with an amine in the presence of hydrogen or another reducing agent to form an alkylated amine. The rhodium complexes that catalyze hydroformylation also catalyze reductive amination (*6*). Sequential hydroformylation and reductive amination of the intermediate aldehyde in the same reaction pot—called hydroaminomethylation—was discovered by Walter Reppe at BASF in the 1940s. A version of this reaction catalyzed by rhodium complexes has been studied more recently in the context of small-scale synthesis (*7*). The reaction starts with inexpensive reagents and generates valuable products, but the potential for commercial chemical synthesis has not been exploited.

The work of Seayad *et al.* begins to demonstrate even greater potential for this reaction, which brings us back to the formation of a single product from a mixture of reactants. Some commercial hydroformylations start from mixtures of isomeric alkenes and generate mostly terminal alcohols or aldehydes (*8–10*). Several new transition metal complexes that catalyze both isomerization and hydroformylation have been reported recently (*11–13*). But most catalysts for the hydroformylation of internal alkenes are ineffective for hydroaminomethylation because amines poison their isomerization activity. The poisoning is probably caused by amines displacing ligands from the metal center of the catalyst.

Seayad *et al.* have found a catalyst composition that is immune to this poisoning, allowing isomerization and hydroformylation to be linked to reductive amination. The soluble compound isomerizes alkenes, catalyzes hydroformylation to form terminal aldehydes, and catalyzes reductive amination to convert the terminal aldehyde to a terminal amine (see the figure).

The catalyst has a simple composition that emerged from modification of structures used previously for hydroformylation and from consideration of a fundamental principle of transition metal chemistry: Ligands with two donor atoms tethered to each other (chelating or bidentate ligands) resist displacement by monodentate ligands (*14*). The catalyst of Seayad *et al.* makes use of this principle of chelation to prevent coordination of amine.

The Seayad *et al.* group, led by Beller, recently modified a ligand used by Eastman Chemicals for hydroformylation (*15*) to create a catalyst for the hydroformylation of internal alkenes to terminal aldehydes (*15*). The modified ligand contains two phosphorus donors that chelate the metal. The rhodium complex with the modified ligand therefore resists coordination of amine and catalyzes both alkene isomerization and the final reductive amination. By luck or design, the catalyst that most effectively isomerizes olefins is also the most active for the final reductive amination.

As exciting as this work is, high hurdles remain before the process can be used to produce millions, thousands, or even hundreds of kilograms of amines. First, the ratio of terminal to internal amine produced by the reaction is much lower than the best ratios of terminal to internal aldehyde produced from commercial hydroformylations, and the rates are slower than would be needed for a commercial process. Second, the most useful amines are terminal primary amines. Beller and co-workers recently reported the selective hydroaminomethylation of terminal alkenes with ammonia (*16*), but the current system apparently does not catalyze additions of ammonia. Finally, diamines, such as those used to generate nylon, are produced on the largest scale. Hydroaminomethylation of dienes, particularly butadiene, will pose additional challenges for the catalyst.

Nonetheless, the work of Seayad *et al.* points the way to useful hydroaminomethylations. It should therefore generate a flurry of activity on this reaction. A commercially viable production of terminal amines from hydrogen, carbon monoxide, and ammonia would constitute a spectacular achievement for homogeneous catalysis and transition metal chemistry.

References

1. P. J. Chenier, *Survey of Industrial Chemistry* (VCH, Weinheim, Germany, ed. 2, 1992).
2. A. Seayad *et al.*, *Science* **297**, 1676 (2002).
3. K. S. Hayes, *Appl. Catal. A* **221**, 187 (2001).
4. B. M. Trost, *Science* **254**, 1471 (1991).
5. P. A. Wender, *Chem. Rev.* **96**, 1 (1996).
6. V. I. Tararov, R. Kadyrov, A. Borner, T. H. Riermeier, *Chem. Commun.* **2000**, 1867 (2000).
7. P. Eilbracht *et al.*, *Chem. Rev.* **99**, 3329 (1999).
8. E. Billig, A. G. Abatjoglou, D. R. Bryant (Union Carbide, 1987) EP213639 [Chem. Abstr. 1987, 107, 7392r].
9. P. M. Burke *et al.* (DSM, Du Pont, 1997) WO 97/33854 [Chem. Abstr. 1997, 127, 294939r].
10. L. H. Slaugh, R. D. Mullineaux (Shell Oil Co., 1966), U.S. Patents 3,239,569 and 3,239,570.
11. L. A. van der Veen, P. C. J. Kamer, P. van Leeuwen, *Angew. Chem. Int. Ed. Engl.* **38**, 336 (1999).
12. B. Breit, R. Winde, K. Harms, *J. Chem. Soc. Perkin Trans. 1* **1997**, 2681 (1997).
13. H. Klein, R. Jackstell, K. D. Wiese, C. Borgmann, M. Beller, *Angew. Chem. Int. Ed. Engl.* **40**, 3408 (2001).
14. J. E. Huheey, E. A. Keiter, R. L. Keiter, in *Inorganic Chemistry: Principles of Structure and Reactivity* (HarperCollins College, New York, 1993), pp. 522–531.
15. T. J. Devon, G. W. Phillips, T. A. Puckette, J. L. Stavinoha, J. J. Vanderbilt, U.S. Patent 4,694,109 (1987).
16. B. Zimmermann, J. Herwig, M. Beller, *Angew. Chem. Int. Ed. Engl.* **38**, 2372 (1999).

