Chlorophyll fluorescence, thermoluminescence, and EPR spectroscopy have been used to investigate the functional properties of the monomeric and dimeric forms of the photosystem II CP47-reaction center (CP47-RC) subcore complex that was isolated (Zheleva, D., Sharma, J., Panico, M., Morris, H. R., and Barber, J. (1998) J. Biol. Chem. 273, 16122–16127). Chlorophyll fluorescence yield changes induced either by the initiation of continuous actinic light or by repetitive light flashes indicated that the dimeric, but not the monomeric, form of the CP47-RC complex showed secondary electron transport properties indicative of QA reduction. Thermoluminescence measurements also clearly distinguished the monomer from the dimer in that the latter showed a Z2 band, which appeared at ~55 °C, following illumination at ~80 °C. This band has been determined to be an indicator of the photoaccumulation of QA'. The ability of the dimeric CP47-RC to show secondary electron transport properties was clearly demonstrated by EPR studies. The dimer was characterized by organic radical signals at about g = 2 induced either by illumination or by the addition of dithionite. The dithionite-induced signal was attributed to QA', but there was no indication of any interaction with non-heme iron. The signal induced by light was more complex, being composed not only of the QA' radical but also of radicals generated on the donor side. Difference analyses indicated that one of these radicals is likely to be due to a D1 tyrosine 161 or D2 tyrosine 161. In contrast, the monomeric CP47-RC complex did not show similar EPR-detectable radicals and instead was dominated by a high yield of the spin-polarized triplet signal generated by recombination reactions between the oxidized primary reductant, pheophytin, and the primary donor, P680. It is also concluded from EPR analyses that both the monomeric and dimeric forms of the CP47-RC subcore complex contain one cytochrome b589 per reaction center. Overall the results suggest that photosystem II normally functions as a dimer complex and that monomerization at the level of the CP47-RC subcore complex leads to destabilization of the bound plastoquinone, which functions as QA.

More than 25 different protein subunits make up the photosystem II (PSII) complex of oxygenic photosynthetic organisms (1). At the heart of this complex is the reaction center (RC) consisting of the D1 and D2 proteins, where primary charge separation occurs (2). Closely associated with the D1 and D2 proteins are two similar chlorophyll a-binding proteins, CP43 and CP47 (3). These proteins serve as an “inner antenna” system that is linked to a secondary light-harvesting system. In higher plants and green algae, the chlorophyll ab-binding proteins (encoded by nuclear located cab genes) act as the secondary light-harvesting system, while phycobilisomes serve the same purpose in other types of oxygenic photosynthetic organisms, such as red algae and cyanobacteria (4). CP43 and CP47 are also distinguished by having a large hydrophilic loop linking putative membrane-spanning regions 5 and 6 (3). These loops are almost certainly located on the luminal surface of the complex and may function in water splitting in some way (5). Treatments with detergents can peel away the various subunits, and it has been shown that during such manipulations CP43 is more readily removed than CP47 (6, 7). It is therefore possible to isolate a CP47-RC complex. Recently a method was described for spinach that yielded a preparation of the CP47-RC complex consisting of a mixture of monomeric and dimeric forms (8). Analyses using mass spectrometry showed that both forms of this subcore complex contained the products of the psbE, psbF, psbI, psbT, and psbW genes as well as the D1 and D2 proteins and CP47. However, the CP47-RC dimer contained, in addition, the products of the psbL and psbK genes. Also of significance was the finding that the dimer and not the monomer contained about one molecule of plastoquinone-9 per RC. Overall, the findings suggest that the monomeric form of the isolated CP47-RC complex was derived by dissociation of the dimer and that the latter conformation is likely to be the in vivo state. Moreover, the finding that the CP47-RC complexes contain several small proteins with putative one transmembrane helices is relevant to de-

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tailed structural studies that are under way on the complex using electron crystallography (8, 9).

In this paper, we characterize the functional properties of the CP47-RC complex isolated from spinach and, in particular, identify differences between the monomeric and dimeric forms. We also address the question of the stoichiometry of cytochrome b_{559} in these preparations.

MATERIALS AND METHODS

Isolation of Monomeric and Dimeric CP47-RC Complexes—The method of obtaining isolated CP47-RC in monomeric and dimeric forms from spinach thylakoids involves solubilization of PSII-enriched membranes using the detergents n-dodecyl β-D-maltoside and heptyl thioglycolysanide, followed by Fractogel ion exchange chromatography and sucrose gradient centrifugation. The procedures have been given in detail previously (8).

