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Spectroscopic characterisation of the reaction centre of photosystem II from higher plants

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Two different photosystem II particles isolated from pea, a core complex and the D1/D2/Cyt *b*-559 reaction centre (RC) complex, have been characterised by absorption, linear dichroism (LD) and circular dichroism (CD) spectroscopy. Only one carotenoid contributes to the LD of the reaction centre, in agreement with the biochemical analysis. This carotenoid is oriented parallel to the long axis of the reaction centre. The chlorophyll Q_Y contribution to the LD is oriented perpendicular to the long axis of the reaction centre. The LD of the reaction centre carotenoid is reversed in the core complex. In addition, the contribution of a second carotenoid species can be observed. In the core, the two pools of carotenoid have a rather different orientation with respect to the membrane plane: one parallel, the other perpendicular. In addition, in the core the sign of the LD in the chlorophyll Q_Y region is reversed and red-shifted as compared to the reaction centre. These observations suggest that the reaction centre is oriented with its long axis perpendicular to the long axis of the PS II core and to the membrane plane. The circular dichroism CD of the reaction centre has intense peaks of opposite sign at 444 nm (negative) and 435 nm (positive), which we attribute to exciton coupling between Chl *a* molecules in the reaction centre. The RC has no CD in the range 460–650 nm; thus there is no exciton coupling between the carotenoid and the other pigments. The lack of CD in this region is consistent with the biochemical analysis of only one carotenoid per reaction centre. The larger core complex exhibits much weaker CD (per Chl). The CD in the Q_Y absorption band indicates exciton coupling between chlorophyll molecules. The sign of the pair of peaks in this region is reversed in the core with respect to the reaction centre.

Reaction center; Photosystem II; Linear dichroism; Circular dichroism; Photosynthesis; Spectroscopy

1. INTRODUCTION

There is much evidence for remarkable similarities between the reaction centres of photosynthetic bacteria and green plants (review [1]). A particular case is the homology between the primary structure of the D1/D2 polypeptide pair of PS II from higher plants and the L/M subunit pair of the reaction centre of photosynthetic purple bacteria. The crystallographic study of the reaction centre of *Rhodopseudomonas viridis* by Deisenhofer et al. [2] showed that the conserved amino acids include those that ligate the special pair of bacteriochlorophyll molecules and the non-haem iron in the bacterial RC. Their work therefore suggested that the prosthetic groups involved in primary charge separation in PS II are located on the D1/D2 heterodimer. This has recently been confirmed by the isolation of a PS II complex containing only D1, D2 and the α- and β-subunits of Cyt *b*-559 [3], which exhibits light-

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Abbreviations: PS II, photosystem II; RC, reaction centre; LD, linear dichroism; CD, circular dichroism; Chl, chlorophyll; BChl, bacteriochlorophyll; Cyt, cytochrome; BBY, PS II-enriched membrane fragment; PAGE, polyacrylamide gel electrophoresis

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induced spectral changes that correspond to the formation of a charge-separated state in this particle [3–6]. The changes include those of a reduced pheophytin molecule and of a spin-polarised triplet state formed via a radical pair intermediate.

The spectroscopic properties of the reaction centre from purple photosynthetic bacteria have been studied extensively, and many features of the pigment organisation in the RC were deduced from such experiments (review [7]). The recent crystallisation of the reaction centres from *Rhodopseudomonas viridis* [2], and from *Rhodobacter sphaeroides* [8–10] has shown that many of these conclusions were correct.

In this communication we present LD and CD spectra of the D1/D2/Cyt *b*-559 complex and a larger oxygen-evolving core complex of PS II isolated from pea. The room temperature spectroscopic properties of the core complex are in reasonable agreement with those reported elsewhere for similar complexes [11–13]. Notably the presence of two types of carotenoid molecule in the core complex is confirmed. However, the spectral properties of the D1/D2/Cyt *b*-559 reaction centre complex differ considerably from those published recently for a reaction centre complex isolated from spinach which was studied at low temperature [14].

