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Minireview/hypothesis

Kok's oxygen clock: What makes it tick? The structure of P680 and consequences of its oxidizing power

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Abstract

New insights in the structure of P680, the primary electron donor in Photosystem II, are summarized and the implications of its oxidizing power for energy transfer and singlet oxygen production are discussed.

Abbreviations: BChl – bacteriochlorophyll; Chl – chlorophyll; LD – linear dichroism; Pheo – pheophytin; PS II – Photosystem II

Introduction

The oxidation of water is most easily and safely accomplished by a concerted 4-electron event, oxidizing two water molecules to one O_2 molecule. Nevertheless, the midpoint potential of +0.8 V and the waste product, O_2, entail considerable risks of damage to the photosynthetic apparatus. Photosystem II (PS II) needs not only a special device to accumulate the four oxidizing equivalents and ensure their concerted action, but also an oxidant strong enough to make it happen. The oxidation potential of the primary photooxidant in PS II, $P680^+$, cannot be measured directly but has been estimated at +1.0 to +1.3 V (Jursinic and Govindjee 1977), at +1.1 V (Klimov et al. 1979) and at +1.2 V (Van Gorkom 1985) by independent approaches. It is much higher than in all other photosystems and suggests an essentially different structure or environment. It is so high that $P680^+$ will oxidize not only the tyrosine which normally acts as the secondary electron donor (Y_Z), but also other amino acid residues and pigments in its vicinity. New insights in the structure and properties of P680 and the consequences of its extremely high redox potential are discussed. For a review of the primary processes in PS II, see Renger (1992).

P680 structure

The structure of P680 and its molecular environment must be responsible for the high potential of $P680^+$/P680. P680 consists of chlorophyll (Chl) *a* and the midpoint potential of monomeric Chl *a* in solution is about +0.8 V (Davis et al. 1979). It has often been proposed that P680 is a monomer, not a special pair like in other photosystems. That may explain why its redox potential is not lower than that of Chl *a* in vitro, but something special in its environment must still be postulated to explain why its potential is higher by 0.3–0.4 V. P680 has many properties that may be taken as evidence for a monomeric structure. The Q_y absorption band is hardly red-shifted relative to that of the antenna Chl *a*; the oxidized state and the triplet state appear to be localized on a single Chl *a* molecule (reviewed by Hoff 1987). Stark effect (Lösche et al. 1988) and hole-burning measurements (Tang et al. 1990) do not show the features characteristic of the special pair in purple bacteria, and most recently LD-ADMR (linear dichroic

absorbance-detected magnetic resonance) measurements showed that the angles between the Q_y transition moment and the triplet x- and y-axis are the same as for Chl *a* in ethanol (Van der Vos et al. 1992). On the other hand, the oxidized-minus-reduced absorbance difference spectrum of P680 is significantly different from that of monomeric Chl *a* in vitro (Borg et al. 1970) and the other properties are found in P700 as well. Thus, if they argue in favor of a monomeric structure, it does not help to explain the high redox potential.

Arguments suggesting a dimeric structure of P680 are the following. The histidines which in the purple bacterial L and M proteins form ligands to the Mg atoms of the special pair, but not those which ligate the accessory BChls, are conserved in the D1 and D2 proteins (Michel and Deisenhofer 1988). At low temperature a conservative CD doublet is observed at the position of the Q_y absorption band of P680 (Otte et al. 1992). Van Kan et al. (1990) found that 4 pigments, presumably 2 Chl and 2 Pheo, contribute to the 680 nm absorption band of the isolated reaction center and Schelvis et al. (1993) found that their selective excitation leads to homogeneous 3 ps kinetics. The initial bleaching upon selective excitation of the long wavelength pigments has at least twice the amplitude expected for excitation of one molecule (Schelvis et al. 1993) and is halved with a time constant of 0.1 ps (Durrant et al. 1992). Schelvis et al. (1993) conclude that P680 is a special pair like in other photosystems, but disguised as a monomer by two features: the Q_y transition moments are nearly (anti)parallel and placed approximately at the magic angle with their connecting axis. This geometry results in a very small exciton splitting with nearly all transition probability in one of the two exciton bands.

