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Carboxylate stretching bands in the S_2/S_1 FTIR spectrum disappear by metal chelator in Ca^{2+}-depleted OEC

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Introduction

The central unit of the oxygen-evolving complex (OEC) of photosystem (PS) II comprises a tetranuclear Mn-cluster, Ca^{2+} and Cl^{-}, which are required for the O_2-evolving activity (Debus 1992, Hoganson and Babcock, 2000, Robblee et al. 2001). In order to probe the structural and mechanistic details of the OEC, Fourier transform infrared (FTIR) spectroscopy has been extensively applied to the studies on OEC in both mid- and low-frequency regions (Noguchi et al. 1995, Chu et al. 2001). The S_2/S_1 FTIR difference spectrum in the mid-frequency region shows characteristic bands that correspond to the carboxylate stretching vibrations in addition to the amide I and amide II bands. These carboxylate bands are believed to be ascribed to acidic amino acid(s) that ligates the Mn-cluster and/or Ca^{2+} (Noguchi et al. 1995, Chu et al. 2001a, Chu et al. 2001b). Therefore, the studies on the carboxylate modes may provide valuable insights for the ligation structure of the Mn-cluster to understand the mechanism of O_2 evolution. Here, we studied the carboxylate modes by focusing on the interactions of Ca^{2+} cofactor. We found that the carboxylate modes were not correlated with Ca^{2+} and the bands were almost completely abolished by EDTA or EGTA in the absence of Ca^{2+}.

Materials and methods

BBY-type PS II membranes prepared from spinach were depleted of Ca^{2+} by 2M NaCl washing under weak light conditions (Ono and Inoue 1986) followed by the treatment with Chelex 100. The membrane samples were incubated in a Chelex-treated buffer medium (20mM Mes/NaOH, 0.4M sucrose, 20mM NaCl, pH6.5) at 0˚C for 5min under darkness in the presence of metal chelators or cations (Ca^{2+} and Sr^{2+}) at the indicated concentration. DCMU was added to the suspension at 0.1mM for measuring the $S_2Q_A^{-}/S_1Q_A$ spectrum, and 0.1mM DCMU and 10mM NH_2OH were added for the Q_A^{-}/Q_A spectrum. The resulting pellet was sandwiched between a pair of ZnSe disks. All wears for sample preparations were rinsed with acid before use. The concentrations of free Ca^{2+} in buffer solutions and sample suspensions were monitored using a Ca^{2+}-sensitive fluorescence probe (Quin2). All Ca^{2+} concentrations were lower than the detectable limit ($< \approx 0.2\mu M$). FTIR spectra were recorded on a spectrophotometer (Bruker IFS-66v/s) equipped with a KBr beamsplitter and an MCT detector. Sample temperature was controlled at 250K in a cryostat (Oxford, Optistat DN1704) with a temperature controller (Oxford, ITC-502). The cold light (HOYA-SCHOTT HL150R) passing through a long-path filter ($\geq$620nm) was used for the sample illumination. Each single-beam spectrum was measured at 4 cm^{-1} resolution by averaging 300scans (130s accumulation). Four spectra in different samples were averaged to improve the signal/noise ratio.

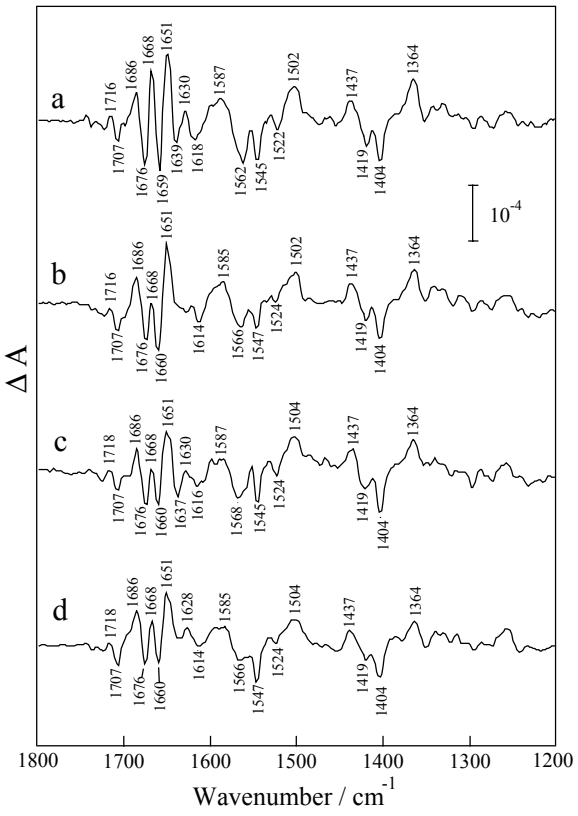

Figure 1. Light-induced S_2/S_1 difference spectra of PS II membranes that are (a) untreated, (b) Ca^{2+}-depleted, replenished with (c) 20mM $CaCl_2$, (d) 20mM $SrCl_2$.

