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Biochimica et Biophysica Acta 1366 (1998) 256–264

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Long wavelength absorption transitions in the D1/D2/cytochrome *b*-559 complex as revealed by selective pigment photobleaching and circular dichroism measurements

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Received 29 April 1998; revised 27 May 1998; accepted 10 June 1998

Abstract

Photobleaching of the isolated D1/D2/cytochrome (cyt) *b*-559 was performed at room temperature and 80K and the absorption spectra analysed. While at room temperature the permanent bleaching of four red absorbing transitions is observed, at 80K the selectivity is greatly increased with bleaching of transitions at 675 nm and 683 nm. The signal associated with the 683 nm structure is sufficiently strong to permit analysis of the bandwidth and band symmetry characteristics, which in the linear electron-phonon coupling assumption indicates an electron-phonon coupling strength (*S*) of about 0.7–1.0 for coupling to bath phonons of frequency $\nu_m = 20–30$ cm^{-1}, and an essentially symmetrical $Q_y(0,0)$ band shape. Circular dichroism (CD) spectra of the D1/D2/cyt*b*-559 complex were measured at room temperature and 150K and analysed in terms of gaussian subbands. A minimal description with three subbands at 668 nm, 674 nm and 682 nm was found, though a more convincing description required four subbands with peak positions at 668 nm, 674–675 nm, 680.5 nm and 683 nm. The 680.5 nm transition is thought to be associated with P680 and has bandwidth characteristics in line with the published electron-phonon coupling strength (D. Tang, R. Jankowiak, M. Seibert, C.F. Yocum, G.J. Small, J. Phys. Chem. 94 (1990) 6519–6522), indicating that this transition has not undergone significant excitonic narrowing. The 683 nm CD subband is probably associated with the same transition which gives rise to the major absorption photobleaching signal on the basis of its wavelength position and band shape characteristics. The probable presence of four transitions in the CD spectrum of the D1/D2/cyt*b*-559 complex is in line with the suggestion that multiple (weak) excitonic interactions may occur between pigments in this chlorophyll-protein complex.

Keywords: D1/D2/cytochrome *b*-559 complex; Absorption spectroscopy; Circular dichroism; Photobleaching; Electron-phonon coupling; Gaussian decomposition

1. Introduction

Primary photochemistry in photosystem II of plants occurs at the level of the pigment-protein complex known as the D1/D2/cyt*b*-559 complex [1] and involves the reduction of an acceptor pigment (pheophytin) by a donor chlorophyll (P680). This complex

Abbreviations: Bchl, bacteriochlorophyll; CD, circular dichroism; chl, chlorophyll; FWHM, full width at half-maximum; P680, primary electron donor of photosystem II; PSII, photosystem II; RC, reaction centre; RT, room temperature

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0005-2728/98/$ – see front matter
PII: S0005-2728(98)00127-3

may be isolated in a purified state which is sufficiently stable to allow both biochemical and spectroscopic analysis. The most commonly reported chlorin stoichiometry is six chlorophyll *a* and two pheophytin molecules per RC complex though preparations with a lower chl/pheophytin ratio have been reported [2,3]. Also some variability in the spectroscopic properties of D1/D2/cyt*b*-559 preparations has been noticed [3,4] which may be correlated with the number of bound chlorophylls.

Despite the extreme spectral crowding of the pigment absorption bands some progress has been made in identifying a number of individual transitions. Thus what is generally considered to be the low energy transition of the special pair dimer (P680) falls near 680 nm [5]. Another transition which is almost isoenergetic with the P680 band and which is thought to be a pheophytin has been detected by hole burning [5], triplet minus singlet absorption spectroscopy [6] and selective pigment photobleaching [7]. Mimuro et al. [8], on the basis of fluorescence spectroscopy measurements, suggest that the second pheophytin may absorb near 670 nm. Absorption difference spectroscopy in (a) selectively photobleached complexes and (b) complexes binding five chl molecules, indicates the presence respectively of antenna or accessory pigment transition near 674 nm and 670 nm [3,7]. The lowest energy transition in the RC complex occurs near 683–684 nm and has been the object of considerable spectroscopic study and speculation in recent years. Thus Kwa et al. [4] and Chang et al. [3] demonstrated its presence by absorption and triplet minus singlet studies at liquid helium temperatures. More recently Konermann et al. [9], using site selection vibrational fluorescence spectroscopy, demonstrated that this transition is associated with a chl rather than a pheophytin molecule. Eijckelhoff et al. [10], on the basis of the earlier suggestion by Tetenkin et al. [11] and Durrant et al. [12] that all or most of the D1/D2/cyt*b*-559 pigments may be excitonically coupled, interpret the redmost (excitonic) band in terms of a primary electron donor transition. On the other hand, the thermal broadening characteristics of the 683 nm gaussian subband seem to be more in keeping with an antenna or accessory chl [13], though this conclusion should not be overstated as it is based on a decomposition analysis of a spectrally crowded system.

