Biochimica et Biophysica Acta, 1016 (1990) 49–54
Elsevier

BBABIO 43164

Characterization of bonding interactions of the intermediary electron acceptor in the reaction center of Photosystem II by FTIR spectroscopy

E. Nabedryk [1], S. Andrianambinintsoa [1], G. Berger [1], M. Leonhard [2], W. Mäntele [2] and J. Breton [1]

[1] *Service de Biophysique, Département de Biologie, CEN Saclay, Gif-sur-Yvette (France) and* [2] *Institut für Biophysik und Strahlenbiologie der Universität Freiburg, Freiburg (F.R.G.)*

(Received 25 July 1989)

Key words: Photosystem II; Reaction center; Pheophytin; Intermediary electron acceptor; FTIR

Molecular changes associated with the photoreduction of the pheophytin *a* intermediary electron acceptor in films of Photosystem II reaction center (D1D2 RC) were characterized by FTIR spectroscopy. Upon accumulation at 240 K of the photoreduced acceptor, three negative carbonyl bands are observed at 1739 cm^{-1}, 1721 cm^{-1} and 1677 cm^{-1} in the light-minus-dark FTIR spectrum of D1D2 RC. The redox-induced FTIR spectrum of the pheophytin *a* anion generated electrochemically in tetrahydrofuran shows only two negative bands at 1743 cm^{-1} and 1706 cm^{-1} which are assigned to changes of absorption of the 10a-ester C=O and 9-keto C=O, respectively. These assignments are based upon the comparison between FTIR data obtained on radicals of pheophytin *a* and its pyroderivative lacking the 10a-ester C=O. Thus, the 1677 cm^{-1} band observed in vivo reflects an interacting 9-keto C=O in D1D2 RC. The close similarity observed between: (i) FTIR spectra obtained on Photosystem II and *Rps. viridis* reaction centers and (ii) amino-acid sequences of the L and D1 polypeptides leads to the assignment of the 1721 cm^{-1} band in D1D2 RC to a protein-bound 10a-ester C=O of the acceptor and the 1739 cm^{-1} band to a contribution from the protonated carboxylic group of Glu D1-130 which is proposed to be H-bonded to the 9-keto C=O of the pheophytin acceptor, in the same way as in the *Rps. viridis* reaction center, Glu L104 is interacting with the 9-keto C=O of H_L. The FTIR data indicate that the interactions of the 9-keto C=O and of the 10a-ester of the intermediary acceptor with the protein are stronger in D1D2 RC than in *Rps. viridis*. These stronger interactions could account, at least in part, for the difference in accessibility to ^1H–^2H exchange of the H-bonded proton of the Glu D1-130 side-chain in D1D2 RC compared to *Rps. viridis* reaction center.

Introduction

The primary photochemistry occurring in the Photosystem II (PS II) of cyanobacteria, algae, and plants is currently thought to involve a charge separation between the chlorophyll(s) constituting the primary electron donor (P680), and a pheophytin acceptor (Phe). This event is then followed by a stabilization step in which the electron proceeds from Phe to the first quinone acceptor. With respect to these primary reactions, which have been extensively characterized both by absorption and EPR spectroscopies (for a review, see Ref. 1), PS II bears striking analogies with the reaction center (RC) from purple photosynthetic bacteria for which the recent crystallographic determination of its structure [2–4] has provided a picture at atomic resolution of the detailed organization of the pigments and protein.

Studies of PS II have made significant progress since the recent purification, using the detergent Triton X-100, of a minimal PS II RC [5] comparable to that of purple bacteria. This photochemically active PS II complex, named D1D2 RC, binds a small number of chlorophylls and contains, in addition to cytochrome *b*-559, two polypeptides, D1 and D2, which display analogy in

Abbreviations: PS II, Photosystem II; Pheo *a*, pheophytin *a*; pyro-Pheo *a*, pyropheophytin *a*; Chl *a*, chlorophyll *a*; pyroChl *a*, pyrochlorophyll *a*; Phe, pheophytin *a* intermediary electron acceptor in PS II; RC, reaction center; H_L, bacteriopheophytin *b* intermediary electron acceptor, *Rps.*, *Rhodopseudomonas*; *C.*, *Chromatium*; FTIR, Fourier transform infrared spectroscopy; THF, tetrahydrofuran.

