The intrinsic stability of the second intermediate following the dioxygen-bound form in the O_2 reduction by cytochrome *c* oxidase

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Abstract Aeration of a two-electron reduced cytochrome *c* oxidase provides a species with two Raman bands at 804 and 356 cm^{-1}, identifying it as the second intermediate following the O_2-bound species in the enzymatic O_2 reduction process. It degrades directly to the fully oxidized form with a half-life time of 70 min at pH 8.0. The stability suggests an effective insulation for the active site in an extremely high oxidation state (Fe^{4+} with one oxidative equivalent nearby) against spontaneous electron leaks, which would dissipate proton motive force. The formation and degradation of the second intermediate are pH-dependent.

Keywords: Cytochrome *c* oxidase; Oxygen activation; Proton pump; P intermediate; Resonance Raman

1. Introduction

Cytochrome *c* oxidase (CcO) is the terminal enzyme of the mitochondrial electron transfer chain and catalyzes the reduction of molecular oxygen [1]. Time-resolved resonance Raman (TR^3) spectroscopy is the most powerful technique for identification of the iron-coordination structures of hemoproteins. In fact, all structural characteristics of the heme a_3–Cu_B binuclear site (the O_2-reduction site) of the O_2 adducts during the enzymatic O_2 reduction, known thus far, have been revealed essentially by RR spectroscopy [2–4]. The reaction system between the fully reduced enzyme (FRCcO) and O_2 has been studied most extensively with various spectroscopic methods including RR spectroscopy, since FRCcO, which contains four electron equivalents sufficient for complete reduction of O_2, is most likely to provide all the intermediates in the catalytic O_2 reduction process.

Five $^{16}O/^{18}O$ isotope-sensitive RR bands have been detected at 804, 785, 571, 450 and 356 cm^{-1} during the course of the reaction between FRCcO and O_2. The initial and last bands at 571 and 450 cm^{-1} are sufficiently well resolved from the other three bands to be assigned as those due to the ν_{Fe-O2} and the ν_{Fe-OH} modes, respectively. The other three bands coexist between the decay of the band at 571 cm^{-1} and the appearance of the band at 450 cm^{-1}, although the bands at 804 and 356 cm^{-1} appear significantly earlier than the 785 cm^{-1} band [5]. The formation and decay of these intermediate species are greatly dependent on the rate of electron supply from heme *a* and Cu_A sites. Experimentally determined order of appearance and disappearance of the intermediate species in the consecutive multi-intermediate reaction system do not always show the order of the intermediates in the reaction sequence. Thus, for the O_2-reduction system of this enzyme, including multi-intermediates, an artificial stop of the O_2 reduction process by limiting the number of electron equivalents (less than four equivalents) for the O_2 reduction would provide critical information for determination of the intermediate species in the reaction sequence. It should be noted that the overall oxidation states of the intermediate species, giving the Raman bands at 804, 785 and 356 cm^{-1}, have not been established conclusively. In other words, these band species in the absence of additional electron equivalent in the enzyme molecule could be transformed spontaneously to either of the other intermediate species, if no oxidation state difference exists between them. Thus, careful examination of the intrinsic stability of these intermediates would solve the issue. Furthermore, a pH effect on the formation and degradation on these bands could provide important insights for mechanism of O_2 reduction and proton pumping.

The finding of the 804 cm^{-1} band by aerating the mixed-valence CcO–CO (MVCcO–CO, Cu_A^{2+} Fe_a^{3+} Cu_B^{1+} Fe_{a3}^{2+}–CO) in which only two electron equivalents are included seems to be evidence for the 804 cm^{-1} band as the second intermediate [6]. However, MVCcO–CO is prepared from treatment of the fully oxidized enzyme (FOCcO) with CO. The mechanism of two-electron reduction of the enzyme by CO is unknown. Thus, the 804 cm^{-1} band species obtained from the MVCcO–CO could have a ligation structure of the O_2-reduction site other than that under turnover conditions. In fact, the normal coordinate analysis of this band is consistent with coordination structure of FeOOH [7] as well as Fe=O. Treatment of the fully oxidized enzyme (FO–CcO) with H_2O_2 also provides Raman bands at 804 and 785 cm^{-1}, depending on the concentration of H_2O_2 [8–10]. A spontaneous transition between these species has been proposed [11]. The 804 cm^{-1} species prepared by the H_2O_2 treatment could have a chemical structure of the O_2-reduction site different from the other two 804 cm^{-1} species.

