transfer between $Q_A$ and $Q_B$, on the other hand, disconnects the Chl *a/b* LHC II-peripheral, effectively converting a PS $II_\alpha$ unit into a PS $II_\beta$ unit and free LHC II in hydrogenated chloroplasts.

In conclusion, our results show specific effects of lipid hydrogenation on PS II organization and function and provide a useful 'handle' in addressing the role of lipids in the thylakoid membrane of photosynthesis.

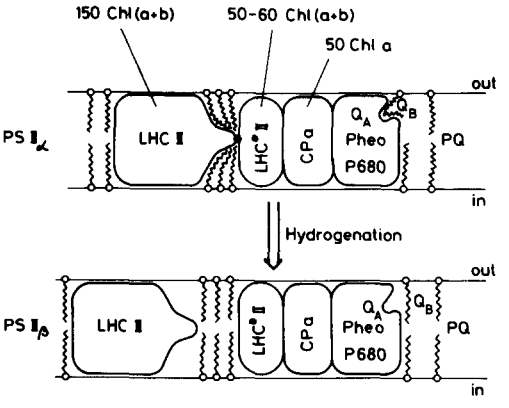


Fig. 8. Schematic of the structural-functional organization of PS II showing the interplay between integral thylakoid membrane complexes and lipids. Note the hydrogenation dependent dissociation of the LHC II-peripheral complement and the inhibition of the $Q_A \rightarrow Q_B$ interaction. (For details, see text.)

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