The membrane-associated tetra-heme cytochrome c_{m552} with unusual heme interactions unique in taking up P-side protons in *Nitrosomonas*

Hyung J. Kim[1], Anup K. Upadhyay[2], Anna Zatsman[2], David Bergmann[1], Mark Whittaker[1], J. Riga[1], Michael P. Hendrich[2] and Alan B. Hooper[1]

[1]*Department of Biochemistry, Molecular Biology and Biophysics University of Minnesota*
[2]*Department of Chemistry, Carnegie Mellon University*

Energy for the growth of the bacterium *Nitrosomonas europaea* is derived from the step-wise oxidation of ammonia to hydroxylamine and to nitrite by a monooxygenase and a dehydrogenase, respectively. We have progressed significantly in purifying, characterizing, and determining the structure of periplasmic redox enzymes including the remarkable 36-heme hydroxylamine oxidoreductase (HAO)-cytochrome c_{554} complex. This complex oxidizes hydroxylamine and directs pairs of electrons for apparent P-side reduction and protonation of ubiquinone mediated by a membrane-bound, tetra-heme *c*-type cytochrome c_{m552}.

The 27 kDa cytochrome c_{m552} was isolated and purified from the membranes of *N. europaea* using Triton X-100 solubilization and subsequent *n*-dodecyl-β-D-maltoside detergent exchange. The cytochrome belongs to the NapC/NirT family of tetra-heme cytochromes implicated in electron transfer between membrane quinols/quinones and periplasmic enzymes, but c_{m552} possesses an unusually long stretch of acidic amino acids at the *C*-terminus (27 of last 44 residues). Based on size exclusion chromatography, c_{m552} purified as a mixture of dimer and tetramer suggesting the presence of a possible four-helix bundle membrane anchor in the latter. c_{m552} exhibited visible maxima at 408 nm (γ peak) when oxidized, and at 420 nm (γ), 524 nm (β) and at 552 nm (α) when reduced. Atypical of NapC/NirT cytochromes, ferrous c_{m552} was found to undergo rapid reaction with O_2 and also to bind CO when reduced. Ferrous c_{m552} could be trapped as the CO derivative, when reduced by hydroxylamine, but only in the presence of catalytic HAO and c_{554}, suggesting that c_{m552} accepts electrons from the HAO/c_{554} complex. Mössbauer spectroscopy with fully reduced ^{57}Fe-enriched samples clearly showed a 1 to 3 ratio of high-spin ferrous doublet to low-spin ferrous doublets. EPR spectra detected the presence of a high-spin/low-spin pair and indicate that two of the three low-spin hemes are spin-coupled and rendered EPR silent.