Mini review

Photosystem II heterogeneity: the acceptor side

Govindjee
Departments of Physiology & Biophysics and Plant Biology, University of Illinois at Urbana-Champaign, 289 Morrill Hall, 505 South Goodwin Ave., Urbana, IL 61801, USA

Abstract

It is well known that two photosystems, I and II, are needed to transfer electrons from H_2O to $NADP^+$ in oxygenic photosynthesis. Each photosystem consists of several components: (a) the light-harvesting antenna (L-HA) system, (b) the reaction center (RC) complex, and (c) the polypeptides and other co-factors involved in electron and proton transport. *First*, we present a mini review on the heterogeneity which has been identified with the electron acceptor side of Photosystem II (PS II) including (a) L-HA system: the PS II$_\alpha$ and PS II$_\beta$ units, (b) RC complex containing electron acceptor Q_1 or Q_2; and (c) electron acceptor complex: Q_A (having two different redox potentials Q_L and Q_H) and Q_B (Q_B-type; Q_B' type; and non-Q_B type); additional components such as iron (Q-400), U ($E_{m,7} = -450\,mV$) and Q-318 (or Aq) are also mentioned. Furthermore, we summarize the current ideas on the so-called inactive (those that transfer electrons to the plastoquinone pool rather slowly) and active reaction centers. *Second*, we discuss the bearing of the first section on the ratio of the PS II reaction center (RC–II) and the PS I reaction center (RC–I). *Third*, we review recent results that relate the inactive and active RC–II, obtained by the use of quinones DMQ and DCBQ, with the fluorescence transient at room temperature and in heated spinach and soybean thylakoids. These data show that inactive RC–II can be easily monitored by the OID phase of fluorescence transient and that heating converts active into inactive centers.
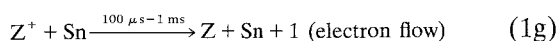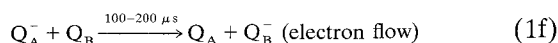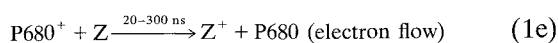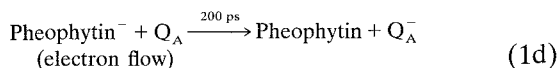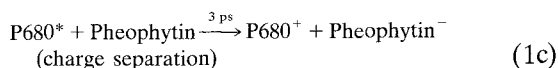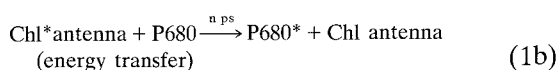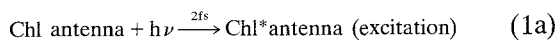
Abbreviations: DCBQ – 2,5 or 2,6 dichloro-*p*-benzoquinone; DMQ – dimethylquinone; Q_A – primary plastoquinone electron acceptor of photosystem II; Q_B – secondary plastoquinone electron acceptor of photosystem II; OIDP – successive fluorescence levels during time course of chlorophyll *a* fluorescence: O for origin, I for inflection, D for dip or plateau, and P for peak

1. Introduction

In oxygenic photosynthesis, electron flow from H_2O ($E_{m,7} = +0.8\,V$) to $NADP^+$ ($E_{m,7} = -0.3\,V$) requires the operation of two photosystems (PS), I and II (see Govindjee and Govindjee 1975, Andréasson and Vänngård 1988). Excitation of reaction center (RC) I leads to reduction of $NADP^+$ and oxidation of plasto-quinol (PQH_2) whereas, the excitation of RC II leads to the oxidation of H_2O to molecular O_2 and the reduction of plastoquinone (PQ) to PQH_2. In this series scheme of electron flow (see Fig. 2 in Demeter and Goindjee 1989), only one PS I and one PS II are required.

In photosystem II, the following reactions are suggested (see e.g., Govindjee and Wasielewski 1989) to occur:

$$Chl\ antenna + h\nu \xrightarrow{2fs} Chl^*antenna\ (excitation) \quad (1a)$$

$$Chl^*antenna + P680 \xrightarrow{n\ ps} P680^* + Chl\ antenna$$
$$(energy\ transfer) \quad (1b)$$

$$P680^* + Pheophytin \xrightarrow{3\ ps} P680^+ + Pheophytin^-$$
$$(charge\ separation) \quad (1c)$$

$$Pheophytin^- + Q_A \xrightarrow{200\ ps} Pheophytin + Q_A^-$$
$$(electron\ flow) \quad (1d)$$

$$P680^+ + Z \xrightarrow{20-300\ ns} Z^+ + P680\ (electron\ flow) \quad (1e)$$

$$Q_A^- + Q_B \xrightarrow{100-200\ \mu s} Q_A + Q_B^-\ (electron\ flow) \quad (1f)$$

$$Z^+ + Sn \xrightarrow{100\ \mu s-1\ ms} Z + Sn+1\ (electron\ flow) \quad (1g)$$

