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S-state Dependent FTIR Difference Spectra for Photosystem II: Examination of Proton Transfer Pathway Intermediates.

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Introduction

Photosynthetic water oxidation is an endogonic reaction driven thermodynamically by the capture of photons and separation of charge within the reaction center. Use of intermediates in the electron transfer pathway provides kinetic stabilization of charge separation and leads to the accumulation of oxidizing equivalents (Diner & Babcock 1996). The photosystem II complex proceeds via 5 intermediate levels of oxidation termed S-states (S_0 - S_4), and upon attaining S_4 the O-O bond is generated and S_0 is regenerated. The actual chemistry of water oxidation is catalyzed by an inorganic cluster of four Mn ions and involves the close coupling of a tyrosine residue (D1 Tyr_Z 161) and the participation of Ca and Cl cofactors. There is considerable debate within the field at present as to the location of the accumulated oxidizing equivalent on each transition (Mn, oxo-bridge or amino acid residue), the formal oxidation states of the cluster and the origin of the expelled protons (Nugent 2001).

The protons that are released during water oxidation provide a means to control the reaction chemistry. Different PSII preparations exhibit different H patterns and essentially two proposals dominate interpretation of this behavior. One proposal is that the proton release is electrostatically modulated by Mn oxidation (Haumann *et. al.,* 1999). The other is that the protons are released to maintain charge neutrality (Tommos & Babcock 2000). In the latter case it has been formulated that the protons leave via a specific pathway. FTIR provides a means to examine the pathway of the proton from a static and kinetic perspective. In this work here we examine H/D exchange effects on the S-state difference spectra to identify proton pathway intermediates in a static sense. Future kinetic work could address the effect of these residues on dielectric relaxation processes following charge separation.

Materials and Methods.

The photosystem II membranes (BBY's) were prepared from spinach and subsequently subfractionated into PSII core complexes with the addition of 0.4 % *n*-octyl-β-D-thioglucoside (Mishra & Ghanotakis 1994). FTIR samples were resuspended in 5 mM MES (pH 6.5), 50 mM sucrose, 5 mM NaCl in the presence of 2/18 mM ferri-/ferro-cyanide redox buffer and either dried onto a window or centrifuged into a pellet and applied to a window. The PSII core-dehydrated samples and BBY–hydrated samples were loaded such that the Amide I/water band (~1652 cm^{-1}) was OD < 1. For D_2O exchange the BBY membranes were incubated for 20 hr in buffer (pL=6.5). The FTIR measurements were performed using a Bruker Equinox 55 spectrometer. The sample was measured in a home made sample cryostat and temperature control of 265 ± 0.003 K was achieved with a Lake Shore 340 temperature controller. A Ge longpass 4 μm filter (Janos) reduced spectral bandwidth and removed the coaxial HeNe beam from the interferometer. Flash excitation was with a single 7 ns Q-switched 532 nm Nd-YAG flash. Flash spacing for the BBY material was 5 s and 0.4 s for the core samples and data was acquired continuously between the flashes.

