

# The NO Reduction Mechanism by *ba*<sub>3</sub>-oxidoreductase from *Thermus thermophilus*

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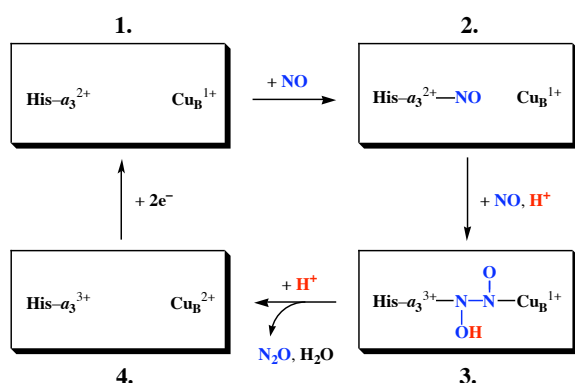
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Reaction pathways in the enzymatic formation and cleavage of the N-N and N-O bonds, respectively, are difficult to verify without the structure of the intermediates, but we now have such information on the primary intermediate in the reaction of *ba*<sub>3</sub>-oxidase with NO from resonance Raman (RR) spectroscopy. We have identified the primary His-heme *a*<sub>3</sub><sup>2+</sup>-NO/Cu<sub>B</sub><sup>1+</sup> species by its characteristic Fe-NO and N-O stretching frequencies at 539 and 1620 cm<sup>-1</sup>, respectively. The Fe-NO and N-O frequencies in *ba*<sub>3</sub>-oxidase are 21 and 7 cm<sup>-1</sup> lower and higher, respectively, than those observed in Mb-NO. We suggest that the reduction of NO to N<sub>2</sub>O by *ba*<sub>3</sub>-oxidase proceeds by the fast binding of the first NO molecule to heme *a*<sub>3</sub> with high-affinity, and the second NO molecule binds to Cu<sub>B</sub> with low-affinity, producing the temporal co-presence of two NO molecules in the heme-copper center. The low-affinity of Cu<sub>B</sub> for NO binding also explains the NO reductase activity of the *ba*<sub>3</sub>-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<sub>B</sub> ligands, the structure of the binuclear heme *a*<sub>3</sub>-Cu<sub>B</sub><sup>1+</sup> 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.