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Abbreviations: CcO, cytochrome *c* oxidase; MV, mixed-valence; RR, resonance Raman; TR^3, time-resolved resonance Raman; FR, fully reduced; FO, fully oxidized

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Thus, it is not clear whether the transition occurs also between the intermediate species, when they appear during the enzymatic O_2 reduction process. Here we report the structure and stability of the 804 cm^{-1} species prepared from MVCcO treated with O_2 at various pH values, examined extensively by resonance Raman techniques.

2. Materials and methods

Bovine heart CcO and $^{16}O^{18}O$ were prepared as described previously [12,7]. A Raman spectrophotometer with a spectral slit width of 10 cm^{-1}, as described elsewhere [7], is sensitive enough for detection of a 0.1 cm^{-1} frequency shift of Raman band through a Raman difference spectrum. The MVCcO–CO was prepared by incubation of FOCcO for 12 h under 1 atm of CO at 22 °C. Before addition of O_2, the container was evacuated to 1×10^{-2} Torr followed by introduction of pure N_2 (99.5%). This procedure was repeated three times to remove excess CO. The resultant enzyme solution gave an absorption spectrum of MVCcO–CO and no significant absorption change was detectable at least for 10 min under anaerobic conditions. TR^3 spectra at a delay time of 1 min after mixing MVCcO–CO with O_2 were obtained as follows. Using a motor-driven syringe pump, MVCcO–CO was passed through a thin silicone tube (diameter 2 mm, wall thickness 0.2 mm) with a glass jacket into which O_2 gas at 1 atm had been introduced. The flow rate through the apparatus was 0.5 ml/min giving 20 s duration for passing through the silicone tube, followed by 40 s duration to arrive at the detection laser beam focused at the flow cell. The complete gas-exchange during the flow in the silicone tube was confirmed by the O_2 concentration of 1.2 mM at the exit point of the silicone tube determined with a Clark-type Oxygen electrode. The laser power at the flow cell was 1.0 mW. The reaction of the MVCcO with O_2 for shorter delay times ($\Delta t = 0.1$–2 ms) was examined by using a mixed-flow apparatus [13] and a double-beam technique [14]. The MVCcO solution (100 μM), saturated with 1 atm of CO, was mixed with O_2-saturated buffer (1.2 mM O_2). (It has been well established that CO-rebinding is negligible within the time scale of the measurement.) The 590.0 nm laser (200 mW) was used to photolyze CO, initiating the reaction, and the 423.0 nm laser (8 mW) was used for Raman excitation. All O_2-adduct species were identified as $^{16}O_2/^{18}O_2$ isotope-sensitive bands as described before [3]. All the spectroscopic measurements were performed at 22 °C.

