BICARBONATE BINDING TO THE WATER-OXIDIZING COMPLEX IN THE
PHOTOSYSTEM II. A FOURIER TRANSFORM INFRARED SPECTROSCOPY
STUDY

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# The authors would like to dedicate this paper to the memory of José V. Ibarra.

Keywords: photosystem II, bicarbonate, donor side, Fourier transform, infrared
spectroscopy.
ABSTRACT

The light-induced Fourier transform infrared difference (FT-IR) spectrum originating from the donor side of O₂-evolving photosystem II was obtained in non-depleted and CO₂-depleted PSII membrane preparations. The observed spectrum free of contributions from the acceptor side signals was achieved by employing 2 mM/18 mM ferri-ferrocyanide as a redox couple. This spectrum showed main positive bands at 1589 and 1365 cm⁻¹ and negative bands at 1560, 1541, 1522 and 1507 cm⁻¹. CO₂-depleted PSII preparations showed a quite different spectrum. The main positive and negative bands disappeared after depletion of bicarbonate. The addition of bicarbonate partially restored again those bands. Comparison between difference FT-IR spectra of untreated and bicarbonate-depleted photosystem II membranes indicated that the positive bands at 1589 and 1365 cm⁻¹ can be assigned to COO⁻ stretching modes from bicarbonate. The higher frequency corresponds to \( \tilde{\nu}_a \) (COO⁻) and the lower frequency to \( \tilde{\nu}_e \) (COO⁻). \(^{13}\)C-labeling FT-IR measurements confirmed these findings and also suggested that the negative band at 1560 cm⁻¹ can be ascribed to \( \tilde{\nu}_a \) (COO⁻). The data are discussed in the frames of suggestion that bicarbonate can be a ligand to the Mn-containing water-oxidizing complex of photosystem II.
INTRODUCTION

Bicarbonate is known to be required for the maximal activity of photosystem (PS) II [1,2]. However, the interpretation of the stimulating effect of bicarbonate on PSII activity and its specific binding site still remain unclear. In the early 1970s, the water-oxidizing side of PSII was considered to be the acting site of bicarbonate [3,4]. Later, the proposal of bicarbonate binding at the acceptor side of PSII between Qa and Qb, the primary and secondary plastoquinone electron acceptors, was supported by numerous experimental data [1,2]. Recently, bicarbonate requirement for the donor side of PSII was shown [5-9]. It was suggested that bicarbonate takes part in the formation of the Mn-cluster capable of water oxidation as an obligatory ligand or through modification of the binding site(s) of Mn [5-9]. Recently, we have provided evidence for a bicarbonate requirement during re-assembly of Mn-containing center capable of water oxidation and showed that bicarbonate is an essential constituent of the water-oxidizing complex (WOC) [10].

The structure of the Mn-cluster has been studied mainly by means of X-ray absorption and electron paramagnetic resonance (EPR) spectroscopies, both of which detect signals directly from Mn ions. At present, the most probable structure model of the Mn-cluster is a tetranuclear cluster in which the Mn ions are connected by oxo and carboxylate bridges [11]. Fourier transform infrared (FT-IR) difference spectroscopy is a powerful method to investigate the structures of proteins and cofactors in the active site of a large protein complex [12]. In the field of photosynthesis, light-induced FT-IR difference spectra of photochemical intermediates were obtained for bacterial reaction centers [13-16] and higher plants photosystems [17-20].
Noguchi et al. [21] investigated the structure of the Mn-cluster in O$_2$-evolving PSII membranes using this technique. They observed the difference spectrum only due to the S$_1$ to S$_2$ transition which reflected the changes in vibrational modes of ligands of the redox-active Mn ions and/or the conformational changes of proteins. Based on these findings they suggested that FT-IR difference spectroscopy is a valid method to investigate the structure of the Mn-cluster and O$_2$-evolving mechanisms. Thus, we have used the FT-IR difference spectroscopy in order to investigate the role of bicarbonate in the WOC of PSII.
STATE BINDING TO THE WATER-OXIDIZING COMPLEX IN THE
PSII. A FOURIER TRANSFORM INFRARED SPECTROSCOPY
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MATERIALS AND METHODS

Preparation of oxygenic and non-oxygenic PSII membranes.- Oxygenic PSII membranes were isolated from spinach by the method of Berthold et al. [22], with the modifications of Yruela et al. [23]. Samples were resuspended in 0.4 M sucrose, 15 mM NaCl, 5 mM CaCl2 and 50 mM Mes-NaOH, pH 6.0, frozen and stored at -80 °C until use. This sample exhibited oxygen evolution rates of 520 μmol O2. mg Chl1. h1 in the presence of 0.5 mM 2,6-dichloro-p-benzoquinone (DCBQ) as artificial electron acceptor.

