Formation and Spectroscopic Characterization of the Carbon Monoxide Adduct of an Alkanethiolate-Coordinated Heme

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The CO adduct of a thiolate-coordinated heme was obtained by two different methods and characterized by UV–vis and resonance Raman spectroscopy. The ν(Fe–CO) band was observed at 428 cm^{-1}. The unusually low frequency indicates the extremely strong σ-donation from the thiolate axial ligand to the heme iron.

The ubiquitous cytochrome P450 isozymes have strong oxidizing ability and a unique structure, that is, the thiolate group of cysteine axially coordinates to heme iron.[1] Much attention has been denoted to these enzymes to date, especially to the relationship between their structures and functions. The CO adduct of cytochrome P450s is characterized by the typical hyperporphyrin spectrum with split Soret bands at 380 and 450 nm. This spectroscopic feature is employed as a criterion for the coordination of a thiolate ligand to a heme. A number of thiolate-coordinated iron porphyrins have been synthesized in order to reproduce the absorption spectrum of the CO adduct and to clarify the effect of the thiolate ligand on the reactivity of the heme.[2]

We have already reported the synthesis and characterization of an alkanethiolate-coordinated heme (**TCP-TB**: Twin Coronet Porphyrin with a ThioBenzyloxy Group, Figure 1).[3,4,5]

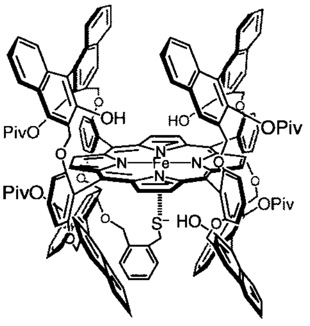
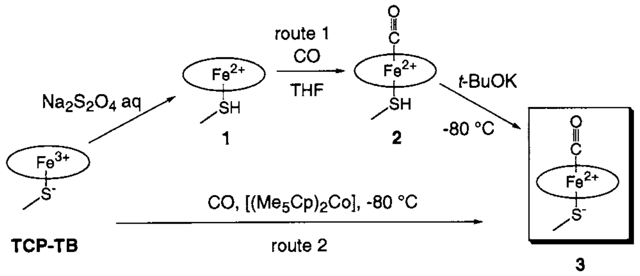


Figure 1. TCP-TB comlpex.

Ferrous **TCP-TB** generated by the usual reduction of the ferric one with an aqueous dithionite solution formed the corresponding CO adduct, which, however, did not show a hyperporphyrin spectrum. We report herein the successful formation of the thiolate-coordinated CO adduct of **TCP-TB** by two different methods and its characterization by UV–vis and resonance Raman (RR) spectroscopy. **TCP-TB**, which has the strongly electron-donating thiolate ligand, can exaggerate the effect of the axial ligand on the vibrational frequencies of the FeCO unit.

At first, we applied the reduction of the ferric form and the deprotonation of the thiol group in the CO adduct **2** (Scheme 1, route 1). The ferrous form **1** obtained by dithionite reduction in aqueous THF exhibited λ_{max} (THF) at 432 and 543 nm, which turned to 422 and 538 nm, respectively, by exposure to CO. The absorption spectrum of the present CO adduct **2** was almost identical with the reported one of the six-coordinate Fe(II)–CO complexes bearing a thiol ligand not a thiolate one.[6] During the reduction of the ferric form in a protic media, the thiolate–Fe(II) complex is tend to be protonated to turn into the thiol-coordinated one with the neutralization of the unstable negative charge. In order to deprotonate the thiol, the addition of various bases to the CO adduct **2** was carried out at –80 °C. Applicable bases should be adequately basic for the deprotonation and less nucleophilic to avoid other side reactions. When a THF solution of *t*-BuOK (ca. 10–20 molar equiv) was added to **2**, the spectrum gradually changed to give split Soret bands (362 and 444 nm), indicating the formation of the CO adduct **3** with the thiolate axial ligand. A large excess of *t*-BuOK was indispensable for the complete deprotonation of the thiol group.



Scheme 1. Formation of the CO adduct **3** via two different routes.

Secondly, we selected decamethylcobaltocene [(Me$_5$Cp)$_2$Co] as a reductant (Scheme 1, route 2).[7] Its redox potential (–1.47 V vs SCE) is low enough to reduce the ferric form of **TCP-TB**: the estimated E_p [Fe(III)/Fe(II)] = –1.01 V (vs SCE) from the observed value, E_p = –1.31 V (*vs.* Ag/Ag$^+$).[4] Since this reduction can be carried out under aprotic conditions, the thiolate group in the ferrous complex is hardly protonated.

The THF solution of ferric **TCP-TB** was cooled to –80 °C under CO atmosphere and then a small excess of the cobaltocene solution (1.5 molar equiv to the amount of **TCP-TB**) was added. This reaction induced an immediate and drastic change in the UV–vis spectrum. A hyperporphyrin spectrum appeared with split Soret bands at 373 and 456 nm (Figure 2A), which was slightly different from that of the CO adduct prepared by the first method. This might be caused by the difference of the conditions between the two measurements. The second method is superior to the first one in simplicity and purity. Therefore, the CO adduct **3** via route 2 was further characterized by RR spectroscopy.