3. Results

3.1. The stability of the O_2 adduct of MVCcO

Manual aeration of MVCcO–CO at pH 8.0 for 5 s provided a fairly stable absorption peak at 607 nm in the difference spectrum against FOCcO, as depicted in Fig. 1((A) 1 min and (B) 8 h). The peak intensity decreased exponentially to a constant level with a 70 min half-time (inset of Fig. 1). Both the half-time and the asymptotic level significantly decreased with pH. At pH 6.0, the half-time was 8 min, and no significant band at 607 nm was detectable at the asymptotic level (data not shown). The $^{16}O_2/^{18}O_2$ isotope-sensitive Raman bands were detectable at 804/768 and 356/342 cm^{-1}, upon excitation at 423.0 nm as depicted in Fig. 1(C) and (D). The band at 356 cm^{-1} was observed upon excitation at 427.1, 426.8 and 423.0 nm but not at 441.6 nm, while that at 804 cm^{-1} was observed upon all the above excitation wavelengths (data not shown). Consistently, the 356 cm^{-1} band was detectable upon 427.1 nm excitation [4] and undetectable upon 441.6 and 413.1 nm excitations [4,6]. The intensities of these Raman bands were roughly proportional to the intensity of the band at 607 nm during the absorption decrease examined at pH 8.0, 7.4 and 6.0. The results indicate that the molecular species giving the absorption band at 607 nm also gives the Raman bands at 804 and 356 cm^{-1}.

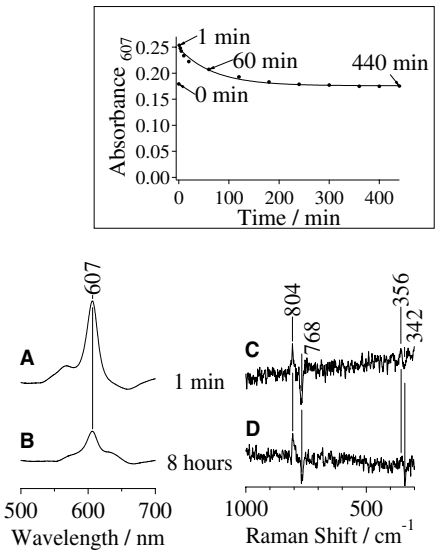

Fig. 1. Absorption difference spectra (P minus FO enzyme) at 1 min (A) and 8 h (B), and Raman difference spectra ($^{16}O_2$ minus $^{18}O_2$) at 1 min (C) and 8 h (D) after mixing O_2 with MVCcO in the absence of CO at pH 8.0.

3.2. Characterization of the present 804 cm^{-1} band species

The oxygen-isotopic shift effects were examined for the 804 cm^{-1} species at pH 8.0, 8 h after aeration of MVCcO. As depicted in Fig. 2, the $^{16}O^{18}O$ isotope-substituted species shows bands at 804 and 768 cm^{-1} in the Raman difference spectra ((A) $^{16}O_2$–$^{16}O^{18}O$, (B) $^{16}O^{18}O$–$^{18}O_2$). The positions of these bands are identical to those of the species prepared from $^{16}O_2$ and $^{18}O_2$ (Fig. 2C), but the intensities are halved. The flat difference pattern calculated by subtracting the average spectrum of $^{16}O_2$ and $^{18}O_2$-substituted species from that of $^{16}O^{18}O$-substituted species (Fig. 2D) indicates that a single oxygen atom (oxide) is at heme a_3 (Fe=O^{2-}). If the band at 804 cm^{-1} is due to the O^-–O^- stretching mode, a band with an intermediate frequency around 786 cm^{-1} should appear in the

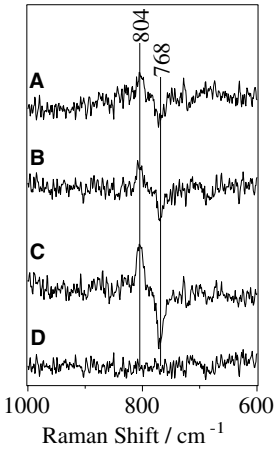

Fig. 2. Effects of $^{16}O^{18}O$ on the Fe=O stretching Raman band (see text).

$^{16}O_2$–$^{16}O^{18}O$ and $^{16}O^{18}O$–$^{18}O_2$ spectra. The results are fully consistent with the results of the same analysis on the bands at 804 cm^{-1} obtained in the reactions of FRCcO with O_2 and of FOCcO with H_2O_2 [7,8,15].

