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Difference in sensitivity to photoinhibition between photosystem II in the appressed and non-appressed thylakoid regions

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Thylakoid vesicles containing photosystem II from either the appressed or non-appressed thylakoid region were subjected to photoinhibitory illumination. Photosystem II from the non-appressed region was found to be less sensitive to photoinhibition compared to photosystem II from the appressed region under both aerobic and anaerobic conditions.

Antenna size; Photosystem II; Electron transport; Photoinhibition; Regulation; Thylakoid membrane organization

1. INTRODUCTION

There is a heterogeneity among PS II centres, both with respect to function and localization along the stacked thylakoid membrane. Most PS II centres are located in the appressed region of the grana stacks but a minor population (20%) is located in the stroma thylakoid regions. This was suggested already in 1977 by Armond and Arntzen [1], based upon analyses of PS II activity in stroma thylakoid vesicles and lactoperoxidase-catalyzed iodination of stacked and destacked thylakoids. Although the presence of PS II in the non-appressed region has been questioned on the basis of recent ultrastructural data [2] and the lack of PS II in certain stroma thylakoid preparations [3,4], the presence of small amounts of PS II proteins in the non-appressed regions has been confirmed by immunogold labelling experiments [5,6]. Moreover, the amount of PS II in stroma thylakoids can be reversibly altered by changing the temperature [7–9].

The function of PS II centres has been shown to be heterogeneous with respect to electron acceptors that quench fluorescence [10–13], the ability to reduce plastoquinone [14,15], the oxidation of water [14–17], and herbicide binding [18–20]. Based upon PS II heterogeneity with respect to fluorescence induction the concept of PS II_α and PS II_β has been introduced [21,22].

In many reports on PS II functional heterogeneity, its relation to the inhomogeneous lateral membrane distribution of PS II is not clear. However, the population of PS II in non-appressed thylakoids was suggested earlier to possess a smaller antenna than granal PS II, since it required higher light intensities for saturation [1] and had a smaller size of EF-particles [23]. The PS II of the non-appressed thylakoids has

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Abbreviations: chl, chlorophyll; Mes, 4-morpholinoethanesulphonic acid; PpBQ, phenyl-*p*-benzoquinone; PS I, photosystem I; PS II, photosystem II

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fluorescence properties typical of PS II_β [24].

We here report on another functional heterogeneity between PS II centres, related to their location in appressed or non-appressed thylakoids. The PS II of isolated stroma thylakoid vesicles was found to be much more resistant to photoinhibition compared to PS II of membrane vesicles derived from appressed thylakoid regions.

2. MATERIALS AND METHODS

2.1. *Preparation of thylakoid vesicles containing PS II representative of either the non-appressed or the appressed thylakoid regions*

Spinach was grown at 20°C under 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. Chloroplast thylakoids were isolated essentially as in [25] and were washed and resuspended in 10 mM sodium phosphate, pH 7.4/5 mM NaCl/5 mM $MgCl_2$/100 mM sucrose. Subfractionation of the thylakoids into stroma thylakoid vesicles and inside-out vesicles representative of the non-appressed and appressed thylakoid regions, respectively [26], was performed by Yeda press treatment and phase partitioning [25] with modifications described in [27].

2.2. *Photoinhibition of thylakoid vesicles*

The photoinhibitory treatment was performed by preillumination of samples in the absence of artificial electron acceptor. Samples (20 μg chl/ml) in 67 mM sucrose/33 mM Mes, pH 6.5/3 mM NaCl were thermostatted to 20°C in a Clark-type oxygen electrode reaction vessel. Preillumination with white light of intensities giving a photon flux density from zero up to 35000 $\mu E \cdot m^{-2} \cdot s^{-1}$ was done for 5 min. Anaerobic conditions were obtained by flushing the sample with nitrogen for 3 min prior to photoinhibitory treatment. The different light intensities were obtained using neutral density filters (Beckman) and by varying the distance between the reaction vessel and the light source.

2.3. *PS II activity measurements*

Immediately after the photoinhibitory preillumination of a sample, 0.2 mM PpBQ was added with a Hamilton-syringe and the PS II activity was measured by the oxygen electrode, at illumination with saturating, or near saturating in the case of stroma thylakoid vesicles, broad red light.

