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Review

Resonance Raman applications in investigations of cytochrome *c* oxidase ☆

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ABSTRACT

Recent applications of resonance Raman (RR) spectroscopy in investigations of cytochrome *c* oxidase (CcO) are reviewed. Red-excited RR spectra for the fully oxidized "as-isolated" CcO tuned to the ligand-to-metal charge transfer absorption band at 655 nm exhibit a Raman band at 755 cm^{-1} assignable to the ν_{OO} stretching mode of a peroxide. Binding of CN^- diminishes the RR band concomitant with the loss of the charge transfer absorption band. This suggests that a peroxide forms a bridge between heme a_3 and Cu_B. Time-resolved RR spectroscopy of whole mitochondria identified a band at 571 cm^{-1} arising from the oxygenated intermediate at $\Delta t = 0.4$, 0.6 and 1.4 ms. Bands at 804 and 780 cm^{-1} of the P and F intermediates were observed at $\Delta t = 0.6$ and 1.4 ms, respectively. The coordination geometries of the three intermediates are essentially the same as the respective species observed for solubilized CcO. However, the lifetime of the oxygenated intermediate in mitochondria was significantly longer than the lifetime of this intermediate determined for solubilized CcO. This phenomenon is due either to the pH effect of mitochondrial matrix, the effect of ΔpH and/or $\Delta\Psi$ across the membrane, or the effect of interactions with other membrane components and/or phospholipids. This article is part of a Special Issue entitled: Respiratory Oxidases.

1. Introduction

Three-dimensional structures provide the basis for clarifying the mechanisms of reactions catalyzed by respiratory enzymes. In such structures, the coordinates of individual atoms can be determined at resolutions as high as 0.1 Å by X-ray crystallography. Vibrational spectroscopy, on the other hand, determines the energy of vibrational levels and it is indirectly correlated with the lengths of bonds between atoms. A correlation between a bond length in a typical molecule and the vibrational frequency of the bond can be determined from data obtained by vibrational spectroscopy and X-ray crystallography. A distance change of 0.01 Å (1 pm) or smaller may be detected by vibrational spectroscopy based on the established correlations (see below). Such a subtle change in bond length reflects a change in the electronic state and thus, the reactivity of a functional group. The ability to provide information with respect to the reactivity of a functional group is unique to vibrational spectroscopy and is essential in order to elucidate the role of the functional group in the reaction mechanism. For example, a linear relationship between the O—O stretching frequency (ν_{OO}/cm^{-1}) and the corresponding O—O bond length (r_{OO}/Å) ($\nu_{OO} = 5098.4 - 2963.3 \times r_{OO}$) is observed for a series of different species of O_2 [1,2] (O_2^+: 1858 cm^{-1} and 1.12 Å, O_2: 1555 cm^{-1} and 1.21 Å, O_2^-: 1108 cm^{-1} and 1.28 Å, O_2^{2-}: 760 cm^{-1} and 1.49 Å) when bound to metal complexes. The O—O and M—O (metal–oxygen) bond orders are inversely correlated. This is rationalized by variable charge transfer from the metal to O_2 through a covalent interaction between the metal d_{xy} orbital and the in-plane π^* orbital of O_2. The degree of charge transfer serves as the basis of the properties and indicates the reactivity of the metal–oxygen adduct. In addition, since vibrational spectroscopy has a time resolution in the picosecond range, it is powerful for determining the structure of transient species in chemical reactions. The advantage of such time resolution is that if there are multiple conformers in a given molecule, the molecule gives multiple signals originating from the respective conformers. In this case, the structures of individual conformers could be determined. However, the three dimensional structure of proteins cannot be determined by vibrational spectroscopy. For many enzymes, both vibrational spectroscopic data and X-ray crystallographic data are now available and this combination of vibrational spectroscopy and X-ray crystallography is becoming more and more important for elucidating reaction mechanisms as a result of the advantages of these two techniques.

