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Rapid Report

Temperature dependence of the $S_1 \rightarrow S_2$ transition in the oxygen-evolving complex of photosystem II studied by FT-IR spectroscopy

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The temperature dependence of the single-turnover redox reaction on the electron-donor side of Photosystem II (PS II) has been investigated by measuring light-induced FTIR difference spectra of DCMU-treated PS II membranes at various temperatures of 80–240 K The negative band at 1404 cm^{-1} in the difference spectra exhibited the same temperature dependence as the S_2 formation in the oxygen-evolving center previously determined by EPR spectroscopy (De Paula, J C , Innes, J B and Brudvig, G W (1985) Biochemistry 24, 8114–8120) This observation strongly supports our previous assignment of the 1404 cm^{-1} band to the structural change of proteins upon the $S_1 \rightarrow S_2$ transition (Noguchi, T , Ono, T and Inoue, Y (1992) Biochemistry 31, 5953–5956) The temperature dependence of the 1660 cm^{-1} band due to cytochrome b_{559} (Cyt b_{559}) oxidation has shown a complementary relationship to that of the 1404 cm^{-1} band, consistent with the view that the oxidation of Cyt b_{559} competes with the S_2 formation on the electron-donor side of PS II The present FTIR data also suggest that the structural difference between the two S_2 states exhibiting the $g = 4$ 1 EPR signal and the multiline signal is not very large as far as the protein moiety around the Mn cluster responsible for the 1404 cm^{-1} band is concerned

The mechanism of oxygen evolution in Photosystem II (PS II) is still one of the biggest riddles in photosynthesis The basic concept we have gained so far is that the oxygen-evolving center consists of four Mn atoms, where a cyclic reaction takes place involving five intermediates called S_i-states ($i = 0$–4) Absorption of one photon advances each of the S-states to the next S-state, and an oxygen molecule is evolved in the course of the $S_3 \rightarrow S_0$ transition (for recent reviews, see Refs 1 and 2) Although many fragments of information about the structure of the Mn cluster have been accumulating through extensive work with EPR and X-ray absorption spectroscopy (for a review, see Ref 3), it is still difficult for us to visualize its real structure and details of the reaction mechanism

As an approach to answer these questions, we have recently applied FTIR spectroscopy to the oxygen-evolving system, and have succeeded in detecting the FTIR difference spectrum upon the $S_1 \rightarrow S_2$ transition free of contribution by the acceptor-side signal [4] The observed spectrum contains information about the structural changes of the protein moiety around the Mn cluster, including the changes in carbonyl groups ligating to redox-active Mn ions and in polypeptide conformations [4]

The temperature dependence of the $S_1 \rightarrow S_2$ transition has so far been studied by means of EPR [5–7] and thermoluminescence [8,9] When PS II membranes in the presence of DCMU are illuminated with continuous light, the S_2 formation fully proceeds above 220 K but is suppressed below 80 K [6] The EPR study showed that two types of S_2 states are formed depending on the illumination temperature, one S_2 state exhibiting the $g = 4$ 1 EPR signal is preferentially formed at about 130 K, whereas another S_2 state exhibiting the $g = 2$ multiline signal is dominant at higher temperatures [6] It has also been known that the S_2 formation competes with oxidation of Cyt b_{559} and the temperature dependence of Cyt b_{559} oxidation exhibits a complementary relationship with the S_2 formation [6]

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Abbreviations Cyt b_{559}, cytochrome b_{559}, DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, FTIR, Fourier transform infrared, Mes, 2-morpholinoethanesulfonic acid, PS II, Photosystem II, Q_A, primary quinone acceptor of PS II, Q_B, secondary quinone acceptor of PS II

In this study, we have investigated the temperature dependence of the redox reaction on the electron-donor side of PS II by means of light-induced FTIR difference spectroscopy The FTIR bands previously assigned to the $S_1 \rightarrow S_2$ transition [4] and to the oxidation of Cyt b_{559} [10] were used to monitor the two reactions

BBY-type PS II membranes [11] capable of oxygen evolution were prepared as in Ref 12 The PS II samples were suspended in Mes-NaOH buffer (40 mM Mes-NaOH (pH 6 5), 400 mM sucrose, 20 mM NaCl) and incubated at 4 °C in the dark for about 24 h, so that all the oxygen-evolving centers are relaxed to the S_1 state DCMU (0 1 mM final) was added into the PS II sample (0 5 mg chlorophyll/ml) in order to insure a single turnover reaction by inhibiting the electron transfer from Q_A to Q_B The sample solution was centrifuged at $150\,000 \times g$ for 15 min, and the resultant pellet was pressed between a pair of BaF_2 plates (13 mm diameter) to be subjected for FTIR measurements

