Ultrafast Photoinduced Ligand Transfer in Carbonmonoxy Cytochrome *c* Oxidase. Observation by Picosecond Infrared Spectroscopy

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The coordination chemistry and ligation dynamics of the cytochrome a_3–Cu_B site of cytochrome *c* oxidase (ferrocytochrome *c*:O_2 oxidoreductase, cytochrome aa_3, or CcO) are essential elements of the function of the enzyme in activating O_2, in effecting its reduction to water, and perhaps in conserving the energy of the redox reaction.[1] Infrared spectroscopy is uniquely useful as a probe for these processes, particularly the behavior of Cu_B^+, which generally is not observable by other spectroscopies.[2,3] Using time-resolved infrared (TRIR) spectroscopy and kinetics measurements we have recently shown that coordination to Cu_B^+ is an obligatory mechanistic step for CO entering the a_3 heme site and departing the protein after photodissociation.[1,4,5] The time scale ($>10^{-7}$ s) of the TRIR measurements, however, precluded our observation of the ligation dynamics immediately following photodissociation. Here we report a picosecond time scale TRIR study of these events. The results reveal that the photoinitiated ligand transfer of CO from Fe_{a3}^{2+} to Cu_B^+ is the fastest ligation reaction yet observed. The rise time of the appearance of Cu_B^+–CO is less than 1 ps. This has profound implications with regard to the structure and dynamics of the cytochrome a_3–Cu_B site, the functional architecture of the protein, and coordination dynamics in general.

Beef heart cytochrome *c* oxidase is isolated and samples are prepared as described previously.[4,6,7] Ultrafast TRIR measurements are obtained in pump–probe experiments using optical delay.[8] The visible pump (photodissociation) pulse (2 ps, 30 μJ) is generated by a Rhodamine 6G dye laser synchronously pumped by a frequency-doubled, mode-locked Nd:YAG laser and then amplified at 30 Hz with 532-nm pulses from a Nd:YAG regenerative amplifier. The infrared probe pulse (2 ps, ~30 nJ) is created by generating the difference frequency between the 532-nm pulses and the amplified dye pulse in a $LiIO_3$ crystal. A typical transient is obtained by scanning the delay line in 0.3-ps steps, at each step integrating 200 shots alternating light on/off. The zero of time and the instrument temporal response (dependent solely on the pump–probe cross-correlation width) are measured directly before and after each series of scans by substituting for the sample a Si wafer in which the pump causes an instantaneous decrease in transmission of the probe.[9]

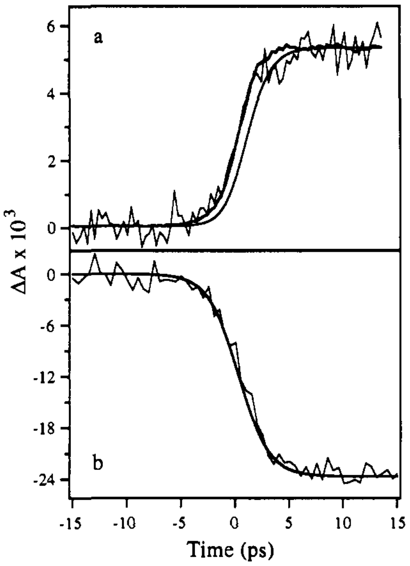

Figure 1. Time-resolved infrared absorption for photodissociated CcO·CO: (a) Transient monitored at the peak of the Cu_B^+–CO absorption at 2062 cm^{-1} (average of 30 scans). The smooth traces are, leftmost, the experimentally determined instrument response function and, rightmost, the convolution of this function with a 1-ps exponential rise. (b) Transient monitored at the peak of the Fe_{a3}^{2+}–CO bleach at 1963 cm^{-1} (average of 20 scans). The smooth trace is the instrument response function.