Respiratory enzymes such as cytochrome *c* oxidase (CcO) pump protons across the membrane [3]. The proton pump is driven by the free energy change accompanied by dioxygen reduction. Respiratory control is a phenomenon in which the electron transfer rate is affected by the proton motive force $\Delta\mu_H^+$, consisting of the proton concentration gradient (ΔpH) and the membrane potential ($\Delta\Psi$). These parameters have been regarded as providing physiologically important control in order to attain the highest efficiency of energy conversion as a whole. Three quarters of the weight of the inner mitochondrial membrane is occupied by membrane proteins and thus the

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respiratory protein complexes are interacting with other proteins and phospholipids. The reactions of the membrane proteins might be affected by these interactions. For solubilized membrane proteins, the interactions with other proteins and the respiratory control are lost. Thus, it is desirable to determine the structure and pursue the reaction of the membrane proteins *in situ* to clarify the reaction mechanisms under physiological conditions. However, the development of such a technique has not yet been attained. The improved RR method has been applied to whole mitochondria and the oxygen activation reaction by CcO has been successfully detected.

Recent applications of RR spectroscopy to investigations of CcO are described in this review. The first application is with respect to the fully oxidized "as-isolated" state. A resonance Raman band assignable to a peroxide molecule was detected, suggesting a Fe—O^-—O^-—Cu_B structure with an O—O distance of 1.5 Å. The second application is with respect to the oxygen activation reaction in whole mitochondria. The results show that essentially the same reaction intermediates are involved and that the lifetime of the oxygenated intermediate is significantly longer than that of solubilized CcO. This indicates the presence of a controlling mechanism in mitochondria which could be related to respiratory control.

2. Cytochrome *c* oxidase

Cytochrome *c* oxidase (CcO) (ferrocytochrome *c*: oxygen oxidoreductase, EC 1. 9. 3. 1) is the terminal component of the mitochondrial respiratory chain and catalyzes the four electron reduction of dioxygen. The reduction of dioxygen is coupled with the process of proton pumping across the mitochondrial inner membrane with a stoichiometry of H^+/e^-. Since a single chemical proton is consumed in the mitochondrial matrix per electron, a proton concentration difference of 3 is generated by transfer of 1 electron. A functional unit of bovine CcO consists of 13 different subunits and its molecular weight is 210 kD [4]. CcO has four redox-active metal centers: the Cu_A site (a binuclear Cu site), heme *a*, heme a_3 and the Cu_B. Cu_A accepts electrons from cytochrome *c* and donates them to heme *a*. Heme *a* delivers electrons to the O_2 reduction site and is proposed to act as the driver of the proton pump. The O_2 reduction site is a binuclear metal center consisting of heme a_3 and Cu_B. The mechanism of dioxygen reduction and the coupling mechanism between electron transfer and proton pumping have been extensively studied. The oxygenated species, the P intermediate, the F intermediate and the O intermediate have been respectively determined to have Fe—O_2, Fe O, Fe O and Fe—OH units at the heme a_3 site based primarily on the iron–oxygen stretching ($\nu_{Fe—O}$) frequencies detected by RR spectroscopy [5-8].

The fully oxidized form of CcO generated under turnover conditions pumps protons upon receiving electrons, while the resting oxidized form does not [9]. Thus, the functions of the two oxidized forms are distinct from each other and the key to understanding the different functions should be found in their different structures. The coordination geometry of the heme a_3 site in the fully oxidized form under turnover conditions has been determined by RR spectroscopy to be Fe^{3+}—OH^- [10,11]. However, the coordination geometry of the resting oxidized form (the fully oxidized "as isolated" form) was not well established. The presence of a bridging ligand between heme a_3 and Cu_B was postulated based on data obtained by EPR spectroscopy [12]. Four electron equivalents should be sufficient for full reduction of the protein, since there are four redox-active metal centers in a functional unit of CcO. However, the "as-isolated" form was shown to require six electron equivalents for full reduction, while the oxidized form under turnover conditions requires four electron equivalents [13]. The results suggested that the bridging ligand is peroxide (O_2^{2-}). The X-ray structure of the resting oxidized CcO showed an area of electron density comparable to that of two oxygen atoms [14]. Although the possibility of attributing the electron density to Cl^- was suggested as an alternative interpretation [15], it was recently confirmed by an X-ray anomalous dispersion analysis that the electron density is due to the presence of O_2^{2-} [16]. The O—O distance of the Fe—O^-—O^-—Cu_B bridged structure was determined to be 1.70 Å. However, the O—O bond distance is too long to represent an ordinary peroxide with a typical O—O distance of 1.5 Å. To our knowledge, no peroxide species with an O—O bond distance of 1.7 Å has been reported. Thus, identifying the factors responsible for the discrepancy should help to clarify the chemical nature of the bridging ligand between heme a_3 and Cu_B.

