Redox State Dependent Axial Ligand Dynamics of Cytochrome $c_{552}$ from *Nitrosomonas europaea*

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*Nitrosomonas europaea* cytochrome $c_{552}$ (*Ne* cyt $c_{552}$) is a member of the cytochrome $c_8$ family, of which *Pseudomonas aeruginosa* cytochrome $c_{551}$ (*Pa* cyt $c_{551}$) is a prototypical member. *Ne* cyt $c_{552}$ and *Pa* cyt $c_{551}$ have high homology in sequence and structure, but their heme substituent $^1\text{H}$ NMR hyperfine shifts differ substantially. Whereas *Pa* cyt $c_{551}$ has the $5\text{-CH}_3 > 1\text{-CH}_3 > 8\text{-CH}_3 > 3\text{-CH}_3$ heme methyl shift pattern with a large spread (~20 ppm) typical of the cyt $c_8$ family, *Ne* cyt $c_{552}$ has a $5\text{-CH}_3 > 8\text{-CH}_3 > 3\text{-CH}_3 > 1\text{-CH}_3$ pattern with a small (~10 ppm) spread. We have proposed that the unusual heme methyl shift pattern of *Ne* cyt $c_{552}$ results from fluxional behavior of the axial Met (1).

The observation of temperature-dependent, $T_1$-independent line broadening of the heme methyl resonances of *Ne* cyt $c_{552}$ supports the proposal that the axial Met is in conformational exchange in oxidized *Ne* cyt $c_{552}$ (1). Interestingly, in the reduced form of *Ne* cyt $c_{552}$, only one configuration of the axial Met is indicated by the NOEs from the Met side chain to the heme substituents (2). The orientation and anisotropy of the $\chi$ tensor for oxidized *Ne* cyt $c_{552}$, calculated from pseudocontact shifts, are compared to *Pa* cyt $c_{551}$. The $\chi_{xx}$ axis for *Ne* cyt $c_{552}$ is oriented at $43^\circ$ relative to the iron-pyrrole II axis, which is significantly different from the value for *Pa* cyt $c_{551}$ ($20^\circ$), but near the value expected if the axial Met is in fast exchange between conformations similar to that seen in *Pa* cyt $c_{551}$ and in the mitochondrial cyts $c$ ($\chi_{xx} \sim 72^\circ$). The magnetic axes calculation also shows that the electronic structure of *Ne* cyt $c_{552}$ is highly axial, supporting the proposal of a fluxional Met in this protein and in agreement with the HALS-type (“large $g_{\text{max}}$”) EPR spectrum reported for *Ne* cyt $c_{552}$ (3). In addition, comparison of the measured and calculated pseudocontact shifts supports the proposal of a redox state-dependent conformational change that may influence axial Met fluxion.