Biochimica et Biophysica Acta, 1057 (1991) 232–238

ADONIS 000527289100097T

BBABIO 43364

Spectroscopic characterisation of the reaction centre of Photosystem II using polarised light: Evidence for β-carotene excitons in PS II reaction centres

W.R. Newell [1,2], H. van Amerongen [1], J. Barber [2] and R. van Grondelle [1]

[1] *Department of Biophysics, Physics Laboratory of the Free University, Amsterdam (The Netherlands) and* [2] *AFRC Photosynthesis Research Group, Department of Biochemistry, The Wolfson Laboratories, Imperial College of Science, Technology and Medicine, London (U.K.)*

(Received 27 July 1990)
(Revised manuscript received 10 December 1990)

Key words: Photosystem II; Reaction center; β-Carotene exciton; Spectroscopy; Charge separation

The absorption and linear dichroism spectra of the isolated D1/D2/cytochrome *b*-559 Photosystem II reaction centre from pea (*Pisum sativum*) measured at 77 K are shown to depend on the concentration of the detergent Triton X-100 (TX-100) in the supporting buffer. This implies that the PS II reaction centre adopts different conformations in different concentrations of this detergent. In the case of the absorption due to β-carotene, additional transitions are observed when the reaction centre is suspended in a medium containing low levels (0.02% TX-100) when compared with higher concentrations (0.20%) of TX-100. Differences are also seen in the lowest energy $Q_{y(0-0)}$ band. Suspension of the PS II reaction centre in higher concentrations of TX-100 results in a slight decrease (7%) in the dipole strength of one or more of long wavelength transitions relative to that measured in 0.01% TX-100, resulting in a large shift of the absorption maximum from 677.5 nm to 672 nm. The linear dichroism spectrum of the PS II reaction centre complex measured in a buffer containing 0.20% (w/v) TX-100 indicates the presence of one spectral form of β-carotene. When measured in a buffer containing 0.02% (w/v) TX-100, the linear dichroism spectrum has more transitions in the β-carotene absorption region. We discuss the possibility that the differences are due to excitonic interactions between molecules of β-carotene which lie close together at lower detergent concentrations. The effect of the detergent TX-100 on the conformation of the PS II reaction centre is discussed in relation to the number of β-carotene molecules in the preparation.

Introduction

It has been established that the primary charge separation of Photosystem II (PS II) can occur in an isolated complex containing Chl *a*, Pheo *a*, β-carotene, and consisting of the D1 and D2 polypeptides, and the α and β subunits of cytochrome *b*-559 [1]. The protein product of the *psb*I gene has also been detected in this reaction centre complex [2,3]. Since the initial isolation many studies have confirmed and extended our knowledge of its photochemical activities [4–10].

It is now quite clear that the reaction centre of PS II has many functional and structural similarities to those of the purple photosynthetic bacteria, and both are classified as RCII-type [11]. This classification is based on the nature of its primary and secondary electron acceptors: pheophytin, and quinones, Q_a and Q_b. However, the two systems differ in the redox potential generated by the oxidised primary electron donor: 0.44–0.50 V in the bacterial system [12], and 1.12 V in PS II [13]. While the structural properties of the protein and cofactors comprising the purple bacterial reaction centre are known in detail from spectroscopic studies (review, Ref. 14), and particularly from the more recent X-ray crystallographic studies [15–17], similar informa-

Abbreviations: BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; CD, circular dichroism; Chl *a*, chlorophyll *a*; DM, *n*-dodecyl β,D-maltoside; HPLC, high-pressure liquid chromatography; LD, linear dichroism; Pheo *a*, pheophytin *a*; PS, photosystem; RC, reaction centre; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; TX-100, Triton X-100.

Correspondence: J. Barber, AFRC Photosynthesis Research Group, Department of Biochemistry, Imperial College, London, SW7 2AY, U.K.

tion is more limited for the reaction centre of PS II. The interpretation of the spectroscopic behaviour of the bacterial reaction centre was aided by the relatively large absorption range of its pigments, bacteriochlorophyll (BChl *a* and *b*), and bacteriopheophytin (BPheo *a* and *b*) and by the clear separations between the wavelengths of their absorption maxima. Results from similar spectroscopic studies of the isolated reaction centre of PS II have proved more difficult to interpret, due to the relatively small separations in the absorption maxima of chlorophyll *a* (Chl *a*) and pheophytin *a* (Pheo *a*) and variability in the spectroscopic properties of the isolated reaction centre [18]. In this paper we use polarised absorption spectroscopy (linear and circular dichroism) to investigate the pigment organisation of PS II reaction centres, in particular that of β-carotene which absorbs in a region where porphyrin absorption is low.