Fig. 1 depicts RR spectra of the fully oxidized "as-isolated" (*A*), fully oxidized "as-isolated" and cyanide-bound CcO (*B*) and their difference spectrum ($C = A - B$) [17]. It is evident in Spectrum 1C that there is a band at 755 cm^{-1}. This means that the band at 755 cm^{-1} for the fully oxidized "as-isolated" form loses its intensity upon binding of CN^-. The inset shows that the absorption band at 655 nm for fully oxidized "as-isolated" CcO (*A′*) loses its intensity upon cyanide binding (*B′*). The RR scattering was excited at 647.1 nm in resonance with the ligand-to-metal charge transfer transition at 655 nm [18,19]. The prolonged laser illumination with higher power (140 mW) diminished the Raman band at 755 cm^{-1} due to photoreduction [20]. The enhancement of only one Raman band at 755 cm^{-1} in Spectrum 1C indicates that the band arises from a diatomic molecule, since we expect only one stretching vibration for a diatomic molecule. These results led to the conclusion that the band at 755 cm^{-1} originates from the ν_{OO} mode of the bridging peroxide. If the peroxide resides at a position different from the binuclear site, then enhancement of the RR band is not expected. There is no RR band assignable to the Fe—O or Cu—O stretching mode in Spectrum 1C, while both the O—O and Fe—O stretching modes are detectable for a side-on peroxy and an end-on hydroperoxy Fe-porphyrin model complexes [21]. Since the RR spectral characteristics of the Fe—O—O—Cu type bridging peroxide has not yet been well established, the reason of the indetectability of neither the Fe—O nor Cu—O stretching mode is not clear at present. As mentioned previously, there is a linear relationship between the O—O stretching frequency and the O—O bond length [1,2]. Based on the relationship ($\nu_{OO} = 5098.4 - 2963.3 \times r_{OO}$), the r_{OO} of the fully oxidized "as-isolated" CcO was calculated to be 1.47 Å, which is close to the bond distance of an ordinary peroxide, but the value (1.7 Å) obtained by X-ray crystallography was significantly larger. The discrepancy between the X-ray and Raman results might be due to inaccuracies in the X-ray structural analysis even at 1.8 Å resolution [14]. However, the $^{18}O_2$-isotopic substitution effects on the RR spectra have not been confirmed because the method to prepare the fully oxidized "as-isolated" form from the fully reduced form under an $^{18}O_2$ atmosphere has not been

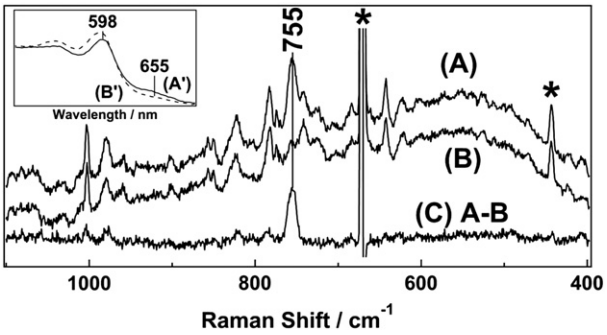

Fig. 1. Resonance Raman spectra of fully oxidized "as-isolated" cytochrome *c* oxidase (A) and its cyanide adduct (B) excited at 647.1 nm. Spectrum C is the Raman difference spectrum (A–B). The laser power was 11 mW. The accumulation period for spectra A and B was 90 min. Spectral features marked with an asterisk are due to emissions of the Kr^+ laser used. The inset shows the visible absorption spectra (*A′*) and (*B′*) for the species corresponding to Raman spectra (*A*) and (*B*), respectively. (Reproduced with permission from Sakaguchi et al., Ref. [17]).

established. An $^{18}O_2$ isotopic analysis of Raman spectra might give insights into the chemical nature of the bridging ligand.

3. *In situ* RR spectroscopic analyses of oxygen activation by CcO in whole mitochondria