2.4. *Determination of PS II antenna size from activity measurements*

In this case PS II activity measurements were performed without prior photoinhibitory preillumination. PS II activity light saturation curves were recorded by varying the intensity of the broad red light used for illumination. The light saturation curves were transformed into Eadie-Hofstee plots where V/I (= PS II activity in μmol $O_2 \cdot (mg\ chl)^{-1} \cdot h^{-1}$ divided by light intensity) is plotted versus V.

3. RESULTS

Fig.1 shows the inhibition of PS II after photoinhibitory preillumination of inside-out (appressed) and stroma thylakoid (non-appressed) vesicles under aerobic conditions. PS II activity in the inside-out vesicles shows a rapid decline in response to the preillumination. After 5 min of preillumination at 6700 $\mu E \cdot m^{-2} \cdot s^{-1}$ 50% of the activity was lost. In contrast the PS II activity in the preilluminated stroma thylakoid vesicles was much more resistant to photodamage and the activity was completely preserved even after preillumination at 35000 $\mu E \cdot m^{-2} \cdot s^{-1}$. The PS II activity in the preilluminated intact thylakoids, which have most of their PS II in appressed regions, showed a clear inhibition but not as severe as in the inside-out vesicles.

Photoinhibitory preillumination of the two thylakoid subfractions under anaerobic conditions led to more pronounced inhibition of the PS II activity in both fractions (fig.2), consistent with other reports demonstrating an enhancement of photoinhibition by anaerobiosis [28–30]. For the inside-out vesicles derived from the appressed region only 20% of the PS II activity remains after preillumination at 2500 $\mu E \cdot m^{-2} \cdot s^{-1}$ compared to 61% after the same preillumination under aerobic conditions. Under anaerobic conditions PS II in the stroma thylakoid vesicles becomes also photoinhibited, although to a lesser extent than PS II from the inside-out thylakoids. The light intensity giving 50% inhibition was 1600 and 530 $\mu E \cdot m^{-2} \cdot s^{-1}$ for the stroma thylakoid and inside-out vesicles, respectively.

In order to elucidate to what extent the differences in photoinhibition sensitivity of the two pools of PS II were a result of differences in anten-

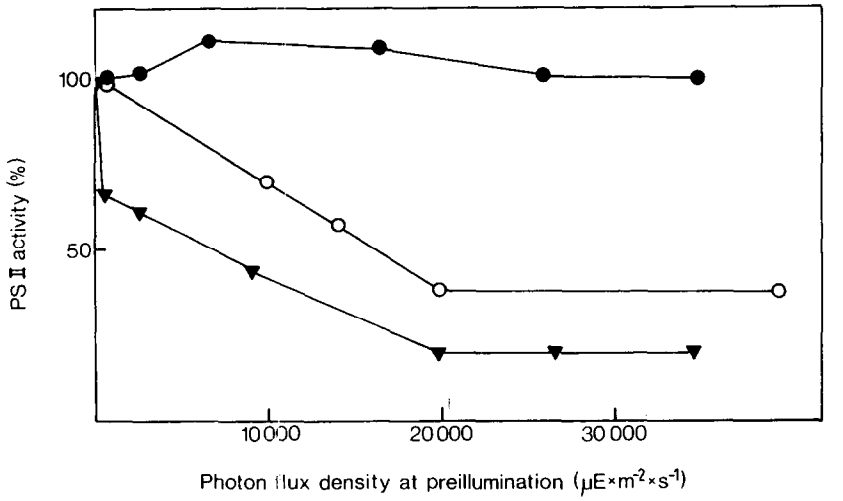

Fig.1. Effect of photoinhibitory preillumination on PS II activity. Stroma thylakoid vesicles (non-appressed region) (●), inside-out thylakoid vesicles (appressed region) (▼) and intact thylakoids (○). Control rates without preillumination were 40, 219 and 92 μmol $O_2 \cdot (\text{mg chl})^{-1} \cdot h^{-1}$, respectively.