Removal of bicarbonate from PSII membranes was achieved by a 250-fold dilution of concentrated PSII preparations with a medium previously depleted of endogenous bicarbonate by means of 60-min flushing with CO2-depleted air and N2. The CO2-depleted air was prepared by passing through a solution of 50% NaOH. The final concentration of the medium was 0.4 M sucrose, 20 mM NaCl, 5 mM CaCl2, 50 mM Mes-NaOH (pH 6.0) [10]. The sample was subsequently incubated in this medium at a Chl concentration of 50 μg mL1 for 2 h at 4°C in the dark and tightly closed. After that the PSII preparation was washed twice with the same buffer in the absence or presence of 1 mM NaHCO3. The centrifugations were done at 30,000 x g for 25 min. The pellets were resuspended in the same buffer with and without NaHCO3, respectively. For exchange with isotopic 13C-bicarbonate, the samples resuspended in buffer at pH 6.0 were incubated in the presence of 0.1 M 13C- bicarbonate for 1 h at 4°C followed by two washing steps in the assay medium at pH 5.5 containing 20 mM 13C bicarbonate.
Oxygen evolution activity.- Oxygen evolution activity was measured in a closed 3-mL cell using a Clark-type electrode under continuous illumination with white light (2,500 \( \mu \text{E}.\text{m}^{-2}.\text{s}^{-1} \)) in the presence of 0.5 mM DCBQ dissolved in ethanol. Samples were resuspended in non-depleted and CO\(_2\)-depleted medium containing 0.4 M sucrose, 15 mM NaCl, 5 mM CaCl\(_2\) and 50 mM Mes-NaOH, pH 5.5 in the absence or presence of NaHCO\(_3\) (for more details see Figure Legends). Oxygen evolution upon the first 1-min illumination by the continuous actinic light was measured.

Chlorophyll fluorescence measurements.- Chl fluorescence yields were measured by using a modulated fluorescence technique (PAM fluorometer, model KS1101, H. Walz, Effeltrich, FRG). The intensity of the modulated measuring light was 12 \( \mu \text{E}.\text{m}^{-2}.\text{s}^{-1} \) and the red actinic light approximately 700 \( \mu \text{E}.\text{m}^{-2}.\text{s}^{-1} \). For measurements samples were resuspended in 0.4 M sucrose, 15 mM NaCl, 5 mM CaCl\(_2\) and 50 mM Mes-NaOH, pH 5.5 at a Chl concentration of 20 \( \mu \text{g}.\text{mL}^{-1} \).

FT-IR measurements.- FT-IR spectra were measured on a Nicolet Magna 550 spectrophotometer equipped with an DQTS detector. For measurements the PSII samples resuspended in 0.4 M sucrose, 15 mM NaCl, 5 mM CaCl\(_2\) and 50 mM Mes-NaOH, pH 5.5, with and without NaHCO\(_3\) (for details see Figure Legends), were supplemented with 2 mM/18 mM potassium ferri-ferrocyanide in order to allow every oxygen evolving complex to relax to the thermally stable S\(_1\) state [24,25]. The \( E_\text{m} \) value of the Mes buffer (pH 5.5) including 2 mM/18 mM potassium ferri-ferrocyanide was 364 mV at which the non-heme iron should stay reduced at pH 5.5 [26]. After that samples
ELECTRON BINDING TO THE WATER-OXIDIZING COMPLEX IN THE photosystem II. A FOURIER TRANSFORM INFRARED SPECTROSCOPY STUDY

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The samples were centrifuged for 3-4 min in a microfuge and the resulting pellet pressed between a pair of BaF$_2$ plates. The absorbance at 1656 cm$^{-1}$ which is due to amide I and some contribution by H$_2$O absorption was maintained between 0.5 and 1.0 for all the samples. Light-induced difference spectra were obtained by subtracting the two spectra taken before and after illumination. Each spectrum was an average of 200 scans and the spectral resolution was 4 cm$^{-1}$. Light illumination was performed at room temperature with red actinic light from a 150-W tungsten lamp powered with a stabilized power supply through several heat-cut filters (KG1 Schott infrared filters) plus a 620 nm cut-off filter. For the final data the characteristic FT-IR spectrum of water vapour was subtracted from all the difference spectra to cancel the water band contributions. For this subtraction spectra were normalized at about 2,200 cm$^{-1}$. The water vapour was measured from empty cavity of the spectrometer.
RESULTS

