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The primary reaction of photosystem II in the D1–D2–cytochrome *b*-559 complex

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Flash-induced absorbance changes in the picosecond and nanosecond time range have been measured in the 'D1–D2–cyt *b*-559 complex' isolated from photosystem II membranes. The results indicate the efficient formation of the primary radical pair $P680^+$ pheophytin$^-$, which had a lifetime of about 36 ns, and the presence of unconnected chlorophyll in this preparation. It is concluded that the complex contains the active photosystem II reaction center, and that this reaction center contains at most 4 chlorophyll *a* molecules.

Reaction center; Photosystem II; Primary reaction; Picosecond spectroscopy

1. INTRODUCTION

Until recently nearly all available evidence seemed to indicate that a 47 kDa chlorophyll-protein is the site of the reaction center of PS II, but this notion has been challenged on the basis of the homology between the D-1 and D-2 proteins and the L and M subunits of the reaction center of purple bacteria (see [1,2] for detailed discussion). The D-1 protein is the well-known herbicide-binding or Q_B protein; the D-2 protein is another intrinsic protein in the 30–40 kDa range, also present in all PS II core preparations. No PS II activity without the 47 kDa protein has been reported, but on the other hand the presence of the D-1 and D-2 proteins in active PS II preparations has never been rigorously excluded [3]. The latter approach was followed by (Ka.) Satoh [4], who recently reported the photoreduction of Q_A in a cyanobacterial PS II particle apparently devoid of the D-1 and D-2 proteins, whereas the former approach was taken by Nanba and (Ki.) Satoh [5], who isolated a pigmented complex from spinach PS II containing only the D-1, D-2 and cytochrome *b*-559 polypeptides [5], the material used here.

This complex was highly enriched in pheophytin *a*, which is known to act as the first electron acceptor in PS II [6–8]. The reversible reduction of about half of the pheophytin was observed upon illumination in the presence of dithionite and methyl viologen. Per 2 pheophytin *a* the preparation contains 4–6 Chl *a*, about 1 β-carotene, and 1–2 cytochrome *b*-559 heme. No plastoquinone could be detected, so Q_A, and perhaps also the secondary donor Z, must be lost if this preparation contains the PS II reaction center. In the absence of secon-

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Abbreviations: Chl, chlorophyll; PS, photosystem; P680, primary electron donor in PS II; Q_A, permanently bound plastoquinone acting as secondary electron acceptor in PS II

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dary donors or acceptors the photochemical activity of the complex should be limited to the formation of the primary radical pair, $P680^+$ pheophytin$^-$, which most likely decays by charge recombination. A light-induced spin-polarized triplet state observed at low temperature in this preparation already indicated the occurrence of a radical pair recombination [9]. We have tried to observe the $P680^+$ pheophytin$^-$ radical pair directly by time-resolved absorbance difference spectroscopy.

2. MATERIALS AND METHODS

The D1–D2–cytochrome *b*-559 complex was isolated as in [5] from a spinach PS II membrane preparation [10]. Before measurement the particles were diluted to an absorbance at 673 nm of 1.7 per cm in 50 mM Tris buffer (pH 7.2), 10% glycerol, and 0.05% Triton X-100 to prevent aggregation. The sample was kept at about 5°C during the measurements and could be used for several hours under 1 Hz flash illumination with little decrease in photochemical activity.

The picosecond absorbance difference measurements were performed with the spectrometer described in [11], using an optical multichannel analyser (OMA-2, EG&G) for detection as in [12]. Additionally, parametric oscillation and frequency doubling [13] were used to generate a 695 nm excitation pulse and, for the kinetic measurements of fig.2, probe pulses at 450 and 680 nm; for the spectral measurements of fig.1, a 'white' probe pulse was generated by focussing the 1064 nm laser output in a D_2O/H_2O mixture. The duration of the exciting and probing pulses was 25 ps, except that of the white pulse which was about 30 ps. The signal-to-noise ratio was enhanced by accumulating 60–150 measurements. To avoid possible effects of slow changes in the sample, the absorbance with and without excitation was measured alternatingly.

3. RESULTS AND DISCUSSION

Fig.1 shows the absorbance difference spectrum of the D1–D2–cyt *b*-559 complex in the region 430–685 nm at 4 ns after a non-saturating 695 nm flash, and around 545 nm also after 24 ns (inset, dashed line). These spectra show features at-

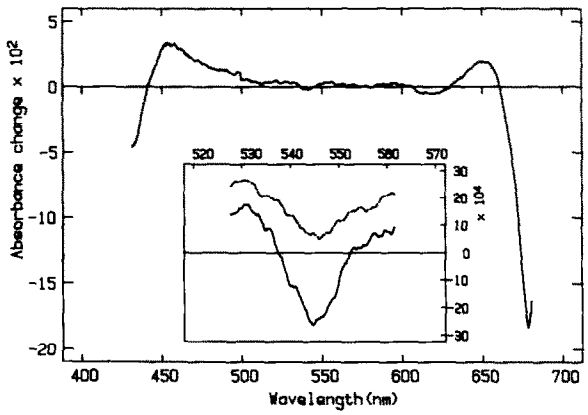

Fig.1. Absorbance difference spectra of the D1–D2–cyt *b*-559 complex 4 ns after excitation by a 25 ps, 695 nm pulse. The flash energy density was about 10^{15} photons/cm^2; the absorbance of the sample was less than 0.3 at the excitation wavelength. (Inset) The 545 nm region enlarged; the dashed line shows the difference spectrum at 24 ns after excitation.

