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Structural Coupling of Extrinsic Proteins with the Oxygen-Evolving Center in Red Algal Photosystem II As Revealed by Light-Induced FTIR Difference Spectroscopy

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Supporting Information

ABSTRACT: Effects of binding of extrinsic proteins (PsbO, PsbQ′, PsbV, and PsbU) on the structure of the oxygen-evolving center (OEC) in photosystem II core complexes from a red alga, *Cyanidium caldarium*, were studied using Fourier transform infrared (FTIR) spectroscopy. S_2-minus-S_1 FTIR difference spectra showed that the protein conformations of the OEC, revealed by the changes in amide I and II bands, were significantly altered upon depletion of all the extrinsic proteins, but mostly recovered when PsbV was rebound with the support of other extrinsic proteins. The recovery of protein conformations correlated well with O_2 evolution activity. This PsbV function of retaining a proper OEC conformation in red algae resembles that of PsbP in higher plants reported previously.

Extrinsic proteins in photosystem II (PSII)[1−3] are bound on the lumenal side of the PSII complex near the oxygen-evolving center (OEC), which consists of the Mn_4CaO_5 cluster, two Cl^- ions, and surrounding protein moieties.[4] Although the intrinsic core proteins of PSII are highly conserved among oxyphototrophs, the components of extrinsic proteins vary across phyla.[1−3] Along with PsbO as a common component in all oxyphototrophs, cyanobacteria have PsbV and PsbU, while green algae and higher plants have PsbP and PsbQ. Red algae have extrinsic proteins similar to those of cyanobacteria, but PsbQ′, which shows a low degree of homology with PsbQ, is additionally attached.[5,6] The extrinsic proteins are known to play roles of stabilizing the OEC by retention of Ca^{2+} and Cl^- cofactors,[1−3] thus regulating the O_2 evolving reaction.[7,8] However, the molecular mechanism for expressing these roles of the extrinsic proteins in oxygen evolution as well as the reason for the dramatic changes in the components of extrinsic proteins during evolution remains to be clarified.

Light-induced Fourier transform infrared (FTIR) difference spectroscopy is a powerful method for detecting the structure of the OEC.[9−12] FTIR difference spectra upon S-state transitions reveal the structural changes in the OEC involving the Mn_4CaO_5 core, surrounding amino acids, polypeptide main chains, and water molecules. Using this method, the effects of extrinsic proteins on the OEC structure have been investigated.[13−15] With PSII membranes from spinach, it was shown that only the binding of PsbP among the three major extrinsic proteins, PsbO, PsbP, and PsbQ, affects the secondary structures of polypeptides around the Mn_4CaO_5 cluster, whereas the structure of the Mn_4CaO_5 cluster itself remains intact as revealed by unchanged interactions of carboxylate groups surrounding the cluster.[13,14] It was suggested that these conformational changes in the OEC induce the function of PsbP, i.e., stabilizing the Ca^{2+} and Cl^- binding in the OEC to optimize O_2 evolution activity.[13,14] A question of whether there are extrinsic proteins with functions similar to that of PsbP in other oxyphototrophs that have different types of extrinsic proteins then arises. To answer this question, here, we studied the structural coupling of the extrinsic proteins with the OEC in a red alga, *Cyanidium caldarium*, which has PsbO, PsbQ′, PsbV, and PsbU but not PsbP, using light-induced FTIR difference spectroscopy.

Figure 1a (black line) shows an S_2-minus-S_1 FTIR difference spectrum of the PSII core complexes from *C. caldarium* (the methods of sample preparation and FTIR measurements are described in the Supporting Information), which is the first report of the FTIR spectrum of the OEC in a red alga. The spectral features are very similar to those of PSII preparations from spinach (Figure S1a of the Supporting Information)[13] and cyanobacteria.[16−18] Prominent bands in the amide I and II (CO stretch and NH bend + CN stretch, respectively, of backbone amides) regions at 1700−1600 and 1600−1500 cm^{-1}, respectively,[16,17] represent the changes in the secondary structures of polypeptide chains around the Mn_4CaO_5 cluster, while the bands in the region of the symmetric COO^- vibrations[16,17] at 1450−1350 cm^{-1} reflect the changes in the interactions of the carboxylate groups as direct ligands to the Mn_4CaO_5 cluster and the constituents of the surrounding hydrogen bond network. The coupled asymmetric COO^- vibrations superimpose the amide II region.[16,17]

