Mechanism and Assembly of the Denitrificatory Nitrous Oxide Reductase

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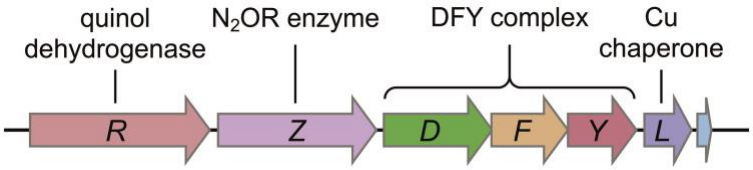
Copper-dependent nitrous oxide reductase is the only known metabolic enzyme that reduces the potent greenhouse gas and ozone-depleting agent N_2O to uncritical N_2 as the final step of denitrification. Its active site comprises the dinuclear Cu_A center, as well as the unique Cu_Z, a [4Cu:2S] cluster that activates the inert gas and shows a substantial degree of structural flexibility [1]. The mechanism of N_2O reduction at this active site remains under debate, with recent results pointing towards proton-coupled electron transfer from Cu_A to the substrate [2], concomitant with a rearrangement of Cu_Z upon binding to N_2O [3].

Recently we reported on the heterologous production of active nitrous oxide reductase from *Pseudomonas stutzeri* in *E. coli* using a re-factored two-plasmid system [4], which also required the production of the entire assembly machinery required to insert the two metal sites. Most unusually, Cu_A and Cu_Z are inserted and matured in the periplasm, while the apoprotein is exported as a fully formed 130 kDa dimer *via* the Tat system. While this strategy avoids problems related to the potential toxicity of copper in the cytoplasm, it poses a series of logistic challenges that include the handling of reduced Cu(I) in the oxidizing extracellular environment, the times and coordinated insertion of the metal and sulfide ions into the protein and the lack of a metabolic energy source such as ATP in the periplasm that can be used to drive the process.



We have produced and analyzed all components of the machinery required for the maturation and operation of N_2O reductase and characterized the players by X-ray crystallography and cryo-EM. This includes the copper chaperone NosL [5] and the unusual ABC transporter NosDFY that interacts with both the chaperone and the enzyme for copper transfer [6]. The emerging, detailed functional picture of copper delivery and copper site assembly in this unique metalloenzyme will be discussed.

References

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