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NITROGENASE MECHANISM

A dynamic tool for nitrogen reduction

Carbon monoxide reveals new possibilities for substrate binding in nitrogenase

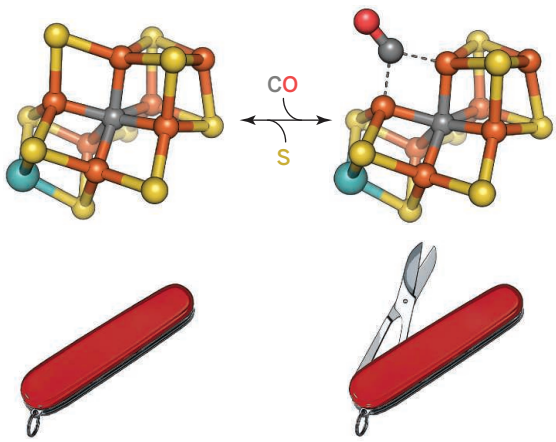
By **Martin Högbom**

Even though nitrogen makes up almost 80% of the atmosphere, it is a limiting nutrient for biomass production. The low reactivity of nitrogen gas (N_2) is a result of its very strong, unpolarized triple bond. Nitrogenase is the only enzyme known that can break this bond to produce compounds such as ammonia (NH_3) for use in biosynthetic pathways. The atomic structure of this amazing system has been known for more than two decades (*1*, *2*), but the chemical mechanism of this central reaction remains unknown. In a biochemical and structural tour de force, on page 1620 of this issue, Spatzal *et al.* (*3*) report the crystal structure of carbon monoxide (CO) bound to the catalytic metal cluster of the enzyme. This work revealed an unexpected structural rearrangement of the cofactor.

The industrial equivalent of the nitrogenase reaction is the Haber-Bosch process developed during the first decades of the 20th century (*4*). In this process, N_2 is reacted with H_2 to produce NH_3. The overall reaction is energetically favorable but has a very high activation barrier. In the Haber-Bosch process, this barrier is overcome with an iron-based inorganic catalyst at high temperatures and pressures (400°C and 200 atm).

Whereas the Haber-Bosch process uses a big hammer to break the triple bond, nitrogenase uses a much more elegant and complicated catalytic tool to disassemble N_2 at ambient temperature and pressure. Enzymes commonly use transition metal ions to perform the most challenging redox reactions. Nitrogenase houses a dauntingly complicated cofactor consisting of no less than one molybdenum, seven iron, and nine sulfur ions, all beautifully organized around a central carbon. It was only a few years ago that the identity of the central atom was finally determined (*5*, *6*).

Although we know what this complicated tool does and looks like, we have not been able to understand how it functions or to obtain snapshots of it in action. A number of mechanistic proposals have been made that suggest either molybdenum or iron as the central catalytic element. Thus, even the key clue of which part of the cofactor directly interacts with the substrate has been missing (*7*). This issue is commonly studied by determining structures of the protein cofactor with various ligands bound, such as inhibitors or substrate analogs. Carbon monoxide (CO) is a particularly attractive ligand to obtain insight into nitrogenase chemistry. It is a diatomic neutral molecule that is isoelectronic with N_2. It also functions as a reversible inhibitor of N_2 reduction and can even act as a substrate and be chemically modified by the enzyme, albeit very slowly.



Uncovered potential. The catalytic Mo/Fe/S metal cluster of nitrogenase is shown (molybdenum in turquoise, iron in orange, carbon in gray, and sulfur in yellow). Binding of carbon monoxide rearranges the cluster, revealing new possibilities for substrate binding and reduction.

Such experiments have proven very difficult to perform. To bind any ligand, the cofactor needs to be in its reduced form. Nitrogenase will only accept its natural reductant, an oxygen-sensitive iron-sulfur protein. Also, CO will only bind to the partially reduced protein generated in a reaction mixture during enzymatic turnover, conditions opposite to the very pure protein solutions usually required for crystallization. This resulted in a wall of technical obstacles that had prevented determination of complex structures and a detailed structural understanding of the mechanism.

Spatzal *et al.* overcame these obstacles with a number of elegant procedures—for example, by succeeding to rapidly crystallize the protein directly from enzymatic reaction mixtures. The structure provided a great surprise in that CO binding actually reorganizes the cofactor structure. X-ray anomalous-scattering measurements show that one of the sulfur atoms is removed from the cluster. This vacancy opens a new binding position on the cofactor where a substrate can coordinate two iron ions simultaneously in a bridging manner. Carbon monoxide binds in this position, where it interacts with both iron ions, and it is also in close proximity to the central carbon atom of the cluster.

The direct interaction with iron indicates that there may be mechanistic similarities to the iron-catalyzed Haber-Bosch reaction. Interestingly, the di-iron bridging position also has similarities to substrate-binding modes proposed in other enzymes performing—for example, reduction of O_2 and H_2, as well as other demanding electron-transfer reactions (*8*–*12*). The rearrangement of the nitrogenase metal cluster thus reveals a new reaction surface that appears to provide possibilities for very challenging chemistry.

Together, the results suggest that the previously available picture of the cofactor actually shows a tool protected for storage rather than ready to perform its function. The mode of N_2 binding cannot be conclusively assigned, but the studies of Spatzal *et al.* establish the structurally dynamic nature of the cofactor. The study does not give a complete and final structural description of nitrogenase catalysis, but it does provide the first crack in the wall. ■

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