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FTIR spectroscopy shows structural similarities between photosystems II from cyanobacteria and spinach

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Photosystem II (PSII), an essential component of oxygenic photosynthesis, is a membrane-bound pigment protein complex found in green plants and cyanobacteria. Whereas the molecular structure of cyanobacterial PSII has been resolved with at least medium resolution [Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauss, N., Saenger, W. & Orth, P. (2001) *Nature (London)* **409**, 739–743; Kamiya, N. & Shen, J.R. (2003) *Proc. Natl Acad. Sci. USA* **100**, 98–103], the structure of higher plant PSII is only known at low resolution. Therefore Fourier transform infrared (FTIR) difference spectroscopy was used to compare PSII from both *Thermosynechococcus elongatus* and *Synechocystis* PCC6803 core complexes with PSII-enriched membranes from spinach (BBY). FTIR difference spectra of *T. elongatus* core complexes are presented for several different intermediates. As the FTIR difference spectra show close similarities among the three species, the structural arrangement of cofactors in PSII and their interactions with the protein microenvironment during photosynthetic charge separation must be very similar in higher plant PSII and cyanobacterial PSII. A structural model of higher plant PSII can therefore be predicted from the structure of cyanobacterial PSII.

Keywords: cyanobacteria; higher plants; photosynthesis; photosystem II; structure.

Photosystem II (PSII) is a membrane-bound pigment protein complex found in plants, algae and cyanobacteria [1]. Light energy is absorbed by light-harvesting complexes and transferred to the primary donor P_{680}, two chlorophyll a molecules. The excitation of P_{680} leads to electron transfer via a pheophytin (H_A) to the primary acceptor Q_A, a plastoquinone-9 molecule (PQ_9). Q_A is a single-electron carrier tightly bound to the protein. In contrast, the secondary acceptor, Q_B, which is also PQ_9, acts as a two-electron gate. The doubly reduced and protonated Q_B is released from the protein complex as plastoquinol and is replaced by another plastoquinone molecule from the plastoquinone pool.

On the donor side, two water molecules as terminal electron donors are oxidized and cleaved into molecular oxygen, electrons and protons by the oxygen-evolving complex (OEC). The OEC contains a tetranuclear manganese cluster, and Ca^{2+} and Cl^- are essential cofactors. The reactions of the OEC proceed through the S-state cycle which comprises five intermediate states, S_0–S_4, where 0–4 are the number of stored redox equivalents. The S_1 state is thermally stable and therefore predominant in dark-adapted PSII. The electrons are transferred from the OEC to P_{680}^+ via the redox-active tyrosine Y_Z. For recent reviews see [2–4].

The molecular structure of this large pigment protein complex is still a matter of debate. Structural models with medium resolution of 3.7–3.8 Å have been published for two cyanobacteria [5,6]. For higher plant PSII, lower resolution structural models are available based on electron cryomicroscopy [7,8]. A comparison of cyanobacterial and higher plant PSII reveals major similarities in general structural arrangement, but there are also obvious differences, such as the presence of smaller and extrinsic subunits [4]. The function of PSII is determined by its cofactors and their precise arrangement within the protein matrix. Thus, it is not known in detail if cyanobacteria can be used as a prototype of oxygenic photosynthesis or if PSII from cyanobacteria differs from that of higher plants in specific aspects. For biotechnological and agricultural use in particular, higher plants are of more interest than cyanobacteria. Therefore, it is important to understand the molecular structure and dynamic function of higher plant PSII.

FTIR difference spectroscopy has proved to be a powerful tool for studying molecular reaction mechanisms of proteins [9–11]. Photochemical reactions in PSII have also been studied by light-induced FTIR difference spectroscopy in mesophilic organisms such as spinach and *Synechocystis* [12–16]. PSII of the thermophilic cyanobacterium *Thermosynechococcus elongatus* is more stable than

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FTIR, Fourier transform infrared; OEC, oxygen-evolving complex; PSII, photosystem II; P_{680}, primary electron donor; PQ_9, plastoquinone-9; Q_A, primary electron acceptor; Q_B, secondary electron acceptor; Y_D, redox-active Tyr160 of the D2 polypeptide; Y_Z, redox-active Tyr161 of the D1 polypeptide.