Correspondence: E. Nabedryk, Service de Biophysique, Département de Biologie, C.E.N. Saclay, 91191 Gif-sur-Yvette Cedex, France.

their primary amino-acid sequence with those of the L and M subunits of bacterial RC. The isolation of this new complex produced proof for the proposals of Trebst [6] and Michel and Deisenhofer [7] that D1 and D2 form the core of the PS II RC, and thus play a role similar to the polypeptides L and M which constitute the scaffold of transmembrane α-helices holding together in a precise geometry the cofactors of the bacterial RC.

Without high-resolution X-ray data for plant photosystems, proposals for the structure and bonding interactions of their primary reactants rely on spectroscopy. In previous studies of bacterial RC and Photosystems I and II (using large chlorophyll-protein complexes containing more than 100 antenna chlorophylls per RC), we have demonstrated that molecular changes associated with the photooxidation of primary electron donors or photoreduction of intermediary acceptors can be investigated by light-induced FTIR difference spectroscopy [8–10]. With this technique, sensitivity can be high enough to detect changes of individual bonds in a large protein complex. In particular, specific changes in the infrared absorption of chlorophyll carbonyl groups have been detected and conclusions have been drawn on the interactions of the pigments with their native environment. This has prompted us to investigate a newly developed D1D2 RC preparation stabilized in the detergent lauryl maltoside, which exhibits a high yield of primary photochemistry. In the present work, we have detected specific light-induced FTIR absorption changes associated with the photoreduction of Phe in D1D2 RC. In order to interpret these FTIR signals, the infrared spectra of isolated pheophytin *a* (Pheo *a*) and pyro-Pheo *a* (lacking the 10a-ester carbonyl) were investigated. Comparison of light-induced spectra obtained in vivo with redox-induced spectra of the (pyro)Pheo *a* anion obtained in vitro leads to the description of the interactions the 9-keto and 10a-ester carbonyls of the acceptor experience with the protein of the D1D2 RC. A preliminary account of this work has been presented [11].

Experimental

The D1D2 RC complex was prepared essentially as in Ref. 5 but chloroplasts from pea rather than spinach were used and Triton X-100 in the NaCl gradient elution buffer was replaced by 0.03% lauryl maltoside as described in Ref. 12. Absorption spectra of the isolated complex as well as light-induced absorption changes upon photoaccumulation of reduced Phe in the presence of 100 mM sodium dithionite were obtained in the visible spectral range [12].

For infrared spectroscopy, a suspension of D1D2 RC was deposited on CaF_2 windows and air-dried. Films were covered with a 150 mM sodium borate buffer (pH 9.5) containing 150 mM sodium dithionite and sealed with another CaF_2 window, constituting microcells (about 10 μm optical pathlength) which were thermostated. For ^1H–^2H exchange experiments, the buffer was made in ^2H_2O and films were left to equilibrate for 1.5 h. Light-minus-dark FTIR spectra were obtained as previously described [9,10].

Pheophytin *a* was prepared from chlorophyll *a* (Chl *a*), in the presence of gaseous HCl diluted with nitrogen bubbling in the chlorophyll solution. PyroPheo *a* was obtained from Pheo *a* by the pyrolysis method of Pennington et al. [13]. Purification of Pheo *a* and its pyro-derivative was achieved by HPLC as described in Ref. 14. Solutions of pheophytins (1–3 mM) were prepared in tetrahydrofuran (THF). The techniques used for solvent drying, for purification of the supporting electrolyte (tetrabutylammonium hexafluorophosphate) and for electrochemistry have been described elsewhere (Ref. 15 and references therein). Using a transparent thin-layer electrochemical cell [15], visible and infrared spectra were recorded before and after anion formation.

