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RESONANCE RAMAN SCATTERING FROM HEME O COMPLEXES AND CYTOCHROME bo_3 OXIDASE

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The cytochromes bo_3 and *bd* are the terminal ubiquinol oxidases in the anaerobic chain of *Escherichia coli*. As deduced from its gene structure in *E. coli*, cytochrome bo_3 is strongly related to the superfamily of heme-copper containing enzymes. In particular, the enzyme catalyzes the two-electron oxidation of ubiquinol and the four-electron reduction of O_2 to H_2O and it couples the free energy of these electron-transfer processes to translocate protons on the periplasmic side of the membrane. Cytochrome bo_3 contains a six-coordinated, low-spin *b*-type heme; a five-coordinated, high-spin oxygen-binding *o*-type heme; and one copper atom (Cu_B). The heme *o* is structurally related to heme *a* with a methyl residue replacing the formyl group at pyrrole ring D [1].

Understanding the molecular mechanism of cytochrome bo_3 function requires the structural characterization of both types of hemes. The electronic spectrum of cytochrome bo_3 doesn't allow clear

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separation of the *b* and o_3 chromophores. Thus, the assignments of the vibrational contributions to the individual hemes is not an easy task. Here we report a vibrational analysis of heme O and its model compounds and compare them to analogous species of heme A and protoporphyrin. With this approach, we have used the model compound data to assign vibrational bands for cytochromes *b* and o_3 in different oxidation and spin states of cytochrome bo_3 [2].

Heme O and its analogues show an intrinsic $\sim 1200\,cm^{-1}$ *x*, *y* splitting of its lowest $\pi-\pi^*$ transition. In addition, we observed enhancement of the vinyl modes in the heme O models, which indicates that a greater amount of conjugation between the vinyl π^* antibonding orbitals and the porphyrin excited π^* Soret orbitals exists in heme O than in heme A. The vinyl influence on the porphyrin skeletal frequencies involves both conjugative and kinetic effects, and the effects of C_α and C_β vinyl deuteration reveal the same pattern of vinyl modes as seen in protoheme complexes. The resonance Raman spectra of high- and low-spin heme O complexes obtained with laser excitation in resonance with the B(0, 0) absorption band show strong enhancement of the ν_{37} and ν_{38} modes that reflect significant lowering effect. Inspection of the RR data of ferric heme O models and ferric heme o_3 derivatives reveals that there are no significant structural differences in the core and peripheral substituents of the heme O and heme o_3 macrocycles. In contrast, there are large spectral differences observed for ferrous heme O models and heme o_3 derivatives. We attribute these differences to a localized region in the binuclear heme o_3/Cu_B center. A weak Raman band arises at $208\,cm^{-1}$ in the spectra of the reduced enzyme. The corresponding Raman spectra of the fully reduced-cyanide and mixed valence cyanide complexes show that the $208\,cm^{-1}$ band has disappeared and that a new band at $230\,cm^{-1}$ has appeared. The $208\,cm^{-1}$ band can be assigned to $\nu(Fe^{2+}\text{-his})$ of reduced heme o_3, and the $230\,cm^{-1}$ band may be a marker characteristic of a six-coordinate low-spin $o_3^{+2/3}$ complex.

References

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