The 804/768 cm^{-1} bands showed 2.3 cm^{-1} upshift upon replacement of H_2O with D_2O (data not shown). This suggests that the bound oxygen atom at heme a_3 iron is hydrogen-bonded to the Cu_B–OH group, in an arrangement analogous to compound II of horseradish peroxidase, in which the Fe–O stretching vibration for the heme iron, with O^{2-} being hydrogen-bonded, shows a slight upshift of about 2 cm^{-1} upon deuteration [16]. This shift has been observed for the 804 cm^{-1} species prepared from FOCcO with H_2O_2 [10].

Fig. 3 depicts the RR spectral change in the initial 2 and 1 ms after the initiation of the reaction of MVCcO with O_2 at pH 8.0 (left panel) and at pH 6.8 (right panel), respectively. The TR[3] spectral changes at pH 6.8 (Fig. 3, right panel) are closely similar to those at pH 8.0, except for the faster decay rate for the 571 cm^{-1} band. Typically the band at 571 cm^{-1} loses its intensity at 400 μs at pH 6.8 (Fig. 3D′), but at 1000 μs at pH 8.0 (Fig. 3E). Fig. 4 plots the relative intensity of the three oxygen-associated modes against time. The decay rate constant of the band at 571 cm^{-1} (k_1), the rise rate constants of the bands at 804 cm^{-1} (k_2) and 356 cm^{-1} (k_3), obtained by fitting a single exponential curve for each intensity change, are $k_1 = 1.1(0.1) \times 10^4$, $k_2 = 9.7(2.8) \times 10^3$, $k_3 = 1.1(0.4) \times 10^4$ s^{-1} at pH 6.8, and $k_1 = 2.2(0.3) \times 10^3$, $k_2 = 5.6(1.0) \times 10^3$ and $k_3 = 4.1(1.7) \times 10^3$ s^{-1} at pH 8.0. The numbers in parenthesis denote standard errors. The rise of the 571 cm^{-1} band in the figure was estimated from the second-order rate constant for the formation of Oxy intermediate, 3.5×10^8 $M^{-1}s^{-1}$ [4], and the oxygen concentration, 0.6 mM. The standard error values of k_1 indicate, with 95% reliability, that the pH dependent decrease in k_1 value is significant. However, the standard errors of k_2 and k_3 at pH 6.8 and 8.0 indicate no statistically significant difference between the four experimental values of

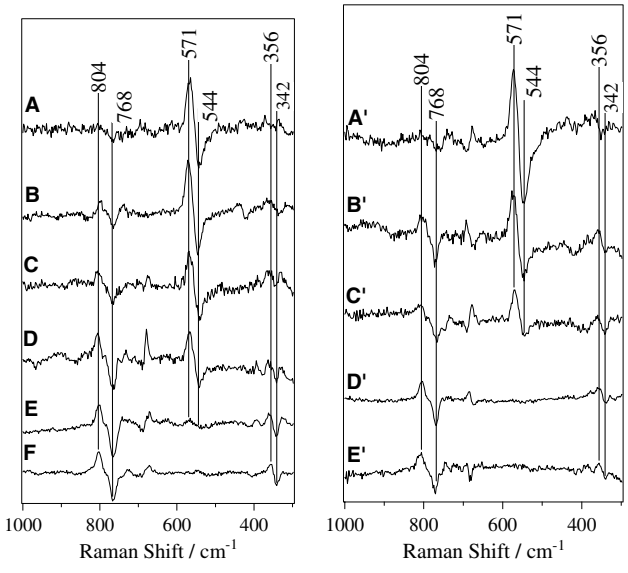

Fig. 3. TR[3] difference spectra ($^{16}O_2$–$^{18}O_2$) at pH 8.0 (left panel) and at pH 6.8 (right panel), excited at 423.0 nm. Delay times are 100 (A and A′), 150 (B and B′), 200 (C and C′), 400 (D and D′), 1000 (E and E′) and 2000 μs (F).