Two previous reports of the polarised absorption properties of the isolated PS II reaction centre complex [19,20] differ considerably, although similar experimental techniques were employed. One report [19] indicated the presence of two spectroscopically distinguishable β-carotene molecules, which are oriented perpendicular to each other in the PS II reaction centre, while the other [20] indicated only one spectral form of β-carotene. In this report, we examine the possibility that the differences observed in the region of β-carotene absorption arise from differences in the conformation of the PS II reaction centre which occur in media differing in their concentration of detergent.

Materials and Methods

PS II reaction centres were isolated from pea (*Pisum sativum*, var. Feltham's First) according to the method of Chapman et al. [9]. Sample purity was checked using SDS-PAGE, and the intactness of the preparation was assessed from the absorption spectrum of the sample measured at 277 K in 50 mM Tris-HCl buffer (pH 7.2). Selected fractions from the first column were applied once again to the ion-exchange column for further purification. Chlorophyll concentrations were estimated spectrophotometrically using the equations given by Lichtenthaler [21].

Absorption spectra were measured with a Cary 219 spectrophotometer, linear dichroism spectra with a modified Cary 61 spectropolarimeter [22], and circular dichroism spectra with a Jasco J-40C spectropolarimeter. The buffer used for measurement of 77 K absorption spectra was 50 mM Tris-HCl (pH 7.2), containing detergent as stated and 70% (v/v) glycerol. The supporting buffer for absorption and CD spectra measured at 277 K was 50 mM Tris-HCl (pH 7.2), containing different concentrations of the detergent TX-100. Dilution of the samples in the supporting media for the different measurements was carried out in dim light, and at temperatures of about 277 K. The presence of 70% glycerol in the suspension medium had no significant effect on the absorption properties of the complex as tested by running spectra with and without glycerol at 277 K.

Orientation of samples was achieved by squeezing a polymerised polyacrylamide gel containing PS II reaction centres, using the technique described by Abdourakhmanov et al. [23]. The final composition of the gel was: 14.5% (w/v) acrylamide, 0.5% (w/v) *N*,*N*-methylenebisacrylamide, 70% (v/v) glycerol, 50 mM Tris-HCl (pH 7.2), polymerised with 0.03% (v/v) *N*,*N*,*N'*,*N'*-tetramethylparaphenylenediamine (TEMED) and 0.05% (w/v) ammonium persulphate (APS). The detergent concentration in the unpolymerised gel mixture was adjusted prior to polymerisation. The PS II reaction centre was equilibrated in the buffer by incubation in the unpolymerised acrylamide solution, on ice, in the dark, for 20 min, before addition of the APS. The polymerised gel was squeezed in two perpendicular directions simultaneously, using a press described by Meiburg [24].

Low-temperature (77 K) spectra were measured in an Oxford cryostat, model DN 1704, connected to an Oxford temperature controller, model ITC4.

Results

Absorption spectra

The absorption spectra of the PS II reaction centre in the presence of 0.20% TX-100 in Tris-HCl buffer (pH 7.2), measured at 277 K and 77 K, are shown in Fig. 1.

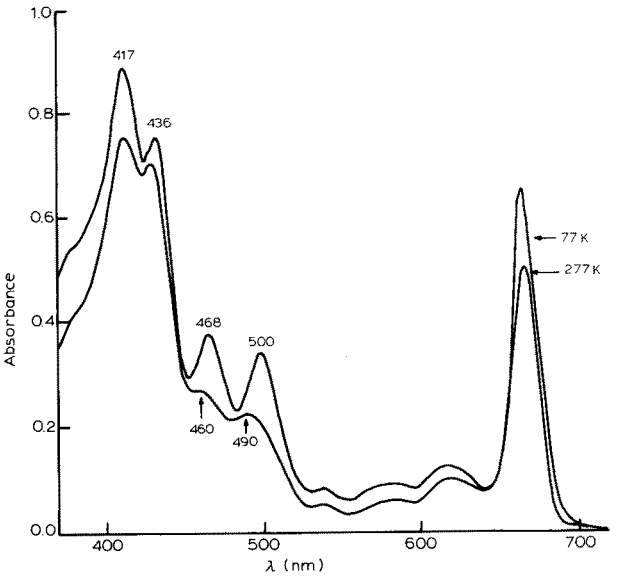

Fig. 1. Absorption spectra of the D1/D2/cytochrome *b*-559 PS II reaction centre complex measured at 277 K (lower curve) and 77 K (upper curve) in a 50 mM Tris-HCl buffer (pH 7.2), containing 0.20% (w/v) TX-100, and in addition 70% (w/v) glycerol for the 77 K spectrum. [Chl] ≈ 5 μg/ml.