Figure 1A shows the light-induced FT-IR spectra in the 1600-1300 cm\(^{-1}\) region of PSII membranes at pH 5.5 in the presence of 2 mM/18 mM ferri-ferrocyanide. This spectrum solely exhibits photoinduced transitions in the donor side of photosystem II [21,24,27]. In this case ferricyanide acts as an exogenous electron acceptor, as shown in the inset (Fig. 1A). The negative band at 2117 cm\(^{-1}\) and the positive band at 2036 cm\(^{-1}\) (Figure 1A, inset) are assignable to the CN stretching modes of ferricyanide and ferrocyanide, respectively [21], indicating that ferricyanide accepts an electron to become ferrocyanide. The absence of a band at 1478 cm\(^{-1}\) associated to Q\(_e^\circ\) [21] confirms that this spectrum involves no contribution from acceptor-side signals. Thus, acceptor side quinone signals were eliminated and redox reactions of the non-heme iron were also suppressed by controlling the redox potential in the sample by the ferri-ferrocyanide couple (1:9) at pH 5.5 [27]. The magnitude of spectral changes in our experiments is about 10\(^{-3}\) - 10\(^{-4}\), i.e. much higher than those published by Noguchi et al. [21,27] since we use saturating continuous light instead of a single flash. In the 1600-1400 cm\(^{-1}\) spectral region negative bands at 1560, 1541, 1522, 1507, 1421 and 1402 cm\(^{-1}\) and positive bands at 1589, 1550, 1531, 1502, 1438 and 1365 cm\(^{-1}\) were observed in control PSII membranes (Fig. 1A, solid line). The shape of this spectrum was drastically modified when bicarbonate was removed from the medium (Fig. 1A, dotted line). It is clear that the main positive bands at 1589 and 1365 cm\(^{-1}\) are not present and the intensity of the negative bands at 1560, 1541 and 1522 cm\(^{-1}\) gets highly reduced. On the other hand, the addition of bicarbonate to the CO\(_2\)-depleted PSII samples partially restored the spectral features of the FT-IR spectrum (Fig. 1A, dashed line). The
observed differences in the spectra cannot be attributed to some local pH shifts since bicarbonate concentration was by two orders lower than that of buffer. Spectra in Fig. 1A were normalized in the ferrocyanide band (2036 cm⁻¹) as shown in the inset. In these conditions the restoration of the main bands was not complete. For example, a 37% restoration of the broad band at 1365 cm⁻¹ with bicarbonate addition was achieved. The fact that the characteristic bands of PSII donor side disappeared and reappeared after depletion and addition of bicarbonate, respectively, suggests that these bands could be assigned to COO⁻ stretching modes of the bicarbonate. The broad shape of some of these bands could be due to the strong band overlapping in this spectral region. Measurements in this spectral region are difficult due to water vapour contribution because its spectrum has a number of sharp bands in the 1500-1800 cm⁻¹ region, however such contribution was eliminated by substraction (see Materials and Methods). On the other hand, the main band at 1364 cm⁻¹ is far from the water bands.