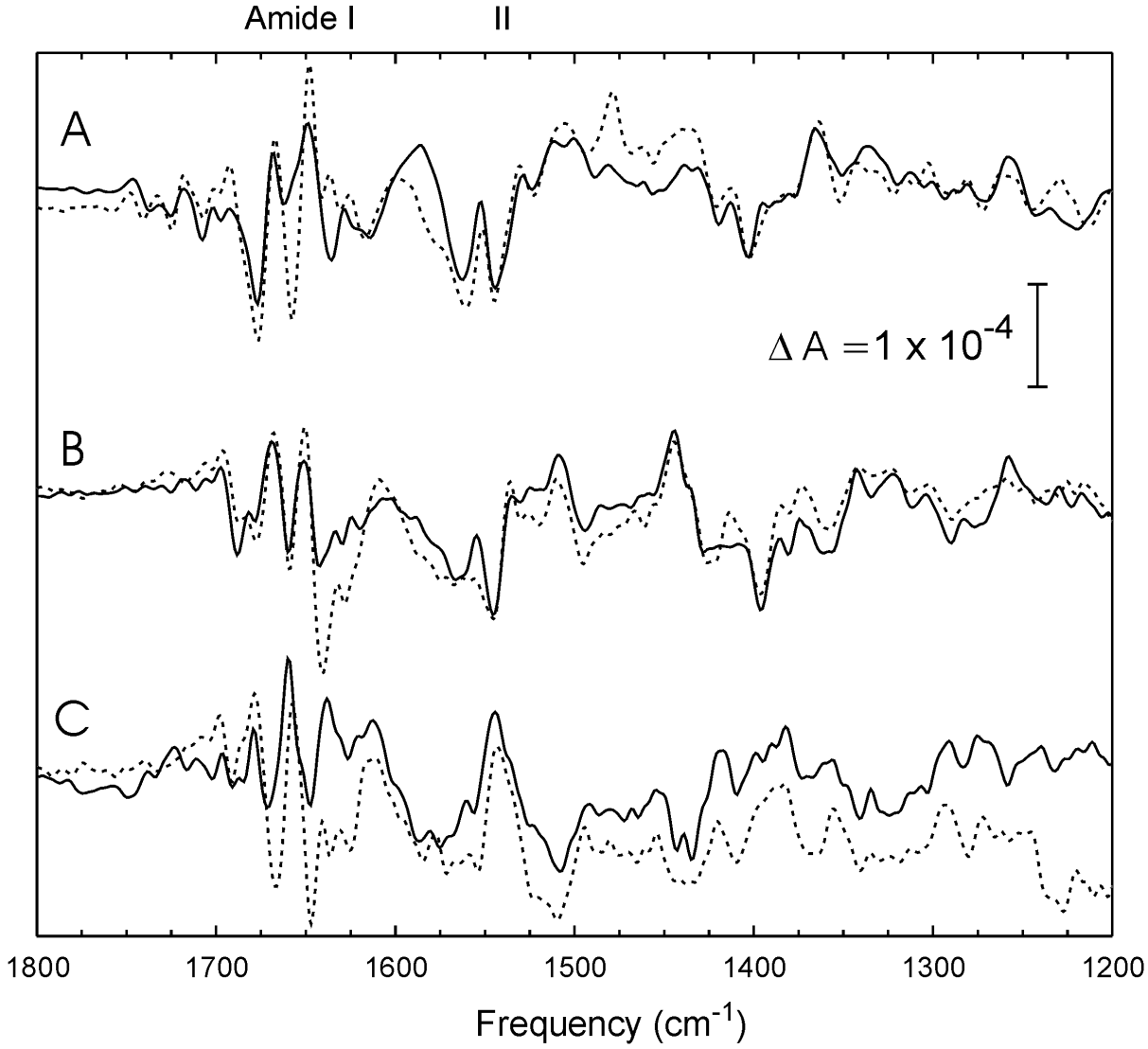

Figure 1: The 265 K FTIR difference spectra for the (A) $S_1 \rightarrow S_2$, (B) $S_2 \rightarrow S_3$ and (C) $S_3 \rightarrow S_0$ transitions in BBY samples suspended in H_2O (solid line) and D_2O (dotted line) buffers at pL 6.5. The data represents that average of ~1000 scans and have been normalized for comparison.

Results & Discussion

FTIR difference spectra in the mid-IR spectra region for the $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions in BBY's are shown in Fig. 1 for H_2O and D_2O buffers. The low frequency regions will be discussed elsewhere (Chu *et al*., these proceedings). The mid-IR spectra reveal numerous spectral modes that undergo H/D dependent shifts. The largest bands appearing are the amide I (1640-1690 cm^{-1}) and amide II (1540 cm^{-1}) bands. These bands indicate that a small structural change arise from changes to the H-bonding backbone of the polypeptides upon S-state advance (Noguchi & Sugiura 2001; Hillier & Babcock 2001). The modes at (+)1552, (-)1544 cm^{-1} attributed to amide II show no real change upon D substitution consistent with this assignment. Both amide regions demonstrate oscillates in sign (0: 0: 1: 1) from the $S_1 \rightarrow S_2$ transition indicative of dynamic changes to the protein. The amide I modes are more complex arising from numerous secondary structural interactions (Noguchi *et. al.,* 1995a). Also seen are intense modes arising from the asymmetric ν_{asym} (COO^-) carboxylate-stretching (1650-1550 cm^{-1}) and the symmetric ν_{sym}