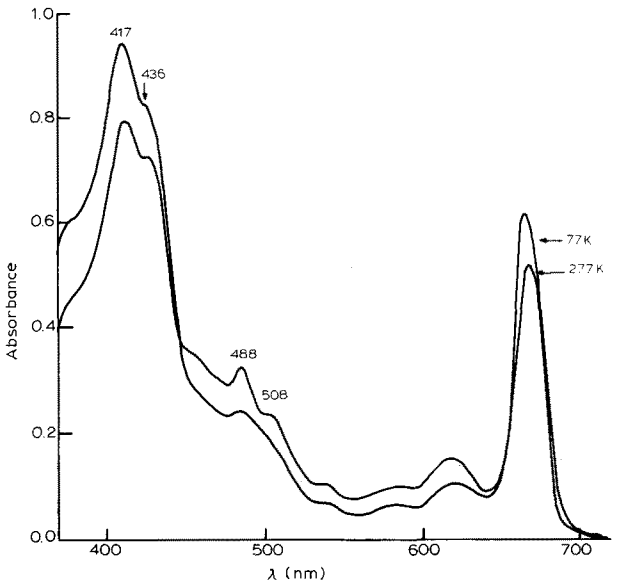

Fig. 2. Absorption spectrum of the D1/D2/cytochrome *b*-559 PS II reaction centre complex measured at 277 K (lower curve) and 77 K (upper curve) in a 50 mM Tris-HCl buffer (pH 7.2), containing 0.02% (w/v) TX-100, and in addition 70% (w/v) glycerol for 77 K spectrum. [Chl] ≈ 5 μg/ml.

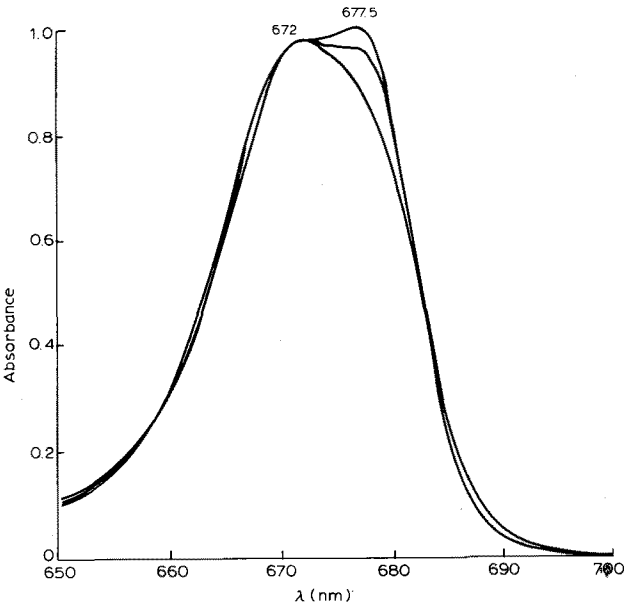

Fig. 3. The $Q_{y(0-0)}$ absorption band of the D1/D2/cytochrome *b*-559 PS II reaction centre complex, measured at 77 K in 50 mM buffer (pH 7.2), 70% (w/v) glycerol, and 0.01% (w/v), 0.10%, and 0.20% (w/v) TX-100. The dipole strength of the lower energy transition decreases at higher concentrations of TX-100. [Chl] ≈ 5 μg/ml.

The most noticeable difference observed by lowering the temperature is the sharpening and red shift of the two lowest energy transitions of β-carotene. The peaks observed at 460 nm and 490 nm in the 277 K spectrum shift to 468 nm and 500 nm, respectively, at 77 K. In addition to this, the Q_y and Soret transitions of the porphyrin pigments also sharpen at lower temperatures.

The equivalent absorption spectra of the PS II RC when suspended in Tris-HCl buffer containing 0.02% TX-100 are different to those measured in the same buffer containing 0.20% TX-100 (see Fig. 2). In both cases the differences occur in the lowest energy porphyrin absorption band, in the carotenoid absorption region, and in the Soret porphyrin absorption peaks. In the low detergent medium the absorption spectrum of the complex remains qualitatively unaltered on lowering the temperature, except for the expected sharpening of the peaks. Of note is that the lowest-energy Q_y transition is broader in the 0.02% TX-100 spectra compared with those measured in the medium containing higher detergent levels due to additional absorption on the red side of the main peak. The carotenoid absorption region now comprises a major peak at 488 nm, a smaller peak at 508 nm, and more than one overlapping transition in the region 440–480 nm. In the Soret absorption bands of the porphyrin molecules, the 436 nm absorption no longer occurs as a distinct absorption peak, but as a shoulder on the main peak at 417 nm.

The effect of TX-100 on the lowest energy absorption band of the PS II reaction centre measured at 77 K is shown in Fig. 3. In all the spectra, the peak clearly comprises at least two transitions. Suspension of the complex in media containing higher concentrations of TX-100 results in a slight decrease in the dipole strength of the longer wavelength transition (the maximum decrease observed is 7%). This results in a rather large change in the position of the absorption maximum from 677.5 nm in 0.01% TX-100 to 672 nm in 0.20% TX-100. A similar effect was reported by Tetenkin et al. [26] except that in their case the long-wavelength absorption transition did not exceed the absorption at 672 nm.