Results

The original preparation of D1D2 RC involves solubilization of PS II membranes in 4% Triton X-100 followed by chromatography and elution in a buffer containing 0.05% Triton X-100 [5]. These particles are rather unstable and rapidly lose their photochemical activity when brought to room temperature or submitted to air-drying for infrared sample preparation. The report that the stability of D1D2 RC was greatly improved when Triton X-100 was replaced by lauryl maltoside after the isolation of D1D2 RC [16] suggested exchanging the detergent on the purification column just before the elution step as described in Ref. 12. These more stable particles (Refs. 12, 16, see also Refs. 17 and 18), when dithionite-treated, exhibit characteristic light-induced absorption changes in the visible spectral range (at 680, 542, 515 and 420 nm), reflecting, the bleaching of Phe [5,19], together with the appearance of the Phe radical anion (at 700, 650, 595 and 450 nm) and the ensuing electrochromic shift of the nearby chromophores (Fig. 1 and Ref. 12).

The light-minus-dark FTIR difference spectra of films of D1D2 RC prereduced with sodium dithionite in H_2O or ^2H_2O buffer and illuminated at 240 K are shown in Figs. 2a and 2b, respectively. Positive bands in these spectra (referred as Phe^-/Phe spectrum) arise from the photoinduced state while the disappearing bands of the neutral state are negative. Due to the decay of Phe^- at room temperature, these signals are fully reversible upon warming the sample in the dark. Three negative bands are detected in the carbonyl stretching domain at 1739 cm^{-1}, 1721 cm^{-1}, and 1677 cm^{-1}. They are assigned to vibrations which disappear or shift after photoreduction

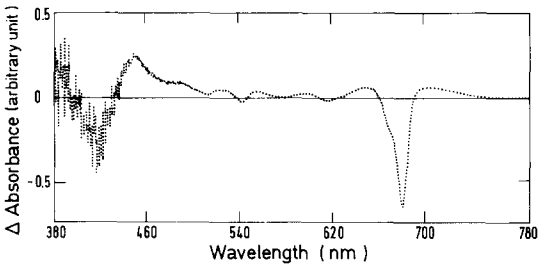

Fig. 1. VIS-NIR light-minus-dark absorption spectrum at 250 K for dithionite-reduced D1D2 reaction centers. Illumination of the sample was performed at 250 K.

of the intermediary acceptor. Comparable signals have been reported for large PS II particles, at 1740 cm^{-1}, 1720 cm^{-1} and 1681–1676 cm^{-1} [10], although the bands are better resolved in D1D2 RC. Signals corresponding to the photoreduction of the intermediary acceptor were similarly observed at 1747 cm^{-1}, 1732 cm^{-1}, 1683 cm^{-1} in *Rps. viridis* bacterial RC [9], and at 1747 cm^{-1}, 1729 cm^{-1}, 1675 cm^{-1} in RC-B875 complexes from *C. vinosum* [20]. These three bands are thus apparently specific to the photoreduction of the intermediary acceptor in bacteria and plants, which suggests

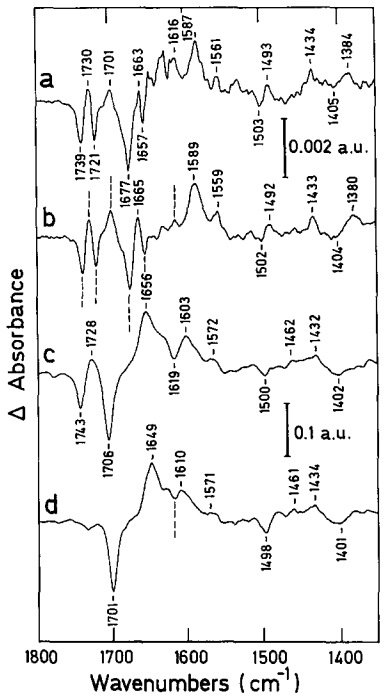

Fig. 2. (a, b) Light-induced FTIR difference spectra (Phe^--minus-Phe) of films of D1D2 RC covered with dithionite (150 mM) redox buffer; (a) ^1H_2O, (b) ^2H_2O; $T = 240$ K, excitation wavelength 665–1100 nm, average obtained from five (a) or six (b) samples, 1024 interferograms co-added for each sample. (c, d) Redox-induced FTIR difference spectra of (pyro)-pheophytin *a* anion formation in tetrahydrofuran. (c) Pheo a^--minus-Pheo *a* at $U = -1$ V; (d) pyroPheo a^--minus-pyroPheo *a* at $U = -1.5$ V; 64 interferograms co-added, $T = 295$ K. The optical pathlength of the cell was 250 μm in (c) while in (d) it was 90 μm. a.u.: absorbance unit. For all spectra the spectral resolution was 4 cm^{-1}.