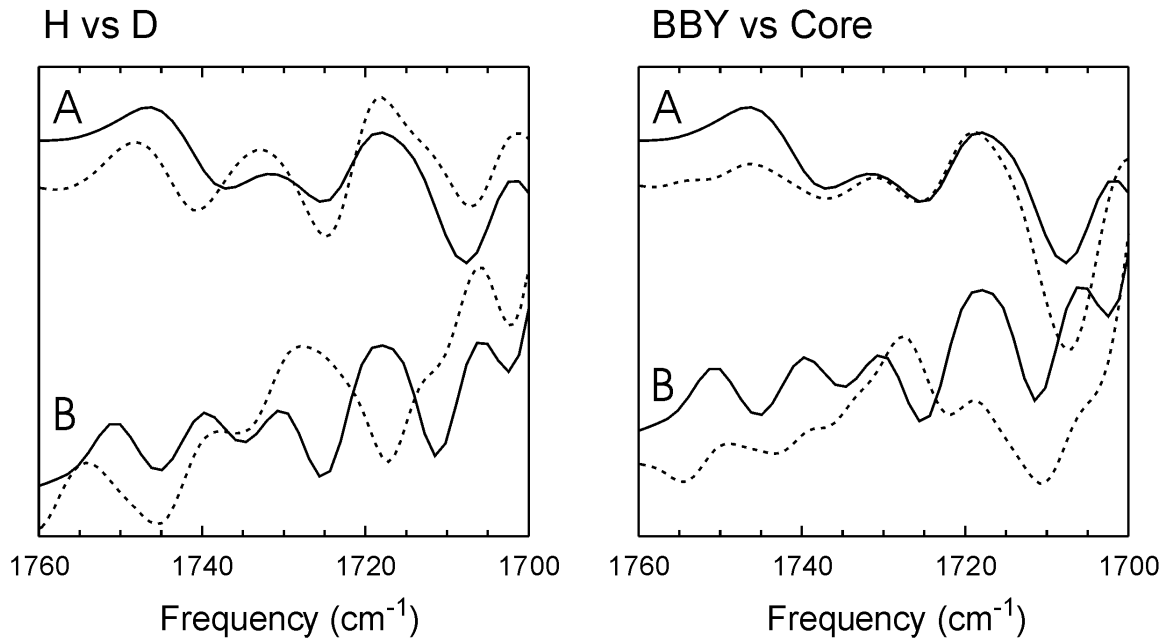

Figure 2: The FTIR difference spectra expanded in the 1760-1700 region for the (A) $S_1 \rightarrow S_2$ and (B) $S_2 \rightarrow S_3$ transitions shown on the left for the BBY samples in H_2O (solid line) D_2O (dotted line) and then on the right for BBY samples in H_2O (solid line) PSII cores in H_2O (dotted line).

