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Spectral properties of stabilized D1/D2/cytochrome *b*-559 photosystem II reaction center complex

Effects of Triton X-100, the redox state of pheophytin, and β-carotene

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Absorption, fluorescence, and CD spectral properties of the isolated D1/D2/cytochrome *b*-559 photosystem II reaction center complex were examined in stabilized reaction center material at 77 K. Spectral properties were dependent on the presence or absence of 0.05% Triton X-100 in the RC suspension medium, on the redox state of pheophytin, and on the state of inactivation of the complex. The specific spectral properties of the PS II RC complex in the red suggest that the primary donor is not a bacterial-type special pair and could be a monomer. Furthermore, the spectral properties in the PS II RC may be the result of excitonic interactions among all the porphyrin molecules in the complex. Interactions between β-carotene and porphyrins indicate a significant role for β-carotene in the PS II RC.

Photosynthesis; Photosystem II; Reaction center; Spectroscopy; Detergent effect; Carotene, β-; (Spinach)

1. INTRODUCTION

The D1/D2/cytochrome *b*-559 reaction center complex of PS II has been purified from spinach [1], pea [2], and *Synechocystis* 6803 [3]. As isolated the preparation contains approx. 4–5 chlorophyll *a*, 2 pheophytin *a*, 1 β-carotene, and 1–2 Cyt *b*-559 heme molecules, and thus it appears to be quite analogous to the bacterial RC [1,2]. Despite its lack of quinones, the PS II RC complex is photochemically active. It exhibits reversible flash-induced absorbance changes [4–6] and performs light-driven diphenylcarbazide to silicomolybdate electron transport [2,7]. Spectral properties of the PS II RC complex have been investigated [1,2,8–10]. However, the RC preparation as originally isolated [1] is not stable [7,10,11], and there are additional concerns that any isolation procedure, especially one that employs detergents, can affect the native state of pigment-protein complexes. Detergents, for example, are known to influence the absorption and fluorescence properties, including peak positions of spectral bands and fluorescence quantum yields, of many isolated Chl-protein complexes [6,10,12,13]. Gulyaev et al. [13] have found in particular that Triton X-100 affects the spectral properties of O_2-evolving PS II core complexes isolated from *A. nidulans*, especially in the long-wavelength region. Triton-extracted PS II RC complex exhibits a room-temperature absorption maximum at 676 nm when freshly isolated [10]. The peak rapidly blue shifts to 674 nm even when the RC is stored at 4°C in the dark, and additional blue-shifting is observed as the complex is inactivated [7,10].

Seibert and co-workers [7,10] have developed procedures that improve the stability of the

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Abbreviations: CD, circular dichroism; Chl, chlorophyll; Cyt, cytochrome; DPC, diphenylcarbazide; LD, linear dichroism; PEG, polyethylene glycol; Pheo, pheophytin *a*; PS II, photosystem II; SiMo, silicomolybdate; RC, reaction center

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D1/D2/Cyt *b*-559 RC complex in the dark and light. Here, we shall reexamine and extend previous spectral studies of the isolated PS II RC complex on stabilized RC material. We have found that the presence of excess detergent in the suspension buffer and exposure of material to inactivating conditions have profound effects on the native state of the PS II RC and hence on the spectral properties of the isolated material. Furthermore, we demonstrate a new relationship involving a spectral band at 490 nm (probably β-carotene), the native state of P_{680}, and the redox state of Pheo.

2. MATERIALS AND METHODS

Reaction center material was prepared from market spinach by the PEG-precipitation procedure detailed in [7]. This method is a modification of the preparation according to Nanba and Satoh [1] which removes excess Triton X-100 and stabilizes the material [7,10]. For best optical properties, the low-speed centrifugation step used to eliminate mostly colorless PEG aggregates from the PS II RC material should be performed at a Chl concentration of about 100 μg/ml. If the Chl concentration is too high, good separation of colorless PEG-aggregates is not observed. Samples used in this study exhibited light-induced DPC to SiMo electron-transport rates of 1400–2300 μmol SiMo reduced · mg Chl^{-1} · h^{-1} and were stored at −96°C for up to 1 month until use. The material was treated with a glucose/glucose oxidase/catalase O_2-scrubbing system [7] prior to use except in the case of the aging experiments. Samples used for studies at 77 K contained 55% glycerol.