that the bonding interactions of the acceptor with the protein are comparable. In the 1620–1760 cm^{-1} spectral region of the light-induced FTIR spectra, carbonyl absorption changes might arise not only from the Phe acceptor (which contains two ester C=O – 10a and 7c – not directly involved in conjugation with the π electron system and one conjugated keto C=O) but also from the protein (peptide and side-chain C=O) of the RC, or from the lipids (two ester C=O per molecule) as well as from the OH bending vibration of water. Thus, in order to determine the contribution of the pigments to the light-induced spectra, a comparison with redox-induced spectra of model compounds is essential [21]. For this purpose, we have investigated the infrared spectra of isolated Pheo *a* and its pyroderivative lacking the 10a-ester C=O, in both their neutral and ionized states.

Using THF as solvent, the Pheo *a* anion radical was generated at $U = -1$ V in a spectroelectrochemical cell transparent in the visible and infrared regions [15]. The optical absorbance spectrum of the Pheo *a* anion (data not shown) is in agreement with published data [22]. The Pheo *a* anion-minus Pheo *a* FTIR difference spectrum, hereafter referred as Pheo a^-/Pheo *a* spectrum, is displayed in Fig. 2c. Compared to the Phe^-/Phe spectrum obtained in vivo (Fig. 2a), the Pheo a^-/Pheo *a* spectrum shows only two negative bands in the highest frequency C=O region, at 1743 cm^{-1} and 1706 cm^{-1}. The FTIR difference spectrum of the bacteriopheophytin *a* (*b*) anion formation [21,23] exhibits similar strong negative bands at 1742 cm^{-1} (1743 cm^{-1}) and 1701 cm^{-1} (1703 cm^{-1}). The infrared absorbance spectrum of Pheo *a* in THF in the electrochemical cell, before electrolysis, also displays two main bands at 1741 cm^{-1} and 1706 cm^{-1} (data not shown) which are due to the C=O stretching vibrations of the 9-keto and ester groups [24,25], respectively (in this solvent, the two ester C=O at the 7c and 10a cannot be distinguished). The frequencies are characteristic of non-interacting C=O groups as expected for a non-hydrogen bonding solvent [26]. Thus, we assign the negative signal at 1706 cm^{-1} in the Pheo a^-/Pheo *a* spectrum to an absorption change of the 9-keto C=O (Table I) the frequency of which is downshifted to 1656 cm^{-1} upon Pheo *a* anion formation. The negative signal at 1743 cm^{-1} can be attributed to either one or both of the 7c- or 10a-ester C=O groups of Pheo *a*, although the 10a-ester C=O should be predominantly affected, due to its closest proximity to the π electron system. Indeed, the 1743 cm^{-1} signal is lacking in the infrared difference spectrum of the pyroPheo *a* anion formation (Fig. 2d). In pyroPheo *a*, the carbomethoxy group at C-10 is replaced by a hydrogen atom. Thus, as already demonstrated for pyrobacteriochlorophyll *a* [27] and pyroChl *a* [28] cations, it appears that the infrared absorption of the 7c-ester C=O in chlorophylls is essentially unperturbed upon radical formation. Therefore, the negative signal at 1743

TABLE I

Carbonyl group assignment

Proposed frequency (cm^{-1}) assignments of the carbonyl groups in the redox-induced (Pheo *a*, pyroPheo *a*, bacterioPheo *b*, bacterioPheo *a*) and light-induced (D1D2 RC, *Rps. viridis* RC, *C. vinosum* RC-B875) FTIR difference spectra.