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that of mesophilic organisms such as higher plants and other cyanobacteria, especially the OEC [17,18]. Therefore, we used this improved *T. elongatus* PSII preparation [18] to compare cyanobacterial and higher plant PSII with respect to their light-induced absorbance changes in the context of charge separation. We discuss the structural and functional implications of the similarities by comparing the FTIR difference spectra of PSII of different species.

Materials and methods

PSII-enriched membranes of spinach (BBY) were prepared as described previously [15].

For $Q_A^- - Q_A$ measurements, the BBY membranes were incubated for 30 min in a buffer containing 50 mM Mes (pH 6.5), 40 mM sucrose, 10 mM NaCl, 0.1 mM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 2 mM phenazine-metasulfate, and 10 mM NH_2OH, which depletes the manganese cluster.

For $S_2Q_A^- - S_1Q_A$ measurements, the BBY membranes were incubated for 30 min in a buffer containing 40 mM Mes (pH 6.5), 400 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 0.1 mM DCMU.

After centrifugation (130 000 ***g***, 15 min, 277 K), the sediment was placed on to a CaF_2 window. The cuvette was closed by a second window and thermostabilized in the FTIR apparatus.

PSII core complexes of *Synechocystis* PCC6803 and *T. elongatus* were isolated and purified as described [16,18]. The core complexes were stored in 20 mM Mes pH 6.5, containing 10 mM $MgCl_2$, 10 mM $CaCl_2$, 0.5 M mannitol and 0.03% β-dodecyl-maltoside.

For $Q_A^- - Q_A$ measurements, the PSII core complexes were incubated for 30 min in a buffer containing 10 mM Mes (pH 6.0), 40 mM sucrose, 2 mM NaCl, 0.1 mM DCMU, 0.1 mM phenazine-metasulfate, and 10 mM NH_2OH to deplete the manganese cluster.

For $S_2Q_A^- - S_1Q_A$ measurements, the PSII core complexes were incubated for 30 min in buffer containing 40 mM Mes (pH 6.5), 400 mM sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 0.1 mM DCMU.

The core complexes were concentrated to a final volume of about 20 μL. Half of this was pipetted on to a CaF_2 window and further concentrated in a gentle stream of nitrogen. With this method, the protein should not completely dry. The cuvette was closed by a second window and thermostabilized in the FTIR apparatus.

The FTIR measurements were performed as described [12,13,15] and modified [19,20].

$Q_A^- - Q_A$ difference spectra were taken at −10 °C; after 100 dark interferograms had been recorded, the sample was illuminated by a halogen lamp for 3 s. After a 2 s delay, 12 times 60 interferograms of the light-induced state were stored. The cycle was repeated after 5 min to improve signal-to-noise ratio.

$S_2Q_A^- - S_1Q_A$ difference spectra were taken at + 16 °C; after 35 dark interferograms had been recorded, the sample was illuminated by a halogen lamp for 1 s. After a 0.5 s delay, six times 10 interferograms of the light-induced state were stored. The cycle was repeated after 5 min to improve signal-to-noise ratio.

Results and Discussion

In Fig. 1 $Q_A^- - Q_A$ difference spectra of BBY membranes from spinach, *Synechocystis* PCC6803 and *T. elongatus* core complexes are presented. The spectra of BBYs (a) and *Synechocystis* (b) are nearly identical with those published [12,15,16]. All major signals of the two difference spectra of spinach and *Synechocystis* are also found in the respective difference spectrum of *T. elongatus* (c) at the same frequencies, for instance, the positive bands at 1719, 1550, 1478, and 1456 cm^{-1} and the negative signals at 1657, 1644, 1632, 1560, and 1519 cm^{-1}, with only minor shifts in frequency (≤ 2 cm^{-1}). Small intensity variations in the amide I region between 1680 and 1600 cm^{-1} can be explained by different sample preparation.