2. MATERIALS AND METHODS

The isolation of the reaction centre of PS II was accomplished by liquid chromatography of Triton X-100-solubilised thylakoid membranes from pea (*Pisum sativum* var. Feltham First) [4]. Particular care was taken to exclude contaminating antenna chlorophyll proteins. Pooled fractions from a first column (20 × 120 mm) containing Fractogel TSK DEAE 650(S) (Merck-BDH) were therefore purified further on a second, smaller column (10 × 60 mm) containing the same DEAE-Fractogel [4]. The purity of each preparation was monitored by SDS-PAGE, spectrophotometric determination of the Cyt *b*-559:Chl *a* ratio, and by the absorption spectrum, using the data in [4] as standard. Core complexes were prepared by the method of Ikeuchi et al. [15] and the same rates of oxygen evolution obtained by these workers were achieved. The polypeptide content was checked by SDS-PAGE.

For LD studies, both types of particle were embedded in polyacrylamide gels [16], whose final composition was 2.5 μg Chl/ml, 14.5% (w/v) acrylamide, 0.5% (w/v) *N,N*-methylenebisacrylamide, 30% (w/v) glycerol, polymerised with 0.03% (v/v) *N,N,N',N'*-tetramethylparaphenylenediamine (TEMED), and 0.05% (w/v) ammonium persulphate (APS). LD in squeezed gels was measured on a modified Cary 61 spectropolarimeter [17], and recorded on a HP85 computer. The gels were compressed in one direction perpendicular to the measuring beam in a press with an optical pathlength of 5.0 mm, from an initial width of 9.0 mm to a final width of 5.5 mm as described in [18]. The optical pathlength of the cell remained constant at 5.0 mm during the squeezing which was carried out in a temperature-controlled cell holder at 4°C.

CD spectra were recorded on a Jasco J-40CS spectropolarimeter, 1 cm-pathlength strain-free cuvettes were used for all CD spectra. The chlorophyll concentration was 10 μg/ml in buffer (50 mM Tris-Cl, pH 7.2). The cell holder was cooled to 4°C.

Absorption spectra were measured on a Cary 219 spectrophotometer interfaced to a HP85 computer. The concentration of chlorophyll was 5 μg/ml. All spectra were measured at 4°C.

3. RESULTS AND DISCUSSION

3.1. *Absorption*

Absorption spectra of reaction centre and core complexes are shown in fig.1. The spectra in the Soret band show different features. The relative magnitudes of the two major peaks in the Soret absorption region are reversed in the reaction centre with respect to the core, the shorter wavelength peak being largest in the reaction centre. We attribute this feature to enrichment in pheophytin *a* in the reaction centre. The positions and relative magnitudes of the shoulders in the carotenoid absorption bands (450–500 nm) differ between the two particles. The peaks in the red region are red-shifted in the core by 4–5 nm relative to the reaction centre.

3.2. *Linear dichroism*

The LD spectra of the two particles are very different (fig.2). An important difference between the LD spectra of the core and reaction centre complex is the reversal of the sign of LD of the carotenoid absorbing at 458 nm and 487 nm, and of the lowest energy Q_Y absorption band of the chlorophylls (positive at 682 nm in the core; negative at 672 nm in the reaction centre). In PS II-enriched membrane fragments (BBY particles [19]) the Q_Y band with a maximum at 682 nm is, like the cores, also positive (not shown). If we assume that the BBYs orient with the plane of the membrane perpendicular to the squeezing direction, we can conclude, as have others [11,13,20], that the longest axis of the core complex lies parallel to the membrane plane. The change of sign

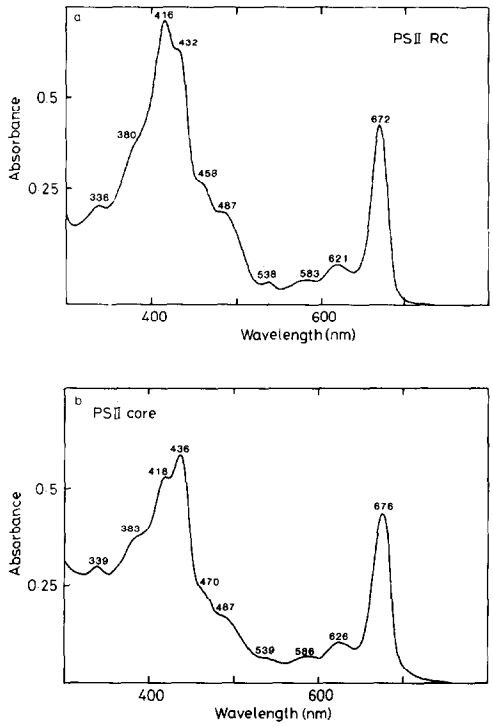

Fig.1. Absorption spectra of (a) D1/D2/Cyt *b*-559 complex and (b) oxygen-evolving core complex. [Chl] = 5 μg/ml in 50 mM Tris-Cl, pH 7.2.