Room-temperature and 77 K absorption spectra were measured using an LOMO (Leningrad) Sph-14M spectrophotometer with an integrating sphere attachment. Fluorescence and fluorescence excitation spectra were obtained with a CKB AMN USSR (Moscow) SPIL spectrofluorometer. CD spectra were recorded on a Jasco J-40 AS dichrograph. Difference spectra (fig.2) were obtained with a Hitachi-557 spectrometer. Deconvolutions were performed assuming that the short-wavelength sides of the bands were Lorentzian shaped and the long-wavelength sides of Gaussian shape. Deconvoluted bandwidths ranged from 9 to 12.5 nm.

3. RESULTS AND DISCUSSION

Fig.1 shows the spectral properties at 77 K of freshly unfrozen PS II RC preparations suspended in the absence or presence of 0.05% Triton X-100. The absorption maximum of the sample without Triton at room temperature is at about 676 nm with a small shoulder at about 680 nm (not shown), but at 77 K (fig.1A) the spectrum exhibits two distinct peaks appearing at 673 and about 680 nm. Deconvolution of the absorption and CD spectra reveal 4 principal narrow bands (668.4, 672.3, 676.1, 681.3 nm) and a minor band at 687 nm. These bands were identified by close examination of amplitudes and signs in the CD spectrum. The fluorescence spectrum (no Triton) peaks at 686 nm with a small shoulder at 692 nm, and the fluorescence excitation spectrum closely coincides with the absorption spectrum. The 692 nm fluorescence shoulder is not due to CP47 contamination and appears to be a phenomenon similar to that observed in *Rps. viridis* RC [14].

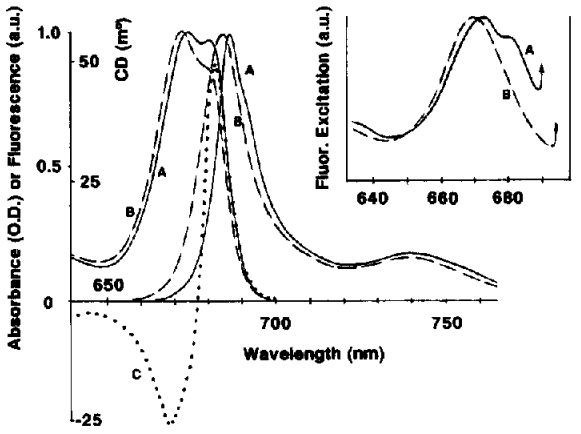

Fig.1. Absorption, fluorescence (λ_{ex} = 435 nm), and CD spectra of isolated PS II RC complex at 77 K. (A) Control sample resuspended in 50 mM Tris-HCl (pH 7.2) buffer (absorption and fluorescence). (B) Same as above except that 0.05% Triton X-100 was also present (absorption and fluorescence). (C) CD spectra for samples (A,B). Both samples have the same CD spectrum, but the amplitude of the maximum at 682 nm is proportional to the absorption at 680 nm; see text. The CD scale indicated is for sample (A). Inset, fluorescence excitation spectra at 77 K for samples (A,B) monitored at 700 nm. The absorption of the fluorescence samples was 0.05 at 670 nm, and the two sets of fluorescence spectra were normalized.

Fig.1B shows that the addition of even a small quantity of Triton X-100 (0.05%) to the preparation causes significant changes in the absorption, fluorescence, and fluorescence-excitation spectra. The amplitude of the 680 nm absorption band decreases and the 673 nm band blue shifts to 670 nm. The fluorescence intensity of the sample increases when Triton is added (fluorescence maxima are normalized in fig.1). Furthermore, the main peak broadens and blue-shifts to 684 nm. No evidence of a 692 nm shoulder is apparent. The CD spectrum of an RC sample resuspended in the

presence or absence of 0.05% Triton (fig.1C) did not change qualitatively; however, the amplitude of the 682 nm CD maximum was proportional to that amplitude of the 680 nm absorption band (not shown). Fluorescence-excitation spectra show loss of the 680 nm component (some samples did show a small shoulder at this wavelength) and a blue shift of the 673 nm component, properties similar to those observed in the absorption spectra when Triton is present. These data suggest that the presence of excess Triton in the suspension medium can distort the native structure of the PS II RC complex though not to the extent that electron transport across the RC is lost [7]. We note that previously published spectra of the PS II RC [2,8–10] correspond more closely to our spectra in the presence of Triton. Thus, the spectra shown in this study suggest that our stabilized PS II RC complex suspended in the absence of Triton X-100 represents a 'more native' state of the PS II RC complex than previously reported. When PS II RC complex in 0.05% Triton was inactivated by aging aerobically in the light for 40 min prior to freezing [7], the absorption band at 680 nm and the CD signal completely vanished (not shown). This result reaffirms the conclusions of a previous study [10] demonstrating that additional spectral changes occur when the PS II RC material degrades.