In the bacterial reaction centre, the vibrations of the quinone cofactors Q_A and Q_B have been definitively assigned using specifically labelled UQ_{10} reconstituted at either Q_A or Q_B [21–24]. The respective data for PSII have not been available so far, and one has to rely on comparisons with model compounds and conclusions drawn from the bacterial reaction centre [12]. Thus, the bands at 1644 and 1632 cm^{-1} have been tentatively assigned to Q_A vibrations and the band at 1478 cm^{-1} to a Q_A^- vibration [12]. These bands in particular agree well in all the spectra, emphasizing that the structures of the respective cofactor, plastoquinone Q_A, and its cofactor–protein interactions are very similar in the three species. In contrast with these great similarities, the carbonyl region above 1680 cm^{-1} shows some obvious differences, which are discussed in detail below (Fig. 3).

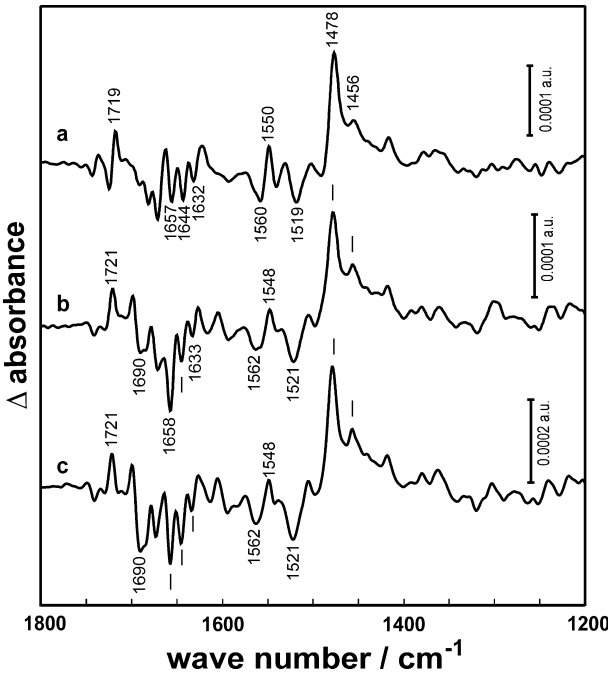

Fig. 1. $Q_A^- - Q_A$ difference spectra of (a) PSII-enriched membranes from spinach (BBY), (b) PSII core complexes from *Synechocystis* PCC6803, and (c) PSII core complexes from *T. elongatus* from 1800 to 1200 cm^{-1}.

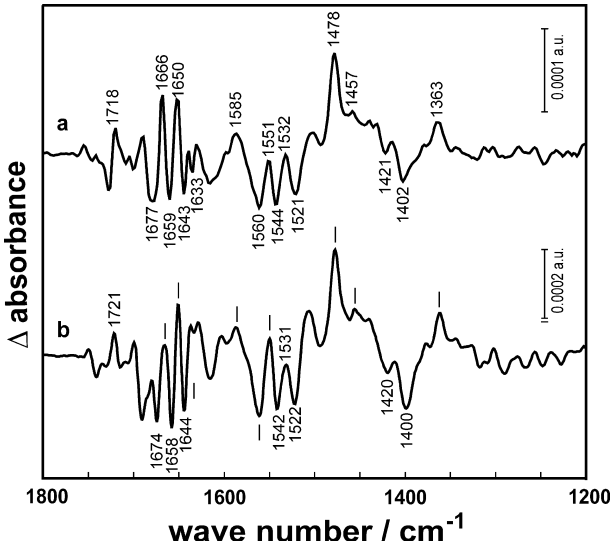

Fig. 2. $S_2Q_A^-$ − S_1Q_A difference spectra of (a) PSII enriched membranes from spinach (BBY) and (b) PSII core complexes from *T. elongatus* from 1800 to 1200 cm^{-1}.