tributable to the reduction of pheophytin *a*. In particular, the negative band at 545 nm (inset) and the positive band at 450 nm strongly suggest that the same absorbance changes which are observed upon photoaccumulation of reduced pheophytin also contribute to this difference spectrum. In addition, one would expect to see the absorbance changes due to P680 oxidation, but unfortunately these are not very distinctive. Large negative changes around 680 and 430 nm are indeed observed and actually the bleaching at 680 nm is larger than might be expected from pheophytin reduction and a stoichiometric amount of P680 oxidation, suggesting that some other Chl *a* must be involved as well. These data show that the photoreduction of pheophytin takes place as a primary process in these particles, and are consistent with the expectation that a Chl *a* absorbing at 680 nm is the electron donor involved.

The kinetics of the absorbance change at 450 nm are shown in fig.2 (●). The rise took place within the 25 ps flash duration (not shown); the decay was exponential with a best-fitting lifetime of 36 ns. This is remarkably longer than the 2–4 ns radical pair lifetimes reported for PS II [7,8], but not unexpectedly: both the presence of Q_A^- and that of antenna Chl *a* might cause a decrease of the observed lifetime. The open circles show the

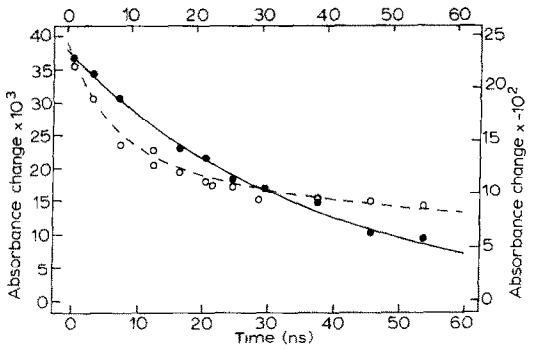

Fig.2. Kinetics of the absorbance changes at 450 nm (●, right-hand scale) and 680 nm (○, left-hand scale). The solid line shows the best-fitting single exponential, decay time (1/*e*) 36 ns. The kinetics at 680 nm were simulated as the sum of two exponentials, the second one with a given 36 ns lifetime, and a stable component; the best-fitting curve (dashed line) has a 5.5 ns lifetime for the first exponential, and relative amplitudes of 0.37, 0.36 and 0.27 for the successive components.

kinetics at 680 nm, plotted upside down to facilitate comparison with the 450 nm data. The recovery is at least biphasic: a rapid phase of about 5 ns is observed, which was not detectable at 450 nm (or 545 nm, not shown), and part of the bleaching appeared to be stable on the time scale studied. Assuming the presence of a 36 ns phase yielded a best fit with a lifetime of 5.5 ns for the fast phase and relative amplitudes of 0.37, 0.36 and 0.27 for the 5.5 ns, 36 ns and long-lived components, respectively. We ascribe the 5 ns phase to the decay of the singlet excited state of some unconnected Chl *a*. This pigment does not transfer its excitation energy to P680, since the lifetime corresponds to that of Chl *a* and this phase was observed also at flash energies well below that required to saturate pheophytin reduction. The relative contribution of the 5 ns phase was larger at 675 than at 680 nm, but the data did not allow an unambiguous spectral deconvolution. The long-lived component presumably is due to the formation of Chl *a* triplet states, both by intersystem crossing in the unconnected Chl and by radical pair recombination in P680.

The quantum yield of pheophytin reduction upon 695 nm excitation was determined by measuring the 680 nm bleaching as a function of the absorbed excitation energy. Based on a differential extinction coefficient of 8 $mM^{-1} \cdot cm^{-1}$ [14] for the bleaching of the 545 nm band upon pheophytin reduction, a quantum yield of about 65% was obtained. What percentage of the absorbed 695 nm quanta is absorbed by the unconnected chlorophyll cannot be determined, because the differential extinction coefficient upon its excitation is uncertain, but evidently it is far from negligible. Thus the quantum yield of pheophytin reduction upon excitation of the reaction center must be substantially higher than 65%.

The preparation used contained about 5 Chl *a* per photoreducible pheophytin and the presence of some unconnected Chl means that the active reaction center contains less Chl. In purple bacteria the reaction center contains, in addition to the special pair which constitutes the primary electron donor, two 'accessory' monomeric BChl molecules, but the histidines which form ligands to the central magnesium atoms in these molecules are not conserved in the D-1 and D-2 proteins [15]. A further purification of the D1–D2–cyt *b*-559 complex may help to establish the number of Chl in the PS II reaction center.

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