Upon depletion of all of the four extrinsic proteins (PsbO, PsbQ′, PsbV, and PsbU) from the PSII core complexes of *C. caldarium*, the spectral features of the amide I and II regions (1700−1500 cm^{-1}) were significantly altered, whereas the bands in the symmetric COO^- region (1450−1350 cm^{-1}) changed little (Figure 1a, red line). When all the extrinsic proteins were rebound to this PSII, the FTIR spectrum mostly recovered

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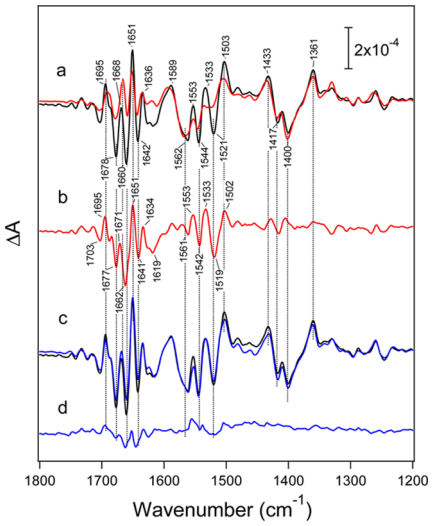

Figure 1. (a and c) S_2-minus-S_1 FTIR difference spectra of untreated PSII core complexes from *C. caldarium* (black lines) and PSII complexes depleted of all the four extrinsic proteins (PsbO, PsbQ′, PsbV, and PsbU) (a, red line) and then reconstituted with all of these extrinsic proteins (c, blue line). (b and d) Double-difference spectra between the two spectra in panel a (b, red line; untreated-minus-all-depleted) and panel c (d, blue line; untreated-minus-all-reconstituted).

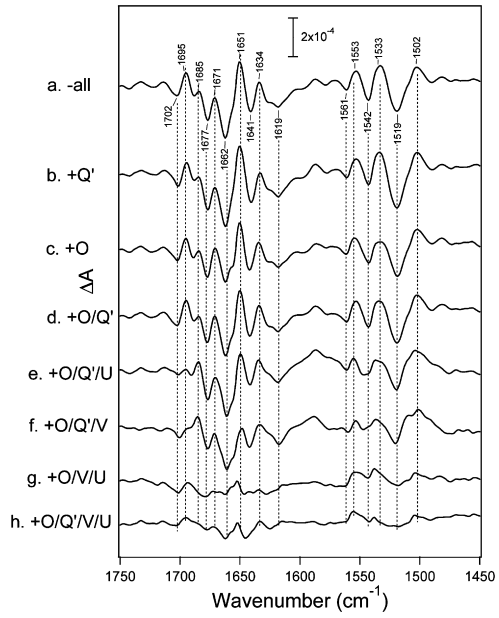

Figure 2. Amide I and II regions of the untreated-minus-treated double-difference FTIR spectra for PSII, in which all the extrinsic proteins were depleted (a) and then reconstituted with PsbQ′ (b), PsbO (c), PsbO/Q′ (d), PsbO/Q′/U (e), PsbO/Q′/V (f), PsbO/V/U (g), and PsbO/Q′/V/U (h).

