Heme Axial Methionine Fluxion in Cytochromes *c*

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Ferricytochromes c_{552} from *Nitrosomonas europaea* (*Ne* cyt c_{552}) and *Hydrogenobacter thermophilus* (*Ht* cyt c_{552}) are unusual for displaying low rhombicity as reflected by a highly compressed range of heme substituent hyperfine ^{1}H NMR shifts (< 10 ppm, compared to > 20 ppm for typical cyts *c*), low $\Delta\Delta_{rh}$ determined from ^{1}H NMR pseudocontact shifts, and "high g_{max}" EPR spectra (1-3). Typically, cyts *c* with His-Met heme axial ligation display have rhombic symmetry at the heme as a result of the interactions of the axial ligands with the heme iron. It has been demonstrated that the unusual properties of *Ht* and *Ne* cyts c_{552} result from fluxionality of the heme axial Met, which results in averaging of two "rhombic" forms to give an "axial" form of cyt *c* (4, 5). The proposed motion is non-dissociative inversion through sulfur, occurring on the μs time scale. In *Ht* cyt c_{552}, this motion has been halted by mutation of a heme pocket Gln to Asn, yielding *Ht* Q64N. This mutant has a hyperfine-shifted ^{1}H NMR spectrum and heme pocket structure strikingly similar to *Pseudomonas aeruginosa* cytochrome c_{551} (*Pa* cyt c_{551}), a structurally homologous cyt *c* that has a fixed axial ligand orientation and rhombicity typical of cyts *c* (6). Structural analysis reveals that this mutation promotes hydrogen bonding in the heme pocket and blocks motion of the axial Met. Subsequently, it has been demonstrated that performing the complementary Asn-to-Gln mutation in *Pa* cyt c_{551} (yielding *Pa* N64Q) induces axial ligand fluxion. Interestingly, *Pa* N64Q is stabilized ($\Delta T_m \sim +8^\circ$C) relative to wild-type, and analysis of unfolding thermodynamics reveals that the stabilization is entirely entropic in nature. It is thus proposed that Met fluxion and release of residue 64 from constraints in the heme pocket increase folded state entropy, stabilizing the protein. Further analysis of cyt *c* heme pocket mutants suggests that axial Met fluxion will occur given enough space in the heme pocket.

1. Timkovich, R.; Cai, M. L.; Zhang, B. L.; Arciero, D. M.; Hooper, A. B. (1994) *Eur. J. Biochem. 226,* 159-168.
2. Arciero, D. M.; Peng, Q. Y.; Peterson, J.; Hooper, A. B. (1994) *FEBS Lett.* 342, 217-220.
3. Karan, E. F.; Russell, B. S.; Bren, K. L. (2002) *J. Biol. Inorg. Chem. 7,* 260-272.
4. Bren, K. L.; Kellogg, J. A.; Kaur, R.; Wen, X. (2004) *Inorg. Chem. 43,* 7934-7944.
5. Zhong, L.; Wen, X.; Rabinowitz, T. M.; Russell, B. S.; Karan, E. F.; Bren, K. L. (2004) *Proc. Natl. Acad. Sci. U.S.A. 101,* 8637-8642.
6. Wen, X.; and Kara L. Bren, K. L. (2005) *Biochemistry 44,* 5225-5233.