	9-keto	10a-ester	Glutamic acid side-chain
Pheo *a*	1706	1743	–
PyroPheo *a*	1701	–	–
D1D2 RC	1677	1721	1739
BacterioPheo *b*	1703	1743	–
Rps. viridis	1683	1732	1747
BacterioPheo *a*	1701	1742	–
C. vinosum	1675	1729	1747

cm^{-1} in the Pheo a^-/Pheo *a* spectrum is assigned to the 10a-ester C=O of the neutral Pheo *a* (Table I) which is shifted to 1728 cm^{-1} upon anion formation. This result, together with previous FTIR observations on cations of bacteriochlorophylls and anions of bacteriopheophytins [21,23], emphasizes that the 10a-ester C=O, which is usually considered not to be directly in conjugation with the π electron system, indeed tends to exhibit orbital interactions with the conjugated system. It can also be noticed that the 9-keto C=O absorbs at lower frequency in pyroPheo *a* (1701 cm^{-1}) than in Pheo *a* (1706 cm^{-1}), as has already been observed for pyro-(bacterio)chlorophyll *a* compared to (bacterio)chlorophyll *a* [27,28]. All these data indicate some coupling between the 10a-ester C=O and the 9-keto C=O of chlorophylls. Additional infrared absorption changes are also observed in the 1630–1350 cm^{-1} region of the Pheo a^-/Pheo *a* spectrum (Fig. 2c) and in particular, the signal at 1619 cm^{-1} can be assigned to the pyrrole C=C vibration of the neutral Phe [24,25].

Discussion

The infrared spectra of the (pyro)Pheo *a* anion radical generated electrochemically in vitro are used as model compound spectra for the interpretation of the light-induced FTIR difference spectra obtained in vivo. Comparison of the Phe^-/Phe spectrum (Fig. 2a) associated with the photoreduction of Phe in D1D2 RC with the Pheo a^-/Pheo *a* spectrum (Fig. 2c) leads to the assignment of the 9-keto C=O of Phe at 1677 cm^{-1} (Table I). Compared to the negative 1706 cm^{-1} signal detected in the Pheo a^-/Pheo *a* spectrum, the 1677 cm^{-1} band observed in vivo thus reflects an interacting 9-keto C=O group in the neutral state of the intermediary acceptor. This assignment confirms our previous observation on large PS II particles [29]. Similarly, a negative band at 1683 cm^{-1} has been observed in the light-induced FTIR difference spectrum of *Rps. viridis* RC (Ref. 9, Table I), upon photoreduction of the bacteriopheophytin *b* acceptor (H_L). Its downshift with respect to the 1703 cm^{-1} keto C=O of the corresponding model compound anion indicates that it is H-bonded in the neutral state of H_L [21]. A similar conclusion concerning the molecular interaction of the bacteriopheophytin *a* acceptor in *C. vinosum* can also be deduced from the comparison of *C. vinosum* [20] light-induced spectra (9-keto C=O vibration at 1675 cm^{-1}) and bacteriopheophytin *a* [23] redox-induced spectra (9-keto C=O at 1701 cm^{-1}). Earlier resonance Raman investigations of D1D2 RC particles have also proposed an H-bonded 9-keto C=O at 1672 cm^{-1} [30] or at 1669 cm^{-1} [18], in addition to free keto groups. However, it was not clear which chromophore, Chl a or Pheo *a*, participates. Very recently, Moënne-Loccoz et al. [31] have assigned a band at 1680 cm^{-1} in resonance Raman spectra of D1D2 RC to the 9-keto C=O of the intermediary acceptor. More generally, both FTIR and resonance Raman spectra of bacterial RCs [9,20,32] and PS II [10,11,29,31] strongly suggest that the 9-keto C=O of the acceptor is H-bonded.

Furthermore, instead of a single negative band in the ester C=O region of the Pheo *a* model compound, two negative bands are found at 1739 cm^{-1} and 1721 cm^{-1} after photoreduction of Phe in D1D2 RC. As mentioned above and summarized in Table I, similar features have been observed for *Rps. viridis* RC (at 1747 cm^{-1} and 1732 cm^{-1}), as well as for *C. vinosum* (at 1747 cm^{-1} and 1729 cm^{-1}). The two bands observed in the high frequency region of the Phe^-/Phe spectrum might have several origins. They certainly involve the 10a-ester C=O which is downshifted after anion formation in vitro. They may involve the 7c ester C=O provided it is bound to the protein and this interaction is altered upon photoreduction. In addition, the carboxylic C=O bond from amino-acid side-chains can also contribute. In *Rps. viridis* RC, possible contributions from Asp or Glu carboxylic C=O in the 1760–1700 cm^{-1} region were probed with ^1H–^2H exchange [33]. After extensive exchange, a decrease of about 30% in intensity for the 1747 cm^{-1} band with respect to the 1732 cm^{-1} band was observed, with hardly any shift of the 1732 cm^{-1} band [33]. This was explained by assuming a partial exchange at a protonated carboxylic group absorbing at 1747 cm^{-1}. In view of the X-ray structural models of *Rps. viridis* [34] and *Rhodobacter sphaeroides* [35] RCs which indicate that the 9 keto C=O of H_L is in close proximity to the carboxylic group of Glu L104, it was proposed that the exchangeable proton is the one from Glu L104 side-chain bonded to the 9-keto C=O of H_L (Ref. 33, see also Refs. 36 and 37). In *Rps. viridis* RC, the 1732 cm^{-1} band was assigned to the 10a ester C=O group whose frequency is downshifted by H-bond-