Adar and Erecinska reported RR spectra of cytochromes in whole mitochondria in the frozen state [22,23]. The quality of the spectra was found to be comparable to that of spectra of solubilized CcO, thus demonstrating the feasibility of using RR spectroscopy for detailed examinations of the structure of the hemes and their environments in cytochromes *in situ*. Application of the technique was extended to the CO-bound form of CcO in mitochondrial suspensions [24]. Fig. 2 depicts RR spectra of whole mitochondria. Since the $\nu_{Fe—CO}$ (the Fe—CO stretching) and $\delta_{Fe—C—O}$ (the Fe—C—O bending) modes are known to serve as sensitive probes of the heme distal environments, CO-bound mitochondria were examined. Upon addition of CO to the fully reduced mitochondria (Spectrum 2A), a new band at 516 cm^{-1} arises (Spectrum 2B). This band undergoes a downshift to 503 cm^{-1} upon replacement of $^{12}C^{16}O$ with $^{13}C^{18}O$ as shown in Spectrum 2C. The results indicate that the band at 516 cm^{-1} is due to the $\nu_{Fe—CO}$ mode. The isotopic frequency shift is evident in the difference spectrum (Spectrum 2D). The isotopic differences are also evident at 576/556 cm^{-1} and 369/349 cm^{-1} for the $^{12}C^{16}O/^{13}C^{18}O$ in Spectrum 2D. The frequencies of the three sets of isotopic difference patterns (576/556 cm^{-1}, 516/503 cm^{-1} and 369/349 cm^{-1}) for $^{12}C^{16}O/^{13}C^{18}O$ are essentially the same as the frequencies determined for solubilized CcO, within experimental error. The bands at 576/556 cm^{-1} are due to the $\delta_{Fe—C—O}$ mode and those at 369/349 cm^{-1} are due to the porphyrin deformation mode that exhibits the $^{13}C^{18}O$ isotope sensitivity [25]. The results show that the heme pocket environment of CcO in mitochondria and solubilized CcO is quite alike. This means that the procedure for preparing solubilized CcO does not alter the heme pocket structure. It is remarkable that only a single vibrational band of an external ligand in mitochondria could be observed and used as a structural marker of the catalytic site of CcO. It is noted that the corresponding frequencies for porcine heart mitochondria, bovine heart mitochondria and solubilized bovine CcO are identical [24]. Based on these results for static CO-bound states, the dioxygen reduction reaction catalyzed by CcO was studied next in intact whole mitochondria.

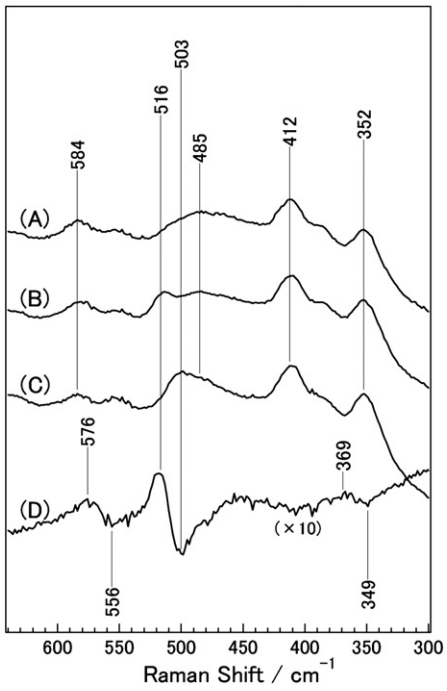

Fig. 2. Resonance Raman spectra of whole mitochondria from porcine heart excited at 427.0 nm. Fully reduced (A), fully reduced and $^{12}C^{16}O$-bound (B), fully reduced and $^{13}C^{18}O$-bound states (C) and the isotopic difference spectrum (Spectrum B–Spectrum C) (D).

