

The Tetraheme Cytochrome c₅₅₄ from *Nitrosomonas europaea* can function as a Bacterial NO Reductase

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The tetraheme cytochrome c₅₅₄ (cyt c₅₅₄) from *Nitrosomonas europaea* can act as an electron acceptor from hydroxylamine oxidoreductase (HAO), in vivo and in vitro. The protein contains one high-spin and three low-spin c-type hemes. HAO catalyzed reduction of cyt c₅₅₄, ligand binding, intermolecular electron transfer, and kinetics of NO and O₂ reduction by cyt c₅₅₄ have been investigated. We detect the formation of a NO bound species in cyt c₅₅₄ by EPR, Mössbauer, and ESI-MS spectroscopies during the HAO catalyzed oxidation of hydroxylamine, indicating that intermediates from HAO readily bind to cyt c₅₅₄. In the half-reduced state, we detect a spin interaction between the [Fe-NO]⁷ state of heme 2 and the low-spin ferric state of heme 1. We find that cyt c₅₅₄ will catalyze the reduction of NO at rates greater than 9 s⁻¹, which is comparable to rates of other known NO reductases. Carbon monoxide or nitrite are shown not to bind to the reduced protein, and previous results indicate that a variety of ligands will not bind in the oxidized state. Thus, the enzymatic site is highly selective for NO. A detailed analysis of EPR spectra taken at X- and Q-band frequencies has been performed to determine the nature of interactions between the hemes of cyt c₅₅₄.