FTIR spectra were measured on a JEOL JIR-6500 spectrophotometer equipped with an MCT detector (EG&G Judson IR-DET101) The sample temperature was controlled in a cryostat (Oxford DN1704) with a temperature control unit (Oxford ITC-4) A Ge filter (OCLI LO2584–9) was placed in front of the sample in order to block the He-Ne laser beam partially leaking into the sample space Light-induced difference spectra were obtained by subtraction between the two single-beam spectra taken before and after illumination Light illumination was performed for 5 s with continuous light (20 mW/cm^2) from a tungsten lamp through several layers of heat-cut filters and a red glass filter ($\lambda > 620$ nm) Each single-beam spectrum was an average of 300 scans (150 s accumulation) The spectral resolution was 4 cm^{-1}

Fig 1 shows light-induced FTIR difference spectra of PS II membranes illuminated and measured at given temperatures (80, 140, 200 and 240 K) Although continuous light was used for illumination, only a single turnover reaction occurred due to the presence of DCMU Under this condition, the quinone electron-acceptor Q_A is fully reduced and stably stored at every temperature examined in the present study (80–240 K) On the other hand, the hole generated on the electron-donor side migrates either to the oxygen-evolving center to form the S_2 state or to Cyt b_{559} depending on the temperature [6]

The difference spectrum at 80 K (Fig 1A) is almost the same as the Cyt b_{559}(ox)Q_A^-/Cyt b_{559}(red)Q_A difference spectrum recently reported by Berthomieu et al [10], which was measured with PS II enriched membranes at 60 K The 1480 cm^{-1} band is ascribed to the typical Q_A^- band, which can be assigned to the carbonyl-stretching mode of semiquinone [13] The 1660 cm^{-1} band is typical of Cyt b_{559} in a high-potential form and is assigned to the structural change in the

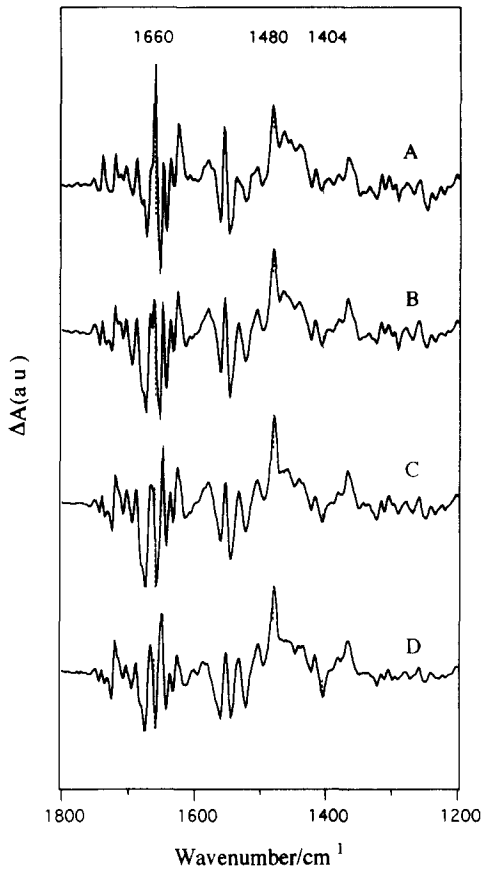

Fig 1 FTIR difference spectra (after minus before continuous light illumination) of PS II membranes in the presence of DCMU illuminated and measured at 80 K (A), 140 K (B) 200 K (C) and 240 K (D) The spectra were normalized on the 1480 cm^{-1} band assigned to the CO stretch of Q^-_A The positions of the negative 1404 and positive 1660 cm^{-1} bands attributed to the $S_1 \rightarrow S_2$ transition and the Cyt b_{559} oxidation, respectively, are marked with dashed lines

apoprotein upon oxidation of Cyt b_{559} [10] Thus, these two bands at 1480 and 1660 cm^{-1} can be used as marker bands for the reduction of Q_A and the oxidation of Cyt b_{559}, respectively

Fig 1D shows the difference spectrum measured at 240 K This spectrum is essentially the same as the $S_2Q_A^-/S_1Q_A$ difference spectrum observed under the same condition but at 250 K in our previous study [4] The band at 1480 cm^{-1} assigned to the CO stretch of Q_A^- is pronounced as in the spectrum measured at 80 K (Fig 1A) The negative 1404 cm^{-1} band can be attributed solely to the S_2/S_1 signal, because the Q_A^-/Q_A difference spectrum involves no band at this position [4,13] In other frequency regions, however, the donor-side (S_2/S_1) [4] and acceptor-side (Q_A^-/Q_A) signals [13] are mutually overlapping in a complex manner, so that the signals in these regions cannot be used to estimate the amount of S_2 formation Thus, we took the 1404 cm^{-1} band as marker for the $S_1 \rightarrow S_2$ transition This band has been tentatively assigned to the symmetric vibration of a COO^- group ligating to a redox-active Mn atom [4]