(Figure 1c, blue line). The spectral changes and recovery are more clearly revealed in double-difference spectra between untreated and treated PSII samples. The double-difference spectrum of PSII complexes depleted of all the extrinsic proteins shows prominent bands in the amide I and II regions (Figure 1b), whereas that of fully reconstituted PSII has only weak features in these regions (Figure 1d). The large amide I and II bands in the former double-difference spectrum with only minor features in the symmetric COO^- region (Figure 1b) indicate that the structure of the polypeptide chains around the Mn_4CaO_5 cluster is drastically altered by depletion of the extrinsic proteins, while that of the Mn_4CaO_5 core with surrounding COO^- groups is mostly unaffected. Which polypeptides are actually subjected to conformational changes and whether these changes are direct or indirect effects are unknown at present. Interestingly, the amide I and II features of the extrinsic proteins depleted PSII of *C. caldarium* (Figure 1a, red line) are significantly different from those of spinach PSII in which all the PsbO, PsbP, and PsbQ extrinsic proteins were depleted (Figure S1b,c of the Supporting Information),[13] suggesting rather different structural effects of the extrinsic proteins on the OEC between red algae and higher plants.

S_2-minus-S_1 FTIR difference spectra were measured using PSII complexes reconstituted with various combinations of extrinsic proteins (Figure S2 of the Supporting Information). The untreated-minus-reconstituted double-difference spectra in the amide I and II regions are shown in Figure 2 (a wider region of 1800−1200 cm^{-1} involving the symmetric COO^- vibrations is presented in Figure S3 of the Supporting Information), in which smaller spectral features indicate more recovery of the protein structure. Enami et al.[5] have shown that PsbO and PsbQ′ can each bind to the PSII core (100 and 61%, respectively) in a manner independent of other extrinsic proteins, whereas binding of PsbV requires the presence of PsbO and PsbQ′ (29% PsbV bound) or PsbO and PsbU (64% PsbV bound). The bands did not recover or only partially recovered by binding of PsbQ′, PsbO, PsbO/Q′, PsbO/Q′/U, and PsbO/Q′/V (Figure 2b−f), whereas the bands significantly recovered by binding of PsbO/V/U (Figure 2g) and PsbO/Q′/V/U (Figure 2h; same as Figure 1d). Other than the changes in the overall intensities, the spectral features are slightly different in the PsbO/Q′/U-reconstituted (Figure 2e) and PsbO/Q′/V-reconstituted (Figure 2f) PSII compared with other samples (Figure 2a−d); the positive peak at 1695 cm^{-1} is lost, and instead, the intensity of the 1685 cm^{-1} peak increases in both spectra. Also, the relative intensity of the strong positive peak at 1651 cm^{-1} is significantly decreased upon PsbO/Q′/V reconstitution. It is thus suggested that some combinations of extrinsic proteins affect the structures of specific polypeptide chains around the Mn_4CaO_5 cluster.

The extents of band recovery (%) in the amide I and II regions, which were estimated by fitting of the double-difference spectra of individual samples (Figure 2a−h) with that of all-depleted PSII (Figure 2a) in the 1720−1480 cm^{-1} region, are presented in Figure 3 (red bars) together with the relative O_2 evolution activities (blue bars) and the amounts of reconstituted extrinsic proteins (in parentheses) reported previously.[5] The recovery rate is expressed as 1 − fitting factor. It should be noted that because spectral features are not exactly the same among the spectra, as mentioned above, and the noise level is relatively high in the amide I and II regions (Figure S3a of the Supporting Information, dotted line), the estimated values have errors of approximately ±5%. It is shown that the tendency of the recovery of FTIR bands correlates well with that of O_2 evolution, suggesting that the activity of the OEC is directly related to the structural changes of the protein conformations around the Mn_4CaO_5 cluster induced by binding of extrinsic proteins.

There was no recovery of FTIR bands upon PsbQ′ binding (Figure 3b). PsbO binding led to a slight recovery of the protein bands [26% (Figure 3c)], whereas further binding of PsbQ′ (Figure 3d) and PsbU (Figure 3e) did not improve the recovery. When PsbV is bound to the PsbO/Q′−PSII complex (Figure 3f), albeit partial reconstitution (29% PsbV),[5] the recovery of FTIR bands showed a meaningful increase (40%). The FTIR bands