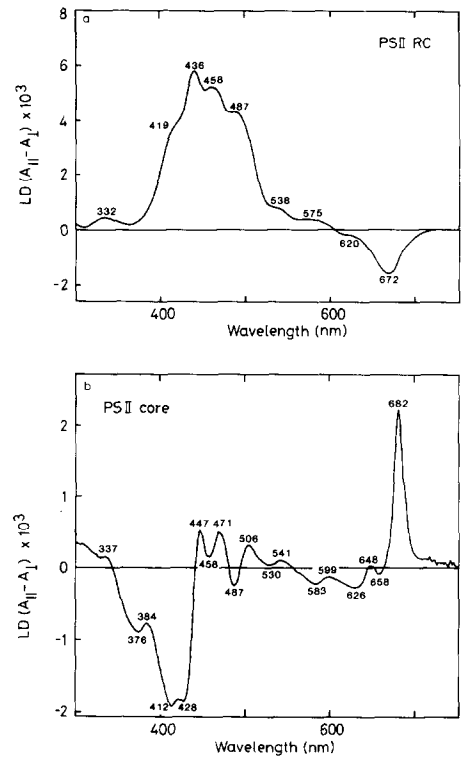

Fig.2. Linear dichroism spectra of (a) D1/D2/Cyt *b*-559 complex and (b) core complex in polyacrylamide gel (see text for composition). A baseline with constant slope of -0.75×10^{-3}/100 nm has been subtracted.

of the carotenoid LD in the reaction centre therefore indicates that this complex has an orientation at right angles to that of the PS II core, that is the longest axis of the PS II reaction centre is at right angles to that of the core complex. Such a conclusion suggests that in vivo the longest axis of the reaction centre is perpendicular to the membrane plane. If this interpretation is correct, then the negative peak at 672 nm in the reaction centre corresponds to Chl molecules perpendicular to the long axis of the RC or parallel to the membrane plane.

The LD spectra of the reaction centre lacks some of the features present in the LD of the core complex. In the 440–510 nm region the pronounced sign changes in the core LD have previously been ascribed to two different carotenoids with dichroism of opposite sign, and slightly different absorption maxima [11–13]. It is suggested [11] that these two carotenoids have different functional roles: (i) as quencher of the triplet state of P-680 [21], and (ii) as accessory electron donor to P-680 [22]. In the core preparation, the carotenoid with positive LD has maxima at 447, 471 and 506 nm; the other carotenoid has minima at 458 and 487 nm. In contrast, in the reaction centre the LD spectrum has only positive peaks at 436, 458 and 487 nm. This indicates that one of the two carotenoids present in the core is lost during the isolation of the reaction centre. This agrees with the biochemical analysis of the reaction centre components, which reveals the presence of only one molecule of β-carotene per reaction centre complex [3,4] (based on normalisation to two pheophytin molecules per complex). It can be further speculated that the carotenoid retained in the isolated reaction centre, unlike the chlorophyll is oriented in a direction which is more parallel to the longest axis of the particle.

Van Dorssen et al. [14] have recently reported the LD spectrum of D1/D2/Cyt *b*-559 reaction centre particles measured at low temperature.

Their spectrum differs markedly from that shown here in that the Q_Y region shows both negative and positive LD, while in the 400–500 nm region two carotenoids with different orientation seem to contribute to the LD. These workers interpret their LD in such a way that the orientation of the D1/D2/Cyt *b*-559 reaction centre complex in the gel is identical to that of the core or membrane in the gel. In this respect we note that Van Dorssen et al. [14] find the carotenoid at 458 and 490 nm to give a negative dichroism, confirming the opposite orientation of the complex in their case.