Fluorescence Measurements—Relative chlorophyll fluorescence yields and time-dependent changes in the yield of chlorophyll fluorescence were measured using a PAM fluorometer (WALZ, Effeltrich, Germany). Samples of 30 μg of Chl ml⁻¹ were illuminated with either continuous, red, actinic light (800 μE m⁻² s⁻¹) or single turnover, saturating xenon flashes. The intensity of the modulated measuring light was 8 μE m⁻² s⁻¹, and the ms induction curves were analyzed using a QA Data acquisition software package (10). In the case of flash excitation, 20 fluorescence traces were averaged in order to increase the signal/noise ratio. Samples were dark-adapted before the measurements for 3 min, and for flash excitation the subsequent flashes were separated by 20-s dark intervals.

Thermoluminescence Measurements—Thermoluminescence curves were measured with a home-built apparatus as described earlier (11). Samples, containing 40 μg of Chl, were dark-adapted for 3 min and then cooled to ~80 °C in darkness. Illumination with continuous white light of 10 watts m⁻² was administered at ~80 °C for 30 s, followed by heating in the dark at a constant heating rate of 20 °C min⁻¹.

EPR Spectrometry—For EPR, 0.3–0.4-ml samples (about 0.3 mg of Chl) were placed in calibrated 3-mm quartz EPR tubes. Dim green lighting was used to minimize photodamage to samples. Identical sets of samples in calibrated EPR tubes were made for each experiment. Samples were dark-adapted for a minimum of 30 min on ice before being frozen to 77 K in the dark. For chemically reduced samples, the addition of dithionite (25 μM/sample) was made from a freshly made concentrated stock solution (1 g/50 ml) in 100 mM Tris/HCl buffer bubbled with oxygen-free nitrogen. During experiments, samples were illuminated at a variety of temperatures from 4 to 273 K as indicated. Illumination in the EPR cavity at ~30 K was accomplished using a 150-watt light source and fiber-optic light guide, while other illumination of the 1000-watt light source, protecting the sample from heating where necessary by a 5-cm water filter. Samples for freezing under illumination to 200 K were made using an ethanol/dry ice bath in a clear glass Dewar. The samples were then quickly transferred to liquid nitrogen in the dark.

Samples were examined by EPR at cryogenic temperatures using a Jeol RE1X X-band spectrometer (9.055 GHz) with 100-kHz modulation and fitted with an Oxford Instruments cryostat. EPR conditions are given in the figure legends. Temperatures within the EPR cryostat were measured with a calibrated thermocouple beneath the sample. Spectra were recorded and manipulated using a Dell microcomputer running Asyst software. No filtering, smoothing, fitting, or background subtraction were required. The vertical scale in figures showing first derivative EPR spectra is arbitrary, with spectra at the same instrument gain unless stated in the figure legend.

The cytochrome b_{559} content of CP47-RC monomers and dimers was measured by reference to a sample of the D1-D2-cytochrome b_{559} RC complex (5.9 Chl/2 Pheo, 242 μg of Chl ml⁻¹). It is assumed that this preparation had one cytochrome b_{559} per reaction center (12). The integrated area of the gₚ peak of this sample was compared with that of two preparations each of PSII monomers (17 and 18.2 Chl/2 Pheo) and dimers (20.8 and 23 Chl/2 Pheo). Accurate measurement of both the chlorophyll concentration and the Chl/Pheo ratios then enabled the ratio of cytochrome b_{559} in the PSII monomers and dimers to be calculated. The chlorophyll levels were measured as described previously (8, 13).