A second light reaction leads to the reduction of Q_A to Q_A^-; this is followed by:
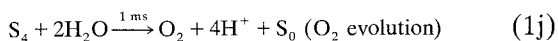
$$Q_A^- + Q_B^- \xrightarrow{400-600\ \mu s} Q_A + Q_B^{2-}\ (electron\ flow) \quad (1h)$$

$$Q_B^{2-} + 2H^+ \longrightarrow Q_B H_2\ (or\ PQH_2)\ (release\ of\ PQH_2)$$
$$(1i)$$
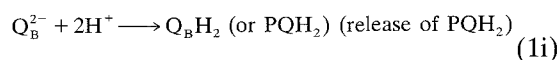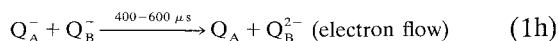
After four light reactions, O_2 is evolved:

$$S_4 + 2H_2O \xrightarrow{1\ ms} O_2 + 4H^+ + S_0\ (O_2\ evolution) \quad (1j)$$

Here, Chl is a chlorophyll a; P680 is a reaction center Chl a of PS II; Q_A is a one-electron acceptor, a bound plastoquinone; Q_B is a two-electron acceptor, secondary plastoquinone; it is tightly bound in its semiquinone from (Q_B^-), but less so in $Q_B H_2$ and Q_B forms; Z is a tyrosine residue (Debus et al. 1988); and S stands for the redox state of the oxygen evolving complex. Steps 1(a) to 1(d) and 1(f), 1(h) and 1(i) are similar to those in photosynthetic bacteria except that bacteriochlorophyll replaces Chl and Q_A and Q_B are ubiquinones instead of plasto-quinones. PS II is unique in its ability to oxidize water to O_2, however (steps 1(g) and 1(j).

Ikegami and Katoh (1973) discovered a component Q400 in samples incubated with ferri-cyanide. Petrouleas and Diner (1986) showed that this Q400 is Fe^{3+}. Thus, Q400 is the Fe between Q_A and Q_B. It may somehow aid in the electron flow form Q_A^- to Q_B, but its functional role is not yet clear.

This picture is further complicated by the evidence, that has built-up over the past 15 years, for the existence of a PS II heterogeneity that includes the light-harvesting antenna (L-HA) system, the RC complex, as well as the electron transport system, the functional significance of which is not yet clear. For review on PS II, see Govindjee (1984), van Gorkom (1985), Diner (1986), Mathis and Rutherford (1987), Velthuys (1987) and Hansson and Wydrzynski (1990). PS II heterogeneity has been discussed by Vermaas and Govindjee (1981), Black et al. (1986) and Melis et al. (1988). PS II is heterogeneous in both its electron donor as well as its electron acceptor side, but we shall discuss only the electron acceptor side here.

2. The heterogeneity of the photosystem II

Table 1 shows the various heterogeneities of photosystem II in order of their discovery.

The Q_L and Q_H

Redox titrations of Q_A show two titration waves (-250 and $0\ mV$) as discovered by Cramer and Butler (1969) (see Horton and Croze 1979). For a review, see Black et al. (1986). There is no evidence that both Q_L and Q_H are on the same reaction center; thus a heterogenous population of PS II, one with Q_L and the other with Q_H is the generally accepted picture. The possibility that this difference can be due to differential accessibility of Q_A has not yet been disproven; an interconvertible heterogeneity of Q_A-Fe^{2+} has been shown to exist (Rutherford and Zimmermann 1984). Evans and Ford (1986) suggest the existence of two tightly bound iron quinones in the PS II acceptor complex. It is generally believed that Q_H is the form that is in the main chain, and Q_L is not (see e.g., Diner and Delosme 1983); Q_L is not electrogenic. However, see van Gorkom (1985) regarding Q_L.