PERSPECTIVES: STRUCTURE

Nitrogenase Reveals Its Inner Secrets

Barry E. Smith

Given sufficient water, plant growth and therefore agricultural productivity is usually limited by the amount of bioavailable (fixed) nitrogen. Biological nitrogen fixation still contributes about half of the total nitrogen input to global agriculture, the rest principally coming from nitrogenous fertilizer produced chemically from the Haber-Bosch synthesis of ammonia. To produce the hydrogen gas together with the high temperatures and pressures needed for this chemical process, about 1% of the world's total annual energy supply has to be consumed. In marked contrast, a similar chemical process requiring only atmospheric temperature and pressure is carried out by nitrogen-fixing bacteria, many of which live in symbiotic association with legume plants. The secret of their success is the enzyme nitrogenase, which transforms atmospheric nitrogen gas (dinitrogen) into ammonia that plants can then use for growth. Many groups have tried for decades to determine how nitrogenase catalyzes this essential process. Now, a high-resolution structure of part of bacterial nitrogenase reported by Einsle *et al.* (*1*) on page 1696 of this issue yields some surprises about the biosynthesis and catalytic activity of this crucial metalloenzyme.

Nitrogenase (*2*) consists of two essential metalloproteins: one, the iron (Fe) protein, is a very specific ATP-activated electron donor to the other, the molybdenum-iron (MoFe) protein. The MoFe protein contains two unique metallosulfur clusters: the P cluster [8Fe-7S] and the [Mo:7Fe:9S]:homocitrate iron-molybdenum (FeMo) cofactor cluster. About a decade ago, the first, relatively low-resolution (2.8 Å) crystal structure of the MoFe protein was reported (*3*). At this level of resolution, there were still some uncertainties about the structures of the metalloclusters. However, subsequent improvements in resolution to 2.0 Å (*4*) and then to 1.6 Å (*5*) yielded what seemed to be the accurate structure of the FeMo cofactor (see the figure). The FeMo cofactor

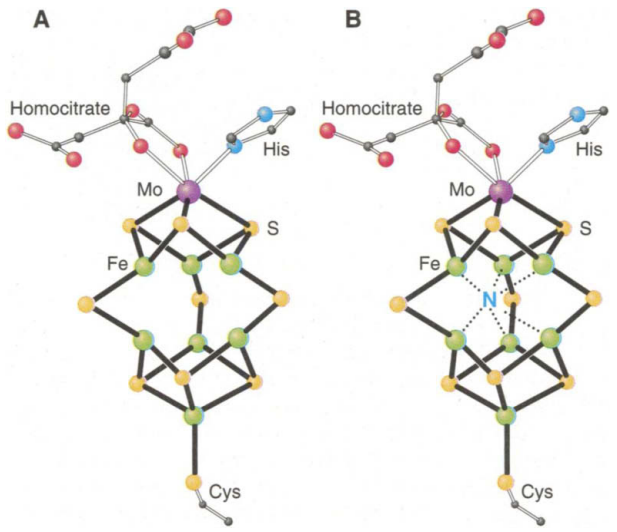
The author is at the John Innes Centre, Colney, Norwich NR4 7UH, UK. E-mail: barry.smith@bbsrc.ac.uk

is bound to the MoFe protein through both a cysteine sulfur ligand (binding to the terminal tetrahedral iron atom) and a histidine ligand (binding to the molybdenum atom, which also binds to the homocitrate through its hydroxyl group and one carboxyl group). One of the features of the structure that has excited considerable interest is the trigonal nature of the other six iron atoms, which appear to be coordinately bound to only three atoms instead of the usual four or more atoms.

Einsle *et al.* report the structure of the MoFe protein of bacterial nitrogenase at an improved resolution of 1.16 Å (*1*). This is a major achievement with a protein of this molecular size (~240,000 daltons). The authors suggest that the analyses of lower resolution data were confused by series termination effects. These are known to lead to resolution-dependent ripples around atomic positions in Fourier analyses of crystallographic data. They conclude that such effects associated with the six central iron atoms and the nine sulfur atoms of the FeMo cofactor would combine, with lower resolution data, to produce a significant negative ripple in the center of the cluster, thus obscuring the electron density of a light atom. These effects are less dominant at high resolution, and their experimental data clearly show electron density at the center of the FeMo cofactor. Therefore, Einsle *et al.* interpret their data in terms of a model that includes an interstitial (internal) hexacoordinate light atom within the FeMo-cofactor cluster that is bonded to each of the six iron atoms (see the figure). Furthermore, they argue that the light atom is most likely to be nitrogen. Carbon or oxygen cannot be ruled out, but sulfur is unlikely. The consequences of these observations for our understanding of nitrogenase catalytic activity are considerable.

