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TITLE

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Picosecond Infrared Study of Carbonmonoxy Cytochrome *c* Oxidase: Ligand Transfer Dynamics and Binding Orientations.

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1. Introduction

Cytochrome *c* oxidase (CcO), an enzyme which catalyzes the reduction of dioxygen to water in the terminal step of the respiratory chain, combines several fundamental chemical processes in performing its function; electron, proton and ligand transfers.[1] The coordination chemistry and ligation dynamics of the cytochrome a_3-Cu_B site, where O_2 and other small molecules such as CO, NO and isocyanates can bind, are essential to the function of the enzyme.[2] The sensitivity of the vibrational frequencies and bandwidths of small molecules to changes in coordination and environment makes infrared spectroscopy uniquely useful as a probe for these processes, particularly at Cu_B^+, which generally is not observable by other spectroscopies.[1,2] Recent time-resolved infrared (TRIR) and visible absorption measurements have shown that coordination to Cu_B^+ is an obligatory mechanistic step for CO entering the cytochrome a_3 heme site and departing the protein after photodissociation.[2] The timescale (> 10^{-7} s) of the TRIR measurements, however, precluded observation of the ligation dynamics immediately following photodissociation. Here we report a picosecond timescale TRIR study of these events. The results reveal that the photoinitiated ligand transfer of CO from Fe_{a3}^{2+} to Cu_B^+, which are believed to lie 4-5 Å apart [1], occurs within 1 ps.

2. Experimental

The experiments reported here were performed on the fully reduced enzyme (Fe_a^{2+}, Fe_{a3}^{2+}, Cu_A^+, Cu_B^+). Beef heart CcO was exchanged into D_2O, reduced with dithionate (pH 7.4) and exposed to ca. 1 atm. CO in an O_2 free environment. The samples were ca. 1 mM in protein, in a 200 μm IR cell. TRIR measurements were obtained in visible pump-IR probe experiments using optical delay. The pump-probe cross-correlation is generally well described by a $sech^2$ function of FWHM = 3.5 ps. The visible pump pulse (ca. 595 nm) is in resonance with the alpha band of the hemes and induces rapid dissociation of CO from Fe_{a3}^{2+}. The IR pulse is tuned to the CO vibrational absorption when bound to Fe_{a3}^{2+} (1963 cm^{-1}) or Cu_B^+ (2062 cm^{-1}). [3] Induced linear dichroism measurements were obtained by rotating the pump polarization relative to the probe polarization. Laser power dependence measurements ensured there was no distortion of the data due to high pump pulse powers. Experimental details are given elsewhere.[4]

3. Results and Discussion

The bleach in the absorption at 1963 cm^{-1} is instantaneous, as expected since photodissociation occurs within 150 fs.[6] The formation of the CO complex of Cu_B^+ was directly observed by monitoring the appearance of the absorption at 2062 cm^{-1} (Figure 1). Surprisingly, this risetime is also instrument response limited. The experimental instrument response and a convolution of this with a 1 ps exponential rise are also shown in Fig. 1. The failure of this convolution to fit the data clearly indicates that the rise of the IR transient is less than 1 ps. In scans out to 250 ps, no further changes in absorption were seen at either probe wavelength. Thus there is no significant recombination of CO with Fe_{a3}^{2+} or further development of the Cu_B^+-CO complex within this time period. This agrees with previous TRIR measurements which determined a 1.5 μs lifetime for the Cu_B^+-CO complex and rebinding to cytochrome *a*3 on the ms timescale.[2]

The low temperature, static extinction coefficient of the CO-Cu_B^+ absorption is 7 times less than for CO coordinated to Fe_{a3}^{2+}.[3] The ratio of the transient ΔA's at 1963 and 2062 cm^{-1} is equal to the static ratio when the two measurements are performed under the same optical conditions (beam overlap, percent photodissociation, etc.) and suggests that complete formation of the Cu_B^+-CO

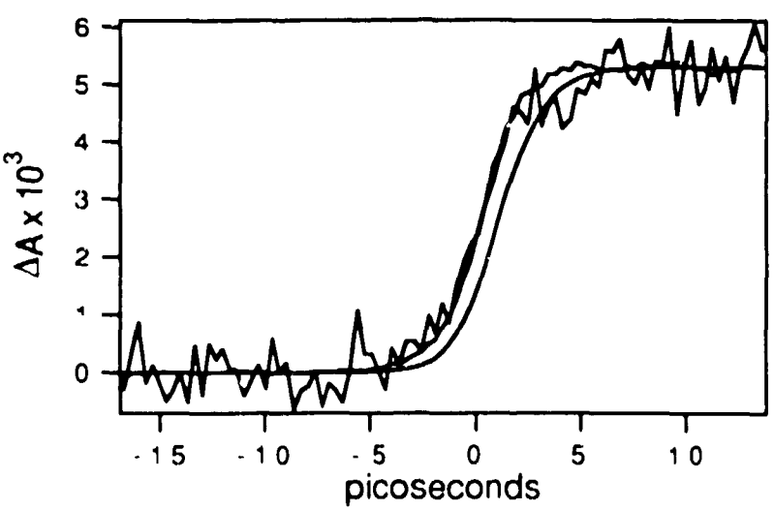

Figure 1. Time-resolved IR absorption for photodissociated CO-CcO monitored at the peak of the Cu_B^+-CO absorption at 2062 cm^{-1}. The smooth traces are; leftmost, the experimental instrument response (3.2 ps $sech^2$) and rightmost, the convolution of this with a 1 ps exponential rise.