RESULTS

Chlorophyll Fluorescence—CP47-RC monomers and dimers were analyzed by measuring variable chlorophyll fluorescence yield to detect their electron transfer activities at room temperature. Fig. 1 shows photoinduced changes of PSII chlorophyll fluorescence yield (ΔF) of dimeric and monomeric CP47-RC subcore complexes. The results clearly show that the quinone within the dimeric form of the complex can act as a secondary electron acceptor indicative of QA activity and because of this exhibits fluorescence characteristics distinctly different from those of the monomeric form of the complex. Saturation actinic light in the absence of any additions induced an increase of PSII chlorophyll fluorescence yield (ΔF) of dimeric CP47-RC (Fig. 1, curve 1). The ΔF/ΔPₚ (Fₚ, so called “constant” F) ratio was about 3, which is similar to the ratio of PSII-enriched membranes (BBYs). The magnitude of ΔF did not increase in the presence of PSII artificial electron donors (data not shown). The real magnitude of Fₚ was confirmed using K-15, a compound that selectively quenches the part of the PSII chlorophyll emission that is due to recombinant luciferase (14). When the actinic light was turned off, the dark relaxation of the ΔF (reflecting the reoxidation of the primary electron acceptor of PSII, QA⁻) was significantly slower for the dimeric CP47-RC compared with that of BBYs (Fig. 1, curves 1, dashed line); less than 20% of the maximal level ΔF was quenched after 150 s in the dark. However, repeated measurements of ΔF on the same CP47-RC sample (after achieving complete reoxidation of QA⁻ in the dark) resulted in a lowering of ΔF. Subsequent additions of artificial electron donors of PSII, Mn⁴⁺ (0.1–20 μM), sodium ascorbate (2 mM), diphenylcarbazide (1 mM), and NH₄OH (1 mM) to the sample did not restore the initial level of photochemical activity (data not shown).

Fig. 1, curve 2, shows the photoinduced chlorophyll fluorescence yield of the dimeric CP47-RC subcore complex measured in the presence of 1 mg ml⁻¹ sodium dithionite, which chemically reduces QA in the dark. In this case, the fluorescence rose to its maximal level (Fₚ) when the sample was illuminated only with measuring light, whereas the application of saturating actinic light caused a reversible decrease of Fₚ (to the level Fₚ,R), related to the photoreduction of pheophytin, as shown earlier.
Comparison of Monomeric and Dimeric CP47-RC Complex

The fluorescence behavior of the monomeric CP47-RC subcore complex differed significantly from dimeric CP47-RC as well as from PSII-enriched membranes and was similar to that of D1-D2-cytochrome $b_{559}$ RC complex. In the absence of actinic light and any additions, switching on the weak measuring light induced an increase of $F$ to its maximal level, $F_m$ (Fig. 1, curve 3), as is also observed for the D1-D2-cytochrome $b_{559}$ RC complex (Fig. 1, curve 5). No positive increase of PSII chlorophyll fluorescence yield was observed when the saturation actinic light was switched on. However, in contrast to the RC preparations, where a short illumination (a few tens of seconds) with saturating actinic light did not induce any significant quenching of $F_m$ in the absence of sodium dithionite (Fig. 1, curve 5), in the monomeric CP47-RC it did induce a significant quenching of $F_m$ (Fig. 1, curve 3). In the presence of sodium dithionite, however, the magnitudes of $F_m$ quenching isolated RC and CP47-RC complexes were comparable (Fig. 1, curves 4 and 6). The addition of a range of PSII artificial electron donors did not induce a $\Delta F$ response (not shown). The addition of K-15 to the monomeric CP47-RC preparation caused quenching of $F$ to the $F_o$ level of the dimeric form of the complexes (data not shown). In the presence of sodium dithionite, illumination of the monomeric CP47-RC by actinic light resulted in fast quenching of $F_m$ to the level of $F_o$ (Fig. 1, curve 4). The time course of the quenching coincides with those recorded for both the dimeric CP47-RC and RC complexes, and the $F_o$ level corresponded to that obtained in the presence of K-15. Also of note is that the light-induced $\Delta F$ quenching in the presence of dithionite for all samples is reversible when the illumination temperature is switched off. However, in contrast to the RC preparations, where a short illumination (a few tens of seconds) with saturating actinic light did not induce any significant quenching of $F_m$ in the absence of sodium dithionite (Fig. 1, curve 5), in the monomeric CP47-RC it did induce a significant quenching of $F_m$ (Fig. 1, curve 3). In the presence of sodium dithionite, however, the magnitudes of $F_m$ quenching isolated RC and CP47-RC complexes were comparable (Fig. 1, curves 4 and 6). The addition of a range of PSII artificial electron donors did not induce a $\Delta F$ response (not shown). The addition of K-15 to the monomeric CP47-RC preparation caused quenching of $F$ to the $F_o$ level of the dimeric form of the complexes (data not shown). In the presence of sodium dithionite, illumination of the monomeric CP47-RC by actinic light resulted in fast quenching of $F_m$ to the level of $F_o$ (Fig. 1, curve 4). The time course of the quenching coincides with those recorded for both the dimeric CP47-RC and RC complexes, and the $F_o$ level corresponded to that obtained in the presence of K-15. Also of note is that the light-induced $\Delta F$ quenching in the presence of dithionite for all samples is reversible when the illumination temperature is switched off. However, in contrast to the RC preparations, where a short illumination (a few tens of seconds) with saturating actinic light did not induce any significant quenching of $F_m$ in the absence of sodium dithionite (Fig. 1, curve 5), in the monomeric CP47-RC it did induce a significant quenching of $F_m$ (Fig. 1, curve 3). In the presence of sodium dithionite, however, the magnitudes of $F_m$ quenching isolated RC and CP47-RC complexes were comparable (Fig. 1, curves 4 and 6). The addition of a range of PSII artificial electron donors did not induce a $\Delta F$ response (not shown). The addition of K-15 to the monomeric CP47-RC preparation caused quenching of $F$ to the $F_o$ level of the dimeric form of the complexes (data not shown). In the presence of sodium dithionite, illumination of the monomeric CP47-RC by actinic light resulted in fast quenching of $F_m$ to the level of $F_o$ (Fig. 1, curve 4). The time course of the quenching coincides with those recorded for both the dimeric CP47-RC and RC complexes, and the $F_o$ level corresponded to that obtained in the presence of K-15. Also of note is that the light-induced $\Delta F$ quenching in the presence of dithionite for all samples is reversible when the illumination temperature is switched off.