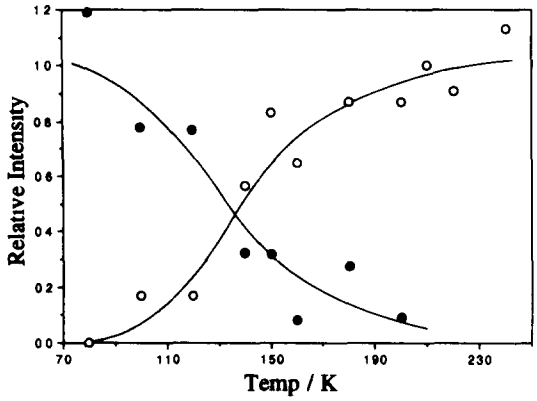

Fig 2 Temperature dependence of the intensities of the 1404 cm^{-1} (S_2/S_1, ○) and the 1660 cm^{-1} (Cyt b_{559}(ox)/Cyt b_{559}(red), ●) bands

The difference spectra at 140 K (Fig 1B) and at 200 K (Fig 1C) also showed an intense Q_A^- band at 1480 cm^{-1} as expected However, the 1660 and 1404 cm^{-1} bands in these spectra exhibited intermediate intensities between the spectra at 80 K and 240 K

Fig 2 shows the effect of illumination temperature on the intensities of the two FTIR bands at 1404 (open circle) and 1660 cm^{-1} (filled circle) These band intensities were estimated after normalization of the spectra on the intensity of the 1480 cm^{-1} Q_A^- band This normalization is based on the fact that the total amount of holes generated on the electron-donor side is necessarily the same as the amount of Q_A^- stabilized on the acceptor side The baselines of the spectra were drawn by connecting the two points at 1800 and 1340 cm^{-1}, where no peak was observed in all the spectra

The intensity of the 1404 cm^{-1} band is nearly zero at 80 K and becomes higher as the temperature increases On the contrary, the 1660 cm^{-1} band is most intense at 80 K and becomes weaker as the temperature increases to be practically undetectable above 200 K Clearly, the decrease in intensity of the 1660 cm^{-1} band is paralleled by the increase in intensity of the 1404 cm^{-1} band The half-maximum temperatures for both the two bands are located at 130–140 K, and the two curves intersect at 135 K

The above temperature-dependent behavior of the 1404 and 1660 cm^{-1} bands is almost identical to those of the S_2 formation and the Cyt b_{559} oxidation, respectively, which were previously studied by de Paula et al [6] by means of EPR under similar experimental conditions The intensity of the multiline EPR signal due to the S_2 state increased while that of the signal at $g = 3\,0$ due to oxidized Cyt b_{559} decreases as the illumination temperature increases from 77 to 220 K, and the two temperature-dependence curves intersected at around 135 K [6] It should be noted that de Paula et al [6] annealed the sample at 200 K for 2 min after illumination in order to convert the $g = 4\,1$ signal to the multiline signal to facilitate the estimation of the overall amount of the S_2 state

Recently, we have reported a single-flash-induced FTIR spectrum which is composed of only the S_2/S_1 difference, being free from acceptor-side signals, by using trypsinized PS II membranes supplemented with an exogenous electron acceptor [4] The spectrum exhibited several bands including the 1404 cm^{-1} band, which were ascribed to the structural changes of the protein moiety around the Mn cluster upon the S_2 formation The correctness of the spectrum was evidenced by the fact that addition of the two spectra (the S_2/S_1 only spectrum and the Q_A^-/Q_A only spectrum) produced a spectrum that is almost identical with the $S_2Q_A^-/S_1Q_A$ spectrum obtained with normal PS II membranes in the presence of DCMU [4] In the present study, we observed that the temperature dependent behavior of the 1404 cm^{-1} band is in good agreement with that of the S_2 formation detected by EPR This observation provides another strong evidence for the correctness interpretation of the S_2/S_1 spectrum exhibiting the 1404 cm^{-1} band as one of the pronounced difference signals

It has been known that there are two types of S_2 states which exhibit different EPR signals, the $g = 4\,1$ signal and the $g = 2$ multiline signal, and the distribution of population among these S_2 states depends on the temperature and cryoprotectants included in the buffer [6,14] The $g = 4\,1$ signal is dominant at lower temperatures (the maximum temperature is 130 K according to de Paula et al [6]) and in the buffer with ethylene glycol or glycerol as a cryoprotectant only the multiline signal is observed at 200 K whereas the $g = 4\,1$ signal is left in the buffer with only sucrose [14] In the present study, where only sucrose is used as a cryoprotectant, both the S_2 states will be formed by illumination The result that the temperature-dependence curve of the 1404 cm^{-1} FTIR band fits that of the total S_2 amount suggests that the difference between the two S_2 states is rather small, at least concerning the protein structure responsible for the 1404 cm^{-1} FTIR band, which arises from a COO^- group provably coordinating to redox-active Mn

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