PS II$_\alpha$ and PS II$_\beta$

An analysis of the rate of growth of the area over the Chl a fluorescence induction curve in the presence of diuron, that blocks electron flow beyond Q_A, revealed another heterogeneity (Melis and Homann 1975, 1976): a portion of PS II does not exist in clusters, i.e., it exists in separate packages without excitation energy transfer between them. These centers were called PS II$_\beta$, whereas the others existing in clus-

Table 1. Photosystem II heterogeneity: The acceptor side

Discovery	Type	Remarks (basis)
Cramer and Butler (1969)	Q_L and Q_H ($-250\,mV$) ($0\,mV$)	Redox potential
Melis and Homann (1975, 1976)	PS II$_\alpha$ and PS II$_\beta$ (Q_α and Q_β)	Antenna size; clustering, etc. (associated quinones for Q_A)
Joliot and Joliot (1979) Eckert and Renger (1980)	Q_1 and Q_2 (Xa)	Absence of relationship to $\Delta\psi$ and C550 for Q_2 and Xa; [DCMU] dependence is different
Thielen and van Gorkom (1981) Lavergne (1982a,b)	Q_B-type centers Q_B'-type centers non-Q_B type centers	Absence of period-2 oscillations for Q_B and non Q_B centers
Graan and Ort (1986)	Active and Inactive centers	PS II activity using DMQ and DCBQ as electron acceptors

ters (i.e., having excitation energy transfer between them) were called PS II$_\alpha$. For a background on Chl a fluorescence, see Govindjee et al. 1986, and for a review on PS II$_\alpha$ and PS II$_\beta$, see Melis et al. (1988). In contrast to PS II$_\alpha$, PS II$_\beta$ has the following characteristics:
a) smaller antenna size;
b) faster Chl a fluorescence rise in the absence of diuron;
c) slower Chl a fluorescence rise in the presence of diuron;
d) a higher proportion of longer wavelength Chl a forms and a smaller proportion of Chl b; and
e) location in unappressed membranes.

It is generally accepted that PS II$_\alpha$ is in the main chain of electron flow. Thus, it would have been a simple picture if Q_β, the acceptor associated with PS II$_\beta$, was Q_L, but it is not since its E_m is $+120\,mV$ (Horton 1981, Thielen and van Gorkom 1981a) not $250\,mV$! Furthermore, PS II$_\beta$ does not use the 2-electron gate route depicted in Eqs. (1) (see Thielen and van Gorkom 1981b). In spite of the objections raised by Hodges and Barber (1986), the concept of PS II$_\beta$ with an acceptor different from Q_A-Q_B has not been disproven. Melis and Duysens (1979) and Melis and Schreiber (1979) showed that Q_β, like Q_α (the quinone acceptor of PS II$_\alpha$), has an absorbance change in the 260–340 nm range and leads to an absorbance change at 550 nm (C550). These data would preclude Q_β from being equivalent to Q_2 (see next section). However, PS II$_\beta$ may itself have a heterogeneous origin. Guen-

ther and Melis (1989) have proposed that PS II$_\beta$ centers are nothing else but damaged PS II$_\alpha$ migrated from appressed to unappressed thylakoid region; they are first repaired to a form that do not reduce Q_B, and then activated to a form that reduces Q_B before being transferred to the appressed membrane to form PS II$_\alpha$.
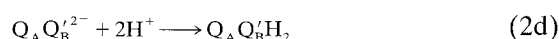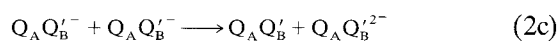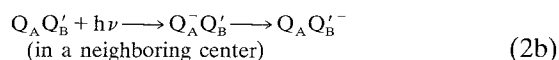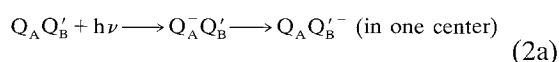
Q_1 and Q_2

On the basis of the fluorescence yield after single-turnover light flashes, Joliot and Joliot (1977, 1979, 1981a,b, 1983) have identified an electron acceptor Q_2 that is different from the main electron acceptor Q_1, the latter being equivalent to Q_A and Q_H. Q_2 is not a quinone, its reduction does not give rise to a membrane potential (ΔA, 515 nm), its turnover is not accompanied by a C550 change (Joliot and Joliot 1981a), and it is equivalent to a component Xa, discovered by Eckert and Renger (1980). It is not certain whether both Q_1 and Q_2 are on the same (Lavergne 1982a,b) or on different reaction centers. When NH_2OH, instead of H_2O, was used as the electron donor, Q_2 reduction (as measured by an increase in the slow Chl a fluorescence yield) was observed (Joliot and Joliot 1981b). Joliot and Joliot (1983) have shown that cytochrome b_6 can be reduced by electrons from reduced Q_2, as well as from reduced Q_1, but the former electron pathway requires higher [DCMU] for inhibition. Microsecond delayed light emission, that was independent of the membrane potential (Jursinic et al. 1978), can now be

154

explained to be due to a back reaction of Q_2^- (or Q_L^-) with P680$^+$ (see Meiburg et al. 1984). Precise measurements on the $E_{m,7}$ of Q_2 and Q_L on the same sample are needed to test the equivalence of these two components.