In an attempt to gain further information on the long wavelength absorption transitions in the PSII RC complex we have studied both the selective photobleaching of long wavelength absorption transitions and the circular dichroism spectra. We demonstrate that limited photobleaching of the RC complex at 80K displays a marked selectivity for the 683 nm transition, which permitted direct analysis of its bandwidth and symmetry characteristics. In addition, evidence is presented which indicates that a CD signal may be associated with this long wavelength transition.

2. Materials and methods

The D1/D2/cyt*b*-559 complex was obtained as previously described [13]. The absorption maximum at room temperature was close to 676 nm and pigment analysis indicates that the preparation used in this study had a ratio of chlorophyll to pheophytin of about 3. Absorption spectra of the complex were measured using an EG&G OMAIII (Model 1460) with an intensified diode array (model 1420) mounted on a spectrograph (Jobin-Yvon HR320) with 150 grooves mm^{-1} grating. The wavelength scale of the instrument was calibrated using a neon spectral calibration source (Cathodeon). The wavelength spacing between pixels is about 0.5 nm. Absorption was measured using light from a halogen lamp attenuated by neutral density filters. The light path was 1 mm. Temperature regulation was achieved with a vacuum-assisted Joule-Thompson refrigerating system (model K-2002T; MMR Technologies). The residual absorption at 730 nm was subtracted from the spectra. Photobleaching was carried out focussing the white light beam from a fiberoptic light source (model Volpi intralux 6000; approx. 500 $W\ m^{-2}$) for 30 min on the sample still residing in its support in the sample chamber. A Jasco-600 spectropolarimeter was used to obtain CD spectra with a bandwidth of 2 nm. For both absorption and CD measurements the sample was diluted to 0.3 OD mm^{-1} in a buffer containing Tricine-NaOH 5 mM, pH 8.0, 0.02% dodecylmatoside and 72% (w/v) glycerol. Measured absorption and circular dichroism spectra were analysed by gaussian subband deconvolution, using a non-linear least squares algorithm as previously described [14].

3. Results

In Fig. 1A the photobleaching spectrum is compared with the absorption spectrum for the D1/D2/cyt*b*-559 complex under RT conditions. The photobleaching spectrum at its maximum represents about 10% of the signal before bleaching. As previously noted [7], the bleaching spectrum peaks near 680 nm being red shifted by about 4 nm with respect to the absorption maximum. In the bleaching spectrum a structure can be seen around 674 nm, as previously noted by Garlaschi et al. [7] in a second derivative analysis.

In Fig. 1B the bleaching spectrum is presented together with the absorption spectrum for the D1/D2/cyt*b*-559 complex under the 80K conditions.

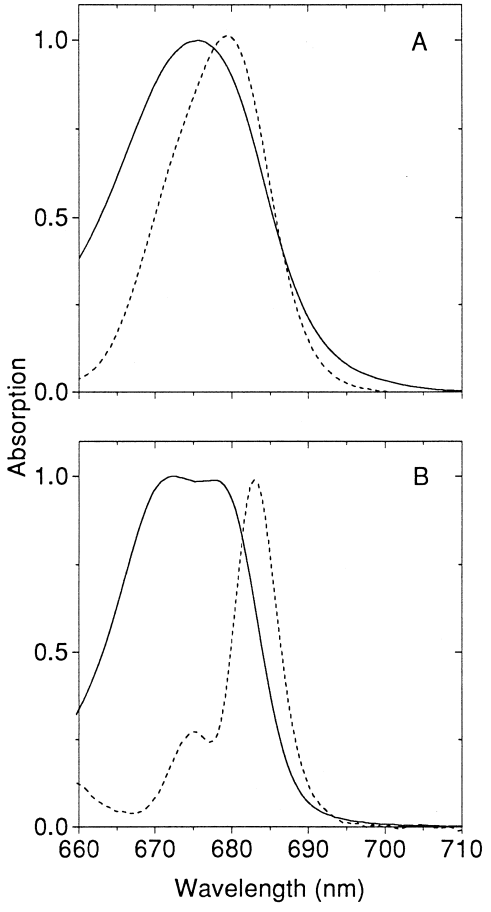

Fig. 1. D1/D2/cyt*b*-559 absorption (arbitrary units, solid line) and photobleaching (dashed line) spectra at room temperature (A) and at 80K (B). In both panels the difference spectra are shown as the control minus bleached spectra. Both spectra are normalised.