In order to examine the bicarbonate effect on the photosynthetic activity of PSII oxygen evolution and chlorophyll modulated fluorescence (Fig. 1B) were measured in the same experimental conditions as in Fig. 1A. Oxygen evolution was measured during the first 1-min illumination by the continuous actinic light. PSII membranes under the control conditions exhibited an oxygen evolution rate of 520 μmol O₂·mg Chl⁻¹·h⁻¹ using DCBQ as artificial electron acceptor. The oxygen evolution activity decreased drastically (up to 81% decrease) when PSII membranes were washed and resuspended in CO₂ depleted buffer (Fig. 1B, line 2) compared with the control (Fig. 1B, line 1). However, the addition of bicarbonate stimulated again the water oxidation activity (Fig. 1B, line 3). The oxygen evolution activity increased up to 60% compared to the control, it means a 41%
Phytochrome binding to the water-oxidizing complex in the
II. A FOURIER TRANSFORM INFRARED SPECTROSCOPY
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restoration which is consistent with that of Fig. 1A, dashed line. These results indicate
that bicarbonate restores the capacity to evolve oxygen. Similar results were obtained
using chlorophyll modulated fluorescence measurements (Fig. 1B) Chlorophyll
fluorescence can be used as an intrinsic sensor of the photosynthetic activity [28]. The
absorption of a quantum of light by the PSII RC results in a fast charge separation
between the primary electron donor, P680, and the primary electron acceptor, Pheo.
This light-induced charge separation process initiates the transport of reducing
equivalents from the PSII RC to the plastoquinone pool via a specially organized
complex of two bound plastoquinones, QA and QB. Chlorophyll fluorescence depends
upon the redox state of QA being minimal (F0) when this quinone is fully oxidized.
However, when QA is fully reduced a maximum fluorescence yield (Fm) is obtained. Fig.
1B shows that the bicarbonate addition is also required for maximum reactivation of ΔF
(ΔF=Fm-F0) related to photoreduction of QA in the bicarbonate-depleted medium. Thus,
the data showed that the reactivation of ΔF upon addition of bicarbonate is
accompanied by restoration of oxygen evolution. This suggests that bicarbonate is
required to activate the WOC in agreement with our previous results [5-10].

In Fig. 2 the light-induced FT-IR spectra showed in Fig. 1 were normalized in the
unaffected 1760-1720 cm⁻¹ spectral region. The normalized spectra also showed that
the main bands affected by the depletion of bicarbonate were those at 1589 and 1365
cm⁻¹ (Fig. 2A, a and b). For a better visualization an expanded view of the 1640-1480
cm⁻¹ region of FT-IR spectra normalized in the 1760-1720 cm⁻¹ region in the presence
and absence of bicarbonate is shown in Fig. 2B, a. The bands at 1541 and 1507 cm⁻¹
were unaffected after bicarbonate depletion. However, it is clear in this superimposed
To determine the specific contributions of bicarbonate we studied the effect of labeling with isotopic $^{13}\text{C}$ bicarbonate on the light-induced FT-IR spectra. Figure 3 compares the donor side FT-IR difference spectra in untreated PSII preparations at pH 5.5 in the presence of $^{13}\text{C}$- (solid line) and $^{13}\text{C}$- (dashed line) bicarbonate. The spectra were quite similar above 1650 cm$^{-1}$, however it was influenced by isotope labeling below this frequency. In particular the main broad positive bands at around 1589 and 1365 cm$^{-1}$ disappeared and some changes at 1600-1400 cm$^{-1}$ were observed. These results are better visualized in the expanded view of the 1650-1400 cm$^{-1}$ region of FT-IR spectra normalized in the 1760-1720 cm$^{-1}$ region in the presence and absence of bicarbonate (Fig. 3B,a). The strong band overlapping described in the 1650-1400 cm$^{-1}$ region [24,25,29] and observed in Fig. 3A mask bicarbonate contributions. Figure 3B,b shows the result of subtracting the FT-IR spectrum of PSII membranes in the presence of $^{13}\text{C}$-bicarbonate (Fig. 3B,a, dashed line) from that in the presence of non-isotopic bicarbonate (Fig. 3B,a, solid line). This difference spectrum should exhibit only the...
modes sensitive to isotopic labeling. Two sharp bands, one negative at 1559 cm\(^{-1}\) and another positive at 1553 cm\(^{-1}\) were observed. The band at 1559 cm\(^{-1}\) corresponds to the negative band observed at 1560 cm\(^{-1}\) in the unlabeled donor side difference FT-IR spectrum (Fig. 3B,a, solid line). This band was clearly shifted down 5 cm\(^{-1}\) upon \(^{13}\)C-bicarbonate substitution, resulting in a new position at 1556 cm\(^{-1}\) (Fig. 3B,a, dashed line). The positive band at 1553 cm\(^{-1}\) confirmed this assignment. Thus the 1560 and 1556 cm\(^{-1}\) may be assigned to modes of \(^{12}\)C- and \(^{13}\)C-bicarbonate, respectively. The \(^{12}\)C-bicarbonate minus \(^{13}\)C-bicarbonate spectrum (Fig. 3B,b) also shows broad positive and negative bands between 1600-1540 cm\(^{-1}\) and 1532-1450 cm\(^{-1}\), respectively. In those regions the assignment of bicarbonate modes is complicated due to the overlapping with strong signals from proteins in each individual FT-IR spectra [24,25,29]. Since the \(^{13}\)C labeling results in downshifts of bicarbonate bands [29] the negative band at around 1532-1450 cm\(^{-1}\) may be interpreted as the downshift upon \(^{13}\)C-bicarbonate substitution of the positive broad band between 1600-1540 cm\(^{-1}\) (Fig. 3B,b). Furthermore, the positive band at 1589 cm\(^{-1}\) in the \(^{12}\)C-bicarbonate spectrum could be tentatively assigned to a mode of \(^{12}\)C-bicarbonate. The corresponding mode of \(^{13}\)C-bicarbonate could be the band at around 1496 cm\(^{-1}\). It is known that only frequency downshifts less than 60 cm\(^{-1}\) are expected for modes of bicarbonate upon \(^{13}\)C-labeling in the 1800-1000 cm\(^{-1}\) spectral region [29]. Our data indicate a 93 cm\(^{-1}\) shift, however, it should be considered that the indicated maxima may not correspond to the exact frequency position of the \(^{12}\)C-bicarbonate modes due to band overlapping.
DISCUSSION