The capability of CP47-RC dimer preparation to reduce Q$_A$ was probed further by measuring the kinetics of chlorophyll fluorescence yield changes when induced by single turnover saturating flashes. As shown in Fig. 2, there was no flash-induced fluorescence yield change in the monomers, as expected from the steady-state fluorescence measurements shown in Fig. 1. In the dimers, however, a flash-induced rise of fluorescence yield was clearly observed, indicating that Q$_A$ can be reduced by a single flash (Fig. 2, curve B). The subsequent decay of the fluorescence yield reflects reoxidation of the semiquinone anion radical Q$_A^-$ which is characterized by a few hundred-ms time constant in the dimers. This decay time is much slower than the 400–500-μs and 3–4-ms values assigned to reoxidation of Q$_A^-$ by forward electron flow to Q$_B$ in fully functional PSII particles or BBVs (see Fig. 1) (16). In addition, the presence of DCMU, which blocks electron transfer between Q$_A$ and Q$_B$, had no effect on the flash-induced fluorescence yield change in the monomers (Fig. 2, curves 4 and 6). The addition of a range of PSII artificial electron donors did not induce a $\Delta F$ response (not shown). The addition of K-15 to the monomeric CP47-RC preparation caused quenching of $F$ to the $F_o$ level of the dimeric form of the complexes (data not shown). In the presence of sodium dithionite, illumination of the monomeric CP47-RC by actinic light resulted in fast quenching of $F_m$ to the level of $F_o$ (Fig. 1, curve 4). The time course of the quenching coincides with those recorded for both the dimeric CP47-RC and RC complexes, and the $F_o$ level corresponded to that obtained in the presence of K-15. Also of note is that the light-induced $\Delta F$ quenching in the presence of dithionite for all samples is reversible when the illumination temperature is switched off.
Comparison of Monomeric and Dimeric CP47-RC Complex

Fig. 4. EPR spectra showing the effects of illumination at cryogenic temperatures. Left, light-minus-dark difference spectra at 4.2 K showing the yield of spin-polarized reaction center triplet in CP47-RC dimers (1.002 mg of Chl/ml) (A) and CP47-RC monomers (0.776 mg of Chl/ml) (B). EPR conditions were as follows: microwave power, 40 microwatts; modulation amplitude, 2 mT. Right, spectra near g = 2, taken dark and then dark after 2-min illumination at 8 K of CP47-RC dimers (C) and CP47-RC monomers (D). EPR conditions were as follows: microwave power, 1 microwatts; modulation amplitude, 0.2 mT. For better comparison between monomer and dimer samples, spectra were corrected for the difference in chlorophyll concentration. For further details, see “Materials and Methods.”

The Zs band may not directly originate from QA but is enhanced by its presence. This possibility is reinforced by the fact that a small Zs band has also been observed with the D1-D2-cytochrome b559 RC complex having no plastoquinone present (11).