Time-resolved RR (TR^3) spectra of dioxygen-bound CcO in mitochondria were recorded by employing the improved artificial cardiovascular system for pursuit of enzymatic reactions [26]. The delay times (Δt) (after initiation of the reaction) were set to 0.4, 0.6 and 1.4 ms using a flow-flash method originally developed by Gibson and Greenwood [27]. The protocol has also been used for TR^3 measurements of solubilized CcO [5–7]. In the TR^3 difference spectra at $\Delta t = 0.4$ ms, the Raman bands at 571/544 cm^{-1} for the $^{16}O_2/^{18}O_2$ isotopomers were detectable. These signals are due to the Fe—O_2 stretching mode of the oxygenated intermediate with end-on geometry [28], which was previously designated compound A by Chance and coworkers [29]. In the TR^3 spectra at $\Delta t = 0.6$ ms, the Raman bands at 804/764 cm^{-1} and 780/750 cm^{-1} were detected for $^{16}O_2/^{18}O_2$. The Raman bands at 804 and 780 cm^{-1} for $^{16}O_2$ are due to the $\nu_{Fe\,O}$ mode of the P and the F intermediates, respectively, and their frequencies were essentially identical to the values of the respective species for the solubilized CcO. The intensities of the three bands with oxygen-isotope-sensitivity at 571, 804 and 780 cm^{-1} at $\Delta t = 1.4$ ms were observed to decrease relative to the intensities observed with shorter delay times. It was noted that the band at 571 cm^{-1} of the oxygenated intermediate was still detectable at 1.4 ms at 11.6 °C. It was not observable at Δt values greater than 1 ms for solubilized CcO at 3 °C. This indicates that the lifetime of the oxygenated intermediate is significantly longer in mitochondria. This is not likely to be due to alterations of the binuclear site in solubilized CcO since RR spectra of the CO-bound form indicate that the heme a_3 site has retained its integrity. If a pH control site exists on the enzyme surface facing the negative side, the slower transition from the oxygenated form to the P intermediate may occur, since the pH value of the mitochondrial matrix is approximately 8. Another possible reason for the long lifetime of the oxygenated intermediate is that it is stabilized by ΔpH and/or $\Delta\Psi$ produced by the transition from P to F intermediates in the other CcO molecules in the same mitochondrial membrane. Note that the F intermediate has already been generated at $\Delta t = 0.6$ and 1.4 ms. One more possibility is that other components of the membrane affect the stability of the oxygenated intermediate. Further study will be required to clarify this possibility. The results demonstrate that the TR^3 technique enables us to probe the oxygen activation reaction by CcO *in situ* in the mitochondrial inner membrane, where many other membrane proteins exist, through analysis of molecular vibrations. This technique is applicable to other physiological reactions in mitochondria.

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References

[1] K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, Applications in Coordination, Organometallic, and Bioinorganic Chemistry, sixth ed. John Wiley & Sons, Inc., Hoboken, 2009.
[2] C.J. Cramer, W.B. Tolman, K.H. Theopold, A.L. Rheingold, Variable character of O—O and M—O bonding in side-on (η^2) 1:1 metal complexes of O_2, Proc. Natl Acad. Sci. USA 100 (2003) 3635–3640.
[3] G.T. Babcock, M. Wikstrom, Oxygen activation and the conservation of energy in cell respiration, Nature 356 (1992) 301–309.
[4] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, S. Yoshikawa, The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å, Science 272 (1996) 1136–1144.