The formation of the CO complex of Cu_B^+ is directly observed by monitoring the appearance of the Cu_B^+–CO absorption at 2062 cm^{-1} (Figure 1a). A calculated curve that represents the convolution of the instrument response function with a 1-ps exponential function describing the appearance of the Cu_B^+–CO absorption is also plotted in Figure 1a. The failure of this convolution to fit the data indicates clearly that the rise of the IR transient is less than 1 ps. The static extinction coefficient of the Cu_B^+–CO absorption is 0.64 mM^{-1} cm^{-1}, a factor of 7 less than the Fe_{a3}^{2+}–CO absorption.[2,3] The ratio of the transient (1 ps) ΔA of Cu–CO to Fe–CO is equal to this static ratio when care is taken to perform the two TRIR measurements under the same optical conditions (beam overlap, fraction of photodissociation, etc.), suggesting that complete development of the static spectrum of Cu_B^+–CO occurs on this time scale. This interpretation was tested by obtaining transients at various probe frequencies within the Cu_B^+–CO absorption band.[8] The spectrum generated in this manner is essentially identical with the static spectrum, except that it is broadened by the 10-cm^{-1} width of the probe pulse. We conclude that the CO is quantitatively transferred and the static Cu_B^+–CO conformation is obtained in less than 1 ps.

In order to understand how the enzyme accomplishes the extremely efficient and rapid transfer of CO between metal centers, we consider three processes (which can be either sequential or concerted) that must occur to produce the observed Cu_B^+–CO transient absorption. These are (1) CO dissociation from Fe_{a3}^{2+}, (2) translation and rotation of CO into position to bind to Cu_B^+, and (3) formation of the Cu_B^+–CO bond. The CO photodissociation is directly observed by means of the transient bleach of the Fe_{a3}^{2+}–CO absorption at 1963 cm^{-1} (Figure 1b). The rise of the bleach is indistinguishable from the instrument response function and must therefore occur in less than 1 ps. Recent femtosecond UV–vis measurements we have made on CcO indicate that CO photodissociation actually occurs in less than 100 fs, probably on the time scale of one vibrational period of the Fe–CO stretch (520 cm^{-1}, 64 fs).[10]

If we assume that photodissociation produces "free" CO, we can estimate the rate at which it moves into position to bind to Cu_B^+ on the basis of the following arguments. Cu_B is generally believed to be within 5 Å of the iron of cytochrome a_3.[11,12]

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Previously, we have measured the orientation of the CO bound to $Fe_{a_3}^{2+}$ as 21° from the heme normal compared to an angle of 51° when it is bound to Cu_B^+.[7] Molecular modeling suggests that the CO vector of Cu_B^+–CO points away from the heme plane. The CO must therefore move approximately 4 Å and rotate 30° to bind to Cu_B^+. We estimate an initial recoil velocity of ~14 Å/ps for the CO on the basis of the energetics proposed for HbCO photodissociation.[13] Accordingly, in free flight, the ejected CO could move 4 Å within 300 fs. It is highly improbable, however, that the CO is able to move this far without colliding with other atoms in the heme pocket. In the case of Hb, the crystal structure indicates that the CO can move no more than 1 Å in free flight[14] and it has been concluded that collisions rapidly (~300 fs) render the motion of the CO diffusive (~1 Å/ps).[13] The unusually narrow infrared line width for CO bound to $Fe_{a_3}^{2+}$ in CcO (half that in HbCO[15]) suggests a rigid, confined heme pocket[15,16] which would likely restrict the motion of "free" CO. Clearly the picture of a "free" CO diffusing through the heme pocket to Cu_B^+ is not consistent with the observed rise time of the Cu_B^+–CO transient, which requires motion of the CO from $Fe_{a_3}^{2+}$ to Cu_B^+ closer to free flight at supersonic velocity than purely diffusive at a thermalized velocity. A concerted mechanism in which the Cu_B^+–CO bond begins to form as the $Fe_{a_3}^{2+}$–CO bond breaks appears a more accurate description of the CO transfer. The close proximity of the Cu_B^+, which is potentially in van der Waals contact with CO bound to $Fe_{a_3}^{2+}$, makes a concerted transfer possible. When CO is bound to $Fe_{a_3}^{2+}$, the CO absorption frequency, bandwidth, and insensitivity to changes at Cu_B^+ suggest that the Cu_B^+–O interaction is weak.[15,16] As the CO dissociates, however, it can begin to interact with Cu_B^+ via the carbon and form a new bond.

