# Spectroscopic Determination of Organic Material Part 4 One Reaction of Oxygen with Cytochrome C Oxidase using Resonance Rman Scattering

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# Spectroscopic Determination of Organic Material
# Part 4 One Reaction of Oxygen with Cytochrome C Oxidase using Resonance Rman Scattering

by

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## Introduction

This experiment is one of the series[1-4] of "Spectroscopic Determination of Organic Material". Especially, as the molecular structure of organic material is very complicate, such a delicate structural analysis requires some new specific unusual techniques, cell, apparatus, instrument(ex. resonance Raman scattering), and others. This experiment is one of typical example.

The one process which oxygen reacted with cytochrome c oxidase showed the photodissociation regarding carbon monoxide. This one process was determined in comparison with this oxygen enzyme reaction and the iron-dioxygen reaction(stretching vibration) which appeared at 567 $cm^{-1}$ (Fe-O stretching mode) finding in oxyhemoglobin or oxymyoglobin. The changes in the intensity of Fe-$O_2$ stretching mode were detected in resonance Raman spectrum.

This experiment was carried on at Institute for Molecular Science partially and Colorado State University partally.

## Experimental and Results

The enzyme of cytochrome c oxidase was isolated with the outstanding method[6] and was stored at liquid nitrogen temperature before the usage for this experiment. The phosphate buffer (100 mM) at pH 7.4 with 1% dodecyl-$\beta$-D-maltoside (200 $\mu$M) solubilized the enzyme which was deoxygenated in an anaerobic chamber. The anaerobic enzyme (200 $\mu$M) was reduced with ascorbic acid (50mM). The cytochrome c (cyt·c,3 $\mu$M) exposed to carbon monoxide for form (cyt·c O-CO).

The sample of (cyt·c O-CO) was placed in one syringe of flow rapid mixing apparatus, and dioxygen saturated (1.4 mM) phosphate buffer (100 mM, pH 7.4) was placed in the other syringe. The solutions were mixed in the above-described apparatus and flowed at the rate varied from 10 $\mu$s to 500 $\mu$s into the special Raman cell where the incident laser both photodissociated the carbon monoxide from the enzyme and probed the resonance Raman spectrum.

The scattered light was detected with an array detection system, 1.25 meter spectrograph (Spex Industries) and linear photodiode array detector (Princeton Applied Research).

To make high sensibity of determination, the reference samples, cyt·c and carboxy myoglobin, were run through this apparatus.

In the case of this determination, an accuracy was $\pm$ 2$cm^{-1}$ on the array detector. The incident laser intensity (100 mV at 413.1 nm) was adjusted to obtain significant photolysis of the CO-bound cytochrome c oxidase. This laser power level assures that the oxygen-bound complex may not be photodissociated by the laser, therefore a mixture of unphotolysed material (cyt·c O-CO), photodissociated but unreacted material (cyt·c O), the former intermediate of the reaction with oxygen (cyt·c O-$O_2$)$_f$ and the latter intermediate (cyt·c $O_2$)$_\ell$ was considered as each important compound for analysis of that reaction process.

(cyt·c O-CO) $\longrightarrow$ cyt·c O + $O_2$ $\rightleftharpoons$ (cyt·c O-$O_2$)$_f$ $\longrightarrow$ (cyt·c O-$O_2$)$_\ell$

These resonance Raman scattering spectra of these

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reaction products of dioxygen with the enzyme in the range of iron-dioxygen mode of heme proteins[7-10] were reported as the related experiment. On the other hand, this new experimental results are shown in Fig. 1.

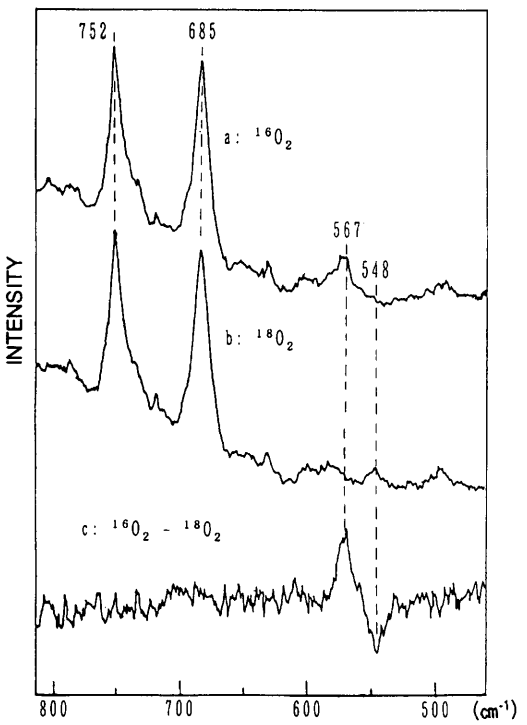


Fig.1 RAMAN SHIFT

The cyt·c O-CO was mixed with either $^{16}O_2$ or $^{18}O_2$ buffer solutions. The specific bands of $^{16}O_2$ and $^{18}O_2$ appeared at 567cm$^{-1}$ and 548cm$^{-1}$ respectively. This isotope shift appeared in Fig.1-c. This spectrum showed the presence of iron-dioxygen stretching mode 567 cm$^{-1}$.