Recently, we have shown that restoration of photoinduced electron flow and oxygen evolution with Mn$^{2+}$ in Mn-depleted photosystem II membrane preparations are considerably increased when bicarbonate is added to a bicarbonate-depleted medium. We concluded, therefore, that bicarbonate is an essential constituent of the WOC of PSII which is important for assembling and maintaining it in the functionally active state. For that, some frequencies in the donor side difference FT-IR spectra can be assigned to bicarbonate modes. The FT-IR difference spectra in the presence and absence of bicarbonate showed clear differences that could not be explained simply by degradation of a sample since removal of bicarbonate was reversible. The main frequencies at 1589 and 1365 cm$^{-1}$ in the light-induced difference FT-IR spectrum of untreated PSII membranes were modified after bicarbonate depletion indicating that bicarbonate modes make a contribution to these bands. Comparison between donor side FT-IR difference spectra in the presence and absence of bicarbonate showed that the positive broad bands at 1589 and 1365 cm$^{-1}$ can be tentatively assigned to asymmetric and symmetric COO$^{−}$ stretching modes of bicarbonate, respectively. Furthermore the $^{13}$C-labeled FT-IR measurements showed downshifts of the 1589 and 1560 cm$^{-1}$ bands. These data confirmed the above results and also indicated that the negative band at 1560 cm$^{-1}$ can be assigned to asymmetric stretching mode of bicarbonate. Previously Noguchi et al. [24] assigned similar bands at 1587, 1560, 1365 and 1403 cm$^{-1}$ to the asymmetric (higher frequency bands) and symmetric (lower frequency bands) COO$^{−}$ stretching modes of a certain carboxylate group in Asp, Glu or the C-termi from amino
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Acid residues present in the surrounding protein, which bridge between Mn²⁺ and Ca²⁺. These assignments were made based on the comparison between the two Sj/S, FT-IR difference spectra using untreated and Ca²⁺-depleted PSII membranes. It is known that Ca²⁺ is an indispensable cofactor for photosynthetic oxygen evolution [30]. The binding site of Ca²⁺ in PSII has not been identified yet although Ca²⁺ is thought to be located in the close vicinity of the Mn-cluster [24,30]. On the other hand, it has been suggested that bicarbonate takes part in the formation of the Mn-center capable of water oxidation as an obligatory ligand [5,6,8,10]. Considering that Ca²⁺ depletion can modify the bicarbonate binding site in the WOC, the above assignment of the bands at 1587, 1560 and 1364 cm⁻¹ can be reconsidered. Since these frequencies were modified after bicarbonate depletion and ¹³C-bicarbonate labeling, our results further indicate that those can be ascribed to stretching modes of the COO⁻ group from bicarbonate. The fact that no change is observed at 1403 cm⁻¹ can be due to the small intensity of this band. Thus, it could be possible that bicarbonate acts as a bridging ligand between the redox active Mn and Ca²⁺. These findings are also consistent with our previous proposal of bicarbonate as a ligand of Mn [5,6,8,10].
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Abbreviations: Chl, chlorophyll; D1, polypeptide of PSII reaction center; DCBQ, 2,6-
dichlorobenzoquinone; FT-IR, Fourier transform infrared spectroscopy; Mes, 2-(N-
morpholino)ethanesulfonic acid; OEC, oxygen evolving complex; Pheo, pheophytin, the
primary electron acceptor; P680, the primary electron donor; PSII, photosystem II; Qa
and Qb, primary and secondary plastoquinone electron acceptors of PSII; RC, reaction
center; WOC, water oxidizing complex.
FUTURE BINDING TO THE WATER-OXIDIZING COMPLEX IN THE
PHOTOSYSTEM II. A FOURIER TRANSFORM INFRARED SPECTROSCOPY
STUDY