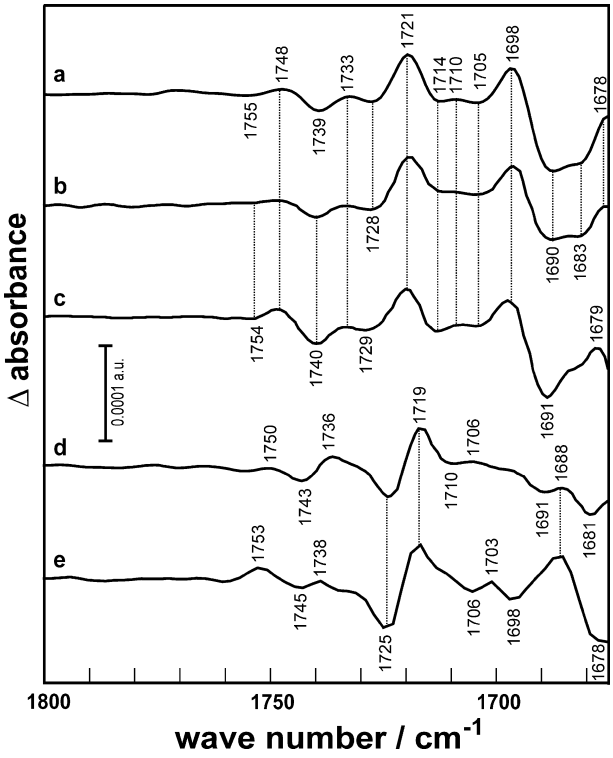

Fig. 3. Carbonyl region of Q_A^- − Q_A and $S_2Q_A^-$ − S_1Q_A difference spectra on enlarged scale. PSII core complexes from (a) *Synechocystis* PCC6803 (Q_A^- − Q_A), (b) *T. elongatus* (Q_A^- − Q_A) and (c) *T. elongatus* ($S_2Q_A^-$ − S_1Q_A); PSII-enriched membranes from spinach (BBY), (d) (Q_A^- − Q_A) and (e) ($S_2Q_A^-$ − S_1Q_A) from 1800 to 1675 cm^{-1}.

In the region above 1750 cm^{-1}, no changes in the protein or cofactors are expected, therefore, this region can be used as a scale of background noise. The difference spectra presented are based on three to five independent samples in each case, and they denote averages of 500 to 10 000 interferograms. All the features of the spectra discussed occur in every difference spectrum and can thus be taken as significant.

The $S_2Q_A^-$ − S_1Q_A FTIR difference spectrum of BBY membranes from spinach (Fig. 2, spectrum a) agrees well with published ones [13,25]. The respective difference spectrum of *T. elongatus* core complexes is shown in Fig. 2 (spectrum b) for comparison. All major features such as the positive bands at 1666, 1650, 1585, 1551, 1532, 1478, 1457 and 1363 cm^{-1} and the negative signals at 1677, 1659, 1643, 1633, 1560, 1544, 1521, 1421 and 1402 cm^{-1} occur in both spectra, with only small shifts in frequency (≤ 2 cm^{-1}). Only the negative band at 1677 cm^{-1} (Fig. 2, spectrum a) is shifted by 3 cm^{-1} to 1674 cm^{-1} in the spectrum of *T. elongatus* (Fig. 2, spectrum b). In addition to the bands tentatively assigned to Q_A (1644, 1632 cm^{-1}) or Q_A^- (1478 cm^{-1}) [12] and already discussed in the context of the Q_A^- − Q_A difference spectra (Fig. 1), signals tentatively assigned to S_1 (1560, 1402 cm^{-1}) or S_2 (1585, 1363 cm^{-1}) [13,26,27] also agree between the two spectra and thus confirm that the structures of the respective cofactors, plastoquinone Q_A and the manganese cluster, and their cofactor–protein interactions are very similar in cyanobacteria and higher plants. This agrees with kinetic investigations of the reaction coordinate of water oxidation in thermophilic cyanobacteria and higher plants [28]. In contrast with these striking similarities, the carbonyl region above 1680 cm^{-1} shows some remarkable differences which are discussed in detail below (Fig. 3).