The results of this experiment demonstrates that the former intermediate in the reaction of oxygen with the enzyme may be formed at room temperature.

In Fig.1, the resonance Raman spectra and difference spectra of the former intermediate shows the reaction of cytochrome c oxidase with the isotopes oxygen observed in a time window of $0 \sim 50\,\mu s$.

a: reaction of fully reduced cytochrome c oxidase with $^{16}O_2$.

b: reaction of fully reduced cytochrome c oxidase with $^{18}O_2$.

c: difference spectrum showing the isotope shift of the Fe-$O_2$ streching vibration mode of former intermediate in the reaction of oxygen with the fully reduced enzyme.

## Discussion

Cytochrome c oxidase is the terminal enzyme in the electron traensport chain. And it catalyses the four electron reaction of oxygen to water in a complex series of steps.[5] For analysis of the complex mechanism of this electron transport process, the molecular structure and property of the former and the latter intermediates at least. The former intermediate in the reaction of oxygen with the fully reduced enzyme (cyt·c O) may be detected at room temperature.

The Fe-$O_2$ stretching mode of oxyhemoglobin or oxymyoglobin was compared to that of the former intermediate with an iron-dioxygen bond analogous to that in the oxygen carrier and transport proteins. The Fe-$O_2$ stretching mode which was detected in the reaction of oxygen with the enzyme showed the same frequency. This isotope shift agreed with that predicted ($\sim$20 cm$^{-1}$) for an iron-dioxygen stretching mode based on a harmonic oscillator approximation. It should be pointed out that the Fe-O stretching from the Fe-O-O-Fe in base-free model complexes has been reported at 574 cm$^{-1}$.

An oxygen isotope sensitive line at 588 cm$^{-1}$ in an early intermediate of the reaction of cytochrome c oxidase with oxygen.[7] However, the sensitive line could not be detected in this experiment. It was considered that the assignment of the Fe-$O_2$ stretching vibration mode at 567 cm$^{-1}$ in the former intermediate of the reaction of oxygen with cytochrome c oxidase was very reasonable and clear description. This resonance Raman scattering and the difference spectrum were very useful method to analyse the result and to draw the conclusion.

The Fe-$O_2$ stretching mode has a characteristic frequency which is uncomplicated by other spectral lines. In addition, the spectrum of the intermediate reacted with $^{16}O_2$ minus that reacted with $^{18}O_2$ corrects for the instrumental artifacts and changes in the spectrum due to the formation of other intermediates leading to very flat difference spectra with major

peaks and valleys at the frequencies of the Fe-$O_2$ stretching modes of the former intermediate. Therefore, it is thought that an accurate determination of the former intermediate may be obtained by following the development and the decay of that mode.

## Conclusion

In the reaction which oxygen reacted with cytochrome c oxidase, the former intermediate was shown photodissociation of carbon monoxide. And the presence of this former intermediate was considered in comparison of the reaction of oxygen with the enzyme and the iron-dioxygen stretching vibration which was detected at 569 $cm^{-1}$. The frequency shown in oxyhemoglobin or oxymyoglobin was same to the iron-dioxygen stretching vibration which was determined with the resonance Raman spectroscopy at room temperature in this experiment.

## Summary

The one process that oxygen reacted with cytochrome c oxidase showed the photodissociation in regard to carbon monoxide. This one process was determined in comparison with this oxygen enzyme reaction and the iron-dioxygen reaction (stretching vibration) that appeared at 569 $cm^{-1}$ (Fe-$O_2$ stretching mode) finding in oxyhemoglobin or oxymyoglobin. The changes in the intensity of Fe-$O_2$ stretching mode were detected in resonance Raman scattering spectrum.

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Footnote - As a pagination for one report was limited by budget, these sections of introduction, experimental and results, discussion, conclusion, and summary should be shortened. And the rigorous restriction was one person one contribution one year.

# 有機物の分光学的測定 （英文）
## 第4報 共鳴ラマン散乱を用いたチトクローム C オキシダーゼと酸素の1つの反応

堀 津 圭 佑

（平成7年9月28日受理）

酸素がチトクローム C オキシダーゼと反応した1つの過程は一酸化炭素に関する光解離を示した．この過程は酸素酵素反応とオキシヘモグロビンやオキシミオグロビンに見つけられる569$cm^{-1}$（鉄・酸素伸縮モード）に現われる鉄・二酸化炭素反応（伸縮振動）との比較において測定された．鉄・酸素伸縮モードは共鳴ラマン散乱スペクトルで検出された．