There is excellent evidence that the FeMo-cofactor clusters act as the enzyme's substrate-binding and -reducing site (*2*), but exactly how and where substrates bind and are activated remains controversial. Attention has focused on the six trigonal iron atoms that seem to have unsatisfied bonding potential. Furthermore, there is increasing evidence from site-directed mutagenesis studies that acetylene and cyanide, which are alternative substrates for the enzyme, bind to one facet of the cluster containing four iron atoms (*6*). However, an alternative view is that dinitrogen binds to molybdenum, possibly after dissociation of the carboxyl ligand from homocitrate. There are good chemical precedents for this proposal (*7*).

If the interstitial atom is nitrogen, there is a possibility that it is inserted into the FeMo cofactor during enzyme turnover, and clearly it is bound to iron. Einsle *et al.* point out that Thorneley and Lowe, in developing a kinetic description of nitrogenase turnover (*8*), found that three electrons had to be transferred, one at a time, from the Fe protein to the MoFe protein before dinitrogen could bind. They suggest that this might reflect the need to replace the electrons used to reduce the interstitial nitrogen to nitride before its release as ammonia. However, if the interstitial nitrogen had to be displaced before a new molecule of dinitrogen could bind, then the pre–steady-state kinetics should show a stoichiometric burst of ammonia being produced after three electrons are transferred. This is not consistent with the data. Furthermore, Thorneley and Lowe found that a partially reduced, enzyme-bound dinitrogen hydride intermediate was formed earlier than ammonia in the reaction cycle, that is, ammonia is not released early in the cycle.



Nitrogen in the middle. Model of the FeMo cofactor of the nitrogen-fixing enzyme nitrogenase (**A**) before and (**B**) after the Einsle *et al.* report (*1*). This report presents a high-resolution structure of the MoFe protein of nitrogenase, which contains the FeMo cofactor. The new work reveals a previously unrecognized interstitial atom in the FeMo cofactor that may possibly be nitrogen (blue N). Carbon, gray; iron, green; sulfur, yellow; molybdenum, purple; oxygen, red; and nitrogen, blue.

Interstitial nitrogen atoms have been observed in rhodium and cobalt carbonyl clusters and are considered to stabilize them, the rhodium clusters being particularly robust (*9*). Therefore, the role of the interstitial atom may be to stabilize the FeMo cofactor. This leads to the question of how it is inserted. The biosynthesis of the FeMo cofactor is extremely complex and involves the products of at least six genes (*2*). The details of the process are not understood, and the need to insert an interstitial nitrogen atom adds a further complication. However, the observation of the interstitial atom opens up a new range of possibilities for the synthetic chemists who thus far have not been able to synthesize a chemical equivalent of the FeMo cofactor (*7*).

There have been several theoretical attempts to model the FeMo cofactor's structure and reactivity (*2*). Most have proposed that the dinitrogen binds to iron, either across or end-on to a face or between a pair of iron atoms, although some calculations indicate that binding to molybdenum is possible. The most sophisticated theories appear to reproduce reasonably successfully the structural and spectroscopic parameters of the FeMo cofactor (*10*). Of course, an interstitial atom has not been considered in any of these theoretical calculations. It seems probable that this atom would alter these models considerably, and would certainly perturb the formal oxidation states of the iron atoms. The six central iron atoms can no longer be considered trigonal, but are closer to tetrahedral in character.

The high-resolution structure of the FeMo cofactor of nitrogenase will force us to think again about how the enzyme is biosynthesized and how it catalyzes the production of ammonia from dinitrogen. The work of Einsle and colleagues provides fresh challenges for synthetic chemists and theoreticians. Once again, nitrogenase has surprised us.

References and Notes

1. O. Einsle *et al.*, *Science* **297**, 1696 (2002).
2. B. E. Smith, in *Advances in Inorganic Chemistry*, A. G. Sykes, R. Cammack, Eds. (Academic Press, London, 1999), vol. 47, pp. 159–218.
3. J. S. Kim, D. C. Rees, *Science* **257**, 1677 (1992).
4. J. W. Peters *et al.*, *Biochemistry* **36**, 1181 (1997).
5. S. M. Mayer, D. M. Lawson, C. A. Gormal, S. M. Roe, B. E. Smith, *J. Mol. Biol.* **292**, 871 (1999).
6. P. M. C. Benton *et al.*, *Biochemistry* **40**, 13816 (2001).
7. C. J. Pickett, *J. Bioinorg. Chem.* **1**, 601 (1996).
8. R. N. F. Thorneley, D. J. Lowe, in *Molybdenum Enzymes*, T. G. Spiro, Ed. (Wiley-Interscience, New York, 1985), vol. 1, pp. 221–230.
9. A. Fumagalli *et al.*, *J. Am. Chem. Soc.* **119**, 1450 (1997).
10. T. Lovell, J. Li, T. Liu, D. A. Case, L. Noodleman, *J. Am. Chem. Soc.* **123**, 12392 (2001).
11. I thank D. Hughes for useful discussions and the Leverhulme Foundation for an Emeritus Fellowship.