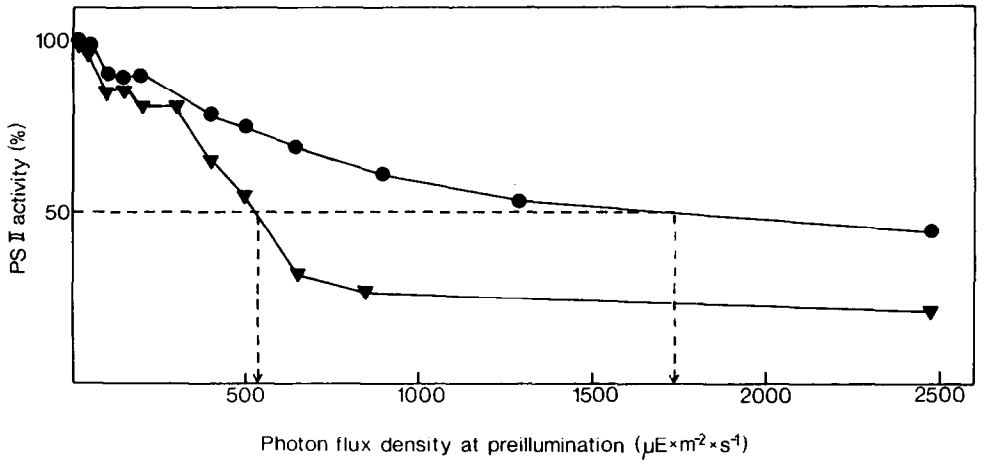

Fig.2. Effect of photoinhibitory preillumination under anaerobic conditions on PS II activity. Stroma thylakoid vesicles (non-appressed region) (●) and inside-out thylakoid vesicles (appressed region) (▼). Control rates without preillumination were 37 and 221 μmol $O_2 \cdot (\text{mg chl})^{-1} \cdot h^{-1}$, respectively.

na size, their electron transport capacities at different light-intensities were determined (fig.3). The slower rise up to V_{max} for PS II from the non-appressed region indicates a smaller antenna size of PS II in these vesicles. To quantify the functional antenna sizes the ratio between PS II activity and the corresponding light intensity (V/I) was plotted versus the PS II activity (V). This gives an Eadie-Hofstee type of plot where the slope is $-1/K_m$. K_m corresponds to the light intensity that is required for half maximal activity, and can therefore be used as an estimate of the functional antenna size. It was found that the ratio between K_m for vesicles from the non-appressed and appressed regions was around 3. Thus, the functional antenna size of PS II in the stroma thylakoid vesicles is only one third of the antenna size of PS II in the inside-out vesicles.