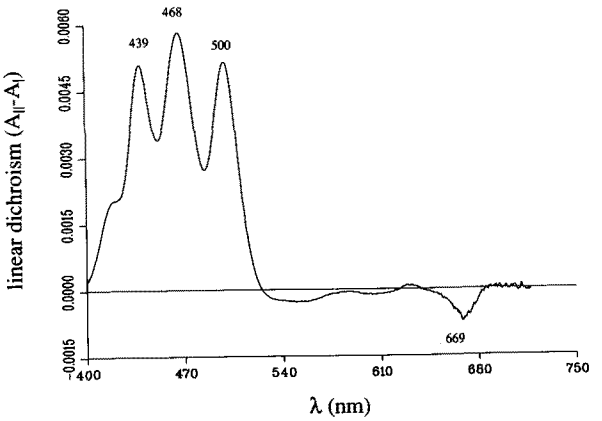

Fig. 4. Linear dichroism spectra of the PS II reaction centre complex measured at 77 K, in 50 mM Tris-HCl buffer (pH 7.2), 70% (w/v) glycerol, and 0.20% (w/v) TX-100. [Chl] ≈ 5 μg/ml.

Linear dichroism spectra

The linear dichroic properties of the isolated PS II reaction centre complex were found to be dependent on the detergent concentration in the supporting buffer. The 77 K linear dichroism (LD) spectrum of the PS II reaction centre complex suspended in buffer containing 0.20% TX-100 (Fig. 4) has positive peaks at 468 nm and 500 nm due to β-carotene absorption, and a peak at 439 nm due to Chl *a* and β-carotene. These peak positions coincide with those observed in the absorption spectrum under the same conditions. We also observed a small negative band extending from about 650 nm to 690 nm in the $Q_{y(0-0)}$ absorption band of the porphyrins. This LD spectrum is qualitatively similar to that measured previously at 277 K [20] except that the magnitudes due to the β-carotene transitions at 468 nm and 500 nm are more intense due to the lower temperature.

The linear dichroism spectrum of the PS II reaction centre measured at 77 K in a medium containing a lower concentration of TX-100 (0.02%) (see Fig. 5) shows significant differences from that measured in 0.20% TX-100 and is similar to that reported originally by Van Dorssen et al. [19]. Major differences between the spectra shown in Figs. 4 and 5 are seen in the region 650–690 nm, and in the transitions below 550 nm. The broad negative band in the red is replaced by strong negative and positive LD extrema at 667 nm and 681 nm, respectively. A similar feature was also observed by Van Dorssen et al. [19], except that in their spectra the red extrema were slightly to the blue of those shown in Fig. 5, possibly due to the difference in the measuring temperatures (4 K versus 77 K). The single positive LD peaks in the carotenoid region observed in 0.20% TX-100 are replaced by large sign changes in the LD measured in 0.02% TX-100. Maxima are seen at 474 nm and 506 nm, and minima at 460 nm and 491 nm. The shoulder observed at 515 nm and peak at 543 nm are the Q_x transitions of Pheo *a*. The LD spectrum of the PS II

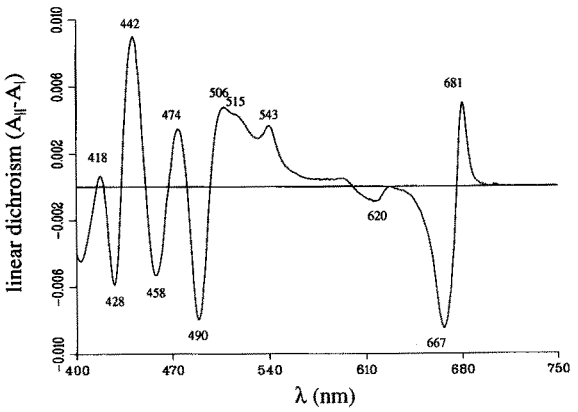

Fig. 5. Linear dichroism spectra of the PS II reaction centre complex measured at 77 K, in 50 mM Tris-HCl buffer (pH 7.2), 70% (w/v) glycerol, and 0.02% (w/v) TX-100. [Chl] ≈ 5 μg/ml.

