An Infrared Study of Carbon Monoxide Bonding to Heme *A* in Cytochrome *c* Oxidase

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Summary Carbon monoxide when bound to reduced cytochrome *c* oxidase of bovine heart has been found to exhibit one C–O stretch band with a frequency and band-width consistent with a terminal CO ligand bound to heme *A* iron within a non-polar environment well-isolated from external solvent.

THE reactions of cytochrome *c* oxidase, the site of oxygen utilization for cellular energy production *via* oxidative phosphorylation, have received much study but the nature of the oxygen binding site *per se* has remained unclear.[1] Oxygen binding at copper, at heme iron, or at more than one metal at the same time are possibilities that have been mentioned. Recently, i.r. bands for CO ligands have been used to probe the oxygen binding sites of hemoglobins and myoglobins.[2,3] We report here the successful extension of this technique to carbonyl cytochrome *c* oxidase although the CO to protein ratio is an order of magnitude smaller in the oxidase than in hemoglobins and myoglobins. CO heme *A* has also been examined.

I.r. spectra were obtained as reported earlier for hemoglobins, myoglobins, and hemins.[3] A solution of cytochrome *c* oxidase isolated from beef heart[4] in 1% Tween 80–0·1M-potassium phosphate, pH 7·4, at a concentration of 0·1mM heme *A* was used to fill the reference cell. The same solution, after treatment with a slight excess of solid sodium dithionite and CO, was used for the sample cell. The difference spectrum for CO oxidase *versus* oxidized oxidase was obtained in calcium fluoride cells, path length 0·025 mm, maintained at 10 ± 2° (Figure). Absorption spectra for the visible and Soret regions were determined in the same CaF_2 cell used in the i.r. study, and found to be the same after the i.r. spectrum had been obtained as

before. Solutions of heme *A* isolated from bovine heart[5] were prepared at 10mM in 0·13M-pyridine in bromoform or at 4mM in 1% Tween 80–0·1M-potassium phosphate pH 7·4.[6]

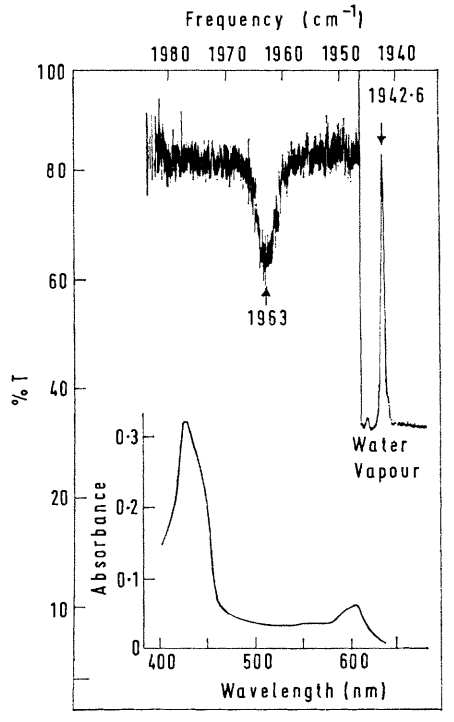

FIGURE. *I.r. difference spectrum of carbonyl cytochrome* c *oxidase* minus *cytochrome* c *oxidase. Water-vapour band at right was used for wavelength calibration. Insert at bottom: Soret and visible spectra of the* CO *derivative in the same filled* CaF_2 *cell used to obtain the i.r. spectrum.*

Spectra for the CO complex were obtained as described earlier for other heme carbonyls.[2]

The heme *A* carbonyl exhibited a broad ν_{CO} band at 1976 cm^{-1} in Tween–phosphate or at 1982 cm^{-1} in pyridine–bromoform.† Upon comparison with spectra for other heme carbonyls, the band widths and frequencies are consistent with expected values of a monomeric CO heme with a strongly electron-withdrawing ring substituent, in this case the 8-formyl.[7]

With the cytochrome *c* oxidase, a single sharp band at 1963 cm^{-1} was found (Figure). The similarity in ν_{CO} for the oxidase and heme *A* is consistent with CO binding to the oxidase at heme iron as a terminal CO ligand. However, this observed ν_{CO} value is inconsistent with CO serving as a bridging ligand (*e.g.*, where carbon is bound to two metal atoms) because in that case a significantly lower frequency would be expected.[8] Another possibility, bonding at both carbon and oxygen atoms of CO,[9] is more difficult to exclude.[10] The extremely narrow half-band width of 3 cm^{-1} (Figure) shows the environment about the CO ligand in the oxidase to be quite different from that of the heme in the same buffer where a half-band width greater than 20 cm^{-1} was found. Thus, the CO ligands, though terminal, do not appear exposed to the environment of the external solvent but rather experience quite uniform and stable solvation interactions. The narrowness of the band is consistent with, but does not absolutely require, a ligand environment of very non-polar character—even less polar than that for CO in hemoglobins and myoglobins.[10] It is reasonable to expect O_2 to bind at the same site as CO—a heme site possibly less polar for the oxidase where oxygen is reduced to water than is the site in hemoglobin where O_2 dissociates without reduction.

We gratefully acknowledge the support of the United States Public Health Service in this work.

(*Received, June 5th,* 1970; *Com.* 874.)

† In pyridine–bromoform, a second less intense band was also found at 2080 cm^{-1}. Both bands were shown to be due to CO in that with $^{13}C^{16}O$ the 1982 cm^{-1} band was shifted to 1937 cm^{-1}, and the 2080 cm^{-1} band to 2033 cm^{-1}. The origin of the two bands in this solvent is being investigated.

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