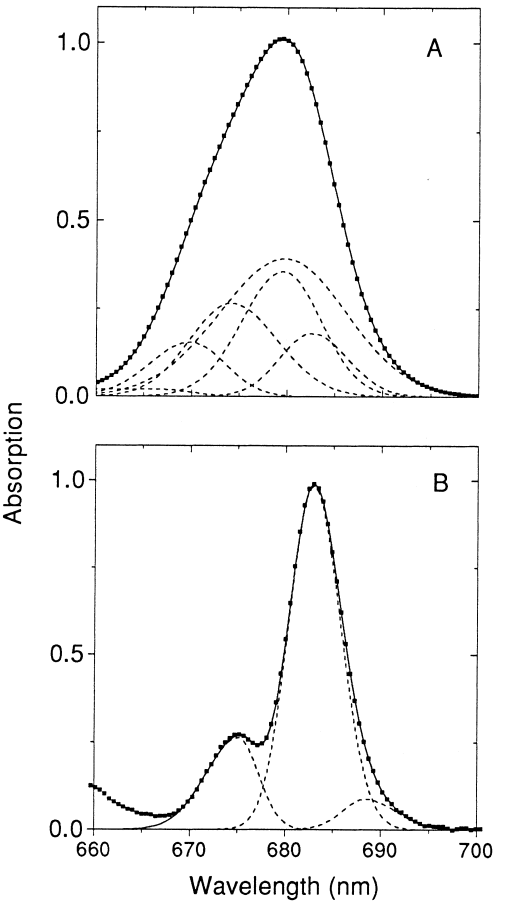

Fig. 2. Decomposition in gaussian subbands of the D1/D2/cyt*b*-559 photobleaching spectra shown in Fig. 1. - - -, gaussian subbands; ——, sum of subbands; ■, experimental spectrum.

The bleaching spectrum is clearly resolved into two bands with maxima at 683 (±0.01) nm and near 675 nm. The major 683 nm band represents bleaching of about 1–2% of the absorption intensity at 683 nm after 30 min irradiation at 80K. This bleaching increases with increasing irradiation time. However, this also leads to decreased selectivity with the development of negative difference spectrum signals which greatly complicates spectral analysis. We have therefore limited our analysis to the 30 min bleached samples. The comparison of the RT and 80K bleaching spectra, taking into account temperature broadening, shows that they have a similar wavelength spread. The major difference is due to the absence of bleaching in the 679–680 nm region at 80K. It is this which allows for the clear resolution of the 675 nm and 683 nm bands. The major 683 nm band has a full width half-maximum (FWHM) of about 7.0 (S.E. ±1 nm,

ten determinations) and is rather symmetrical within the errors ($FWHM_H = 3.4 \pm 0.6$ nm, $FWHM_L = 3.6 \pm 0.5$ nm, the subscripts refer to the high and low energy side of the band).

Both RT and 80K bleaching spectra have been analysed by gaussian decomposition (Fig. 2). In this analysis we were not guided, as is usually done, by an error minimisation procedure. This was due to an inadequate signal/noise ratio in the difference spectra, as can be seen by the above cited errors associated with the mean FWHM. Instead a trial and error procedure was used in which the sum of the subbands was visually compared with the experimental curve. The RT subband description is based on the thermal broadening decomposition of the absorption spectrum study of Cattaneo et al. [13] and shows major contributions by all four long wavelength subbands with maxima near 675 nm, 680 nm (two subbands) and 683 nm. The broad 680 nm gaussian represents P680 and its FWHM is determined by strong electron-phonon coupling [5]. The narrow band near 680 nm has been previously identified as a pheophytin [7]. In the 80K experiment these two subbands are completely absent, with significant gaussian intensity associated with subbands near 675 nm and 683 nm. In addition a minor red shifted subband is required to describe the red tail. The major 683 nm subband has a FWHM of 7.0 nm and is symmetrical within experimental errors.

Fig. 3 shows the CD spectra at 300K, 150K and 110K of the unbleached PSII RC complex in the Q_y absorption region. We have estimated the standard error distribution between 650 and 710 nm which fall in the interval between 0.05 and 0.1 mdeg. As previously reported by others the markedly non-conservative spectrum at room temperature has a positive maximum near 681 nm and a negative peak near 665 nm [11,15,16]. Upon lowering the temperature the negative lobe increases in intensity and shifts to the red by several nanometers, leading to a small red shift of the zero crossover point. This behaviour is expected for the thermal narrowing of a non-conservative spectrum. There is also a small red shift of the positive maximum upon lowering the temperature, which cannot be explained in terms of thermal narrowing and may indicate some minor band position or intensity changes. In addition we note that a clear CD structure becomes apparent in the 674 nm region

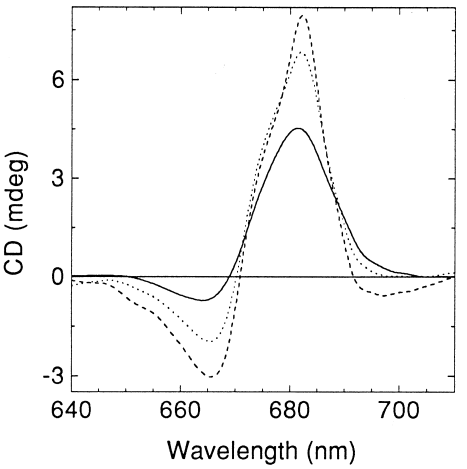

Fig. 3. D1/D2/cyt*b*-559 circular dichroism spectra at 300K (solid line), 150K (dotted line), 110K (dashed line).

upon lowering the temperature while the long wavelength side of the positive lobe remains rather structureless.