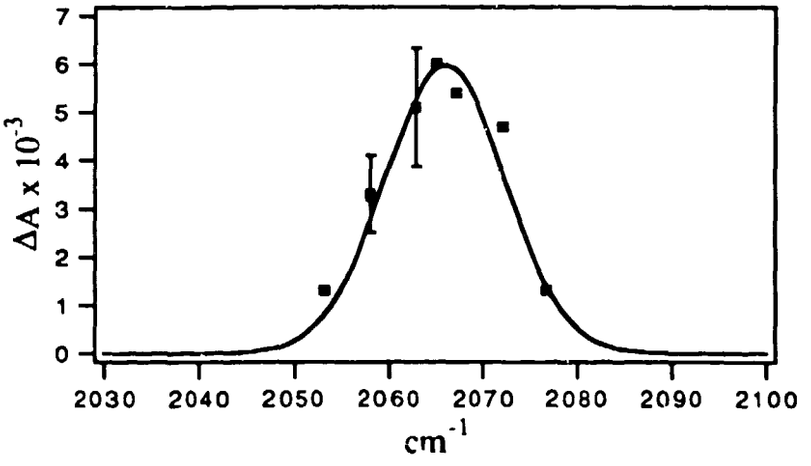

Figure 2. Picosecond transient IR spectrum of the 2062 cm^{-1} stretch of CO when bound to Cu_B^+. The gaussian (FWHM = 12 cm^{-1}) is not fit to the data.

complex occurs within one picosecond. This interpretation was tested by obtaining transients at various probe frequencies within the Cu_B^+-CO absorption band. The spectrum generated from these transients is identical to both the low temperature spectrum and that obtained at 1 μs [6], except that it is broadened by the 10 cm^{-1} width of the probe pulse (Figure 2). No wavelength dependence to the absorption rise was observed. We conclude that CO is quantitatively transferred and the Cu_B^+-CO complex is formed in 1 ps or less.

Induced linear dichroism experiments can provide a measure of the average angle of the CO dipole relative to the heme plane normal.[7,8] In the limit of 0% photodissociation, the polarization ratio at 1963 cm^{-1} is 1.75±0.06, yielding an average angle of 20±3° for CO bound to Fe_{a3}^{2+}. This result was obtained previously on the μs timescale [8] and is similar to values obtained for Hb-CO (18°) and one of the Mb-CO conformers (20°).[7] A polarization ratio of 1.00±.05 was obtained at 2062 cm^{-1}. This corresponds mathematically to either an average angle of 55±3° or an isotropic orientation for CO when bound to Cu_B^+. An isotropic distribution could result either from molecular reorientation on the experimental timescale or an orientation of the CO species formed at 2062 cm^{-1} which is uncorrelated to the orientation of the photoselected hemes. The rotation time for CcO is on the microsecond timescale and cannot contribute significantly to depolarization in a few picoseconds. Furthermore, the narrow bandwidth of for the CO vibration at 2062 cm^{-1} indicates that the CO is in a single, unique

environment. For example, "free" CO in the hemoglobin pocket has a 30 cm^{-1} wide band [9] as compared to 7 cm^{-1} when coordinated to Cu_B^+. Consequently, we conclude that the measured polarization ratio of 1.0 means that the CO dipole in the Cu_B^+-CO complex is oriented at an angle near 55° to the cytochrome a_3 heme normal.

The remarkable rate of CO transfer from Fe_{a3}^{2+} to Cu_B^+ provides new insight into the structure of CcO and suggests an unhindered pathway or channel is required to facilitate ligand transfer from one metal center to the other. The rapidity of this transfer does not allow for any barriers to CO translation or rotation nor for any ligand reorganization at Cu_B^+. It is possible that the ligand tranfers via a concerted-like mechanism. The close proximity of the two metals (4-5 Å) means that the CO is potentially within van der Waals contact with Cu_B while bound to Fe_{a3}. When CO is coordinated to Fe_{a3}^{2+}, the CO vibrational frequency, bandwidth, and insensitivity to changes at Cu_B^+ suggest that the Cu_B^+-O interaction is weak. As the CO dissociates, however, it can begin to interact with Cu_B^+ and form a new bond. In any event, the heme pocket must be constructed in such a manner that it restricts the motion of bound CO but expedites rapid transfer of the photodissociated CO between metal centers. This feature of the protein is significant to the role of Cu_B as a ligand shuttle to Fe_{a3} in the functional dynamics of the protein.

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References

1. B. G. Malmstrom, Chem. Rev. **90**, 1247 (1990) and references therein.
2. W.H. Woodruff, O. Einarsdottier, R. B. Dyer, K. A. Bagley, G. Palmer, S. J. Atherton, R. A. Goldbeck, T. D. Dawes, and D. S. Kliger, Proc. Natl. Acad. Sci. USA. **88**, 2588 (1991) and references therein.
3. F. G. Fiamingo, R. A. Altschuld, P. P. Moh, and J. O. Alben, J. Biol. Chem. **257**, 1639 (1982).
4. R. B. Dyer, K. A. Peterson, P. O Stoutland, and W. H. Woodruff, (a) J. Am. Chem. Soc. (1991) in press, (b)Proc. SPIE Soc. Opt. Eng. **1432**, 502 (1991).
6. P. O. Stoutland, J.-C. Lambry, J.-L. Martin and W. H. Woodruff, J. Phys. Chem. (1991) submitted.
7. J. N. Moore, P. A. Hansen, and R. M. Hochstrasser, Proc. Natl. Acad. Sci. USA. **85**, 5062 (1988).
8. R. B. Dyer, J. J. Lopez-Garriga, O. Einarsdottier, W. H. Woodruff, J. Am. Chem. Soc. **111**, 8962 (1989.
9. P. A. Anfinrud, C. Han, and R. M. Hochstrasser, Proc. Natl. Acad. Sci. USA. **86**, 8387 (1989).