Q_B-type, Q_B'-type and non-Q_B type centers

Both Q_2 and PS II$_\beta$ involve non-Q_B type centers, i.e., centers that do not have the functional 2 electron gate normally involved in the reduction of the plastoquinone pool (Eqs. (1f) and (1h)). The absence of the two electron gate does not necessarily imply the absence of Q_B since it is feasible that Q_B may also function as one electron acceptor:

$$Q_A Q_B' + h\nu \longrightarrow Q_A^- Q_B' \longrightarrow Q_A Q_B'^- \text{ (in one center)} \tag{2a}$$

$$\begin{aligned} Q_A Q_B' + h\nu &\longrightarrow Q_A^- Q_B' \longrightarrow Q_A Q_B'^- \\ &\text{(in a neighboring center)} \end{aligned} \tag{2b}$$

$$Q_A Q_B'^- + Q_A Q_B'^- \longrightarrow Q_A Q_B' + Q_A Q_B'^{2-} \tag{2c}$$

$$Q_A Q_B'^{2-} + 2H^+ \longrightarrow Q_A Q_B' H_2 \tag{2d}$$

Such centers are called Q_B'-type centers, and those in which the two electron gate function of Q_B is absent are called non-Q_B type centers (see Thielen and van Gorkom 1981b, Lavergne 1982a,b). Thus, by this definition non-Q_B centers may include Q_B' type centers. The non-Q_B type centers are assumed to be the 'inactive' centers because the electron flow to the plastoquinone pool is very slow. However, only a portion of non-Q_B centers, i.e., those missing both Q_B and Q_B', may be inactive. They may be the PS II$_\beta$ centers and the Q_2 centers.

Lavergne (1987) has shown the reduction of Q_A, by UV absorbance measurements, in non-Q_B' centers; however, the spectra obtained had a poor fit with the known spectra of Q_A.

Inactive and active centers

Graan and Ort (1986) observed that there are about 1.7 mmol of active PS II reaction centers per mol chlorophyll for electron flow from H_2O to the native PQ molecules or to dimethylbenzoquinone (DMQ) although a total of 3 mmol of PS II centers are revealed when certain halogenated benzoquinones (2,5, or 2,6 dichloro-p-

benzoquinone, DCBQ) are used as electron acceptors or when herbicide terbutryn binding sites are counted. Thus, about 60% of centers (1.7/ 3×100) are normally active, and the other 40% are normally inactive. By independent methods the existence of these inactive PS II centers, as slowly (about 2 s) recovering, has been established by Chylla et al. (1987) in thylakoids and by Chylla and Whitmarsh (1989) in intact leaves. These inactive centers, perhaps, include most of the non-Q_B centers (both Q_2 and PS II$_\beta$). Jursinic and Dennenberg (1988a) have suggested that in their experiments, the enhanced yields caused by DCBQ are due to double turnovers of PS II centers. It is difficult to imagine why DCBQ, but not DMQ, should induce double turnovers in their experiments for the same flash. Although further research is needed to reconcile the differences between the conclusion of Jursinic and Dennenberg and Graan and Ort, it is suggested that their criticisms probably do not apply under other conditions. In section 5 it will be shown that with μM [DCBQ], but not with [DMQ], the OID Chl a variable fluorescence is quenched. However, μM [DMQ] is able to quench only the DP variable fluorescence. At the light intensities used in this work, we have no reason to believe that the use of DCBQ would give double hits per center. We suggest that the OID phase measures PS II inactive and the DP phase PS II active centers. Melis (1985) had earlier speculated that the OID phase (also called OPl) measures PS II$_\beta$ and DP phase PS II$_\alpha$ centers. Furthermore, independent of all of the above, the existence of inactive and active centers cannot be dismissed in view of the existence of PS II$_\beta$, Q_2 and non-Q_B centers (see Lavergne 1982a,b, Black et al. 1986).

The existence of inactive PS II was recently challenged by Chow et al. (1989). Using 3 μs repetitive flashes at 4 Hz, they have obtained in spinach leaf discs, a ratio of 2.8–3.0 mmol active PS II mol^{-1} Chl to 1.6 mmol active PS I mol$^-$ Chl, leaving no room for the existence of inactive PS II. This result can be understood only if we accept that either there is 100% double hit in PS I or the turnover of PS I is twice as fast as that of PS II, a conclusion that is in disagreement with the available kinetic data (see e.g., Rutherford and Heathcote 1985).

Other acceptors

Evans et al. (1985) have suggested the existence of another component 'U' ($E_{m,10} = -450\,mV$) between Pheophytin and Q_A; its reduction enhances the triplet yield from P680. Jursinic and Dennenberg (1988b) have recently suggested the existence of an ancillary quinone that they call Aq ($E_{m,7} = +318\,mV$) in 40% of the centers. However, their result is difficult to reconcile with that of Brettel et al. (1985) who seem to have ruled out the existence of an ancillary electron acceptor functioning with a quantum yield > 15% when Q_A is reduced. Thus, Q_2 (=Xa)/U/Aq phenomena may involve a low quantum yield side path. The question of the heterogeneity of PS II acceptors is far from being solved.