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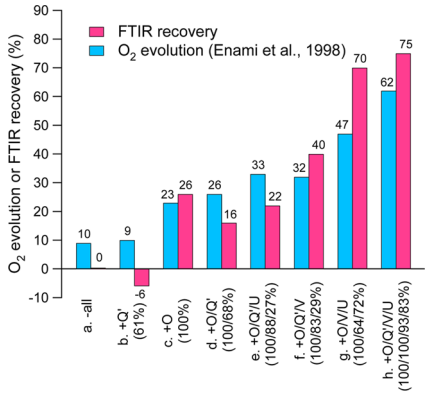

Figure 3. Extents of recovery (%) of the FTIR bands in the amide I and II regions (red bars) in PSII core complexes, in which all the extrinsic proteins were depleted (a) and then reconstituted with PsbQ′ (b), PsbO (c), PsbO/Q′ (d), PsbO/Q′/U (e), PsbO/Q′/V (f), PsbO/V/U (g), and PsbO/Q′/V/U (h). The estimated values have errors of approximately ±5%. The relative O_2 evolution in the presence of 50 mM $CaCl_2$ (blue bars) and the amounts of reconstituted extrinsic proteins (figures in parentheses), which were taken from ref 5, are also shown.

mostly recovered (70−75%) when PsbV and PsbU were bound together in addition to PsbO (Figure 3g) or PsbO/Q′ (Figure 3h). This dramatic recovery by PsbV and PsbU seems to indicate that both of these extrinsic proteins are the major components for restoring the structure of the OEC. However, taking into consideration the medium increase in the FTIR recovery by partial (29%) binding of PsbV (Figure 3f) but no effect of PsbU (Figure 3e), we concluded that PsbV binding mainly contributes to the restoration of the proper protein conformation of the OEC, while PsbU plays a role of supporting the proper binding of PsbV.

This FTIR property of PsbV in *C. caldarium* is very similar to that of PsbP in spinach, which was revealed in previous FTIR studies showing that proper binding of PsbP is required for restoration of the protein conformation of the OEC.[13,14] Thus, the FTIR data in this study provide a structural basis for the similarity of the function of PsbV in red algae and that of PsbP in higher plants, which has been proposed on the basis of O_2 evolution activity.[5] It is presumed that the change in the protein conformation of the OEC by binding of PsbV or PsbP affects the stability of the Mn_4CaO_5 cluster, perhaps by altering the binding constants of Ca^{2+} and Cl^-, or changing the energy barrier of their release or the accessibility of reductants. The function of PsbU in stabilizing PsbV binding may also be similar to that of PsbQ in higher plants. Although PsbQ is not required for PsbP binding and also does not affect the FTIR spectrum in normal PSII from spinach,[13] PsbQ supports proper binding of a modified PsbP such as Δ15-PsbP in which 15 N-terminal residues have been truncated.[15] It is interesting to note that the structural effect of PsbO is slightly different between red algae and higher plants; there was a minor effect of PsbO on the OEC conformation in *C. caldarium* (Figure 3c−e), whereas binding of PsbO did not affect the amide I bands of the FTIR spectrum in spinach.[13]

In conclusion, this FTIR study of *C. caldarium* strongly supports the view that the function of PsbV in red algae, as a main component for regulating the protein conformation of the OEC, corresponds to that of PsbP in higher plants, while PsbU that supports PsbV binding corresponds to PsbQ with slight modifications. Red algae and higher plants are both thought to be evolved from cyanobacteria,[19,20] which have PsbV and PsbU and also have cyanoP and cyanoQ, homologues of PsbP and PsbQ, respectively.[1−3] How the functions of extrinsic proteins were transferred from cyanobacteria to red algae in the red lineage and from cyanobacteria to higher plants in the green lineage during evolution of oxyphototrophs will be answered by further FTIR studies of cyanobacterial extrinsic proteins. Such a study is currently under way.

■ ASSOCIATED CONTENT

Supporting Information

Details of the materials and methods, comparison of the FTIR spectra between *C. caldarium* and spinach, S_2-minus-S_1 difference spectra of all of the reconstituted samples, and untreated-minus-treated double-difference spectra in the 1800−1200 cm^{-1} region. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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