These spectra have been analysed by gaussian spectral decomposition and the results are presented in Fig. 4 and Table 1. Owing to the presence of both positive and negative lobes it is possible to find a number of gaussian descriptions which provide a satisfactory numerical description, particularly if asymmetric bands are allowed. We have therefore been guided in our search by a number of line broadening characteristics, in addition to the purely numerical one, and in the assumption that the shapes of CD bands are similar to those of absorption bands. In this respect it is important to point out that the FWHM of the low energy side of the positive lobe ($FWHM_L$) at RT and 150K is 7.5 nm and 5.4 nm respectively. This is in good agreement with the expected values for P680 based on linear electron coupling of intensity $S = 2$ to protein phonons of mean frequency $\nu_m = 25$ cm^{-1} [5] and thus indicates that the long wavelength side of the positive CD lobe may be approximated by a single band the intensity of which is comparable with that of the CD spectrum itself. In addition, only (nearly) symmetrical bands were accepted (see Section 4) and the temperature dependence of the FWHM was required to be in agreement with linear electron coupling to a phonon bath with a mean frequency of approx. 25 cm^{-1} [5]. In this way we have found that a minimal description

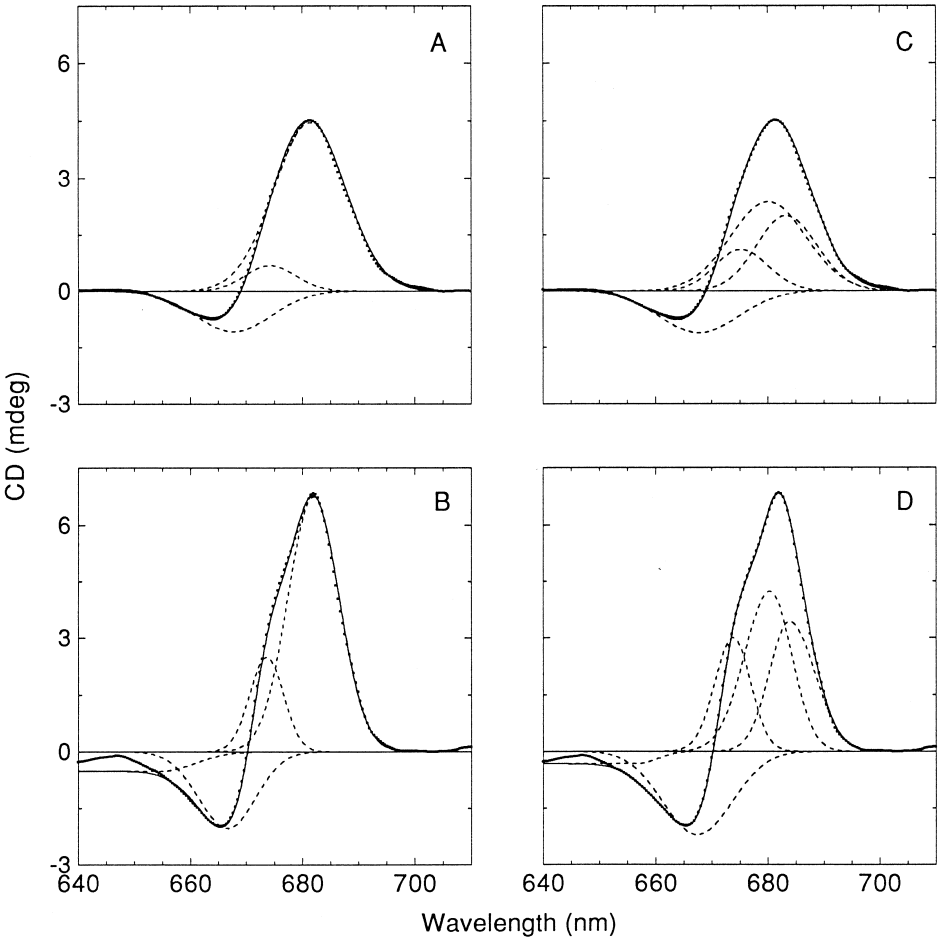

Fig. 4. Gaussian subband decomposition of D1/D2/cyt*b*-559 CD spectra measured at 300K (A,C) and 150K (B,D). (A,B) Three subband decomposition; (C,D) four subband decomposition. - - -, gaussian subbands; ——, sum of subbands; ■, experimental spectrum.