For a discussion of PS II heterogeneity in a marine diatom *Phaeodactylum*, see Owens (1986) and in a green alga *Dunaliella*, see Guenther et al. (1988).

3. A working model of the PS II heterogeneity

We consider the following model as a working hypothesis for PS II acceptor side heterogeneity. There is evidence for the existence of Q_L and Q_H; PS II$_\alpha$ and PS II$_\beta$ (Q_α and Q_β); Q_1 and Q_2 (or Xa); and Q_B, Q_B' and non-Q_B-type centers (see section 2). The Active (fast) and Inactive (slow) centers must be related to the above heterogeneity. In view of the 4 different types of heterogeneity, there are, in principle, at least 16 possible types of PS II, ignoring, for the time being Q_B' centers. However, certain types can be easily eliminated because of (1) the equivalence of most of PS II$_\beta$ with non Q_B-type centers in terms of the absence of 2-electron gate function and (2) the absence of evidence that Q_2, that is *not* a quinone, has two different redox potentials. See possible types of PS II in Eq. (3).

PS II$_\alpha$-Q_1-Q_H-Q_B-type is suggested to be in the main pathway of PS II and comprises most of the active centers. However, some Q_B' centers, that are included in the non Q_B-type in this scheme, may also be included in the active centers. On the other hand, most of the inactive centers include most of PS II$_\beta$ (with the possible exception of some Q_B' centers, included in PS II$_\beta$-Q_1-Q_H-non Q_B-type centers) and most or all of PS II$_\alpha$-Q_2-non Q_B type centers. Further research may reduce the number of heterogeneous centers predicted to exist here. It is now necessary to make all the measurements for the different types of centers on the same samples of appressed and unappressed mem-

$$\tag{3}$$
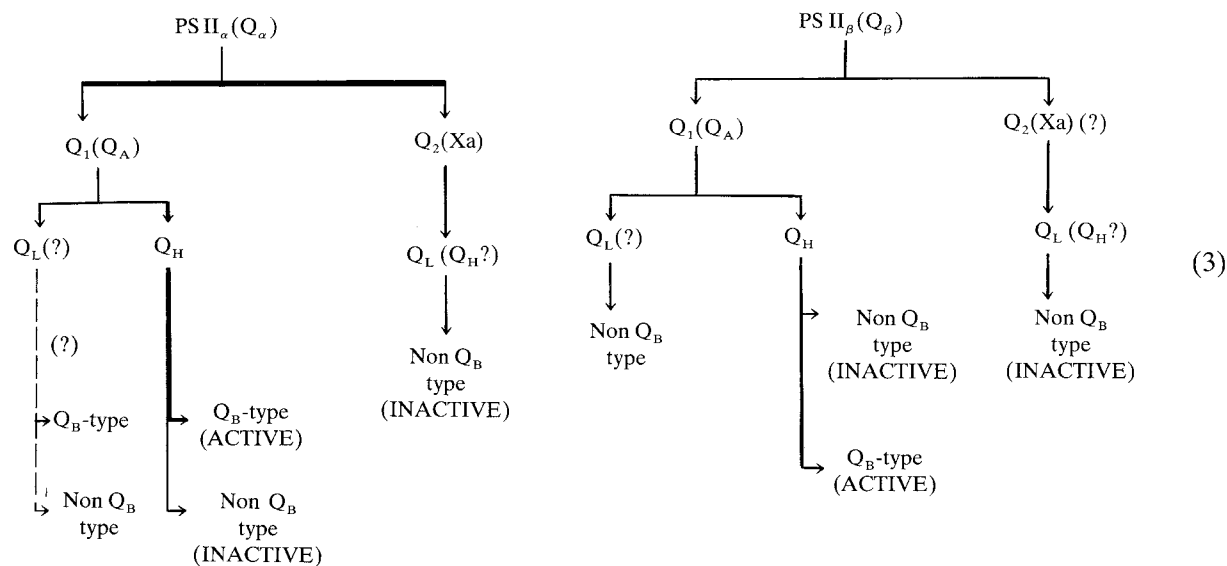
branes in order to obtain a clear picture of the heterogeneity of PS II acceptors. We must eliminate the possibility of heterogeneous samples before obtaining the final answer and we must test where and how Evans' U and Jursinic's Aq (Q318) fit (or do not fit) in this picture. Aq cannot be $Q_2(Xa)$ or Q_L due to its positive redox potential; Jursinic concludes that Aq is different from Q400 but one wonders if Q400 is also heterogeneous and if Q318 is a component of it.