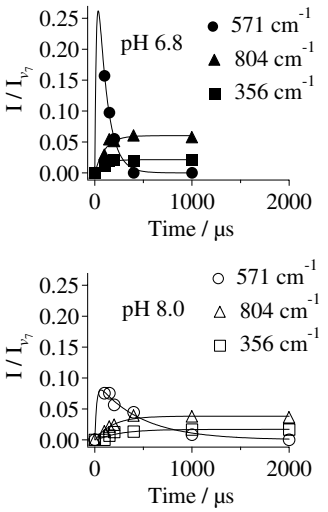

Fig. 4. Time-dependent intensity change of the Raman bands at 571, 804 and 356 cm^{-1}. Upper and lower panels are for pH 6.8 and 8.0, respectively. Intensity of oxygen isotope-sensitive Raman bands is normalized to that of the porphyrin ν_7 mode at 683 cm^{-1} under the assumption that the intensity of the ν_7 mode is little affected by pH and delay time.

the rate constants. No statistical difference is detectable between the three rate constants at pH 6.8. That is the case at pH 8.0. However, the standard errors for these rate constants indicate that k_1 value at pH 6.8 is statistically larger than those of k_2 and k_3 as well as k_1 at pH 8.0. Therefore, transition from the 571 cm^{-1} species to the 804/356 cm^{-1} species at pH 6.8 is significantly faster than that at pH 8.0. At 0.4 ms, both the 804 and 356 cm^{-1} bands attain the maximal levels essentially identical with those observed at 1 min after O_2 treatment of MVCcO–CO, as given in Fig. 1. The complete measurements for the transition from the 571 cm^{-1} species to the 804 cm^{-1} species for MVCcO as given in Fig. 3 have not been reported thus far, although this transition has been examined by two other groups. Han et al. reported only the decay of 571 cm^{-1} band. They followed the rise of the next intermediate formation with absorption spectroscopy [17]. Although they reported two small decay constants (5×10^{-3} and 5×10^{-4} s^{-1}), their experimental conditions in the presence of CO are not suitable for examination of the stability of the intermediate species. Proshlyakov et al. [18] did not observe the full growth of the band at 804 cm^{-1} because of the limitation of their method. Thus they did not show that the bands at 804 and 356 cm^{-1} arise from a single intermediate. The signal/noise ratio of the spectra given in Fig. 3 is much higher than those previously reported (cf., Fig. 6 in [17] and Fig. 3 in [18]).

4. Discussion

(1) The two-electron reduced enzyme prepared from the MV enzyme–CO complex.

The absorption spectrum of MVCcO–CO complex indicates that both heme *a* and Cu_A are in the oxidized state. The initial intermediate (the 571 cm^{-1} species) and the final product (the 804/356 cm^{-1} species) formed in the reaction between O_2 and the two-electron reduced enzyme prepared from MVCcO–CO

complex provide RR bands (Fig. 3) strictly identical with those that appear in the reaction between O_2 and FRCcO, in terms of both the band positions and the $^{18}O_2/^{18}O^{16}O/^{16}O_2$ isotopic shift effects (Fig. 2) [3]. These results indicate that CO produces the two-electron reduced enzyme strictly identical with one present during the catalytic turnover.

(2) The reactivity of the two-electron reduced enzyme with O_2.