EPR Spectrometry—CP47-RC monomers and dimers were also analyzed by EPR to detect their electron transfer capabilities at cryogenic temperatures. Fig. 4 shows that the CP47-RC monomers gave a high yield of spin-polarized reaction center triplet (Fig. 4B) compared with PSII dimers (Fig. 4A). The high yield of spin polarized triplet observed in the monomer is reminiscent of that recorded previously with the isolated D1-D2-cytochrome b559 RC complex (19). The CP47-RC dimers showed a maximum of 12.5% triplet yield compared with the monomers. The g = 2 region of the EPR spectrum was examined for organic radicals. The formation of a radical by illumination showed that the CP47-RC dimers were capable of stable charge separation (Fig. 4C), while the monomers showed much less activity (Fig. 4D). No tyrosine radical was present, and no rapidly reversible light-induced signals were detected in either preparation. The light-induced g = 2.003 signal (Fig. 4C) in CP47-RC dimers had characteristics suggesting it was a mixture of at least two radical species. Taken together, these results indicate that the CP47-RC monomers show a similar capability to the D1-D2-cytochrome b559 RC complex with electron transfer at cryogenic temperatures restricted to the forward and back reactions between the primary donor P680 and primary acceptor phaeophytin (19, 20). However, CP47-RC dimers are capable of electron transfer beyond phaeophytin, thereby reducing the triplet yield and producing the g = 2 radical on illumination.

Fig. 5, A and B, shows that low spin cytochrome b559 was present in both CP47-RC monomers and dimers, the latter having a broader gss peak near 220 mT. In both preparations, the cytochrome b559 was in the low potential, fully autoxidized form, and no light-induced changes were observed. As outlined under “Materials and Methods,” the content of cytochrome b559 was estimated by comparison with the D1-D2-cytochrome b559 RC preparation, which is assumed to have one heme per reaction center (13). This comparison shows that CP47-RC monomer preparations (0.92 and 0.81 per RC) and dimers (0.97 and 0.90 per RC) had slightly less than one cytochrome b559 per RC. An additional peak near g = 6 was observed in both monomer and dimer preparations (not shown). This was larger in the preparations with lower ratios and may indicate that some conversion of low spin heme to high spin heme occurs. We conclude that there is one cytochrome b559 per reaction center in both preparations.

The EPR results confirm the data obtained from chlorophyll fluorescence and thermoluminescence indicating that the additional electron transfer component is present in the dimeric form of the CP47-RC complex and that this component is likely to be a plastoquinone functioning as QA. However, careful analysis of EPR radical spectra attributed to this component showed almost no formation of any type of iron-semiquinone EPR signal (21–23). The use of PSII membranes (i.e. BBY-type) of similar reaction center concentration was used to confirm that a <10% reaction center concentration of iron-semiquinone would have been detected. A possible explanation was that the non-heme iron, normally located between the QA and Qb sites in PSII, was absent in the CP47-RC dimer.

Fig. 5 (right side) shows that in CP47-RC dimers following chemical reduction, an organic radical was detected at g = 2. The radical (Fig. 5C) had Hpp = 0.95 mT, g = 2.005 (where Hpp represents peak to trough line width of the EPR spectrum) and was microwave power-saturated above 1 microwatts. This is characteristic of an anionic semiquinone such as might be expected if QA were present but the non-heme iron was absent. A similar signal was observed in PSII membranes treated with cyanide to remove the interaction between QA and the non-heme iron (24, 25). By contrast, the PSII monomer sample shows no chemically induced radical under these conditions (Fig. 5D).

The results of Figs. 4 and 5 therefore show that CP47-RC dimers retain QA and are capable of electron transfer to it, forming the semiquinone. The non-heme iron seems to have been lost, showing that this cofactor can be removed before QA and before isolation of the D1-D2-cytochrome b559 RC complex, where it is known to be absent (26). The mixture of radicals obtained in Fig. 4C can now be assigned to the QA semiquinone and the chlorophyll/carotenoid usually found to be an electron donor at these temperatures (19, 20). The presence of QA as electron acceptor may allow other electron donors to function. No tyrosine radical was detected in dark-adapted samples of PSII monomers or dimers. However, by freezing a PSII dimer sample under illumination, a complex spectrum at g = 2 was...
obtained (Fig. 6A), which decayed rapidly upon dark adaptation at 273 K (Fig. 6B). The presence of a QA semiquinone radical superimposed on signals from electron donors such as chlorophyll makes analysis of this complex spectrum difficult. However, a crude subtraction of the signal from the QA semiquinone (chemically reduced; Fig. 6C), assuming an equal yield from chemical and photochemical reduction, produces Fig. 6D. Comparison with the characteristic spectrum of the tyrosine YD radical from PSII membranes (Fig. 6E) shows that hyperfine peaks corresponding to those of the tyrosine radical are observed in Fig. 6D. This indicates that a tyrosine radical can be trapped in PSII dimers in addition to the narrower Chl$^\pm$ or Car$^+$ species, producing the mixed spectrum in Fig. 6D. Since the spectra of tyrosine YZ and YD radicals are very similar, we cannot distinguish between these species.