Another question deals with the equivalence of Q_2 and Q_L (Joliot and Joliot 1983). We need to know $E_{m,7}$ of Q_2 before we can associate Q_2 only with Q_L. If this equivalence can be proven, we will be left with only 5 types of PS II centers: PS II_α–Q_1–Q_H–Q_B-type (the major type); PS II_α–Q_1–Q_H–non Q_B-type; PS II_α–Q_2–Q_L–non Q_B-type; PS II_β–Q_1–Q_H–non Q_A-type; and PS II_β–Q_2–Q_L–non Q_B-type.

4. Stoichiometry of PS II to PS I

It has always been assumed in the past that in higher plants, the ratio of PS II to PS I was 1. Whitmarsh and Ort (1984) showed that in spinach this ratio was indeed 1 when functional PS II was measured. On the other hand, Melis and coworkers (see e.g., Melis et al. 1988) reported a value of approximately 2 for PS II : PS I ratio based on the Q_A (for PS II) and P700 (for PS I) contents. Graan and Ort (1986) have provided an excellent solution to the difference between the ratios of 1 and 2 (see Ort and Whitmarsh 1990). As noted earlier, they have shown, using two types of quinone acceptors (DMQ and DCBQ), that about 60% of centers are active and accessible to DMQ. The other 40% that are inactive (slow) in reducing native plastoquinone efficiently donate electrons to DCBQ. Briantais et al. (1988) also suggest that the ratio of active PS II to inactive (slow) PS II is 60:40, but they believe that these centers are in the same antenna bed, not PS II_β. There is a general agreement that the P700 content is about 1.6 mmol per mol Chl. The total PS II, as measured by the content of and by terbutryn binding sites, is about 3.0 mmol per mol Chl suggesting that the ratio of total PS II to PS I is about 2, and

the ratio of active PS II to PS I is about 1. The former is confirmed in a recent measurement of the ratio of 1.9 ± 0.5 for P680/P700 assuming the existence of 2 pheophytin molecules/P680 (Kobayashi et al. 1988). On the other hand, the same ratio was 0.65 ± 0.20 in cyanobacteria. The ratio of functional RC2/RC1 was about 1.3 in the green alga *Chlorella* (Myers and Graham 1983). Thus, different organisms may have different PS II : PS I ratios, functional as well as total (see Chow et al. 1988, Jursinic and Dennenberg 1989).

The existence of inactive PS II may not have a significant effect on the maximum quantum yield of photosynthesis because of the smaller antenna size of PS II_β (Thielen and van Gorkom 1981c) and, perhaps, of other inactive units in PS II_α. Also, one should not ignore the possibility of excitation energy transfer from some inactive to active centers. A heterogeneity in PS II_α has recently been suggested to exist in appressed membranes (see Albertsson and Yu 1988). For a discussion of PS II heterogeneity through measurements on O_2/flash, in thylakoids and inside out vesicles, see Delrieu and Rosengard (1988).

5. Chlorophyll *a* fluorescence transient as an indicator of active and inactive PS II in soybean and spinach thylakoids

Melis (1985) measured the quenching of Chl *a* fluorescence rise (labeled as Fo to Fpl) by adding high concentrations (100–200 μM) of DMQ and TMQ in spinach thylakoids. Melis suggested that the Fo to Fpl rise is due to the prompt reduction of the primary plastoquinone Q_A of PS II_β (Q_β) and is the consequence of the inability of PS II_β to donate electrons, on a fast scale, to secondary electron acceptors. However, no firm evidence was provided on the basis of differential effects on PS II_α and PS II_β. As noted above, Graan and Ort (1986) showed that the number of PS II centers capable of active water oxidation was increased by μM concentration of DCBQ and closely matched the number of terbutryn binding sites. However, the number of PS II centers active in water oxidation was much lower when μM concentration DMQ was used as an electron acceptor. It was suggested that DCBQ-supported

water oxidation measures both the normally active (fast) and the inactive (slow) centers, whereas DMQ-supported water oxidation measures only the active centers. Cao and Govindjee (1990), using the protocol of Graan and Ort, have investigated the differential effects of DCBQ and DMQ on the Chl a fluorescence transient (OIDP, for a definition, see Munday and Govindjee, 1969). It was shown that approx. 1–2 μM DMQ quenched 50% of the DP phase, whereas 20 μM DMQ quenched 100% of the DP phase without any effect on the OID phase. In contrast, approx. 2.5–3 μM DCBQ quenched not only all the DP phase but also 50% of the OID phase, whereas 15 μM DCBQ quenched 100% of the OID phase. No effect on Fo was observed at these concentrations of DCBQ. Thus, 15–20 μM DCBQ addition can now be used to measure true Fo levels of thylakoids. These μM concentration dependencies of quinones on Chl a fluorescence rise phases OID and DP are in quantitative agreement with the observations of effects observed by Graan and Ort (1986) on inactive and active centers, respectively. Thus we suggest that the OID phase of Chl a fluorescence is a monitor of PS II inactive centers and DP phase that of PS II active centers. Such a proposal had also been made by Briantais et al. (1988) for *Chlamydomonas reinhardtii* cells treated with chloramphenicol.