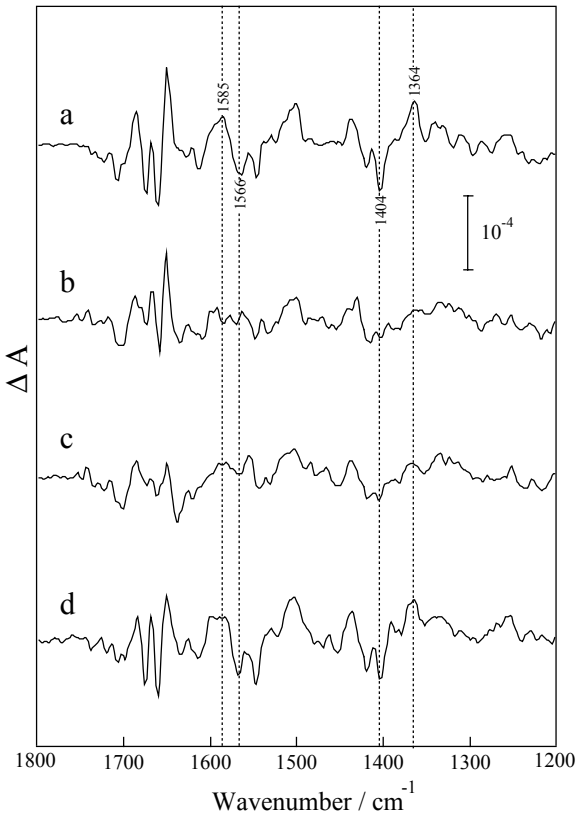

Figure 2. Light-induced S_2/S_1 difference spectra of Ca^{2+}-depleted membranes with (a) no-addition, (b) 1mM EDTA, (c) 1mM EGTA, and (d) 1mM EDTA followed by 20mM $CaCl_2$.

Results

Figure 1 shows the effects of Ca^{2+} depletion on the S_2/S_1 difference spectrum of PS II membranes. The S_2/S_1 spectra were obtained by subtracting the Q_A^-/Q_A difference spectrum from the $S_2Q_A^-/S_1Q_A$ difference spectrum after normalization with respect to the intensity of the CO stretching band of Q_A^- at 1479cm^{-1}. The double difference spectrum of the non-treated control PS II membranes (spectrum a) showed the characteristic vibrational features of the S_2/S_1 difference spectra at 1364(+)/1404(–)cm^{-1} and at 1587(+)/1562(–)cm^{-1} for the symmetric and the asymmetric stretching vibrations of the carboxylate ligand for the Mn-cluster. The pronounced differential bands in the 1690–1630cm^{-1} (amide I) and 1590–1515cm^{-1} (amide II) regions correspond to the conformational change of the protein backbone upon the S_1 to S_2 transition. In the previous report, the carboxylate stretching bands were not induced in the membranes that were depleted of Ca^{2+} using low-pH treatment (Noguchi et al. 1995). To the contrary, the carboxylate bands were decisively evident in the Ca^{2+}-depleted membranes (spectrum b), although the O_2-evolving activity was inhibited down to 16% relative to that of the control membranes. Replenishment of Ca^{2+} restored the inhibited O_2 evolution (up to 80%), but did not affect the carboxylate bands (spectrum c), indicating that Ca^{2+} is not involved in the changes of the ligation structure of the Mn-cluster upon the S_2 formation. This view was further supported by the finding that the carboxylate bands were equally induced in the Sr^{2+}-supplemented PS II membranes (spectrum d). The amide I and II

bands were slightly modified by Ca^{2+} depletion but were not restored by the replenishment of Ca^{2+} (spectrum c), indicating that these changes may be attributed to some anomalous effects induced by the high-salt washing.