The two-electron reduced enzyme reacts with O_2 to form the Raman band at 571 cm^{-1} followed by the two Raman bands at 804 and 356 cm^{-1}, keeping an essentially constant intensity ratio of the latter two bands. The two bands decay very slowly and synchronously to the fully oxidized form without any intermediate. The $^{18}O_2/^{18}O^{16}O/^{16}O_2$ isotopic shift effects show no O–O bond for the 804 cm^{-1} band species. These results conclusively determine the reaction sequence as the 571 cm^{-1} band species (Fe–O_2) – the 804/356 cm^{-1} band species (P) (Fe=O) – the 785 cm^{-1} band species (F) (Fe=O) – the 450 cm^{-1} band species (Fe–OH) in the enzymatic O_2 reduction process. It should be noted that the origin of the 356 cm^{-1} band has long been under debate [2–4]. The proposed assignment of the band at 356 cm^{-1} to the N(His)–Fe=O bending mode [3,5] is consistent with a theoretical calculation [19]. Furthermore, these results prove that the 804/356 cm^{-1} band species is in an oxidation state higher (most likely by one oxidative equivalent) than that of the 785 cm^{-1} band species. The appearance of the 804 cm^{-1} band is somewhat earlier than that of the 785 cm^{-1} band during the course of the reaction between FRCcO with O_2 [5]. However, the finding is not sufficient to conclude the position of the 804 cm^{-1} species in the enzymatic O_2 reduction process, as described in Introduction. The link between the 804 and 356 cm^{-1} bands have not been established. In fact, the results given by Proshlyakov et al. [18] are not sufficiently accurate for concluding the link. Furthermore, Han et al. [4] stated "the intensity of the line at 355 cm^{-1} correlates roughly with the intensity of the ferryl line we detect at 786 cm^{-1}".

(3) The 804 cm^{-1} band species in the reaction between O_2 and the fully FR enzyme.

Kitagawa and co-workers [3,5] observed clearly the 804 cm^{-1} band during the course of the complete reduction of O_2 with FRCcO. However, Babcock's group and Rousseau's group [2,4] have not observed (or reported) the 804 cm^{-1} band for H_2O solution. As shown in the spectra of Kitagawa's group [5], the 804 cm^{-1} band overlaps significantly with the 785 cm^{-1} band. Thus, the discrepancy between the results of Kitagawa's group and those of the other groups may be derived from the difference in the signal/noise levels of the spectra. In fact, some of the reported spectra of Rousseau's group (cf. Figs. 3 and 5 in [4] and Fig. 2d in [20]) indicate the presence of the 804 cm^{-1} band. Another possible reason for the discrepancy is the difference in the excitation wavelength for the RR measurement that is, 423.0 nm for Kitagawa's group vs. 413.1 and 416.0 nm for the other groups [15,20].

(4) The two P forms, P_m and P_r.

The enzyme species with Raman/absorption bands at 804 cm^{-1}/607 nm and at 785 cm^{-1}/580 nm are denoted as P and F forms, respectively [8–10,21]. P form, prepared from aeration of MVCcO and FR CcO are defined as P_m and P_r forms, respectively. In the reaction system of FRCcO with O_2, heme *a* is oxidized with the rate constant essentially identical with that of the degradation of the O_2-bound form [22]. Furthermore, the degradation rate of the 571 cm^{-1} band in the FRCcO–O_2 system is several fold faster than that in the MVCcO–O_2 system [4,17]. Thus, it has been proposed that P_r, which is the second intermediate produced directly from the O_2-bound form, is a three electron reduced O_2-bound form. That is, heme *a* reduces the bound O_2 at the O_2-reduction site before the electron transfer from Cu_B to the bound O_2 [23]. Despite the difference in the overall oxidation state, P_m and P_r forms are thought to have essentially identical Raman/absorption spectra. However, no structural evidence for discriminating P_r from P_m has been obtained. The kinetic evidence is not conclusive for the oxidation state of P_r, which is one equivalent lower than P_m. The electron transfer from Cu_B to the bound O_2 is likely to be controlled by the oxidation state of heme *a*, so that reduction of the bound O_2 in FRCcO is faster than that in MVCcO. In both cases, the second intermediate following the O_2-bound form is P_m (the two electron reduced O_2-bound form). In the FRCcO–O_2 system, oxidation of heme *a* produces F form, not P_r, which has the cuprous Cu_B, which is consistent with a detailed kinetic analysis of the absorption spectral changes in the system [24].

(5) The stability of the 804/356 cm^{-1} species.

