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CO PHOTOLYSIS OF CYTOCHROME OXIDASE INVESTIGATED BY PS RESONANCE RAMAN SPECTROSCOPY

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Low-power picosecond resonance Raman spectroscopy was used to investigate the identity of the axial ligand of heme a_3 and relaxation processes in the heme a_3 pocket of cytochrome oxidase after CO photolysis. Our results show that the proximal histidine remains ligated to heme a_3 after CO photolysis excluding the transient ligation of a photolabile, endogenous ligand. Furthermore, the relaxation of the heme a_3 macrocycle modes occurs on the sub ps time scale, while relaxation of the heme pocket to its equilibrium conformation takes place on the μs time scale.

Keywords: Picosecond; resonance Raman; cytochrome oxidase; CO; photolysis

INTRODUCTION

Cytochrome oxidase contains 4 redox active metal centers: heme *a*, heme a_3, Cu_A and Cu_B. The catalysis takes place at the binuclear center formed by heme a_3 and Cu_B. The dynamics in the heme a_3 pocket have been studied extensively using CO photolysis and two models have been proposed. In one model [1] the proximal histidine remains ligated to heme a_3, while in the other model [2] an endogenous ligand dissociates from Cu_B and transiently binds to heme a_3

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displacing the proximal histidine after CO photolysis. We have applied low-power ps resonance Raman difference spectroscopy to identify the axial ligand of heme a_3 directly after CO photolysis.

METHODS

Cytochrome oxidase was isolated from beef heart. Raman difference spectroscopy was done with two synchronously pumped dye lasers with 7 ps pulses at a 153 kHz repetition rate. Photolysis pulses were 36 nJ at 592 nm and probe pulses were 16 nJ at 441 nm.

RESULTS AND DISCUSSION

The low-frequency resonance Raman spectrum of the fully reduced enzyme (Fig. 1a) contains contributions from both hemes, while the spectrum of its CO-complex (Fig. 1b) only shows peaks of heme a^{2+} using 441 nm excitation. The a_3^{2+} vibrations that disappear upon CO ligation, reappear in the Raman difference spectra (Fig. 1c–f) after CO photolysis. Although the a_3^{2+} macrocycle modes at 332, 366 and 400 cm^{-1} are unchanged in the photolysis product, the Fe-his frequency is upshifted from 214 to 220 cm^{-1} as was observed in ns experiments [1, 2]. The intensities of the modes in the difference spectra were not sensitive to attenuation of the probe power by a factor of 8, indicating that we do not observe additional photolysis due to the probe pulses. Since the Fe-his mode is observed at 0 ps delay time and substantial protein movement to accommodate ligation of a distal ligand is not expected on this fast time scale, we conclude that the proximal histidine remains ligated to heme a_3. Furthermore, the a_3^{2+} macrocycle modes show no frequency shift on the time scale of our experiment, so most likely they assume their equilibrium frequencies on the sub ps time scale. The heme a_3 pocket relaxes to its equilibrium conformation on the μs time scale as indicated by the relaxation of the Fe-his vibration.

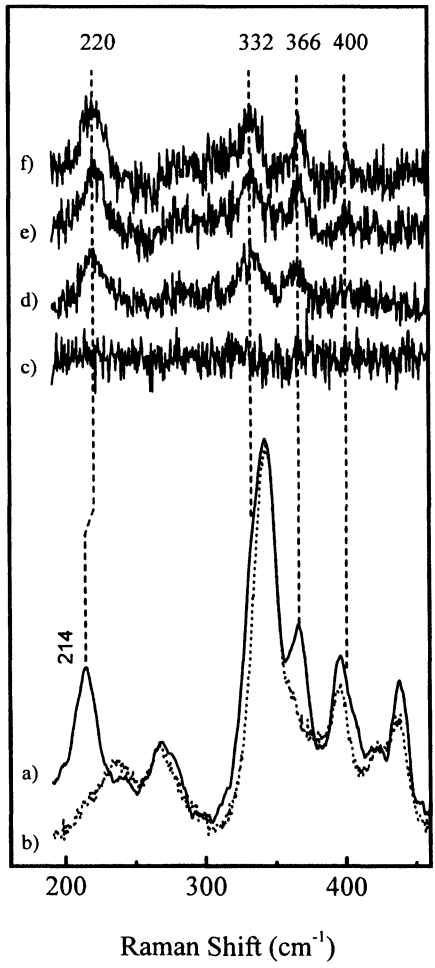

FIGURE 1 Low-frequency Raman spectra of cytochrome *c* oxidase from beef heart obtained with 441 nm excitation. The equilibrium fully reduced enzyme (a) and its CO complex (b). Difference spectra taken at −20 ps (c), 0 ps (d), 30 ps (e), and 1 ns (f) after CO photolysis. The difference spectra were obtained by subtracting the probe-only spectrum from the pump-probe spectrum after normalization on the 438 cm^{-1} heme a^{2+} mode. In spectra 1c–f, CO was photolyzed with 592 nm pulses.

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