[5] S. Ferguson-Miller, G.T. Babcock, Heme/copper terminal oxidases, Chem. Rev. 96 (1996) 2889–2908.
[6] T. Kitagawa, T. Ogura, Oxygen activation mechanism at the binuclear site of heme-copper oxidase superfamily as revealed by time-resolved resonance Raman spectroscopy, Prog. Inorg. Chem. 45 (1997) 431–479.
[7] S. Han, S. Takahashi, D.L. Rousseau, Time-dependence of the catalytic intermediates in cytochrome *c* oxidase, J. Biol. Chem. 275 (2000) 1910–1919.
[8] K. Oda, T. Ogura, E.H. Appelman, S. Yoshikawa, The intrinsic stability of the second intermediate following the dioxygen-bound form in the O_2 reduction by cytochrome *c* oxidase, FEBS Lett. 570 (2004) 161–165.
[9] D. Bloch, I. Belevich, A. Jasaitis, C. Ribacka, A. Puustinen, M.I. Verkhovsky, M. Wikstrom, The catalytic cycle of cytochrome *c* oxidase is not the sum of its two halves, Proc. Natl Acad. Sci. USA 101 (2004) 529–533.
[10] S. Han, Y.-C. Ching, D.L. Rousseau, Ferryl and hydroxy intermediates in the reaction of oxygen with reduced cytochrome *c* oxidase, Nature 348 (1990) 89–90.
[11] T. Ogura, S. Hirota, D.A. Proshlyakov, K. Shinzawa-Itoh, S. Yoshikawa, T. Kitagawa, Time-resolved resonance Raman evidence for tight coupling between electron transfer and proton pumping of cytochrome *c* oxidase upon the change from the Fe^{V} oxidation level to the Fe^{IV} oxidation level, J. Am. Chem. Soc. 118 (1996) 5443–5449.
[12] B.F. Van Gelder, H. Beinert, Studies of the heme components of cytochrome *c* oxidase by EPR spectroscopy, Biochim. Biophys. Acta 189 (1969) 1–24.
[13] M. Mochizuki, H. Aoyama, K. Shinzawa-Itoh, T. Usui, T. Tsukihara, S. Yoshikawa, Quantitative reevaluation of the redox active sites of crystalline bovine heart cytochrome *c* oxidase, J. Biol. Chem. 274 (1999) 33403–33411.
[14] H. Aoyoma, K. Muramoto, K. Shinzawa-Itoh, K. Hirata, E. Yamashita, T. Tsukihara, T. Ogura, S. Yoshikawa, A peroxide bridge between Fe and Cu ions in the O_2 reduction site of fully oxidized cytochrome *c* oxidase could suppress the proton pump, Proc. Natl Acad. Sci. USA 106 (2009) 2165–2169.
[15] M. Fabian, L. Skultety, C. Brunel, G. Palmer, The cyanide stimulated dissociation of chloride from the catalytic center of oxidized oxidase, Biochemistry 40 (2001) 6061–6069.
[16] M. Suga, N. Yano, K. Muramoto, K. Shinzawa-Itoh, T. Maeda, E. Yamashita, T. Tsukihara, S. Yoshikawa, Distinguishing between Cl^- and O_2^{2-} as the bridging element between Fe^{3+} and Cu^{2+} in resting-oxidized cytochrome *c* oxidase, Acta Crystallogr. D67 (2011) 742–744.
[17] M. Sakaguchi, K. Shinzawa-Itoh, S. Yoshikawa, T. Ogura, A resonance Raman band assignable to the O—O stretching mode in the resting oxidized state of bovine heart cytochrome *c* oxidase, J. Bioenerg. Biomembr. 42 (2010) 241–243.
[18] H. Beinert, R.E. Hansen, C.R. Hartzell, Kinetic studies on cytochrome *c* oxidase by combined EPR and reflectance spectroscopy after rapid freezing, Biochim. Biophys. Acta 423 (1976) 339–355.
[19] R. Mitchell, P. Mitchell, P.R. Rich, The assignment of the 655 nm spectral band of cytochrome oxidase, FEBS Lett. 280 (1991) 321–324;
Erratum in, FEBS Lett. 282 (1991) 449.
[20] T. Ogura, S. Yoshikawa, T. Kitagawa, Resonance Raman study on photoreduction of cytochrome *c* oxidase—distinction of cytochrome *a* and cytochrome a_3 in the intermediate oxidation states, Biochemistry 24 (1985) 7746–7752.
[21] J.-G. Liu, T. Ohta, S. Yamaguchi, T. Ogura, S. Sakamoto, Y. Maeda, Y. Naruta, Spectroscopic characterization of a hydroperoxo-heme intermediate: conversion of a side-on peroxo to an end-on hydroperoxo complex, Angew. Chem. Int. Ed. 48 (2009) 9262–9267.
[22] F. Adar, M. Erecinska, Resonance Raman spectra of whole mitochondria, Biochemistry 17 (1978) 5485–5488.
[23] F. Adar, M. Erecinska, Photoreductive titration of the resonance Raman spectra of cytochrome oxidase in whole mitochondria, Biochemistry 18 (1979) 1825–1829.
[24] T. Takahashi, T. Ogura, Resonance Raman spectra of cytochrome *c* oxidase in whole mitochondria, Bull. Chem. Soc. Jpn. 75 (2002) 1001–1004.
[25] C. Rajani, J.R. Kincaid, Resonance Raman studies of hemoglobin with selectively deuterated hemes. A new perspective on the controversial assignment of the Fe–CO bending mode, J. Am. Chem. Soc. 120 (1998) 7278–7285.
[26] T. Takahashi, S. Kuroiwa, T. Ogura, S. Yoshikawa, Probing the oxygen activation reaction in intact whole mitochondria through analysis of molecular vibrations, J. Am. Chem. Soc. 127 (2005) 9970–9971.
[27] Q.H. Gibson, C. Greenwood, Reactions of cytochrome oxidase with oxygen and carbon monoxide, Biochem. J. 86 (1963) 541–555.
[28] T. Ogura, S. Takahashi, S. Hirota, K. Shinzawa-Itoh, S. Yoshikawa, E.H. Appelman, T. Kitagawa, Time-resolved resonance Raman elucidation of the pathway for dioxygen reduction by cytochrome *c* oxidase, J. Am. Chem. Soc. 115 (1993) 8527–8536.
[29] B. Chance, C. Saronio, J.S. Leigh Jr., Functional intermediates in the reaction of membrane-bound cytochrome oxidase with oxygen, J. Biol. Chem. 250 (1975) 9226–9237.