Role of Asn64 in Regulation of Axial Met Orientation and Redox Function of *Pseudomonas aeruginosa* Cytochrome c_{551}

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Pseudomonas aeruginosa cytochrome c_{551} (PA) and its counterpart *Hydrogenobacter thermophilus* cytochrome c_{552} (HT) exhibit high sequence identity (56%), and their main-chain folding is almost identical. However, the axial Met orientation that is crucial for heme electronic structure is different in these proteins.[1] X-ray analysis of PA indicated that Asn64 of PA forms a hydrogen bond with Ile48 and is anchored in close proximity of axial Met. On the contrary, NMR analysis of HT suggested that Gln64 of HT is not hydrogen bonding to Ile48 and points toward the outside of the protein. In order to evaluate the role of Asn64 in determining axial Met orientation of PA, we replaced the Asn64 by Gln or Ala. The observation of similar ^{1}H NMR spectra patterns for the oxidized forms of PA-N64Q and PA-N64A mutants and HT suggested that the axial Met orientation in these proteins is similar to each other, and that the steric hindrance between Asn64 and axial Met is crucial for the axial Met orientation of PA.

Electrochemical study of the proteins revealed that Asn64 is also important for regulation of redox potential ($E^{o\prime}$) of PA. Among the proteins, $E^{o\prime}$ value decreased in order of PA > PA-N64Q > PA-N64A. This result implied that the positively polarized N atom of Asn64 of PA destabilizes cationic ferric heme selectively and raises its $E^{o\prime}$ value.

The present study demonstrated that Asn64 of PA plays a significant role in regulation of not only the axial Met orientation, but also $E^{o\prime}$ value which correlate closely with thermodynamic stability of the oxidized form of the protein.[2]

[1] Tachiiri, N.; Hemmi, H.; Takayama, S. J.; Mita, H.; Hasegawa, J.; Sambongi, Y.; Yamamoto, Y., *J. Biol. Inorg.Chem*., 2004, 9(6), 733-742.

[2] Takayama, S. J.; Mikami, S.; Terui, N.; Mita, H.; Hasegawa, J.; Sambongi, Y.; Yamamoto, Y., *Biochemistry*, published online.

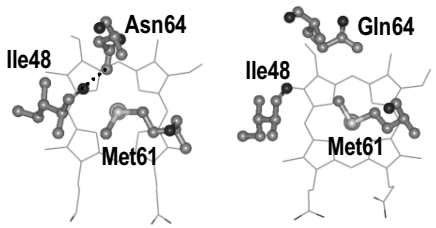

Figure Schematic representation of the structure around axial Met of PA(left, PDB:351C) and HT(right, PDB:1AYG)