Van Mieghem et al. (1991) have found an angle of 30° between the molecular plane of the Chl molecule in the triplet state and the membrane plane. On that basis, LD-ADMR measurements by Van der Vos et al. (1992) indicated that the Q_y absorption moment and the normal to the membrane plane make an angle of $54 \pm 8°$. That is the magic angle at which no LD is observed. The absence of LD of the Q_y absorption of P680 explains the apparent inconsistency noted by Otte et al. (1992) that photoaccumulation of $Pheo^-$ is accompanied by a blue-shift of 680 nm absorbance and CD, but not of LD. The positive LD band at about 681 nm must then be attributed to another pigment. The 'active' Pheo has negative LD (Breton 1990), so the most likely origin of the positive LD band is the 'inactive' Pheo (Van der Vos et al. 1992). The presence of such a pigment is also suggested by the fluorescence polarization measurements of Kwa et al. (1992). These cannot be explained by a dimer with a large angle between the Q_y transition moments of the constituent monomers, as LD-ADMR shows (Van der Vos et al. 1992), and must be due to energy transfer between pigments with non-parallel Q_y transitions. On the other hand, since the fluorescence yield may easily be dominated by a very small fraction of the sample in which a long-lived excited state is formed, the fluorescence polarization data may not be representative for reaction centers capable of charge separation.

If the Q_y transitions of the two Pheos have opposite LD and the two constituent monomers of P680 have (anti)parallel Q_y transitions at the magic angle with the normal to the membrane, this part of the reaction center is clearly not C_2-symmetrical around an axis perpendicular to the membrane. On the basis of its orientation, Van Mieghem et al. (1991) proposed that P680 is in fact homologous to the accessory BChl in the bacterial reaction center, rather than to the special pair. Also the 2–3 ps charge separation time (Wasielewski et al. 1989a,b) requires that the edge-to-edge distance between P680 and Pheo is much smaller (about 5 Å) than that between the bacterial special pair and BPheo (Moser et al. 1992), if no accessory pigments intervene. The above mentioned indications for the presence of a special pair in PS II would then suggest that the other P680 Chl is homologous to one BChl of the bacterial special pair, and that the other BChls, those in the 'B-branch', have no counterpart in PS II. A substantial shift and rotation would still be needed to obtain the specific geometry of P680 described above and the pattern of conserved histidines does not help to support this model.

The spectroscopy of P680 is further complicated by spectral heterogeneity. Van Kan et al. (1990) found that the Q_y absorption band of P680 at 10 K has a distinct shoulder on the long-wavelength side. Otte et al. (1992) showed that this is due to the occurrence of two different spectral forms of P680 in the preparation. Van der Vos et al. (1992) could distinguish 3 forms in their triplet-minus-singlet difference spectra, but the additional one peaking at

shorter wavelength may not be due to P680. No relation of the spectral heterogeneity to a possible functional heterogeneity has been reported and it is not clear if the spectral heterogeneity is induced by the isolation of the reaction centers; site-selection spectroscopy on more intact Photosystem II preparations is needed on this point.

P680 excitation

Since the redox potential of $P680^+/P680$ is higher than that of antenna Chls, there is a problem involved in energy transfer to P680: The nearest antenna Chls must be at a safe distance to avoid their oxidation by $P680^+$. The rate of exciton transfer decreases with the 6th power of the center-to-center distance; the rate of electron transfer decreases exponentially with the edge-to-edge distance. Figure 1 shows the consequences of this situation for the quantum yield of photosynthesis as a function of distance between P680 and the nearest antenna Chl. Even at optimal orientation and spectral overlap the center-to-center distance (curve marked C–C) must be smaller than 20 Å due to the competition between energy transfer to P680 and the exciton loss rate of $(2\ ns)^{-1}$. Using the relation between optimal electron transfer rate and distance given in Moser et al. (1992), the edge-to-edge distance (curve E–E) should be larger than 20 Å to avoid Chl oxidation in competition with Y_Z oxidation. Actually Chl oxidation takes about 50 ms at low temperature (Visser et al. 1977), suggesting an E–E distance of 23 Å.

The problem is solved if in vivo P680 receives its excitation energy via Pheo, which cannot be oxidized by $P680^+$, and Pheo is excited via a Chl at a C–C distance of < 20 Å from Pheo and an E–E distance of > 20 Å from P680. This Chl cannot be one of the accessory Chls present in the isolated PS II RC, because their energy transfer to Pheo/P680 takes 30 ps (Schelvis et al. 1993) and must take nanoseconds if the excitation is thermally distributed over the intact PS II antenna. An interesting candidate for energy transfer to the 'active' Pheo could be the F695 emitting Chl in the core-antenna protein CP47, because both have negative LD (Breton 1990, Van Dorssen et al. 1987). A similar orientation might be expected for pigments optimized for long-distance energy transfer. One might speculate that, on the other side of the reaction center, CP43 provides a Chl at the right distance and orientation for energy transfer to the 'inactive' Pheo.