ing to Trp L100 which is located at a suitable distance to interact with the 10a-ester C=O of H_L [34].

Analysis of amino-acid sequence indicates that the Glu residue proximal to the 9-keto C=O of H_L (Glu L104) is conserved between the L subunits of RCs from various purple bacteria and the D1 polypeptide (Glu D1-130) of PS II [6,38]. In addition, the Trp residue (Trp L100) which is located near the 10a ester C=O of H_L in *Rps. viridis* RC [34], is replaced by a Tyr residue in the D1 polypeptide (Tyr D1-126) and, hence, molecular interaction should be possible between the 10a-ester C=O of Phe in D1D2 RC and Tyr D1-126. Thus, the striking similarity observed between (i) FTIR spectra obtained on bacterial RC and D1D2 RC upon photoreduction of the intermediary acceptor (see also Table I) and (ii) the amino-acid sequence of the L and D1 polypeptides leads us to assign the 1721 cm^{-1} band in the Phe^-/Phe spectrum to the protein bound 10a-ester C=O of the acceptor and the 1739 cm^{-1} band to a contribution from the C=O of the Glu D1-130 carboxylic group. Indeed, the infrared frequency (1677 cm^{-1}) of the 9-keto C=O of the Phe acceptor in D1D2 RC indicates that this group is strongly H-bonded in the neutral state. In view of the sequence homology between the D1 and L polypeptides, we propose that the 9-keto C=O of Phe is H-bonded to the side-chain of Glu D1-130, in the same way as, in *Rps. viridis* RC, the 9-keto C=O of H_L is bound to Glu L104. This proposal implies that Glu D1-130 is protonated in the relaxed state of the D1D2 RC. However, several differences in the light-induced infrared spectra of D1D2 RC and bacterial RC have to be emphasized. First, we do not detect any deuterium isotope effect in the 1750–1670 cm^{-1} region of the Phe^-/Phe spectrum when the photoreduction of Phe is performed on films of D1D2 RC covered with a deuterated buffer (Fig. 2b). Under our ^1H–^2H exchange conditions, which lead to 60–70% deuterated peptide protons in both D1D2 and *Rps. viridis* RCs [33], a noticeable decrease of intensity of the 1747 cm^{-1} band was observed for *Rps. viridis* RC [33]. Secondly, the extent of the frequency downshift of the C=O bands in vivo with respect to that observed in vitro is larger in D1D2 RC than in bacterial RC. More specifically, as depicted in Table I, the 9-keto C=O is downshifted by 29 cm^{-1} in D1D2 RC (1677 cm^{-1}) with respect to Pheo *a* (1706 cm^{-1}), while it is only downshifted by 20 cm^{-1} in *Rps. viridis* RC (1683 cm^{-1}) with respect to bacteriopheophytin *b* (1703 cm^{-1}). A similar observation is made for the 10a-ester C=O, which is downshifted by 22 cm^{-1} in D1D2 RC (1721 cm^{-1}) with respect to Pheo *a* (1743 cm^{-1}) and by 11 cm^{-1} in *Rps. viridis* RC (1732 cm^{-1}) with respect to bacteriopheophytin *b* (1743 cm^{-1}). Thus, the comparison of our infrared data on the photoreduction of the intermediary acceptor in bacteria and PS II RCs suggests that the strength of the intermolecular interactions assumed by the carbonyls (both 10a-ester and 9-keto C=O) of the acceptor with the protein is larger in PS II than in bacterial RC. Although the strength of the H-bonding interaction between the glutamic acid residue and the 9-keto C=O of the intermediary acceptor may play a direct role in the different ^1H–^2H exchangeability of the glutamic side-chain proton when comparing D1D2 and *Rps. viridis* RCs, a large part of this difference is more probably related to the solvent accessibility. The exchange data suggest that, in the vicinity of the glutamic residue, local breathing motions of the D1 polypeptide are more restricted than those of the L polypeptide of *Rps. viridis* RC. In this respect, our FTIR data can be taken to indicate that the strength of the glutamic H-bond contributes both to hinder and to modulate the solvent accessibility to this important region of the RC.