Despite all the similarities described above, both the Q_A^- − Q_A (Fig. 1) and $S_2Q_A^-$ − S_1Q_A (Fig. 2) FTIR difference spectra reveal distinct differences between the cyanobacterial PSII and that of spinach, but in particular in the carbonyl region above 1680 cm^{-1}. Carbonyl stretching vibrations of protonated carboxylic acids are often observed in this spectral region. Therefore, Fig. 3 shows the respective difference spectra of Figs 1 and 2 on an enlarged scale from 1800 to 1675 cm^{-1}. The Q_A^- − Q_A difference spectra of the two cyanobacterial species compared here (Fig. 3, spectra a and b) show close similarities to prominent positive signals at 1721 and 1698 cm^{-1} and negative ones at 1690 and 1683 cm^{-1} (shoulder). In addition, there are reproducible positive bands at 1748, 1733 and 1710 cm^{-1} as well as negative bands at 1754/1755, 1739/1740, 1728, 1714 and 1705 cm^{-1}. All these bands are also observed in the $S_2Q_A^-$ − S_1Q_A FTIR difference spectrum of *T. elongatus*, with no more than 1 cm^{-1} variation in frequency. This is of great interest because the $S_2Q_A^-$ − S_1Q_A FTIR difference spectrum does not only contain signals from the acceptor side (Q_A/Q_A^-), but also from the donor side (S_1/S_2). The fact that there are no additional features that could be correlated with S_1 or S_2 indicates that the donor side (S_1/S_2) does not contribute at all to difference signals above 1680 cm^{-1} and that the clear differences between cyanobacterial and spinach PSII do not result from differences in the donor side between the respective species, but from the acceptor side only.

The respective difference spectra of BBY membranes are presented in Fig. 3 (spectra d and e). The Q_A^- − Q_A difference spectrum (d) mainly agrees with the $S_2Q_A^-$ − S_1Q_A FTIR difference spectrum (e), but there are slight

differences. Both spectra show prominent difference signals at 1725 cm^{-1} (negative) and 1719 cm^{-1} (positive). In addition, positive bands are observed at 1750–1753, 1736/1738, 1703–1706, and 1688 cm^{-1} and negative bands at 1743/1745, 1706–1710, and 1678–1681 cm^{-1}, which agree between the $Q_A^- - Q_A$ and the $S_2Q_A^- - S_1Q_A$ FTIR difference spectra. The three bands at 1750, 1743 and 1736 cm^{-1} are shifted by 2–3 cm^{-1} to higher frequencies in the $S_2Q_A^- - S_1Q_A$ FTIR difference spectrum (e); the small bands between the major signals at 1719 and 1688 cm^{-1} are near the limit of resolution and should be interpreted with caution.