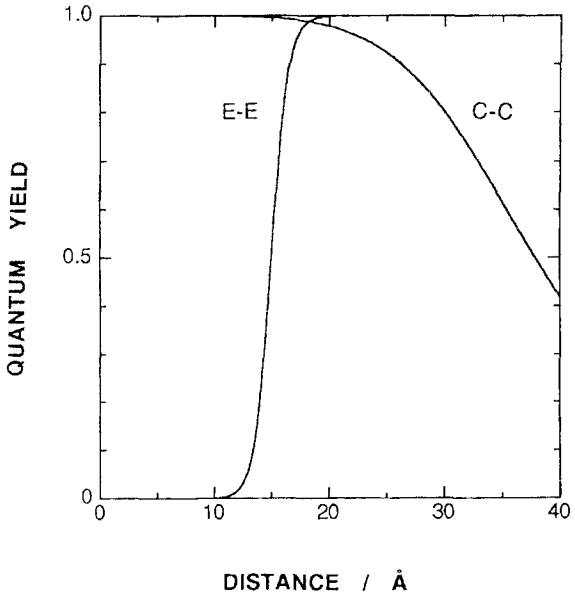

Fig. 1. Limitation of the quantum yield of Photosystem II by losses due to Chl oxidation at too short edge-to-edge distance from P680 (E–E) and by losses due to the limited lifetime of the excited state at too large center-to-center distance between Chl and P680 (C–C). The maximum quantum yield allowed by these limitations was calculated as $Q_Y = k_1 / (k_1 + k_2)$. For curve E-E, k_1 represents the photosynthetic use of $P680^+$, taken to be $10^6\ s^{-1}$, and k_2 represents the rate of Chl oxidation by $P680^+$ which was assumed to follow the (free energy optimized) distance dependence given in Moser et al. (1992) ($\log k_2 \approx 15 - 0.6\ r$, with r the E–E distance in Å). For curve C–C, k_2 represents losses of excitations in the antenna, taken to be $5.10^8\ s^{-1}$, and k_1 is the Förster energy transfer rate calculated assuming that the excited state spends 1% of its 2 ns lifetime on the Chl nearest to P680 and can be transferred to P680 only from there, the orientation factor and overlap integral in the Förster equation are maximal, the refractive index is 1.4 and the effective maximum extinction coefficient is 150 000 at 680 nm. Reasonable errors in these assumptions will not shift the curves by more than a few Å.

P680 triplet decay

The redox potential of $P680^+$ is also high enough to oxidize β-carotene. The main function of carotenoids in photosynthesis is to prevent the production of singlet oxygen by the Chl triplet states inevitably formed by intersystem crossing in the antenna and by charge recombination in the reaction center (Cogdell and Frank 1987). This function should be especially important in PS II, which is the source of oxygen. Isolated PS II reaction centers contain one

or two β-carotene molecules, but the triplet state of P680 is not transferred to carotene and instead decays in 33 μs to the ground state (Durrant et al. 1990) by generating singlet oxygen (Macpherson et al. 1993). The result is an inactivation of the reaction center in seconds, accompanied by an irreversible bleaching of P680 and Pheo (De Las Rivas et al. 1993). Triplet-triplet transfer requires orbital overlap and a sufficiently short distance between carotene and P680 would imply an extremely fast oxidation of carotene by $P680^+$. Apparently, evolution has not thought of using carotene as the normal secondary electron donor and instead carotene is kept at a safe distance. Carotene does become photooxidized in experimental conditions leading to accumulation of $P680^+$ and may help to avoid or delay accumulation of $P680^+$ in vivo (De Las Rivas et al. 1993). Such extreme stress conditions should be rare, however, and we propose that the normal function of carotene in the reaction center more likely is the quenching of singlet oxygen. In intact PS II the reaction center triplet is not produced when electron transport is inhibited and Q_A^- accumulates, but during normal PS II activity the continuous formation of $P680^+Pheo^-$ by reversed electron transport, in Boltzmann equilibrium with more stabilized states, is thermodynamically unavoidable and charge recombination to $P680^T$ cannot be prevented (Van Gorkom 1985). As observed in the isolated PS II RC, the P680 triplet state will decay by generating singlet oxygen, which can be trapped by the many β-carotene molecules in the PS II core and reaction center, but not in the close vicinity of P680. As a consequence, oxidative destruction of P680, Pheo, or nearby amino acid residues by singlet oxygen will destroy the reaction center sooner or later. Replacement then is the only solution. Indeed, the selective turnover of the D1 protein during light-limited photosynthesis (Prasil et al. 1992) and the very existence of a circadian rhythm in its synthesis in cyanobacteria (Kondo et al. 1993) seem to confirm that photosynthetic oxygen evolution is intrinsically suicidal. The selectivity for D1 might suggest that the triplet state in $P680^T$ is localized on a Chl molecule associated with D1 only, but it could also be accessible to oxygen from the D1 side only and the site of oxygen production (see Svensson et al. 1991) could well be the main origin of this selectivity.

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