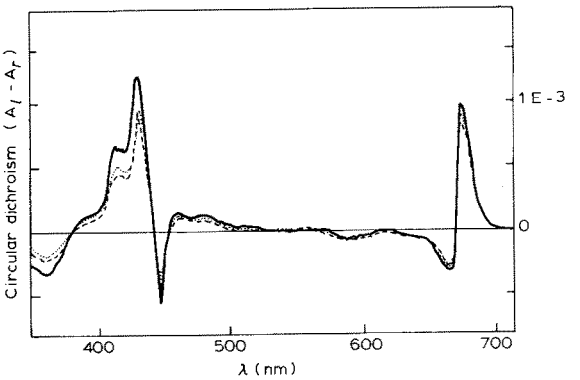

Fig. 6. Circular dichroism spectra of the PS II reaction centre complex measured at 77 K, in 50 mM Tris-HCl buffer (pH 7.2), containing 0.02% (———), 0.20% (· · · · · ·), or 1.0% (- - - - - -) TX-100. [Chl] ≈ 10 μg/ml.

reaction centre in 0.03% *n*-dodecyl β-D-maltoside at 277 K is almost identical to that observed with 0.02% TX-100 in both peak position and magnitude, both in the carotenoid and in the porphyrin Q_y-region (data not shown, also see Ref. 25).

Circular dichroism spectra

The CD spectra of the PS II reaction centre suspended in buffer containing 0.02%, 0.20%, and 1.0% TX-100 (measured at 277 K) are shown in Fig. 6. The shape of these spectra is the same as that observed by us previously [20] and confirmed by others [18,26] but significantly different to that reported by Van Dorssen et al. [19]. Here we note the very small CD signals associated with β-carotene. Only slight oscillations above the broad positive CD in this region (450–500 nm) are observed. Slightly stronger CD signals in the carotenoid absorption region were observed by Tetenkin et al. [26] but their measurements were conducted at 77 K. The spectrum remains qualitatively unaltered on raising the detergent concentration, apart from a flattening of the spectrum in the carotenoid absorption region. At 1.0% TX-100, the magnitude of the positive CD band at 682 nm is reduced by approx. 20%, compared to the spectra at 0.02% TX-100.

Discussion

The results presented above show that the absorption properties of the pigments of the PS II reaction centre complex are dependent on the concentration of the detergent TX-100 in the suspension medium. It is likely that the changes observed in the absorption properties of the PS II reaction centre pigments are a consequence of changes induced in the organisation of the PS II reaction centre proteins, rather than through a direct effect of detergent on the pigment molecules. The most obvious detergent dependencies are seen in the absorp-

tion and LD spectra in the lowest energy absorption band of the porphyrin molecules (650–690 nm), and in the absorption region of β-carotene (450–510 nm).

The $Q_{y(0-0)}$ absorption band

The changes in the absorption spectra between 650 and 690 nm are difficult to interpret, since at least six porphyrin molecules have transitions in this region, the wavelength maxima of which have not yet been resolved absolutely in the absorption spectrum. However, the absorption spectra in Fig. 3 indicate that suspension of the PS II reaction centre complex in buffers containing higher concentrations of TX-100 leads to a slight decrease in the dipole strength of one or more of the component transitions absorbing at the red side of the main Q_y absorption band. The reduction in absorbance at 677.5 nm is small: 3% in 0.10% TX-100, and 7% in 0.20% TX-100, relative to the absorbance of the complex when suspended in 0.01% TX-100. Since the magnitudes of the two resolvable transitions in the region 650–700 nm are very similar, a small decrease in the longer wavelength absorption is sufficient to bring about a shift in the overall observed absorption maximum from 677.5 nm to 672 nm. This observation may explain the variability in the wavelength of the absorption maximum of the PS II reaction centre complex reported by different authors [1,6,18–20,25] and already emphasised by Tetenkin et al. [26] and Seibert et al. [27].

The 681 nm positive LD peak observed when the isolated complex is suspended in low levels of detergent is probably caused by a transition which absorbs at slightly higher energy, since no resolvable component is present in the absorption spectrum at this wavelength. We suggest that the shift of the observed peak position to a longer wavelength is most likely due to a strongly negative LD signal at a shorter wavelength. Consequently, the observed LD spectrum is a composite of the two overlapping transitions. A similar LD feature was observed by Van Dorssen et al. [19] for isolated PS II reaction centres at 4 K. The reason for the loss of the long wavelength LD signals with increased TX-100 is unclear. A possible contribution to this effect is the likely difference in the shape of the complex at the two different detergent concentrations, giving rise to different orientation properties of the reaction centre in the squeezed gel. We consider it unlikely, however, that the loss of the 685 nm LD peak results from large changes in the organisation of the porphyrin molecules within the complex, since at even higher concentrations of TX-100 (1.0%), the rotational strength in this region is reduced only slightly (Fig. 6).