Acknowledgements

We thank E. Barillot, A. Wollenweber and J. Kléo for their help in some phases of this project and D. Thibodeau for a careful reading of the manuscript. Part of this work was funded by the European Community (contract No. ST2J-0118-2-D).

Note added in proof (Received 23 January 1990)

After submission of this article, detailed ENDOR studies of the photoreduced intermediate electron acceptor in PS II have been reported by Lubitz et al. (Biochim. Biophys. Acta 977 (1989) 227–232). The distance between Glu D1-130 and the oxygen of the photoreduced pheophytin acceptor (Phe^-) was estimated to be 1.57 Å. In bacterial reaction centers, the corresponding distance was calculated to be 1.8 ± 0.2 Å (see Ref. 36). It thus appears that the interaction between the Glu proton and the pigment is stronger in PS II than in bacterial reaction center, both for the neutral (as seen by FTIR studies) and the photoreduced state of the intermediate electron acceptor (as seen by ENDOR studies).

References

1 Mathis, P. and Rutherford, A.W. (1987) in Photosynthesis (Amesz, J., ed.), pp. 63–96, Elsevier, Amsterdam.
2 Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature (Lond). 318, 618–624.
3 Chang, C.-H., Tiede, D., Tang, J., Smith, U., Norris, J. and Schiffer, M. (1986) FEBS Lett. 205, 82–86.
4 Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1987) Proc. Natl. Acad. Sci. USA. 84, 5730–5734.
5 Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA. 84, 109–112.
6 Trebst, A. (1986) Z. Naturforsch. 41c, 240–245.
7 Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.

8 Mäntele, W.G., Nabedryk, E., Tavitian, B.A., Kreutz, W. and Breton, J. (1985) FEBS Lett. 187, 227–232.

9 Nabedryk, E., Mäntele, W., Tavitian, B.A. and Breton, J. (1986) Photochem. Photobiol. 43, 461–465.

10 Tavitian, B.A., Nabedryk, E., Mäntele, W. and Breton, J. (1986) FEBS Lett. 201, 151–157.

11 Barillot, E., Nabedryk, E., Andrianambinintsoa, S., Mäntele, W. and Breton, J. (1989) Biophys. J. 55, 180a.

12 Breton, J. (1990) in Perspectives in Photosynthesis (Jortner, J. and Pullman, B., eds.), Vol. 22, pp. 23–38, Kluwer, Dordrecht.

13 Pennington, F.C., Strain, H.H., Svec, W.A. and Katz, J.J. (1964) J. Am. Chem. Soc. 86, 1418–1426.

14 Berger, G., Wollenweber, A., Kléo, J., Andrianambinintsoa, S. and Mäntele, W. (1987) J. Liquid Chromatogr. 10, 1519–1531.

15 Mäntele, W., Wollenweber, A., Rashwan, F., Heinze, J., Nabedryk, E., Berger, G. and Breton, J. (1988) Photochem. Photobiol. 47, 451–455.

16 Seibert, M., Picorel, R., Rubin, A.B. and Connolly, J.S. (1988) Plant Physiol. 87, 303–306.

17 Chapman, D.J., Gounaris, K. and Barber, J. (1988) Biochim. Biophys. Acta 933, 423–431.

18 Ghanotakis, D.F., De Paula, J.C., Demetriou, D.M., Bowlby, N.R., Petersen, J., Babcock, G.T. and Yocum, C.F. (1989) Biochim. Biophys. Acta. 974, 44–53.