3.3. *Circular dichroism*

The reaction centre has a strong CD (fig.3) probably due to exciton coupling between the pigments [23]. The CD spectrum of the reaction centre around 670 nm is non-conservative, suggesting a strong contribution due to interactions between non-degenerate excited states on different molecules. The positive contribution to the spectrum at the long wavelength side may be due to the special pair and is possibly analogous to the P-870 part of the CD spectrum of BChl *a*-containing purple photosynthetic bacteria [24]. Similarly, the CD at 666 nm may correspond to the pheophytin contribution in the RC. The main difference from the CD spectrum of the purple bacterial RC would then be the absence of the contribution mainly due to the 'voyeur' Chl *a* molecules of the RC. If these are almost degenerate and interact weakly their contribution to the CD might cancel.

The reaction centre exhibits no CD in the carotenoid absorption region. This is in contrast to reaction centres from *Rb. sphaeroides*, which show strong CD of the carotenoid, in this case spheroidene [25]. Since only one carotenoid is present in bacterial reaction centres, it was concluded that the binding site of the carotenoid is asymmetric. The lack of carotenoid CD in the reaction centre of PS II strongly suggests that only one carotenoid is present in the reaction centre and that its structure is not distorted by interaction with the protein.

This CD spectrum for the PS II reaction centre is remarkably different from that given by Van Dorssen et al. [14] for a similar particle from spinach. Although we have no good explanation for these differences it indicates that the D1/D2/Cyt *b*-559 reaction centre complex can give

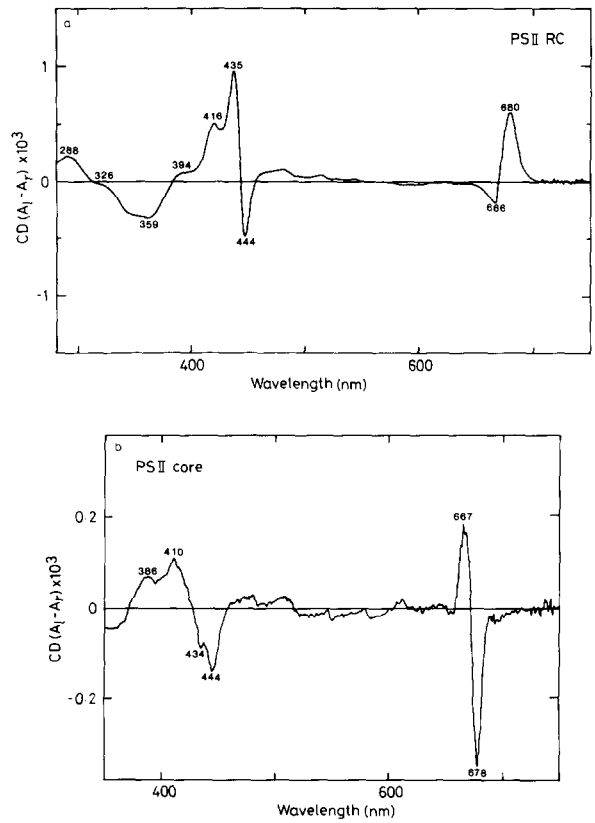

Fig.3. Circular dichroism spectra of (a) D1/D2/Cyt *b*-559 complex and (b) core complex. [Chl] = 10 μg/ml in 50 mM Tris-Cl, pH 7.2.

rise to inconsistencies in spectral features (Breton, J., personal communication). In our hands, however, the OD, LD and CD were remarkably reproducible between different preparations.

The CD of the core complex is much weaker (per Chl) than the reaction centre CD (about 3 × in the red absorption maximum, and 7 × in the Soret region). The core also shows no CD arising from the carotenoid molecules. Since the LD suggests the presence of two carotenoids, we must conclude that these pigments are quite distant from each other thus reducing dipolar interaction to an undetectable level, or that their mutual orientation excludes the possibility of exciton interaction. A notable difference from the reaction centre CD is the reversal of the sign of the pair of peaks at the red end of the spectrum. This may be a useful feature to test the purity of reaction centre preparations.

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