It has been reported that the photochemical activity of the PS II reaction centre complex and its resistance to damage by strong illumination, is preserved when the detergent TX-100 is exchanged for dodecyl β,D-maltoside (DM) [9,27] or digitonin [28], or when TX-100 is removed by precipitation of the protein with poly(ethylene glycol) [27], or when the use of TX-100 for membrane solubilisation is completely replaced by DM [29]. It is suggested in these reports that TX-100 has a damaging effect on PS II, which is related to a shift in the Q_y absorption maximum of the complex to shorter wavelengths, and loss of photochemical activity. The spectra shown in Fig. 3 above indicate that the blue-shift of the $Q_{y(0-0)}$ absorption maximum may arise from a small reduction (less than 7%) in the dipole strength of one or more transitions in the region above 672 nm. We note that all the above procedures, apart from that of Ghanotakis et al. [29] employ 4.0% TX-100 for the initial membrane solubilisation, followed by ion-exchange chromatography in the presence of 0.20% TX-100. We therefore suggest that TX-100 itself does not cause irreversible damage to the PS II reaction centre, as long as sufficient care is taken to keep the preparations in the dark, and at low temperatures (4°C). The 'low' detergent spectrum of the $Q_{y(0-0)}$ region measured in 0.01% TX-100, which shows two resolvable transitions and a maximum at 677.5 nm (Fig. 3), was obtained following dilution of sample stored in buffer containing 0.20% TX-100. We note here that even at 1.0% TX-100, the rotational strength of the bands in the Q_y absorption region is reduced only slightly (CD spectra, Fig. 6). Therefore, the conformation of the pigments that gives rise to the observed CD is little affected by higher TX-100 concentrations.

It has recently been reported [28] that the stability and photochemical activity of the isolated PS II reaction centre is significantly prolonged when suspended in a medium containing the detergent digitonin, even at 30 °C. The absorption spectrum of this sample resembles that in other reports but is characterised by an absorption maximum at 670 nm in the red measured at 77 K. This observation, together with findings presented here (Fig. 3), suggests that the precise position of the maximum absorption in the red is sensitive to the presence of detergent but is not necessarily a reflection of large structural changes leading to loss of activity.

Absorption properties of β-carotene in the PS II reaction centre

We now discuss in more detail the differences observed in the region of β-carotene absorption in the linear dichroism spectra of the PS II reaction centre in low (0.02%) and high (0.20%) concentrations of TX-100.

The initial report of the polarised absorption properties of the isolated D1/D2/cytochrome *b*-559 PS II reaction centre complex measured at 4 K [19] seemed to indicate the presence of two spectrally distinct forms of β-carotene, which were oriented perpendicular to each other in the pigment-protein complex. A subsequent report by ourselves [20] showed only two transitions in the carotenoid region in the absorption and linear di-

chroism spectra measured at 277 K. The results presented here show that both spectra can be obtained with the isolated PS II reaction centre, and that the observed differences arise from differences in the supporting medium, and not from differences in the biochemical preparations.

The first report [19], suggested that the two spectrally distinguishable β-carotene molecules occupy different positions in the PS II reaction centre complex, and that different interactions between these two molecules with the protein matrix give rise to the different absorption maxima of the two species (458 nm and 490 nm for one pool, 474 nm and 506 nm for the other). However, the observation of only one spectral form of β-carotene observed under certain conditions Ref. 20, and this report is not consistent with the conclusion that the PS II reaction centre contains two spectrally distinct forms of β-carotene. Here we examine another possibility, namely that the greater number of transitions observed by Van Dorssen et al. [19] and by us, in the absorption and LD spectra of the complex in lower concentrations of the detergent TX-100, arise as a result of excitonic interactions between individual molecules of β-carotene.

The absorption properties of a pigment dimer coupled by excitonic interaction are altered with respect to the absorption properties of the component monomers [30]. It is possible that the two pairs of LD extrema at 506 nm (positive), 490 nm (negative), and at 474 nm (positive) and 458 nm (negative) observed in the PS II reaction centre complex in 0.02% TX-100 (Fig. 5) arise as a result of excitonic coupling of the two lowest energy transitions of β-carotene. The Davydov splitting would then be 645 cm^{-1} for the first pair of transitions and 737 cm^{-1} for the second pair. The band-centres of these two exciton-split transitions lie at 498 nm and 466 nm. These positions correspond with the absorption and LD maxima of the PS II reaction centre complex observed at 77 K, when measured in buffer containing a higher concentration (0.20%) of TX-100. From the average energy separation, and the dipole strength of the two carotenoid transitions observed in organic solvent, we estimate that the distance between the two carotenoid molecules is approx. 10Å.

