The NO Reduction Mechanism by *ba*$_3$-oxidoreductase from *Thermus thermophilus*

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Reaction pathways in the enzymatic formation and cleavage of the N-N and N-O bonds, respectively, are difficult to verify without the structure of the intermediates, but we now have such information on the primary intermediate in the reaction of *ba*$_3$-oxidase with NO from resonance Raman (RR) spectroscopy. We have identified the primary His-heme a_3^{2+}-NO/Cu$_B^{1+}$ species by its characteristic Fe-NO and N-O stretching frequencies at 539 and 1620 cm^{-1}, respectively. The Fe-NO and N-O frequencies in *ba*$_3$-oxidase are 21 and 7 cm^{-1} lower and higher, respectively, than those observed in Mb-NO. We suggest that the reduction of NO to N_2O by *ba*$_3$-oxidase proceeds by the fast binding of the first NO molecule to heme a_3 with high-affinity, and the second NO molecule binds to Cu$_B$ with low-affinity, producing the temporal co-presence of two NO molecules in the heme-copper center. The low-affinity of Cu$_B$ for NO binding also explains the NO reductase activity of the *ba*$_3$-oxidase as opposed to other heme-copper oxidases. With the identification of the primary nitrosyl intermediate in which the bound NO is not hydrogen-bonded to one of the Cu$_B$ ligands, the structure of the binuclear heme a_3-Cu$_B^{1+}$ center in the initial step of the NO reduction mechanism is known. Evidence for formation of N–N bond in the NO reduction mechanism by the heme-copper bimetallic center will be discussed on the basis of RR spectroscopy and a density functional theory calculation.