Novel Metal Binding Sites of Denitrifying Enzymes

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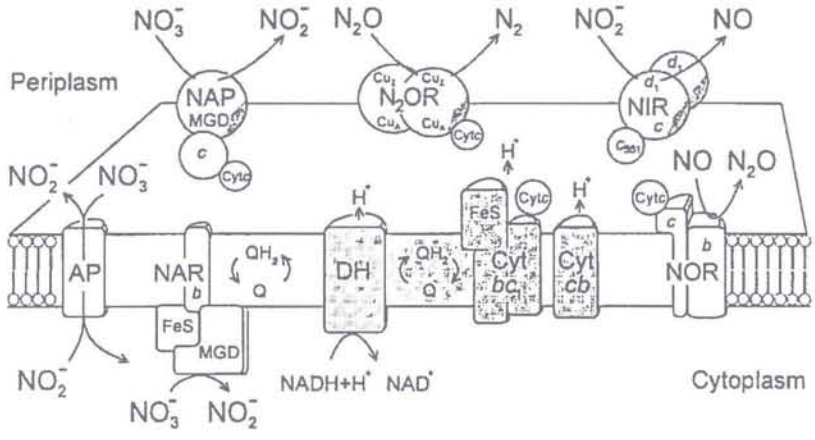
Denitrification is defined as dissimilatory or respiratory transformation of nitrate (NO_3^-) to gaseous nitrogen (N_2) for the purpose of energy conservation in bacterial cells.[1,2]

$$NO_{3\,(aq)}^- \rightarrow NO_{2\,(aq)}^- \rightarrow NO_{(g)} \rightarrow 2N_2O_{(g)} \rightarrow N_{2(g)}$$

The N oxyanions and the N oxides are utilized in place of oxygen as the terminal electron acceptors during anaerobic electron transport phosphorylation. The importance of denitrification arises from the fact that it constitutes one of the main branches of the global nitrogen cycle.[1] Recently, denitrificaiton process is examined more closely due to growing concerns about the environment. Nitrous oxide, also know as laughing gas, has become third most significant contributor to the global warming.[3,4] Nitrate, regardless to its role in plant growth, is now believed to cause surface and ground water contamination.[1]

All of the denitrifying enzymes are metalloproteins, and each enzyme carries different types of metal binding sites that are essential to its specific catalytic activity. Nitrate reductase binds molybdopterin guanine dinucleotide (MGD) and an iron-sulfur cluster in one of the subunits.[5,6] Nitrite reductase and nitrous oxide reductase are copper containing proteins.[7,8] Nitric oxide reductase is an integral membrane protein that binds heme *b* and *c* in subunit B and C, respectively.[9,10]



The anaerobic electron transport chain of a denitrifying enzyme (*P. stutzeri*).[1]

Nitrate reductase catalyzes the first step of denitrification. The crystal structure of the first respiratory nitrate reductase has been solved.[11] However, characterization of the catalytic cycle seems rather challenging. EPR (electron paramagnetic resonance) spectroscopy and EXAFS (extended X-ray absorption fine structure) spectroscopy are widely used techniques in the study of molybdoenzymes.[12] A few years ago, a series of distinctive Mo(V) EPR spectra (High g [resting], High g [nitrate], Low g [split]) were identified from periplasmic nitrate reductase isolated under different conditions.[13,14] Further studies on the catalytic cycle of nitrate reductase suggests that a Mo(IV) mono-oxo to Mo(VI) di-oxo cycle is most prefered.[6,13,14] The proposed catalytic cycle shows an unusual coordination of the Mo(VI) metal which binds seven ligands. This type of Mo(VI) coordination is also shown in the crystal structure of dimethyl sulfoxide reductase (DMSOR).[15] Recently, several inorganic model compounds have been synthesized and provide valuable information regarding nature of the catalytic intermediates during enzyme turnover.[16]