of the main spectral features requires three subbands with wavelength maxima near 668 nm, 674 nm and 682 nm (Fig. 4A,B). From a purely numerical point of view this description is satisfactory within the measurement errors. Both the major, positive band at 682 nm and the negative 668 nm band have similar bandwidth characteristics (about 15–16 nm at RT and 11 nm at 150K) while the 674 nm band is somewhat narrower. In this minimal description the major positive subband, assumed to represent P680, has its wavelength maximum near 682 nm. This is red shifted by 1.5–2 nm with respect to the peak value of the primary donor, as indicated by oxidised minus reduced difference spectra [17], hole burning [5] and triplet minus singlet [6] measurements. We have therefore examined the possibility of including a more red shifted subband in the decomposition description even though no clear CD structure is apparent in the red wing. The most satisfactory description of this kind which we have been able to find is presented in Fig. 4C,D and Table 1, where positive subbands with peak wavelength values near 674–675 nm, 680.5 nm and 683–684 nm and a negative band near 668 nm are found. The principal subband at 680.5 nm has bandwidth characteristics which are substantially unchanged with respect to the main band in the minimal, three subband description. An unexpected feature of these descriptions is the rather large increase in intensity of the 674 nm (positive) and 668 nm (negative) subbands upon lowering the temperature (Table 1). We are unable at present to explain this, though it could in principle be associated with temperature induced changes in pigment-pigment dipole orientation and/or distance.

Table 1
Gaussian parameters for the subband decompositions of circular dichroism spectra of D1/D2/cyt*b*-559 complex, measured at 300 and 150K

	300K	150K
Three band description		
Band I (−)		
λ_{max} (nm)	668	666.9
FWHM (nm)	16.1	11.9
Area (%)	26.0	36.1
Band II (+)		
λ_{max} (nm)	674.4	673.8
FWHM (nm)	10.6	7.1
Area (%)	10.8	26.6
Band III (+)		
λ_{max} (nm)	681.7	682.2
FWHM (nm)	15.2	10.8
Area (%)	100	107.6
Four band description		
Band I (−)		
λ_{max} (nm)	667.9	667.6
FWHM (nm)	16.8	14.6
Area (%)	28.5	48.0
Band II (+)		
λ_{max} (nm)	675.4	674.0
FWHM (nm)	11.1	7.6
Area (%)	18.1	33.8
Band III (+)		
λ_{max} (nm)	680.4	680.6
FWHM (nm)	15.9	9.9
Area (%)	56.0	62.0
Band IV (+)		
λ_{max} (nm)	683.5	684.1
FWHM (nm)	12.7	9.5
Area (%)	37.7	32.1

The areas of the gaussians have been normalised to the area of the 681 nm positive subband at 300K, which is set at 100.

4. Discussion

Irradiation of aerobic suspensions of the D1/D2/cyt*b*-559 complex with white light at RT brings about the selective bleaching of long wavelength absorbing pigments [7,18–20]. In the present study we have extended this photobleaching technique to 80K and noticed that a high degree of bleaching selectivity is attained. Thus, while at RT significant bleaching of four pigment pools with wavelength maxima near 675 nm, 680 nm (two subbands) and 683 nm occurs, at 80K only the 675 nm and 683 nm pools are degraded, with a particularly high degree of selectivity for the 683 nm pool. We exclude that these irreversible bleaching changes are associated with the charge separated state, as this decays on a nanosecond time scale and is blue shifted by about 2 nm with respect to the major 683 bleaching [21–23]. Interestingly, Visser et al. [24] performed a subpicosecond transient absorption study at 77K where they observed a transient difference spectrum after about 1 ps very similar to the permanent photobleaching structure at 683 nm presented here, both in terms of wavelength position and band shape. It therefore seems possible that the spectrum of Visser et al. [24] represents a ground state bleaching of essentially the same transition which we report on here. Also, van Kan et al. [22] suggested the presence of a minor gaussian subband near 683 nm in the long wavelength tail of their difference spectrum, peaking at 680 nm, of the charge separated state. However, in our 80K photobleaching spectrum no structure is apparent in the 680 nm region. It also seems unlikely that the photobleaching spectrum we observe may be associated with electrochromic band shifts of the kind studied by Mulkidjanian et al. [25], given its stability and the absence of the characteristic positive and negative signals. Furthermore, the marked band symmetry of the 683 nm bleaching structure argues against the possibility of it being due to band shifts. We, therefore, interpret the photobleaching signal in terms of irreversible pigment photodestruction of the kind already observed [18,19,26,27]. The 683 nm-photobleached band probably represents photodegradation of the same pigment previously observed in 6K absorption measurements after photobleaching at RT [19] of the PSII core complex. As mentioned in Section 1 an absorption transition near 683 nm has been detected by a variety of spectroscopic approaches [3,4,9,13] and it is considered to be the lowest energy transition of the PSII RC complex. We interpret the 683 nm bleaching structure observed here at 80K to represent the homogeneously and inhomogeneously broadened $Q_y(0,0)$ transition of this redmost spectral form.

