Mechanism of Nitrous Oxide Reduction with Activated N_2O Reductase from *Achromobacter Cycloclastes*

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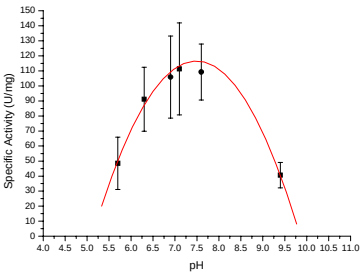
Denitrification is the reductive cascade from nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) to dinitrogen (N_2) and therefore is an intrinsic process of the global nitrogen cycle. N_2O is kinetically inert but is thought to contribute to ozone depletion and global warming. Therefore, it is important to achieve a comprehensive understanding of N_2O metabolism. N_2O reductase (N_2OR) catalyzes the two electron reduction of N_2O to N_2 and H_2O. This is the terminal step of the denitrifying process. According to the recent crystallographic results on homodimeric N_2ORs from *Pseudomonas nautica*[1] and *Paracoccus denitrificans*[2], this enzyme contains two copper centers, Cu_A and Cu_Z. The Cu_A site in N_2OR is quite similar to that in cytochrome c oxidase and is implicated in electron transfer to the active site. On the other hand, the Cu_Z site is a unique structural motif consisting of μ_4-sulfide bridged tetranuclear Cu cluster. Although the Cu_Z site is thought to function as a catalytic center to reduce N_2O, the mechanism of N_2O reduction is not well understood. Recently, we isolated a recombinant N_2OR from *Achromobacter cycloclastes* (*Ac*) and found it is significantly activated towards N_2O reduction by reduced methyl viologen (MV). Moreover, the activated *Ac* N_2OR is less colored and has a low intensity EPR signal after preincubation with reduced MV.[3] These results indicate that the catalytically active state of Cu_Z in *Ac* N_2OR is 4[Cu(I)].

Herein, pH dependence of the activation and the reactivity of the activated *Ac* N_2OR towards the reaction with N_2O will be shown in order to shed light on the mechanism of N_2O reduction in the N_2OR. Preliminary results of the pH profile suggest the activity of the *Ac* N_2OR is enhanced under neutral pH conditions. The plausible mechanism of N_2O reduction with N_2OR will be discussed on the basis of the data. In addition, influence of anion species such as chloride and azide towards the activity of N_2ORs will be taken into consideration.



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