Although fluorescence intensities cannot be used to assay proportions of PS II centers without conversion into [Q_A], they can be used to obtain qualitative information. Thus, we have used the idea of using the OID phase as a qualitative measure of inactive centers in studying the effect of elevated temperatures. Sundby et al. (1986) found that above 30°C there is a disassociation of peripheral light-harvesting chlorophyll a/b complex (LHC–II) from photosystem II and a migration of LHC–II to the PS I-rich non appressed thylakoid regions. This mimics the conversion of PS II$_\alpha$ into PS II$_\beta$ centers. Cao and Govindjee (1990) have shown that heating of spinach thylakoids at 55°C for 1 min raises the fluorescence level at 50 ms after illumination, and, that this raised level is quenched by 15–20 μM DCBQ, but not by 20 μM DMQ. These results suggest that heat treatment converts PS II active centers into PS II inactive cen-

ters among other things. We note that Buknov et al. (1990) have independently observed quenching of weak-light fluorescence in heated *Amaranthus* thylakoids by ferricyanide treatment.

Recently, Guenther et al. (1990), also using OIDP fluorescence transient, have shown that a light-dependent conversion of PS II$_\beta$, non-Q_B centers to PS II$_\alpha$, Q_B centers occurs in dark grown *Chlamydomonas reinhardtii*; the ratio of OID to DP phase was found to drastically decrease within an hour of illumination.

6. Concluding remarks

In order to clarify the PS II heterogeneities, it is recommended that parallel measurements be made on the existence and proportions of PS II$_\alpha$ and PS II$_\beta$; Q_1 and Q_2; Q_L and Q_H; Q_B- and non Q_B-type centers; and active and inactive centers on the same thylakoid samples, as well as separated appressed and unappressed membranes. Such a parallel study involving chlorophyll a fluorescence measurements, redox titrations, period 2 oscillations, and PS II activity with low concentrations of DMQ and DCBQ should yield information on the equivalence or non-equivalence of the various PS II heterogeneities. Care will have to be taken to differentiate between the dark-adapted state and the state in which the 'slow' centers are closed when evaluating these results.

The question of the ratio of active PS II to PS I seems to have been solved, in the first approximation, as far as mature spinach thylakoids are concerned. However, this does not preclude variations in this ratio or total PS II to PS I ratio under certain experimental conditions and in other organisms (see Chow et al. 1988, Jursinic and Dennenberg 1989).

Chlorophyll a fluorescence transient ratios for OID and DP phases may be used as a quick and approximate tool to monitor the ratio of inactive and active centers in thylakoids provided care is taken to monitor true 'O', 'I' and 'P' levels. Precise quantitation will involve inclusion of considerations such as the size of the antenna and the probability of energy transfer among individual PS II units. The use of 15–20 μM

158

DCBQ is suggested as a means of measuring true Fo that is necessary for the interpretation of most Chl *a* fluorescence transient data.

Acknowledgements

The author thanks the Interdisciplinary McKnight Grant for financial support, J. Cao for experiments described in section 5, Don Ort and Roger Chylla for discussions, and Trish Bright for typing this manuscript.

References

Albertsson P-Å and Yu S-G (1988) Heterogeneity among Photosystem II$_\alpha$. Isolation of thylakoid membrane vesicles with different functional antennae size of Photosystem II$_\alpha$. Biochim Biophys Acta 936: 215–221

Andréasson L-E and Vänngård T (1988) Electron transport in Photosystem I and II. Ann Rev Plant Physiol Plant Mol Biol 39: 379–411

Black MT, Brearley TH and Horton P (1986) Heterogeneity in chloroplast photosystem II. Photosynth Res 8: 193–207

Bouges-Bocquet B (1973) Electron transfer between the two photosystems in spinach chloroplasts. Biochim Biophys Acta 314: 250–256

Brettel K, Schlodder E and Witt HT (1985) Evidence for only one stable electron acceptor in the reaction center of Photosystem II in spinach chloroplasts. Photobiochem Photobiophys 9: 205-213