Fig.2 shows absorption spectra of control RC complex (no Triton) and that exposed to reductant

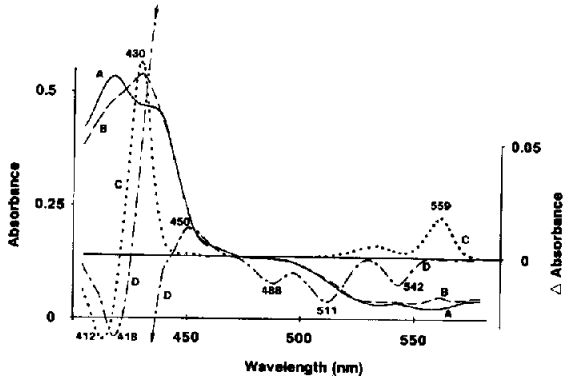

Fig.2. Absorption spectra (A,B) and absorption difference spectra (C,D) of PS II RC complex at room temperature (no added Triton X-100). (A) Control sample. (B) The sample after addition of 2 mg/ml dithionite. (C) Dithionite minus control sample (Cyt *b*-559 reduced). (D) Light minus dark sample, both with dithionite (Pheo reduced). The scale of (*D*) is expanded by a factor of two vs (C).

(2 mg/ml dithionite added under anaerobic conditions in the dark; $E_h < -400$ mV). The dithionite-minus-control difference spectrum shows that the treatment reduces Cyt *b*-559. When the dithionite sample is further pre-exposed to saturating steady illumination, reduced Pheo is photo-accumulated irreversibly (fig.2D). This photoaccumulation step lowers the fluorescence yield from 2.3 tot 0.7%. Using the $\Delta\epsilon$ for the Cyt *b*-559 α-band (15 $cm^{-1} \cdot mM^{-1}$) [15] and that for the Q_x-band of Pheo *a* (6.6 $cm^{-1} \cdot mM^{-1}$) (calculated from [1]), we obtain a $Pheo^-$:Cyt *b*-559 heme ratio of 0.77. This is very close to the value of 0.76 reported previously [1].

Although there are apparent structural similarities between the bacterial and PS II reaction centers, the spectral properties of the two are quite different. For example, there is considerable optical overlap of the PS II RC pigments in the red region that is not the case in bacteria [16]. Thus, it is important to determine whether there are other peculiarities regarding pigment interactions in the PS II RC. Fig.3 shows 77 K absorption and CD spectra of PS II RC complex (no added Triton) under conditions where Pheo is either oxidized or pre-reduced. A decrease in absorption at 683 nm accompanies the reduction of Pheo. The CD and absorption spectra show that this is associated with a decrease in the general dipole strength and a 2–3 nm blue shift of the long-wavelength band. Additional changes also occur in the β-carotene region where splitting of the 490 nm absorption band in the CD spectra disappears upon reduction of Pheo (fig.3B, inset). Since the RC contains only one β-carotene [1,2], the splitting might be the result of interactions between the electronic transitions of β-carotene and the transitions of Pheo probably at around 510 nm. On the other hand, the existence of a specific absorption band at 490 nm itself might be the result of interaction of β-carotene not only with Pheo but also with other Chl molecules in the native PS II RC complex structure. The fact that the 490 nm absorption band disappears upon inactivation of the RC complex, as does the 680 nm absorption band characteristic of the native complex (not shown), lends support to this idea.

Since LD spectra of the PS II RC show that the 490 nm transition moment is perpendicular to the plane of the original PS II membrane and that pigments absorbing in the 680 nm region are