In the following discussion we will assume that thermal broadening of the 683 nm transition is determined by linear electron-phonon coupling of strength S to bath phonons of mean frequency ν_m. In this case the band width (FWHM) is given by

$$\mathrm{FWHM}^2 = \mathrm{FWHM}^2_{\mathrm{hom}} + \mathrm{FWHM}^2_{\mathrm{inh}} \quad (1)$$

where the first term represents the so-called homogeneous broadening and the second term is the inhomogeneous site distribution generally associated with statistical fluctuations of pigment site energies. This second term is thought to be temperature insensitive. From an extensive hole burning analysis (2–4K) of antenna pigments and RC primary donor pigments Small and co-workers [5] have concluded that the mean thermally active phonon frequencies, ν_m, fall in the 20–30 cm^{-1} range for chls bound to chl-protein complexes, including the D1/D2/cyt*b*-559 complex [5]. They furthermore show that the electron-phonon coupling strength, S, is quite weak ($S \approx 0.5$–1) for antenna pigments and strong ($S \geq 2$) for primary donor pigments. Thermal broadening analysis of absorption subbands in the 80–300K range [13] is in agreement with these conclusions and indicates that the linear electron-phonon assumption is reasonable. From Fig. 2 the 683 nm subband has a FWHM of 7.0 nm (150 cm^{-1}). If we assume a $FWHM_{inh} = 100$ cm^{-1} and $\nu_m = 20$–30 cm^{-1} this measured FWHM value yields an electron-phonon coupling strengths between 0.7 and 1.0. For a coupling strength of 2 ($\nu_m = 25$ cm^{-1}) the expected FWHM is about 200 cm^{-1} (9 nm), well outside the errors of our measurement. This suggestion of a relatively weak electron-phonon coupling strength for the 683 nm transition is in agreement with the fluorescence, hole burning studies of Groot et al. [28]. The very recent suggestion of Peterman et al. [29] that an additional phonon mode around 80 cm^{-1} is seen in site selected fluorescence measurements with excitation in this long wavelength transition has not been considered in the present calculations. However, if this mode were to carry significant coupling strength, also in absorption, it would lead to thermal broadening, with the result that our calculation of S (for coupling to the main low frequency phonon modes) would be an overestimate. We therefore conclude that the 80K photobleaching difference spectrum bandwidth suggests rather weak electron-phonon coupling for the 683 nm transition, of the kind which characterises monomer, antenna type chls. The earlier suggestion that the 683 nm transition has weak electron-phonon coupling [13], based on a thermal broadening analysis of the gaussian decomposition subbands, is therefore confirmed. This conclusion is also in agreement with Konermann et al. [9] who demonstrated that the fluorescence vibrational structure of the long wavelength transition is that of a chl monomer.

We now address the question of band symmetry. The bleaching spectrum of the 683 nm transition is essentially symmetrical within the experimental errors. This is in reasonable agreement with the skewness coefficient calculations of Zucchelli et al. [30] for an absorption transition which is fairly weakly coupled ($S = 0.5$–0.8) to phonon frequencies in the 20–30 cm^{-1} interval. In this case for 80K the high energy side of the absorption band was estimated to be about 10% broader than the low energy side when a symmetrical gaussian inhomogeneous distribution of 100 cm^{-1} was used. A similar result is apparent in the chl absorption calculations of Peterman et al. [31] at this temperature. Our inability to detect this slight asymmetry on the blue side of the 683 nm band could be due to experimental errors or possibly to an asymmetry in the site distribution function.

It should be mentioned that the high energy side might also be slightly broadened by the presence of Franck-Condon active vibrational modes. However, if the lowest lying vibrational mode has a frequency of 260 cm^{-1} and is weakly coupled, as suggested by hole burning of PSI antenna chl [32] (however see [31]), no significant effect is expected on the homogeneously and inhomogeneously broadened zero-zero transition at 80K (unpublished data).

Konermann and Holzwarth [9,33] have proposed that the D1/D2/cyt*b*-559 chls have very asymmetric bands with the high energy side about twice as broad as the low energy side at 80K. The present data, which demonstrate substantial band symmetry for the 683 nm transition of the PSII RC complex, are clearly in disagreement with this suggestion.

Tetenkin et al. [11] initially suggested that the transition dipole moments of the D1/D2/cyt*b*-559 complex pigments might interact to form an excitonically coupled cluster. This was subsequently taken up by Durrant et al. [12] and analysed by assuming structural analogy with the bacterial RC. In this case the D1/D2/cyt*b*-559 complex is envisaged as binding four chls and two pheophytins in a (weakly) interacting 'core' cluster with the remaining two chls being bound more 'externally'. In this hypothesis the absorption transitions are suggested to be determined primarily by multiple excitonic interactions. It is