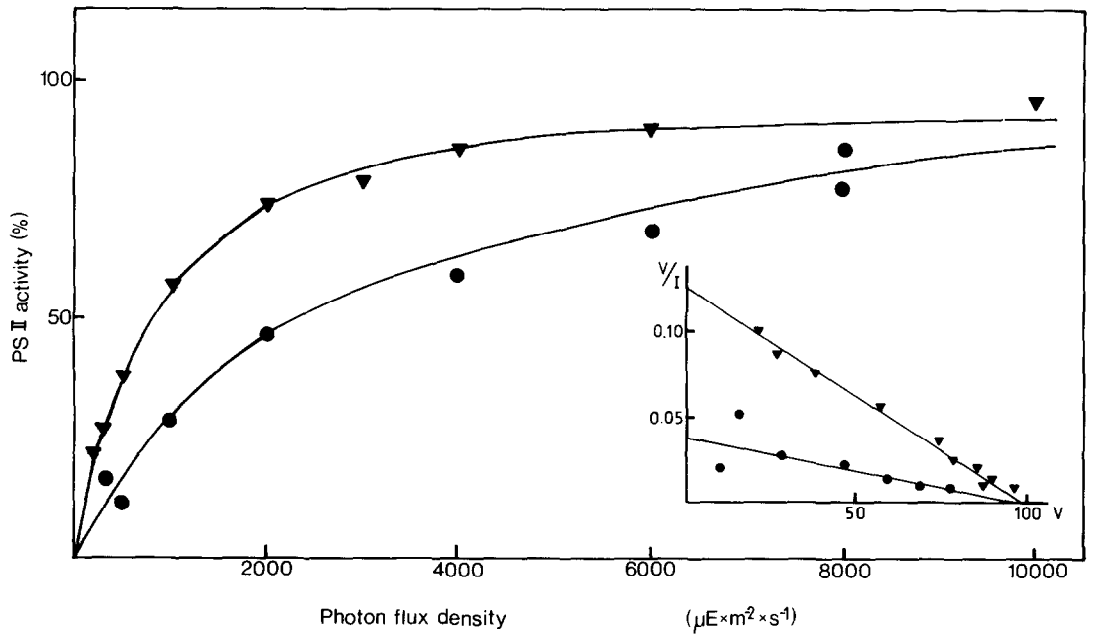

Fig.3. PS II activity as a function of light intensity. Stroma thylakoid vesicles (non-appressed region) (●) and inside-out vesicles (appressed region) (▼). Inset: Eadie-Hofstee plot where V/I (= PS II activity in μmol $O_2 \cdot$(mg chl)$^{-1} \cdot h^{-1}$ divided by light intensity) is plotted versus V. The intercept on the ordinate is V_{max}/K_m and that on the abscissa is V_{max}. The slope is $-1/K_m$ where K_m is the light intensity that gives half-maximal activity. K_m gives therefore a measure of the functional antennae size. V_{max} was 47 and 231 μmol $O_2 \cdot$(mg chl)$^{-1} \cdot h^{-1}$ for stroma thylakoid and inside-out vesicles, respectively, and K_m was 2553 and 790, respectively.

4. DISCUSSION

The present results demonstrate that PS II in stroma thylakoid vesicles is less sensitive to photoinhibition than PS II in thylakoid vesicles derived from the appressed region. It is likely that this points to a difference between PS II in the native non-appressed and appressed thylakoid regions. The resistance of non-appressed PS II against light damage would strengthen our suggestion [7,31] that the temperature-dependent increase in the amount of PS II in non-appressed thylakoids [7–9] is a regulatory mechanism to prevent overexcitation and photodamage to PS II under conditions of high light and temperature.

The mechanism behind the different sensitivity to photoinhibition between the two PS II populations is not clear. Under anaerobic conditions the 3-times smaller sensitivity to photoinhibition of the stroma PS II centres (fig.2) correlates well with their 3-times smaller antenna size (fig.3). However, antenna size differences are not sufficient to explain the complete insensitivity of stroma PS II manifested under aerobic conditions (fig.1) and some other mechanism therefore has to be considered. It could be argued that oxygen could have a protective effect on stroma PS II through a Mehler reaction in the PS I-rich stroma thylakoids. However, we recorded only a very poor oxygen consumption during the photoinhibitory treatment (not shown).

Recent data show that newly synthesized PS II Q_B-protein is first inserted into the non-appressed thylakoid regions [19,32], before being transported to the granal membrane regions. It could therefore be argued that newly synthesized and unassembled PS II would not be susceptible to photoinhibition. However, PS II from stroma thylakoids is not completely insensitive to photoinhibition since under anaerobic conditions it does become photoinhibited. Besides, newly synthesized PS II protein would possibly only contribute to a minor part

of the total PS II in the stroma thylakoids which comprise some 20% of all PS II reaction centres. In addition, the amount of PS II in the stroma thylakoids can be rapidly and reversibly changed with temperature [7,9], which also argues against stroma thylakoid PS II being only in a premature form.

The pool of PS II which is located in the non-appressed thylakoid regions is considered to be PS II_β [9,24]. It is possible that one or other of the unique properties ascribed to PS II_β centres could explain the absolute resistance to photoinhibition of PS II in stroma thylakoids under aerobic conditions. PS II_β, apart from having a smaller antenna size, differs from PS II in the primary electron acceptor [10–13] and it lacks a charge accumulating component functioning like Q_B in the two-electron gate mechanism [13]. There is evidence that a part of PS II, possibly identical to PS II_β, is inactive and unable to reduce plastoquinone [14,15], although it can reduce artificial quinone electron acceptors with concomitant oxygen evolution [14–17]. Accordingly, the PpBQ used in our electron transport measurements should induce oxygen-evolving electron transport in PS II from both appressed and stroma thylakoid regions, whether the latter is regarded as an inactive pool of PS II or not. On the other hand, during the photoinhibitory preillumination, in the absence of added artificial acceptor, neither of the two types of PS II would perform electron transport, unless a cyclic electron flow is assumed. If a PS II cycle [10,33–36] is typical for the PS II centres located in stroma thylakoids, it could apart from the smaller antenna size account for their lower sensitivity to photoinhibition.

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