A pair of excitonically coupled pigment molecules must lie about a C_2 symmetry axis. Theory predicts [30] that the directions of the excitonic transitions associated with these pigments are perpendicular to each other about this axis. In the low-detergent (0.02% TX-100) LD spectrum (Fig. 5), the proposed exciton-split transitions of β-carotene have LD of different signs, which is consistent with the hypothesis that the transitions in the low-detergent spectrum arise as a result of excitonic coupling between two β-carotene molecules. Since the signs of the transitions observed in an LD spectrum are determined by the orientation properties of the entire complex in the squeezed gel, it seems that the C_2 symmetry axis associated with the two interacting β-carotene molecules corresponds with one of the macroscopic orientation axes of the particle in the squeezed gel. Since the magnitude of the positive and negative peaks of each of the LD absorption pairs are about equally strong, the original monomeric β-carotene transitions will make an angle of approx. 45° with one of the orientation axes of the PS II reaction centre in the gel. At 277 K the CD in the carotenoid absorption region is detectable, but very weak above the broad positive CD observed in the region 460–500 nm (see Fig. 6). Lowering the temperature to 77 K improves this CD only slightly, as shown in Ref. 26. For an excitonically interacting pair of chromophores to have weak associated CD requires that they lie almost in the same plane.

In the original report of the isolation of the PS II reaction centre [1], the ratio of Pheo *a* : β-carotene was estimated to be 2 : 1. If the reaction centre of PS II contains two molecules of Pheo *a*, as seems likely by comparison with the reaction centres from different species of purple bacteria [31,32], then it may also be expected to contain one β-carotene molecule. This seemed to be supported by our linear dichroism spectrum reported earlier, which was measured at 277 K in 0.2% TX-100 [20]. However, in the most recent reports [33,34], the ratio of Pheo *a* : β-carotene was measured as approx. 2 : 2 from HPLC separation of the PS II reaction centre pigments. This implies that the PS II reaction centre contains two molecules of β-carotene, a suggestion made previously by Van Dorssen et al. [19]. based on their 4 K linear dichroism spectrum.

Assuming this stoichiometry (Pheo : β-carotene = 2 : 2) is correct, we consider possible reasons for the loss of excitonic coupling between β-carotene molecules when the PS II reaction centre is suspended in a medium containing higher concentrations of TX-100. The proposed excitonic coupling could arise if the isolated complex forms dimeric structures, or larger aggregates, when suspended in a medium containing low levels of detergent, such that β-carotene molecules on different PS II reaction centres come sufficiently close to each other to experience excitonic coupling. Indeed, dimeric PS II particles have been suggested to occur in vitro and in vivo [35–37]. If this is the case for the isolated reaction centre, the coupling would be abolished by dispersal of the aggregates in media containing higher concentrations of detergent. Alternatively, the structure of the PS II reaction centre proteins themselves could be altered in higher TX-100 concentrations. This alteration may result in disruption of the excitonic coupling by increasing the distance and changing the angle between the interacting β-carotene monomers.

The characteristic sign changes in the LD spectrum of the PS II reaction centre in the region of β-carotene

absorption in low concentrations of TX-100 are also observed in the LD of larger PS II core complexes [20,38–40]. These sign changes were also attributed to the existence of two spectrally different forms of β-carotene. There is some correspondence between the LD extrema observed in the D1/D2 complex in low concentrations of TX-100, and in the larger PS II core complexes. The extrema occur in the isolated PS II reaction centre at 458, 474, 490 and 506 nm, and in the PS II core complex at 458, 476, 488 and 506 nm. Since PS II core complexes from *Synechococcus* sp. have been shown to contain only one chemical species of carotenoid (β-carotene) [41], we suggest that the origin of these LD sign changes is the same in the two systems; i.e., they may arise from the same excitonic interactions between two β-carotene molecules in the reaction centre.

Acknowledgements

Acknowledgement is made of financial support from the Agriculture and Food Research Council. W.N. is most grateful to Drs. David Chapman and Kleoniki Gounaris (Imperial College) for instruction in the preparation of the PS II reaction centre complex, and to John De-Felice for preparing the PS II-enriched membranes. We would like to thank Dr. Alex Drake, Department of Chemistry, Birkbeck College, London, for making available the Jasco J40-C spectropolarimeter for the measurement of the CD spectra. R.v.G. acknowledges valuable discussions with J. Breton. W.N. was in receipt of a SERC research studentship at time of work and acknowledges current financial support from the Royal Society, U.K. (Leverhulme William and Mary Research Fellowship).