It is well known that the CO molecule ligated to ferrous cytochrome P450$_{cam}$ is readily photodissociable and that the mixture of the CO complex and the CO-free ferrous form is observed during the photo-excitation.[8] In the present case with laser beam power of 5 to 50 mW, no prominent Raman line was detected near 1340 cm^{-1}, which could be assignable to the oxidation marker band (ν_4) of the CO-free ferrous form.

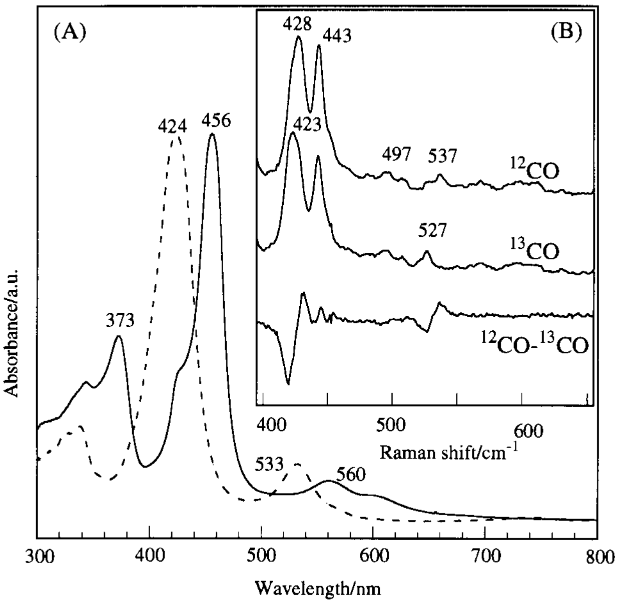


Figure 2. (A) UV-vis spectra of ferric **TCP-TB** and its **CO** adduct **3** (-80 °C, THF) through route 2. Solid line: CO complex; broken line: ferric **TCP-TB**. (B) RR spectra in the low frequency region of the ^{12}CO and ^{13}CO adducts of **TCP-TB** (-80 °C, THF). Ex. 457.8 nm; power 20 mW.

The RR spectrum of the CO adduct **3** exhibited a strong line at 428 cm^{-1} (Figure 2B), which shifted to 423 cm^{-1} by replacement of ^{12}CO with ^{13}CO. The observed isotopic shift upon ^{13}CO substitution is in good agreement with the value (5 cm^{-1}) calculated from the harmonic oscillator approximation of Fe–CO stretching vibration. Therefore, this band is assigned to ν(Fe–CO) mode. Compared with the reported ν(Fe–CO) frequencies of substrate-unbound CO-cytochrome P450s (471, 464 cm^{-1})[9] and modeling CO adduct (479 cm^{-1}),[10] the observed value (428 cm^{-1}) is extremely low. The ν_4 band of CO-**TCP-TB** is located at 1360 cm^{-1}, which is considerably lower than that of CO-cytochrome P450$_{cam}$ (1366 cm^{-1}).[9a] A strong σ-donor trans to CO induces an increase of the population of Fe dz^2 orbital, which is not favorable for the Fe–C σ bonding, evidenced by the decrease of ν(Fe–CO). In contrast, a good π-donor increases ν(Fe–CO), allowing the enhancement of Fe–CO π-back bonding. Hence, the present observations, especially the anomalously low frequency of ν(Fe–CO), could be attributed to the extremely strong σ-donation mainly from the thiolate axial ligand to the iron atom. Electrochemical studies support this argument: the reduction potential, Fe(III)/Fe(II) couple, of **TCP-TB** is more negative by ca. 0.5 V than that of substrate-unbound cytochrome P450$_{cam}$.[1]

The other weak CO sensitive band was also observed at 537 cm^{-1} (Figure 2B) and shifted to 527 cm^{-1} on ^{13}CO substitution. This band showed the two similarities with δ(Fe–C–O)[11] of the CO complexes of P450s (558 cm^{-1}),[9] hemoglobin (578 cm^{-1})[12] and myoglobin (577 cm^{-1});[12] (i) higher in frequency and weaker in intensity than the corresponding ν(Fe–CO), (ii) a larger ^{12}CO/^{13}CO shift (Δ = 10–15 cm^{-1}) than that of ν(Fe–CO). Therefore, we assigned it to δ(Fe–C–O) mode. Previously, the simultaneous enhancement of ν(Fe–CO) and δ(Fe–C–O) was observed in the CO adduct of cytochrome P450s.[9] It has been known that the activation of δ(Fe–C–O) mode in RR spectrum requires symmetry lowering.[11c] The observation of δ(Fe–C–O) mode in CO-**TCP-TB**, which has no symmetry, is consistent with this argument. In contrast, δ(Fe–C–O) mode was not detected in the other thiolate-coordinated model of high symmetry.[10] Further characterization of the present and relating CO adducts is now under way.

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