The last step of denitrification is nitrous oxide reduction to nitrogen. Nitrous oxide reductases are homodimeric proteins carrying two copper binding sites, Cu_A and Cu_Z, in each subunit.[2,17,18] Previously, both copper sites were considered to be dinuclear copper centers. However, recent crystallographic data revealed that the structure of Cu_Z site contains 4 copper atoms bridged by one hydroxo or sulfur ligand.[2,19] However, the stretching mode of Cu-S in resonance Raman spectroscopy strongly suggests that the bridging ligand is sulfur.[8]

References

1. Zumft, W. G. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 533-616.

2. Rosenzweig, A. C. *Nat. Struct. Biol.* **2000**, *7*, 169-171.

3. Robertson, G. P.; Paul, E. A.; Harwood, R. R. *Science (Washington, D. C.)* **2000**, *289*, 1922-1925.

4. Dickinson, R. E.; Cicerone, R. J. *Nature (London)* **1986**, *319*, 109-115.

5. Guigliarelli, B.; Magalon, A.; Asso, M.; Bertrand, P.; Frixon, C.; Giordano, G.; Blasco, F. *Biochemistry* **1996**, *35*, 4828-4836.

6. Butler, C. S.; Charnock, J. M.; Bennett, B.; Sears, H. J.; Reilly, A. J.; Ferguson, S. J.; Garner, C. D.; Lowe, D. J.; Thomson, A. J.; Berks, B. C.; Richardson, D. J. *Biochemistry* **1999**, *38*, 9000-9012.

7. Suzuki, S.; Kataoka, K.; Yamaguchi, K.; Inoue, T.; Kai, Y. *Coord. Chem. Rev.* **1999**, *190-192*, 245-265.

8. Rasmussen, T.; Berks, B. C.; Sanders-Loehr, J.; Dooley, D. M.; Zumft, W. G.; Thomson, A. J. *Biochemistry* **2000**, *39*, 12753-12756.

9. Moeenne-Loccoz, P.; Richter, O.-M. H.; Huang, H.-w.; Wasser, I. M.; Ghiladi, R. A.; Karlin, K. D.; de Vries, S. *J. Am. Chem. Soc.* **2000**, *122*, 9344-9345.

10. Hendriks, J.; Oubrie, A.; Castresana, J.; Urbani, A.; Gemeinhardt, S.; Saraste, M. *Biochim. Biophys. Acta* **2000**, *1459*, 266-273.

11. Dias, J.; Than, M.; Humm, A.; Bourenkov, G. P.; Bartunik, H. D.; Bursakov, S.; Calvete, J.; Caldeira, J.; Carneiro, C.; Moura, J. J.; Moura, I.; Romao, M. J. *Structure (London)* **1999**, *7*, 65-79.

12. Bray, R. C. *Q. Rev. Biophys.* **1988**, *21*, 299-329.

13. Bennett, B.; Berks, B. C.; Ferguson, S. J.; Thomson, A. J.; Richardson, D. J. *Eur. J. Biochem.* **1994**, *226*, 789-798.

14. Bennett, B.; Charnock, J. M.; Sears, H. J.; Berks, B. C.; Thomson, A. J.; Ferguson, S. J.; Garner, C. D.; Richardson, D. J. *Biochem. J.* **1996**, *317*, 557-563.

15. McAlpine, A. S.; McEwan, A. G. *J. Biol. Inorg. Chem.* **1997**, *2*, 690-701.

16. Thapper, A.; Christian, L.; Fryxelius, J.; Behrens, A.; Nordlander, E. *J. Inorg. Biochem.* **2000**, *79*, 67-74.

17. Prudencio, M.; Pereira, A. S.; Tavares, P.; Besson, S.; Cabrito, I.; Brown, K.; Samyn, B.; Devreese, B.; Beeumen, J. V.; Rusnak, F.; Fauque, G.; Moura, J. J. G.; Tegoni, M.; Cambillau, C.; Moura, I. *Biochemistry* **2000**, *39*, 3899-3907.

18. Zumft, W. G.; Matsubara, T. *FEBS Lett.* **1982**, *148*, 107-112.

19. Brown, K.; Tegoni, M.; Prudencio, M.; Pereira, A. S.; Besson, S.; Moura, J. J.; Moura, I.; Cambillau, C. *Nat. Struct. Biol.* **2000**, *7*, 191-195.