References

1 Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
2 Ikeuchi, M. and Inoue, Y. (1988) FEBS Lett. 241,99–104.
3 Webber, A.N., Packman, L., Chapman, D.J., Barber, J. and Gray, J.C. (1989) FEBS Lett. 242, 259–262.
4 Satoh, K. and Nanba, O. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 69–72, Martinus Nijhoff, Dordrecht.
5 Okamura, M.Y., Satoh, K., Isaacson, R.A. and Feher, G. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. I, pp. 379–381, Martinus Nijhoff, Dordrecht.
6 Barber, J., Chapman, D.J. and Telfer, A. (1987) FEBS Lett. 220, 67–73.
7 Takahashi, Y., Hansson, O., Mathis, P. and Satoh, K. (1987) Biochim. Biophys. Acta 893, 49–59.
8 Danielius, R.V., Satoh, K., Van Kan, P.J.M., Plijter, J.J., Nuijs, A.M. and Van Gorkom, H.I. (1987) FEBS Lett. 213, 241–244.
9 Chapman, D.J., Gounaris, K. and Barber, J. (1988) Biochim. Biophys. Acta 933, 423–431.
10 Wasielewski, M.R., Johnson, D.G., Seibert, M. and Govindjee (1989) Proc. Natl. Acad. Sci. USA 86, 524–528.
11 Pierson, B.K. and Olson, J.M. (1987) in Photosynthesis (Amesz, J., ed.), New Comprehensive Biochemistry, Vol. 15, Elsevier, Amsterdam.
12 Loach, P.A., Androes, G.M., Maksim, A.F. and Calvin, M. (1963) Photochem. Photobiol. 2, 443–454.
13 Klimov, V.V., Allakhverdiev, S.I., Demeter, Sh. and Krasnovsky, A.A. (1979) Dokl. Akad. Nauk. SSSR 249, 227–230.
14 Parson, W.W. (1987) in Photosynthesis (Amesz, J., ed.), New Comprehensive Biochemistry, Vol. 15, Elsevier, Amsterdam.
15 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
16 Chang, C.H., Tiede, D., Tang, J., Smith, U., Norris, J. and Schiffer, M. (1986) FEBS Lett. 205, 82–86.
17 Allen, J.P., Feher, G., Yeates, T.O., Komiya, Y. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 6162–6166.
18 Braun, P., Greenberg, B.M. and Scherz, A. (1990) Biochemistry 29, 10376–10387.
19 Van Dorssen, R.J., Breton, J., Plijter, J.J., Satoh, K., Van Gorkom, H.J. and Amesz, J. (1987) Biochim. Biophys. Acta 893, 267–274.
20 Newell, W.R., Van Amerongen, H., Van Grondelle, R., Aalberts, J.W., Drake, A.F., Udvarhelyi, P. and Barber, J. (1988) FEBS Lett. 228, 162–166.
21 Lichtenthaler, H.K. (1987) Methods Enzymol. 148, 350–382.
22 Bokma, J.T., Johnson, W.C. Jr., and Blok, J. (1987) Biopolymers 26, 893–909.
23 Abdourakhmanov, I.A., Ganago, A.O., Erokhin, Y.E., Solevev, A.A. and Chugnov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186.
24 Meiburg, R.F. (1985) Thesis, State University of Leiden, The Netherlands.
25 Breton, J. (1990) in Perspectives in Photosynthesis (Jortner, J. and Pullman, B., eds.), Kluwer, Dordrecht.
26 Tetenkin, V.L., Gulyaev, B.A., Seibert, M. and Rubin, A.B. (1989) FEBS Lett. 250, 459–463.
27 Seibert, M., Picorel, R., Rubin, A.B. and Conolly, J.S. (1988) Plant Physiol. 87, 303–306.
28 Satoh, K. and Nakane, H. (1990) in Progress in Photosynesis Research (Baltcheffsky, M., ed.) Vol. I, 271–274, Martinus Nijhoff, Dordrecht.
29 Ghanotakis, D.F., De Paula, J.C., Demitriou, D.M., Bowlby, N.R., Peterson, J., Babcock, G.T. and Yocum, C.F. (1988) Biochim. Biophys. Acta 974, 44–53.
30 Tinoco, I. (1963) Radiation Res. 20, 133–139.
31 Barber, J. (1987) Trends Biochem. Sci. 12, 321–326.
32 Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
33 Kobayashi, M., Maeda, H., Watanabe, T., Nakane, H. and Satoh, K. (1990) FEBS Lett. 260, 138–140.
34 Gounaris, K., Chapman, D.J., Booth, P., Crystall, B., Giorgi, L.B., Klug, D.R. Porter, G. and Barber, J. (1990) FEBS Lett. 265, 88–92.
35 Dekker, J.P., Boekema, E.J., Witt, H.T. and Rogner, M. (1988) Biochim. Biophys. Acta 936, 307–318.
36 Rogner, M., Dekker, J.P., Boekema, E.J. and Witt, H.T. (1987) FEBS Lett. 219, 207–211.
37 Morschel, E. and Schatz, G.H. (1987) Planta 172, 145–154.
38 Tapie, P., Choquet, Y., Wollman, F.-A., Diner, B. and Breton, J. (1986) Biochim. Biophys. Acta 850, 156–161.
39 Van Dorssen, R.J., Plijter, J.J., Dekker, J.P., Den Ouden, A., Amesz, J. and Van Gorkom, H.J. (1987) Biochim. Biophys. Acta 890, 134–143.
40 Breton, J. and Katoh, S. (1987) Biochim. Biophys. Acta 893, 99–107.
41 Ohno, T., Satoh, K. and Katoh, S. (1986) Biochim. Biophys. Acta 825, 1–8.