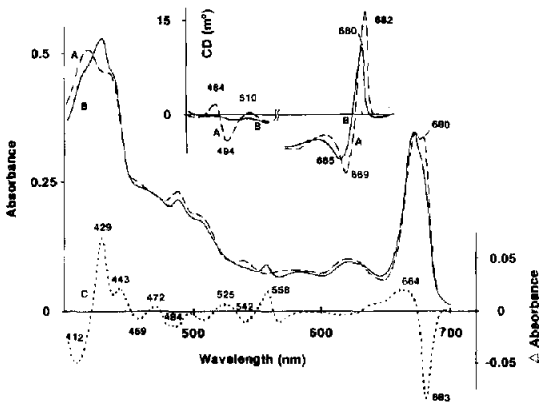

Fig.3. Absorption (A,B) and absorption difference spectra (C) of the PS II RC complex at 77 K (no added Triton X-100). (A) Control sample. (B) Sample with reduced Cyt *b*-559 and pheophytin (dithionite plus light). (C) Spectrum (B) minus spectrum (A). The inset shows CD spectra at 77 K of samples (A,B) in the chlorophyll and β-carotene spectral regions.

parallel [8,9,12], we suggest that the β-carotene molecule might be situated between the D1 and D2 proteins approximately along the C_2-axis where it could interact with porphyrin molecules. This general arrangements appears different from that reported for the bacterial RC [17].

The special pair in the bacterial RC complex is formed by two closely overlapping BChl molecules [18,19]. The spectral features of the special pair, characterized by a broad, strongly red-shifted absorption band, are due in a large part to exchange-resonance interactions between the two molecules [20]. Our data and those of others [1,2,6,8,10,16] show that these features do not exist in the PS II RC complex. Thus, it appears that there is little overlap between the π-electron systems of the porphyrin rings of donor molecules in the PS II RC complex. Consequently, it may be necessary in the case of the PS II RC to reexamine the existence of a special pair exactly analogous to that in the bacterial RC. The fact that the triplet state of Chl in the PS II RC seems to be localized on a single molecule rather than delocalized over two Chls as in the bacterial RC is consistent with this although other explanations are possible [21]. On the other hand, the apparent lack of π-electron overlap and the monomeric nature of the triplet do not preclude delocalization of singlet excited states between pigment molecules by excitonic interactions. In fact, strong rotational strength (the positive CD bands in figs.1, 3 have 3-times the rotational strength compared to that of the bacterial special pair [22]) and conservative CD of the PS II RC indicate the existence of such interactions. However, using the 681.3 and 668.4 nm bands deconvoluted from our absorption and CD spectra (fig.1) as the major extreme bands, we calculate a maximum interaction energy of about 142 cm^{-1} [23] for PS II RC pigments. This value is much lower than that for the bacterial special pair but is similar to those reported for interactions among the other bacterial RC pigments [20,24].

Thus, if there are similarities between the geometries of the pigments found in the bacterial and PS II RCs, they do not seem to coincide in the region of the bacterial special pair. This could mean that the Chl *a* molecules in the PS II RC (corresponding to the bacterial special pair) (i) are much further apart, (ii) their Q_y transitions are close to perpendicular, or (iii) the angle formed by the vector connecting the two Mg atoms of the special pair and the vector perpendicular to the two π-rings is close to 54.7° [23,25]. Any of these three conditions could result in the absence of pair interactions that lead to large red shifts. We also suggest in the absence of strong pair interaction that the spectral properties of the PS II RC are governed by excitonic interactions among all six porphyrin molecules (more recent studies indicate that the PS II RC contains 4 Chl *a* molecules [8] rather than five as originally determined) in the complex and that no bands in the red region of the absorption or CD spectra belong exclusively to the primary donor or Pheo molecules per se. This is consistent with the fact that all changes which disturb excitonic interactions in the RC, including the presence of detergent (which could be broadening the band at 680 nm) or degradation of the complex, seem to produce similar spectral changes, such as the disappearance and blue-shifting of long-wavelength absorption bands. This could also explain why the spectral changes induced by excitation of the pigment molecules, oxidation of the primary donor, or reduction of Pheo are so similar [6]. On the other hand, it again raises questions about the exact nature of the primary donor, whether it is a Chl *a* monomer (as has been suggested in the past [26]) or some other structure different from the bacterial special pair. Furthermore, the strong correlation of the 490 nm band of β-

carotene with the 680 nm band and the fact that β-carotene could be located between the D1 and D2 proteins along the C_2-axis of the RC, suggest that a special interaction between β-carotene and perhaps two Chl molecules on the D1 and D2 proteins may form the primary donor of PS II. Wasielewski, for example, has observed interporphyrin interactions between two porphyrins connected by a carotene despite the large porphyrin-porphyrin distance involved (Wasielewski, M., personal communication). Finally, the apparent interaction of β-carotene with the photoactive Pheo also suggests the possibility of a rôle for β-carotene in electron transfer in PS II.

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