therefore of interest that our gaussian analysis of CD spectra indicates the presence of three and possibly four subbands with wavelength maxima near 668 nm, 674 nm, 680–682 nm and possibly 683 nm. Due to the rather large experimental errors associated with CD measurements it is not possible to distinguish between the three and four subband descriptions on numerical criteria. However, the wavelength position of the major positive subband, near 680.5 nm in the four band description, favours this one. The major positive band is rather broad at RT (FWHM about 16 nm) and displays a pronounced temperature sensitivity (10–11 nm at 150K). In the linear electron-phonon coupling approximation these band widths are in good agreement with $S \approx 2$, $\nu_m = 25\ cm^{-1}$, $FWHM_{inh} = 100\ cm^{-1}$ as determined by hole burning [5]. Braun et al. [16] described the PSII RC CD spectrum at RT in terms of two main gaussians near 680 nm (positive) and 668 nm (negative) which were interpreted respectively as the low and high energy exciton bands of a P680 dimer. These authors also suggested the presence of an extremely weak transition near 675 nm, thought to be associated with vibrational bands of the P680 electronic transition. While our description of the 668 nm and 680–682 nm transitions are consistent with the interpretation of Braun et al. [16], it is evident from the low temperature analysis that the 674 nm band is quite intense. We therefore suggest that this CD band may be associated with the electronic transition detected near this wavelength in absorption photobleaching experiments (Fig. 2). We are at present performing parallel CD and absorption photobleaching experiments to investigate this possibility.

As pointed out above, the bandwidth of the principal CD subband near 680 nm in both the three and four subband descriptions is rather broad and in excellent agreement with the electron-phonon coupling characteristics described by Tang et al. [5] from hole burning experiments on P680. Furthermore there is close agreement with the oxidised minus reduced difference spectrum for P680 of Witt et al. [17]. This suggests that this transition, thought to be associated with either a chlorophyll dimer or multimer, is not subject to significant excitonic narrowing [34].

In order to position the major positive subband near 680 nm a CD gaussian with significant intensity near 683–684 nm is required. While we feel that the assigned bandwidths for this putative CD subband should be regarded with caution due to the rather large measurement errors and the lack of structure in this spectral region, for a $FWHM_{inh} = 100\ cm^{-1}$ and $\nu_m = 25\ cm^{-1}$ a value of $S = 1.1$ is calculated. This value is rather similar to that calculated above for the 683 nm absorption transition from photobleaching experiments. The correspondence of the redmost CD subband with the photobleaching structure at 683 nm both in terms of wavelength position and apparent electron-phonon coupling lends weight to the four subband CD description. This description of the D1/D2/cyt*b*-559 CD spectrum is quite different from the previous interpretation of Braun et al. [16] which was exclusively in terms of a P680 dimer, i.e. with two electronic transitions, and may be in line with the multimer suggestion of Tetenkin et al. [11] and Durrant et al. [12]. We are presently investigating this aspect by performing selective photobleaching experiments and analysing the CD spectra for subband correlations.

It is well known that there is considerable sequence homology between the D1 and D2 polypeptides of PSII and the L and M subunits of the structurally well-characterised reaction centre of *Rhodopseudomonas viridis* [35,36]. This has prompted the suggestion that there may also be a close steric relation between the pigments. However, whereas the bacterial reaction centre binds four Bchl molecules that of PSII binds six. This led to the suggestion that the two extra PSII chlorophylls may be 'external' to the inner core of four chlorophyll molecules [3,37]. As the lowest energy transition in the bacterial reaction centre is associated with the primary donor [38–40] it has been suggested that the 683 nm transition in the D1/D2/cyt*b*-559 complex is associated with one of the 'external' pigments [3,37]. The present observation that this long wavelength transition may be irreversibly photobleached with quite high selectivity and without apparent absorption changes in the 680 nm region does in fact suggest that it may be located externally to the primary donor pigment(s).

References

[1] O. Nanba, K. Satoh, Proc. Natl. Acad. Sci. USA 84 (1987) 109–112.