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photosystem II, bicarbonate, donor side, Fourier transform, infrared

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HETEROGENEOUS BINDING TO THE WATER-OXIDIZING COMPLEX IN THE
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STUDY

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Photosystem II, bicarbonate, donor side, Fourier transform, infrared
FIGURE LEGENDS

Fig. 1.- (A) Light-induced FT-IR difference spectra of PSII membranes at pH 5.5. Control experiment (solid line); bicarbonate-depleted medium (dotted line); after addition of 1 mM NaHCO$_3$ to the bicarbonate-depleted medium (dashed line). Samples were illuminated with continuous light at room temperature in the presence of 2 mM/18 mM ferri-/ferrocyanide. Spectra were normalized at the CN stretching region of ferri- and ferrocyanide as shown in the inset. (B) Kinetics of oxygen evolution activity and photoinduced changes of chlorophyll fluorescence yield in PSII membranes in control medium (1) and in bicarbonate-depleted medium with no addition (2) and after addition of 1 mM NaHCO$_3$ (3).

Fig. 2.- (A) Light-induced FT-IR difference spectra of PSII membranes in control medium (a) and in bicarbonate-depleted medium with no addition (b) and after addition of 1 mM NaHCO$_3$ (c) at pH 5.5. Spectra were normalized at the unaffected 1750-1700 cm$^{-1}$ spectral region. (B) a) Expanded view (1640-1480 cm$^{-1}$) of the light-induced FT-IR difference spectra of PSII membranes in non-depleted bicarbonate medium (solid line) and after depletion of bicarbonate (dashed line); b) double difference spectrum by subtracting the FT-IR difference spectrum in bicarbonate-depleted buffer (a, dashed line) from that in non-depleted bicarbonate buffer (a, solid line). The experimental set up was as described in Fig. 1A.
Fig. 3. (A) Light-induced FT-IR difference spectra in the 1650-1350 cm$^{-1}$ region of PSII membranes in control medium (solid line) and in the presence of 1 mM ($^{13}$C)NaHCO$_3$ (dashed line) in the medium at pH 5.5. Spectra were normalized at the 1750-1700 cm$^{-1}$ region. (B) a) Expanded view (1640-1480 cm$^{-1}$ region) of the light-induced FT-IR difference spectra of PSII membranes in control medium (solid line) and in the presence of 1 mM ($^{13}$C)NaHCO$_3$ (dashed line) in the medium at pH 5.5; b) double difference spectrum by substracting the FT-IR difference spectrum in the presence of ($^{13}$C)NaHCO$_3$ buffer (a, dashed line) from that in the presence of unlabeled bicarbonate buffer (a, solid line). Experimental conditions were as described in Fig. 1A.
II. A FOURIER TRANSFORM INFRARED SPECTROSCOPY STUDY

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The following authors would like to dedicate this paper to the memory of José V. Ibarra.

Keywords: photosystem II, bicarbonate, donor side, Fourier transform, infrared
A study of the effects of rhombohedral-spinel phase transitions on the magnetic properties of Fe(III)-based compounds in the presence of Ni(II) and Mn(II) is not fully described in the text. The diagram labeled 'Fig. 2' shows spectral data with wavenumbers in the range of 1800 to 1300 cm\(^{-1}\). The text mentions the role of rhombohedral-spinel phase transitions in influencing the magnetic behavior of Fe(III)-based compounds, particularly in the context of Ni(II) and Mn(II) interactions. Further details are required to fully understand the study's findings.
LIGAND BINDING TO THE WATER-OXIDIZING COMPLEX IN THE
PHOTO SYSTEM II. A FOURIER TRANSFORM INFRARED SPECTROSCOPY
STUDY

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