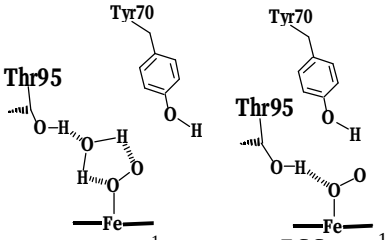
The Specific Sensing of O_2 by the Oxygen Sensor Protein HemAT

Hideaki Yoshimura[1, 2], Shiro Yoshioka[1], Takehiro Ohta[1], Teizo Kitagawa[1] and Shigetoshi Aono[1]

[1]Okazaki Institute for Integrative Bioscience, [2]The Graduate University for Advanced Studies

HemAT is the oxygen sensor protein controlling the aerotaxis of *B. subtilis.* HemAT should distinguish O_2 from CO and NO for functioning precisely as an oxygen sensor. In this work, we have studied about the molecular mechanism of specific sensing of O_2 by HemAT using spectroscopic and mutagenesis methods.

We have reported that the resonance Raman (RR) spectrum of O_2-bound HemAT shows three Fe-O stretching bands at 554, 566, and 572 cm^{-1}. These bands are derived from three species with different heme distal structures as follows; the first species with hydrogen bonds on both oxygen atoms of the heme-bound O_2 gives the 554 cm^{-1} band, the second one with a hydrogen bond only on the proximal oxygen atom gives the 566 cm^{-1} band, and the third one with no hydrogen bonds on heme bound O_2 gives the 572 cm^{-1} band[1]. RR spectroscopy with O_2-bound T95A and Y70F mutants has revealed that Thr95 is the amino acid residue making hydrogen bonds to the heme-bound O_2.



Figure

RR spectra of CO and NO-bound HemAT show the Fe-C, C-O and N-O stretching bands at 496, 1966, and 1636 cm^{-1}, respectively. T95A and Y70F mutants exhibit these three bands at almost the same positions. These values are similar to those of myoglobin H64L mutant (490, 1965, and 1635 cm^{-1}, respectively), but not to those of wild type myoglobin (512, 1944, and 1613 cm^{-1}, respectively). While hydrogen bonds to heme-bound ligands exist in wild type myoglobin, there are no such hydrogen bonds in myoglobin H64L mutant. These results indicate that no hydrogen bonds are made to the heme-bound CO and NO in HemAT. These results suggest that HemAT distinguishes O_2 from CO and NO with using the hydrogen-bonding network containing Thr95.

1. Ohta T. et al. *J. Am. Chem. Soc.* 2004, 126(46), 15000