In comparison with the three cyanobacterial difference spectra (Fig. 3, a–c), a completely different pattern is found in the BBY spectra (Fig. 3, d–e). As mentioned above, these differences cannot be correlated with light-induced changes at the donor side because there are no differences between the $Q_A^- - Q_A$ and $S_2Q_A^- - S_1Q_A$ FTIR difference spectra of the respective species. As mainly carboxylic acids absorb in this region, we measured $Q_A^- - Q_A$ difference spectra in D_2O instead of H_2O (data not shown). This isotopic exchange should induce typical frequency shifts of $\approx$ 5–10 cm^{-1} in such protonated carboxylic acids [29,30]. Unexpectedly, we did not observe any shifts. One cannot exclude the possibility that carboxylic acids buried deep in the protein, far away from the bulk water phase, may not exchange their hydrogen atoms with deuterium. However, the fact that none of the several distinct features are changed by this isotopic exchange makes it very unlikely that all the difference signals above 1680 cm^{-1} belong to nonexchanging carboxylic acid groups. Thus, we conclude that, in the context of light-induced charge separation in PSII, no protonation changes occur in carboxylic acids in the environment of Q_A to compensate for the negative charge of Q_A^-. This is, nevertheless, in agreement with the situation in the bacterial photosynthetic reaction centre where, on the one hand, Q_A reduction is accompanied by significant proton uptake [31], but, on the other hand, no carboxylic acids become protonated [32]. It has recently been shown for the bacterial reaction centre that the observed proton uptake may lead to the protonation of histidines at the entrance of the proton uptake channel to Q_B [33]. Even though comparable data are not yet available for PSII, one can imagine a similar mechanism here, but this has still to be confirmed by further experiments.

One has to take into account that the structure of the bacterial reaction centre differs from that of PSII in this region in particular, because its cytoplasmic H-subunit is missing in PSII. This may, of course, influence the method of proton uptake, but the function has, nevertheless, to be performed, namely the uptake of protons to compensate for the negative charge transferred to the primary quinone. In this light, the results presented here are really remarkable: both systems obviously accomplish this function without protonating a carboxylic amino acid. This suggests a mechanistic similarity.

If the distinct difference signals above 1680 cm^{-1}, which are very different in cyanobacterial and spinach PSII, do not result from protonation events, they may be related to the protein–cofactor interactions of the pheophytin H_A, which is very close to the plastoquinone Q_A in PSII [6,7]. In the bacterial reaction centre, the relevant bacteriopheophytin has been shown to be responsible for the difference signals above 1680 cm^{-1} in the $Q_A^- - Q_A$ difference spectra [32] and to undergo typical changes related to reoxidation of Q_A^- in the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ transition [33]. The ester vibrations of bacteriopheophytin in particular, seem to be involved [32]. Therefore, we suggest that the different patterns of difference signals in cyanobacterial and spinach PSII may be due to different protein–cofactor interactions of the pheophytin close to Q_A. As the $Q_A^- - Q_A$ and $S_2Q_A^- - S_1Q_A$ difference spectra of the different species agree in most details apart from this small spectral region, we conclude that the general structure of the PSII complex is very similar in cyanobacteria and higher plants such as spinach.

One should take into account the fact that the cyanobacterial PSII preparations were used as core complexes, whereas spinach was used in the form of PSII-enriched BBY membranes. This may result in differences in charge separation and consequently also in the difference spectra. However, the fact that all the spectra presented show close similarity over most of the middle infrared spectral region and distinct differences only in the small region above 1680 cm^{-1} strengthens our conclusion that the photosystems are very similar in structure at the key residues, indicating a very similar reaction mechanism.

Conclusion

Comparison of the FTIR difference spectra from the cyanobacterial core complexes of *T. elongatus* and *Synechocystis* PCC6803 and the higher plant PSII-enriched membranes of spinach reveals almost identical difference spectra for the different organisms. As FTIR is very sensitive to even small changes in bond length, angle, strength and hydrogen bonds, our results indicate no large differences between cyanobacterial and higher plant PSII. The structure of the key residues of PSII and their protein–cofactor interactions must therefore be very similar. This can be stated definitely for the manganese cluster and the protein–cofactor interactions of the donor side, at least, and for the plastoquinone Q_A and protein–cofactor interactions of the acceptor side. The only exception appears to be the pheophytin cofactors, which seem to carry out different protein–cofactor interactions in cyanobacteria and higher plants. This could be further investigated by site-directed mutagenesis and isotopic labelling of pheophytin.

Overall, the structure and function of PSII are similar in higher plants and cyanobacteria, and FTIR difference spectroscopy allows prediction of strong structural similarities between these photosystems, even though a structural model of higher plant PSII is not yet available.

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