**DISCUSSION**

Chemical analyses has previously shown that the dimeric, but not the monomeric, form of the isolated CP47-RC complex contained plastoquinone-9 measured to be 1.5 ± 0.3 molecules/RC (8). The fluorescence, thermoluminescence, and EPR data presented in this paper show that this plastoquinone can act as a secondary electron acceptor, indicating that the dimeric form of this complex is closer to being a functional PSI complex than the monomeric form. Indeed, the CP47-RC dimer contains more protein subunits than the monomer (see Introduction) and binds slightly more chlorophyll (21 ± 2.5 compared with 18 ± 1.5 molecules/RC (see accompanying paper (8)). However, the EPR data show that the signal due to photoinduced plastoquinone reduction in the dimer is not that of the iron-semiquinone typically found in more intact PSI systems. It seems highly likely that although the CP47-RC maintains QA activity, the complex has lost its non-heme iron during isolation or that the iron-quinone interaction has been disturbed. The monomeric complex, which lacks QA, is capable of primary charge separation and charge recombination between the radical pair P680$^-$ Pheo$^-$ as shown by the high fluorescence yield in weak measuring light and by the high yield of spin-polarized P680 triplet detected by EPR.

The capability of CP47-RC dimers to reduce QA upon illumination in the absence of added exogenous electron donors implies the presence of active endogenous donor(s) in the complex. The dimers do not evolve oxygen and thus do not contain a functional manganese cluster that serves as a final electron donor for QA reduction in active PSI centers. Treatment of the dimeric complexes with EDTA plus Tris, which completely removes manganese from PSI, had no effect on the variable fluorescence (not shown), excluding the possibility that some residual, nonfunctional manganese could act as electron donor. In the case of the D1-D2-cytochrome b$_{559}$ RC complex, the accessory chlorophylls and $\beta$-carotene can donate electrons to P680$^+$ (27, 28). The observation that the fluorescence relaxation curves can be induced by repetitive flashes (Fig. 2) indicates that the endogenous donor rapidly recovers after its oxidation. This makes it very unlikely that the accessory...
chlorophylls or β-carotene are the only endogenous electron donors, since they are quite stable in the oxidized state. The possibility that cytochrome b558 acts as a donor is also unlikely, given that this heme protein is present in its low potential oxidized state in both forms of the complex. Since the EPR data indicate light-induced formation of a tyrosine radical (Fig. 6), which could be either Yz* or Yb*, it is more likely that Tyr-Z, which has a much shorter lifetime in the oxidized radical form than Tyr-D, is the electron donor for QA reduction. In that case, the relaxation of the flash-induced fluorescence yield increase would correspond to a reoxidation of QA* via recombination with Yb*. This idea is in agreement with the 600-ms relaxation time of Yz* and a similar reoxidation half-time of QA* in Ca2+-depleted PSII particles, which, like the CP47-RC dimers, lack a functional manganese cluster and have impaired QA to Qb electron transfer (16). Thus, the detection of a tyrosine radical in the CP47-RC dimer indicates that this complex is functionally active on the donor side as well as the acceptor side. The perturbations in its functions compared with more intact PSII systems are probably linked to the absence of CP43 and the extrinsic proteins of the oxygen-evolving complex as well as to the loss of the non-heme iron from the acceptor side and the manganese cluster from the donor side. Furthermore, a distinct difference between the fluorescence properties of the dimeric CP47-RC and BBYs was the fast oxidation of QA in BBYs compared with the CP47-RC dimer when the actinic light was turned off (Fig. 1, trace 1). This is expected, since BBYs maintain a plastoquinone pool and Qb activity.

There has been discussion as to whether PSII normally exists as a monomer or dimer in vivo (1). Although the experiments presented in this paper were not conducted to enter into this debate, they do strongly suggest that the PSII complex in the membranes from which the CP47-RC complex was isolated are dimeric. The starting point for the isolation procedure are PSII-enriched membranes of the BBY type (29). These membranes are derived from the grana that contain the majority of PSII-enriched membranes of the BBY type (29). These membranes are dimeric. The starting point for the isolation procedure are the membranes from which the CP47-RC complex was isolated this debate, they do strongly suggest that the PSII complex in

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