Fig.2 shows the effects of chelator on the S_2/S_1 difference spectra of the Ca^{2+}-depleted PS II membranes. The addition of 1mM EDTA significantly diminished the carboxylate bands at 1364(+)/1404(–)cm^{-1} for symmetric and 1585(+)/1566(–)cm^{-1} for asymmetric vibrations concomitant with considerable abolishment of the amide I and II bands (spectrum b). Similar effects were observed by the supplementation with 1mM EGTA (spectrum c), suggesting that the chelating functions of these chemicals are responsible for their effects on the spectra. The characteristic vibrational features on the S_2/S_1 spectrum, both the carboxylate and the amide bands, were recovered by the subsequent replenishment with 20mM Ca^{2+} after the chelator treatement (spectrum d), indicating that chelator effects are fully reversible. The apparent K_m value for the inhibitory effects of EDTA was estimated to be 0.49mM.

Discussion

It was proposed that a carboxylate bridging between the redox-active Mn and Ca^{2+} was responsible for the FTIR bands and the coordination mode of the carboxylate changed from the bidentate to the unidentate structure in the S_1 to S_2 transition (Noguchi et al. 1995), based on the Ca^{2+}-dependence and the frequency difference between the asymmetric and symmetric vibrational modes. However, our present results clearly demonstrate that Ca^{2+} is not associated with the carboxylate stretching bands in the S_2/S_1 spectrum, indicating that the putative carboxylate bridging between Ca and Mn is not responsible for the carboxylate bands found in the S_2/S_1 spectrum. This view is consistent with the finding that Sr^{2+} substitution did not induce any changes in band position of the carboxylate vibrations in the S_2/S_1 spectrum (Figure 1). The absence of the carboxylate bands in the low-pH treated Ca^{2+}-depleted membranes is probably caused by the difference in the procedures for Ca^{2+} depletion, and/or by the presence of EDTA which was included in the sample suspension. Based on these results, we propose that the carboxylate bridges between two Mn ions or, alternatively, chelates one Mn ion in bidentate manner in the S_1 state, then, one of the coordination bonds is selectively released to cause the appearance of the unidentate structure upon the S_2 formation.

The FTIR results suggest that the ligation structure of the Mn-cluster does not depend on Ca^{2+} in both the S_2 and S_1 states. In fact, the redox and magnetic properties of the S_2 state Mn-cluster in the Ca^{2+}-depleted PS II are indistinguishable from those of the normal S_2 state, as indicated by the normal $S_2Q_A^-$ TL band and the normal S_2 state multiline EPR signal in the depleted PS II (data not shown). To summarize, the Mn-cluster in the Ca^{2+}-depleted and native PS II membranes has inherently identical properties in the S_1 and S_2 states, except for the inhibition of O_2-evolving capability. Therefore, Ca^{2+} is likely to play key roles, in states higher than S_2, which control the ligation structure and redox chemistry of the Mn-cluster necessary for the oxygen evolution as well as the binding of the substrate water.

Light-induced changes in the FTIR spectra upon the S_1 to S_2 transition were largely suppressed by the addition of EDTA or EGTA to the Ca^{2+}-depleted PS II membranes without appearance of any new band (Figure 2). The absence of the characteristic S_2/S_1 bands is not due to the inhibition of the oxidation of the Mn-cluster to the S_2 state in the presence of the chelator, since the S_2 formation was evident by the generation of the normal $S_2Q_A^-$ TL band and S_2 multiline EPR signal (data not shown). These also indicate that the redox and magnetic properties of the S_2 state Mn-cluster are largely normal in the chelator-supplemented membranes. Our FTIR results indicated that the chelators interact with the Mn-cluster at the S_1 and/or S_2 states, although such associations do not significantly influence the properties of the cluster. The possible association of a chelator to the Mn-cluster has been suggested by EPR studies also (Boussac et al. 1990).

Based on the present results, we propose that a chelator interacts with the Mn-cluster as a replacement for the native carboxylate ligand responsible for the generation of the carboxylate stretching bands. Since the carboxylate stretching bands were not induced in the chelator-supplemented membranes, the S_1 to S_2 transition is not accompanied by the change of the ligation mode of the chelator. Taking the normal properties of the S_2 state Mn-cluster into consideration, the ligation geometry of the Mn-cluster in the S_2 state may not be significantly altered even in the presence of the chelators. If this is the case, the chelator carboxylate may be associated in an unidentate manner with the Mn-cluster in the both S_1 and S_2 states.

Acknowledgments

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