

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

Ravinder Kaur and Kara Bren

*Department of Chemistry, University of Rochester*

*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 ( $\sim 20$  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  $g_{max}$ ") 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.

1. Bren, K. L.; Kellogg, J. A.; Kaur, R.; Wen, X. *Inorg. Chem.* **2004**, *43*, 7934-7944.
2. Timkovich, R.; Bergmann, D.; Arciero, D. M.; Hooper, A. B. *Biophys. J.* **1998**, *75*, 1964-1972.
3. Arciero, D. M.; Peng, Q. Y.; Peterson, J.; Hooper, A. B. *FEBS Lett.* **1994**, *342*, 217-220.