[2] M. Seibert, in: J. Deisenhofer and J. Norris (Eds.), The Photosynthetic Reaction Centre, vol. 1, Academic Press, New York, 1993, p. 317.
[3] H.C. Chang, R. Jankowiak, N.R.S. Reddy, C.F. Yocum, R. Picorel, M. Seibert, G.J. Small, J. Phys. Chem. 98 (1994) 7725–7735.
[4] S.L.S. Kwa, C. Eijckelhoff, R. van Grondelle, J.P. Dekker, J. Phys. Chem. 98 (1994) 7702–7711.
[5] D. Tang, R. Jankowiak, M. Seibert, C.F. Yocum, G.J. Small, J. Phys. Chem. 94 (1990) 6519–6522.
[6] R. van der Vos, P.J. van Leeuwen, P. Braun, A.J. Hoff, Biochim. Biophys. Acta 1140 (1992) 184–198.
[7] F.M. Garlaschi, G. Zucchelli, P. Giavazzi, R.C. Jennings, Photosynth. Res. 41 (1994) 465–473.
[8] M. Mimuro, Plant Cell Physiol. 34 (1993) 321–327.
[9] L. Konermann, I. Yruela, A.R. Holzwarth, Biochemistry 36 (1997) 7498–7502.
[10] C. Eijckelhoff, F. Vacha, R. van Grondelle, J.P. Dekker, J. Barber, Biochim. Biophys. Acta 1318 (1997) 266–274.
[11] V.I. Tetenkin, B.A. Gulyaev, M. Seibert, A.B. Rubin, FEBS Lett. 250 (1989) 459–463.
[12] J.R. Durrant, D.R. Klug, S.L.S. Kwa, R. van Grondelle, J.P. Dekker, Proc. Natl. Acad. Sci. USA 92 (1995) 4798–4802.
[13] R. Cattaneo, G. Zucchelli, F.M. Garlaschi, L. Finzi, R.C. Jennings, Biochemistry 34 (1995) 15267–15275.
[14] G. Zucchelli, R.C. Jennings, F.M. Garlaschi, J. Photochem. Photobiol. B Biol. 6 (1990) 381–394.
[15] W.R. Newell, H. van Amerongen, J.E. Barber, R. van Grondelle, Biochim. Biophys. Acta 1057 (1991) 232–238.
[16] P. Braun, B.M. Greenberg, A. Scherz, Biochemistry 29 (1990) 10376–10387.
[17] H.T. Witt, E. Schlodder, K. Brettel, O. Saygin, Ber. Bunsen-Ges. Phys. Chem. 90 (1986) 1015–1024.
[18] A.N. Macpherson, A. Telfer, J. Barber, T.G. Truscott, Biochim. Biophys. Acta 1143 (1993) 301–309.
[19] N.P. Mishra, C. Francke, H.J. van Gorkom, D.F. Ghanotakis, Biochim. Biophys. Acta 1186 (1994) 81–90.
[20] T. Tomo, M. Mimuro, M. Iwaki, M. Kobayashi, S. Itoh, K. Satoh, Biochim. Biophys. Acta 1321 (1997) 21–30.
[21] G. Hastings, J.R. Durrant, J. Barber, G. Porter, D.R. Klug, Biochemistry 31 (1992) 7638–7643.
[22] P.J.M. van Kan, S.C.M. Otte, F.A.M. Kleinherenbrink, M.C. Nieven, T.J. Aartsma, H.J. van Gorkom, Biochim. Biophys. Acta 1020 (1991) 146–152.
[23] B. Hillmann, K. Brettel, F. van Mieghem, A. Kamlowski, A.W. Rutherford, E. Schlodder, Biochemistry 34 (1995) 4814–4827.
[24] H.M. Visser, M.L. Groot, F. van Mourik, I.H.M. van Stokkum, J.P. Dekker, R. van Grondelle, J. Phys. Chem. 99 (1995) 15304–15309.
[25] A.Y. Mulkidjanian, D.A. Cherepanov, M.S. Haumann, W. Junge, Biochemistry 35 (1990) 3093–3107.
[26] A. Telfer, J. Barber, FEBS Lett. 246 (1989) 223–228.
[27] A. Telfer, Wei-Z He, J. Barber, Biochim. Biophys. Acta 1017 (1990) 143–151.
[28] M.L. Groot, J.P. Dekker, R. van Grondelle, F.T.H. den Hartog, S. Volker, J. Phys. Chem. 100 (1996) 11488.
[29] E.J.G. Peterman, Doctorate Thesis, Ch. 7, 1998, pp. 91–103.
[30] G. Zucchelli, F.M. Garlaschi, R.C. Jennings, Biochemistry 35 (1996) 16247–16254.
[31] E.J.G. Peterman, T. Pullerits, R. van Grondelle, H. van Amerongen, J. Phys. Chem. 101 (1997) 4448–4457.
[32] J.K. Gillie, G.T. Small, J.H. Golbeck, J. Phys. Chem. 93 (1989) 1620–1627.
[33] L. Konermann, A.R. Holzwarth, Biochemistry 35 (1996) 829–842.
[34] A.S. Davydov, Theory of Molecular Excitons, Plenum Press, New York, 1971.
[35] P. Mathis, A.W. Rutheford, in: J. Amesz (Ed.), Photosynthesis, Elsevier Science, Amsterdam, 1987, pp. 63–96.
[36] J. Deisenhofer, O. Epp, K. Mike, R. Huber, H. Michel, Nature 318 (1985) 618.
[37] H.C. Chang, G.J. Small, R. Jankowiak, Chem. Phys. 194 (1995) 323–333.
[38] R. Friesner, Y. Won, Biochim. Biophys. Acta 977 (1989) 99–122.
[39] W.W. Parson, in: H. Sheer (Ed.), Chlorophylls, CRC Press, Boca Raton, FL, 1991, pp. 1153–1180.
[40] N.R.S. Reddy, S.V. Kolaczkowski, G.J. Small, Science 260 (1993) 68–71.