Recently, RR and absorption spectral analysis of P_m form has been studied under CO and O_2 atmosphere [6]. The experimental conditions, including CO and O_2 that regenerate the P_m form, were not designed for examination of the stability of P_m. In the present conditions for the analysis of the stability of the species, CO was removed carefully as described in Section 2. The stability of P_m has not been examined so extensively as in the present work, though a brief description on decay of P_m has been reported [11]. The intrinsic stability of P_m largely contributes to the complete stability of P_m in the presence of CO [6,11], though the regeneration of P_m by CO may not be negligible as has been suggested [11].

The high overall oxidation state of P_m (formally $Fe^{4+}=O^{2-}$ with one extra oxidation equivalent) suggests its extremely high oxidation state, which is produced by the free energy released upon the bond cleavage of the bound O_2. In fact, an E_m of 1.2 V for P has been proposed [21]. The highly reactive iron atom is likely to extract electrons spontaneously from protein moiety, which would not only dissipate the free energy but also provide serious damage in the enzyme molecule. The amazingly high stability of the P species indicates the presence of an effective protection (insulation) system against the spontaneous electron transfer to the O_2-reduction site. The insulation is likely to contribute to the specific and effective electron transfer to heme a_3 in the P state, which is indispensable for the effective energy transduction by this enzyme.

The location of the extra oxidative equivalent has been a matter of chemical concern [16]. One possibility is on the iron, establishing an Fe^V electronic state. Since heme A has the formyl group, which has a strong electron withdrawing ability, it has a distinct electronic structure compared with other kinds of iron porphyrin [3]. RR measurement of high-valent state of a formyl-substituted heme is underway. Other possibilities, including porphyrin π-cation radical, Tyr^{244} radical and Cu_B^{3+} have been proposed [3,16,25,26].

(6) Effects of pH.

The final level and rate of disappearance of the P-species are dependent on pH. The results suggest that some pH-dependent spontaneous electron transfers exist on a time scale much longer than that of the physiological reaction. The pH sensi-

tivity detectable in the TR^3 spectral change in the initial 2 ms is physiologically relevant, suggesting proton extraction during the Oxy to P transition. Time-resolved absorption studies suggested that there was no pH dependence of the decay of the Oxy or the rise of the P intermediate in the pH range 6–9, although the reaction became 1.4 times slower in D_2O than in H_2O [27,28]. A prominent pH dependence of the decay rate of Oxy has only been detected in the present TR^3 spectroscopy, which separately follows the decay of Oxy and the rise of P_m intermediates, using different Raman bands as markers. The P_m formation involves the O–O bond break giving at least one OH^-. Thus, proton extraction at the O_2-reduction site must be coupled with the Oxy to P_m transition, consistently with the present pH dependence. However, the protons could be available from some groups near the O_2-reduction site but not equilibrated with the bulk water phase during the transition.

The maximal population of Oxy at pH 6.8 is 2–3-fold larger than that at pH 8.0 (Fig. 4). One of the possible interpretations for the finding is as follows; the Raman scattering cross section of the ν_{Fe-O_2} mode is dependent on protonation (or hydrogen bonding) state of the bound O_2, which is influenced by the external pH. Establishment of this factor would provide a new insight for the mechanism of the O_2 activation by the enzyme.