(COO^-) carboxylate-stretching (1450-1300 cm^{-1}) modes for the $S_1 \rightarrow S_2$ transition (Noguchi *et. al.,* 1995a,b) and new modes for the $S_2 \rightarrow S_3$ transition (Noguchi & Sugiura 2001; Hillier & Babcock 2001). The large changes seen on the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions in these carboxylate-stretching regions are consistent with coordination changes involving the Mn_4Ca cluster involved in water oxidation. Several regions of the FTIR spectra are useful for identifying amino acids candidates involved in H-bonding networks and H-wires. The protonated carboxylic acid (C=O) modes (1750-1700 cm^{-1}) are largely separated from protein vibrational modes and provide a means to monitor aspartate and glutamate residues. An expansion of this region for the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ difference spectra is shown in Figure 2. Close inspection of the $S_1 \rightarrow S_2$ difference spectra in Fig. 2A(left) reveals three modes that are H/D sensitive. These modes tentatively may arise from the interactions of three carbonyl groups. Peaks at (+)1747, (-)1737 & (+)1732 cm^{-1} in H_2O upshift upon deuteration by about 1-3 cm^{-1} to (+)1748, (-)1741 & (+)1733 cm^{-1} in D_2O. This upshift is not attributable to H/D exchange of the COOH group, which is typified by a 10-15 cm^{-1} downshift (Barth 2000), but rather suggests interactions involving deuterium and H-bonding networks. Accordingly, the $S_1 \rightarrow S_2$ difference spectra does not directly identify modes attributable to Asp or Glu residues that undergo H/D exchange behavior expected for an intermediate in a proton expulsion pathway. This is surprising as these residues typically play important roles in proton pathways in other light activated systems. To account for why there are not spectral features undergoing a strong deuterated downshift it is possible that the side chain COOH is nonexchangeable after 20 hr. However, recent pH titration of EPR signals suggests that the Y_Z can be rapidly titrated by the external media (Geijer *et. al.,* 2001). Thus if Y_Z is titratable then *via infra* the surrounding amino acids too are likely titratable. The more likely interpretation is that these modes are not structurally perturbed during the transition. They will therefore not appear in the FTIR difference spectra for this transition.

The $S_2 \rightarrow S_3$ transition is shown in Fig. 2B(left) and indicates several modes appear to shift upon H/D exchange. The S_2 mode at (+)1718 cm^{-1} and the S_1 modes at (-)1715 and (-)1725

cm^{-1} disappear upon D exchange and are replaced with a S_1 modes at (-)1715 cm^{-1} and a broad S_2 mode at (+)1725 and a shoulder at (+)1712 cm^{-1}. The spectral changes upon deuteration are complex and there is likely to be a number of amino acids involved in this difference spectrum. At least one residue from Asp or Glu is likely involved. The data does indicate that there is the possibility for a down shift in the S_2 1718 cm^{-1} mode. Other shifts may also be apparent but are complicated by H/D exchange of the COOH group and of H-bond networks.

The $S_1 \rightarrow S_2$ transition for a PSII core preparation is shown in Fig 2(right) in comparison to a BBY sample. The $S_1 \rightarrow S_2$ transition in this spectral region is very similar for the two sample preparations. One spectral feature that is notably stronger in the core preparation is the intensity of the (-)1710 cm^{-1} band. The variation between samples may be attributable to a difference in the p*K* of an Asp or Glu residue. The increased intensity in the core samples may therefore suggest that the p*K* for this group is lower in the BBY material. The strong asymmetric feature is indicative of a net deprotonation of an Asp or Glu residue during the $S_1 \rightarrow S_2$ transition and may represent some internal polarization or dipole change. However, this feature would not be consistent with the involvement of Asp or Glu residues in the accumulation of net charge suggested by electrochromic band shifts. The $S_2 \rightarrow S_3$ transition in Fig. 2b(right) reveals that core complexes have peaks that are in a similar position to BBY's yet reside on a slightly different baseline. The notable exception are the S_2 1725 cm^{-1} and S_3 1730 cm^{-1} modes in BBY samples which are downshifted in PSII core preparations.

Conclusion

This study has been a preliminary investigation into the proton pathways associated with the oxidation of water. The static approach used here revealed FTIR changes that appear at the formal S-state level. The data indicate H/D changes to modes that characteristically arise from Asp and Glu residues in proteins. In particular the $S_2 \rightarrow S_3$ transition appears to show a number of modes potentially arising from the carbonyl region of Asp or Glu residues. For the $S_1 \rightarrow S_2$ transition these changes are less apparent and indicate that the overall S-state advance does not structurally perturb these residues. The question then becomes the origin of these changes. From this data alone it is not possible to pinpoint the groups involved. However mutational changes to amino acid residues can be used for this purpose and will need to be explored. The reason then for the structural changes during the S-state transition is also an interesting question. It is likely that the answer lies in small changes that result from (small) changes in local charge. Future work will need to examine these modes for all the S-states to observe the reversal of the changes. Another approach is to utilize the kinetic aspects of FTIR spectroscopy to enable the resolution of transiently generated species. This then has the exciting possibility of resolving the fast proton hopping motions of the substrate protons along a proton wire.

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