Briantais J-M, Cornic G and Hodges M (1988) The modification of Chlorophyll fluorescence of *Chlamydomonas reinhardtii* by photoinhibition and chloramphenicol addition suggests a form of photosystem II less susceptible to degradation. FEBS Lett 236: 226–230

Bukhov NG, Sabat SC and Mohanty P (1990) Analysis of chlorophyll *a* fluorescence changes in weak light in heat-treated *Amaranthus* chloroplasts. Photosynth Res 23: 81–87

Cao J and Govindjee (1989) Chlorophyll *a* fluorescence transient as an indicator of active and inactive Photosystem II in thylakoid membranes. Biochim Biophys Acta 1015: 180–188

Chow WS, Anderson JM and Hope AB (1988) Variable stoichiometries of Photosystem II to Photosystem I reaction centers. Photosynth Res 17: 277–281

Chow WS, Hope AB and Anderson JM (1989) Oxygen per flash from leaf disks quantifies Photosystem II. Biochim Biophys Acta 973: 105–108

Chylla RA and Whitmarsh J (1989) Inactive Photosystem II complexes in leaves: turnover rate and quantitiation. Plant Physiol 90: 765–772

Chylla RA, Garab G and Whitmarsh J (1987) Evidence for slow turnover in a fraction of Photosystem II complexes in thylakoid membranes. Biochim Biophys Acta 894: 562–571

Cramer WA and Butler WL (1960) Potentiometric titrations of the fluorescence yield of spinach chloroplasts. Biochim Biophys Acta 172: 503–510

Debus RJ, Barry BA, Sithole I, Babcock GT and McIntosh L (1988) Directed mutagenesis indicates that the donor to P680$^+$ in Photosystem II is tyrosine-161 of the D1 polypeptide. Biochemistry 27: 9071–9074

Delrieu M-J and Rosengard F (1988) Characterization of two types of oxygen-evolving Photosystem II reaction center by the flash-induced oxygen and fluorescence yield. Biochim Biophys Acta 936: 39–49

Demeter S and Govindjee (1989) Thermoluminescence in plants. Physiologia Plantarum 75: 121–130

Diner BA (1986) The reaction center of Photosystem II. In: Staehlin LA and Arntzen CJ (eds) Encyclopedia of Plant Physiology; Photosynthesis III (NS), Vol 19, pp 442–436. Springer-Verlag, Berlin

Diner B and Delosme R (1983) Oxidation-reduction properties of the electron acceptors of Photosystem II. I. Redox titration of the flash-induced caroteroid band shift, of C550 and of the variable fluorescence yield in spinach chloroplasts. Biochim Biophys Acta 722: 443–459

Eckert JH and Renger C (1980) Photochemistry of the reaction centers of system II under repetitive flash group excitation in isolated chloroplasts. Photochem Photobiol 31: 501–511

Evans MCW and Ford RC (1986) Evidence for two tightly bound iron quinones in the electron acceptor complex of Photosystem II. FEBS Lett 195: 290–294

Evans MCW, Atkinson YE and Ford RC (1985) Redox characterization of the Photosystem II electron acceptors. Evidence for two electron carriers between pheophytin and Q. Biochim Biophys Acta 806: 247–254

Govindjee (1984) Photosystem II: the oxygen evolving system of photosynthesis. In: Sybesma C (ed) Advances in Photosynthesis Research, Vol I, pp 227–238. Martinus Nijhoff/Dr W Junk, the Hague

Govindjee and Govindjee R (1975) Introduction to Photosynthesis. In: Govindjee (ed) Bioenergetics of Photosynthesis, pp 1–50. Academic Press, New York

Govindjee and Wasielewski M (1989) Photosystem II: from a femtosecond to a millisecond. In: Briggs GE (ed) Photosynthesis, C.S. French International Symposium. Alan Liss Publishers, N.Y., pp 71–103

Govindjee, Amesz J and Fork DC (eds) (1986) Light Emission by Plants and Bacteria. Academic Press, Orlando FL

Graan T and Ort DR (1986) Detection of oxygen-evolving Photosystem II centers inactive in plastoquinone reduction. Biochim Biophys Acta 852: 320–330

Guenther JE and Melis A (1989) The physiological significance of photosystem II heterogeneity in chloroplasts. Photosynth Res 23: 105–109

Guenther JE, Nemson JA and Melis A (1988) Photosystem II stoichiometry and chlorophyll antenna size in *Dunaliella salina* (green algae). Biochim Biophys Acta 934: 108–117

Guenther JE, Nemson JA and Melis A (1990) Development of Photosystem II in dark grown *Chlamydomonas reinhardtii*. A light dependent conversion of PS II$_\beta$. Q$_B$-nonreducing centers to the PS II$_\alpha$, Q$_B$ reducing form. Photosynth Res 24: 35–46