19 Klimov, V.V., Klevanik, A.V., Shuvalov, V.A. and Krasnovsky, A.A. (1977) FEBS Lett. 82, 183–186.

20 Nabedryk, E., Tavitian, B.A., Mäntele, W., Kreutz, W. and Breton, J. (1987) in Progress in Photosynthesis Research, Vol. I (Biggins J., ed.), pp. 177–180, Martinus Nijhoff, Dordrecht.

21 Mäntele, W., Wollenweber, A., Nabedryk, E. and Breton, J. (1988) Proc. Natl. Acad. Sci. USA. 85, 8468–8472.

22 Fujita, I., Davis, M.S. and Fajer, J. (1978) J. Am. Chem. Soc. 100, 6280–6282.

23 Mäntele, W., Wollenweber, A., Nabedryk, E., Breton, J., Rashwan, F., Heinze, J. and Kreutz, W. (1987) in Progress in Photosynthesis Research. Vol. I, (Biggins, J., ed.), pp. 329–332, Martinus Nijhoff, Dordrecht.

24 Katz, J.J., Dougherty, R.C. and Boucher, L.J. (1966) in The chlorophylls (L.P. Vernon and G.R. Seely, eds.), Ch. 7, Academic Press. New York.

25 Fujiwara, M. and Tasumi, M. (1986) J. Phys. Chem. 90, 250–255.

26 Ballschmiter, K. and Katz, J.J. (1969) J. Am. Chem. Soc. 91, 2661–2677.

27 Leonhard, M., Wollenweber, A., Berger, G., Kléo, J., Nabedryk, E., Breton, J. and Mäntele, W. (1989) in Techniques and New Developments in Photosynthesis Research (Barber J., and Malkin R., eds.), Vol. 168, pp. 115–118, NATO ASI Series, Plenum, New York.

28 Leonard, M., Nabedryk, E., Berger, G., Breton, J. and Mäntele, W. (1990) Proceedings of the VIIIth International Congress on Photosynthesis, Stockholm, 6–11 August 1989, in press.

29 Tavitian, B.A., Nabedryk, E., Wollenweber, A., Mäntele, W. and Breton, J. (1988) in Spectroscopy of Biological Molecules New Advances (Schmid E.D., Schneider, F.W. and Siebert, F., eds.), pp. 297–300 Wiley & Sons, Chichester, U.K.

30 Fujiwara, M., Hayashi, H., Tasumi, M., Kanaji, M., Koyama, K., and Satoh, K. (1987) Chem. Lett. Ser. C, 2005–2008.

31 Moënne-Loccoz, P., Robert, B. and Lutz, M. (1989) Biochemistry 28, 3641–3645.

32 Lutz, M. (1980) Proceedings of the 7th International Conference on Raman spectroscopy (Murphy, W.F., ed.), pp. 520–523, North-Holland, Amsterdam.

33 Nabedryk, E., Andrianambinintsoa, S., Mäntele, W. and Breton, J. (1988) in The Photosynthetic Bacterial Reaction Center: Structure and Dynamics (Breton, J. and Verméglio, A., eds.), Vol. 149, pp. 237–250. NATO ASI Series, Series A: Life Sciences, Plenum, New York.

34 Michel, H., Epp, O. and Deisenhofer, J. (1986) EMBO J. 5, 2445–2451.

35 Yeates, T.O., Komiya, H., Chirino, A., Rees, D.C., Allen, J.P. and Feher, G. (1988) Proc. Natl. Acad. Sci. USA. 85, 7993–7997.

36 Feher, G., Isaacson, R.A., Okamura, M.Y. and Lubitz, W. (1988) in The Photosynthetic Bacterial Reaction Center: Structure and Dynamics (Breton, J. and Verméglio, A., eds.), Vol. 149, pp. 229–235, NATO ASI Series, Series A: Life Sciences, Plenum, New York.

37 Lubitz, W., Lönnewitz, B., Plato, M., Isaacson, R.A., Okamura, M.Y. and Feher, G. (1990) Proceedings of the VIIIth International Congress of Photosynthesis, Stockholm, 6–11 August 1989, in press.

38 Komiya, H., Yeates, T.O., Rees, D.C., Allen, J.P. and Feher, G. (1988) Proc. Natl. Acad. Sci. USA 85, 9012–9016.