Whether the reaction is concerted or stepwise, the CO likely arrives at Cu_B^+ with considerable excess energy which must be dissipated along with the energy of the Cu_B^+–CO bond formation. It is difficult to estimate the rates of the intramolecular vibrational energy transfer processes which dissipate this excess energy, since the magnitude of the excess energy and the structure and vibrational coupling of the Cu_B^+–CO center are not known. The observed rise time of the Cu_B^+–CO absorption, however, requires that these processes be very rapid, at most hundreds of femtoseconds.

The remarkable rate of CO transfer from $Fe_{a_3}^{2+}$ to Cu_B^+ provides new insight into the structure of CcO. The heme pocket must be elegantly designed to restrict the motion of bound CO but to facilitate rapid transfer between metal centers of photodissociated CO. The rapidity of this transfer does not allow for any barriers to CO translation and rotation nor for any ligand reorganization at Cu_B^+. These features of the protein are significant to the role of Cu_B as a ligand shuttle to Fe_{a_3} in the functional dynamics of the protein.[1] This reaction is also of interest from a fundamental standpoint in understanding the elementary steps involved in coordination and atom-transfer dynamics, in the same sense that chlorophyll reaction centers have become a paradigm for understanding ultrafast electron transfer.

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$Ag(CO)B(OTeF_5)_4$: The First Isolable Silver Carbonyl

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From an academic or an industrial point of view, carbon monoxide is one of the most important ligands in transition-metal chemistry. Only a handful of metals in the d block have failed to yield an isolable carbonyl complex, and silver is one of them.[1-3] Despite this, there has been interest in $Ag(CO)_n^+$ species as catalysts for the carbonylation of alkanes, arenes, alkenes, aldehydes, alcohols, and amines.[4] Until now, $Ag(CO)_n^+$ species have only been detected in zeolite hosts[5] or in strongly acidic media such as concentrated H_2SO_4 or $BF_3{\cdot}H_2O$ (generally at low temperatures and high pressures).[6] In this communication we report the synthesis, structure, and IR spectrum of $Ag(CO)B(OTeF_5)_4$. Not only is this the first isolable silver carbonyl, it also exhibits one of the highest CO stretching frequencies of *any* metal carbonyl species (2204 cm^{-1}).

When $AgOTeF_5$,[7] $B(OTeF_5)_3$ (which is volatile),[8] and excess CO (final pressure = 200 Torr) were mixed in a glass vessel at 25 °C, 2 equiv of CO was taken up (all CO uptake and evolution experiments were carried out quantitatively (±0.05 equiv) by using high vacuum line manometry):

$$AgOTeF_5 + B(OTeF_5)_3 \xrightarrow[25\ ^\circ C,\ 36\ h]{excess\ CO} Ag(CO)_2B(OTeF_5)_4$$

When this compound was placed under vacuum, 1 equiv of CO was evolved within minutes, leaving $Ag(CO)B(OTeF_5)_4$, which slowly (hours) lost the remaining equivalent of CO and re-formed $AgOTeF_5$ and $B(OTeF_5)_3$. Both carbonyl complexes are extremely hygroscopic: water vapor displaced the CO ligands and reacted with the $B(OTeF_5)_4^-$ counterions, liberating $HOTeF_5$. When $AgOTeF_5$ alone was treated with CO (P_{CO} = 320 Torr), only 1 equiv of CO was taken up (this process was also reversible).

The solid-state IR spectrum of $Ag(CO)B(OTeF_5)_4$ exhibited $\nu(CO)$ at 2204 cm^{-1} ($\nu(^{13}CO)$ = 2154 cm^{-1}). The carbon–oxygen stretching frequency is significantly higher than in free CO (2143 cm^{-1}),[9] a clear indication that CO is acting merely as a Lewis base

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(1) We define a carbonyl complex as a stoichiometric $ML_x(CO)_y$ compound with at least one demonstrable M—C≡O linkage. In this vein, complexes such as $Pt(CO)(AuPPh_3)_8Ag(NO_3)_3$[2a] and $Cp(CO)_2Mo(\mu\text{-}Ag(PPh_3))(\mu\text{-}PPh_2)Mn(CO)_4$,[2b] with all Ag—C(O) distances ≥2.7 Å, are not silver carbonyls.

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