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References

[1] Babcock, G.T. and Wikstrom, M. (1992) Nature 356, 301–309.
[2] Ferguson-Miller, S. and Babcock, G.T. (1996) Chem. Rev. 96, 2889–2907.
[3] Kitagawa, T. and Ogura, T. (1997) in: Progress in Inorganic Chemistry (Karlin, K.D., Ed.), vol. 45, pp. 431–479, Wiley, New York.
[4] Han, S., Takahashi, S. and Rousseau, D.L. (2000) J. Biol. Chem. 275, 1910–1919.
[5] Ogura, T., Hirota, S., Proshlyakov, D.A., Shinzawa-Itoh, K., Yoshikawa, S. and Kitagawa, T. (1996) J. Am. Chem. Soc. 118, 5443–5449.
[6] Kim, Y., Shinzawa-Itoh, K., Yoshikawa, S. and Kitagawa, T. (2001) J. Am. Chem. Soc. 123, 757–758.
[7] Ogura, T., Takahashi, S., Hirota, S., Shinzawa-Itoh, K., Yoshikawa, S., Appelman, E.H. and Kitagawa, T. (1993) J. Am. Chem. Soc. 115, 8527–8536.
[8] Proshlyakov, D.A., Ogura, T., Shinzawa-Itoh, K., Yoshikawa, S., Appelman, E.H. and Kitagawa, T. (1994) J. Biol. Chem. 269, 29385–29388.
[9] Proshlyakov, D.A., Ogura, T., Shinzawa-Itoh, K., Yoshikawa, S. and Kitagawa, T. (1996) Biochemistry 35, 76–82.
[10] Proshlyakov, D.A., Ogura, T., Shinzawa-Itoh, K., Yoshikawa, S. and Kitagawa, T. (1996) Biochemistry 35, 8580–8586.
[11] Juneman, S., Heathcote, P. and Rich, P.R. (2000) Biochim. Biophys. Acta 1456, 56–66.
[12] Yoshikawa, S., Choc, M.G., O'Toole, M.C. and Caughey, W.S. (1997) J. Biol. Chem. 252, 5498–5508.
[13] Ogura, T., Yoshikawa, S. and Kitagawa, T. (1985) Biochim. Biophys. Acta 832, 220–223.
[14] Ogura, T., Maeda, A., Nakagawa, M., and Kitagawa, T. (1987) in: Kobayashi, T. (Ed.), Primary Processes in Photobiology, vol. 20, Proceedings in Physics, Springer, Berlin, pp. 233–241.
[15] Varotsis, C., Zhang, Y., Appelman, E.H. and Babcock, G.T. (1993) Proc. Natl. Acad. Sci. USA 90, 237–241.
[16] Kitagawa, T. and Mizutani, Y. (1994) Coord. Chem. Rev. 135/136, 685–735.
[17] Han, S., Ching, Y.-C. and Rousseau, D.L. (1990) J. Am. Chem. Soc. 112, 9445–9451.
[18] Proshlyakov, D.A., Pressler, M.A. and Babcock, G.T. (1998) Proc. Natl. Acad. Sci. USA 95, 8020–8025.
[19] Ghosh, A. and Skancke, A. (1998) J. Phys. Chem. B 102, 10087–10090.
[20] Han, S., Ching, Y.-C. and Rousseau, D.L. (1990) Nature 348, 89–90.
[21] Wikstrom, M. and Morgan, J.E. (1992) J. Biol. Chem. 267, 10266–10273.
[22] Han, S., Ching, Y.-C. and Rousseau, D.L. (1990) Proc. Natl. Acad. Sci. USA 87, 8408–8412.
[23] Morgan, J.E., Verkhovsky, M.I., Palmer, G. and Wikstrom, M. (2001) Biochemistry 40, 6882–6892.
[24] Suchera, A., Szundi, I. and Einassdottir, O. (1998) Biochemistry 37, 17905–17914.
[25] Proshlyakov, D.A., Pressler, M.A., DeMaso, C., Leykam, J.F., DeWitt, D.L. and Babcock, G.T. (2000) Science 290, 1588–1591.
[26] Fabian, M. and Palmer, G. (1999) Biochemistry 38, 6270–6275.
[27] Oliveberg, M., Brzezinski, P. and Malmstrom, B.G. (1989) Biochim. Biophys. Acta 977, 322–328.
[28] Karpeforts, M., Adelroth, P., Namslauer, A., Zhen, Y. and Brzezinski, P. (2000) Biochemistry 39, 14664–14669.