A newly discovered role for iron-sulfur clusters

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Nature has exploited the structural and electronic plasticity of small inorganic structures called [Fe-S] clusters (Fig. 1) in at least five fundamental ways: (i) electron transfer, (ii) Lewis acid-assisted enzyme catalysis, (iii) radical generation, (iv) sulfur donation, and (v) control of protein conformational changes associated with signal transduction (1). In their role as agents of electron transfer, it is known that polypeptide environments that surround [Fe-S] clusters modulate their midpoint potentials so that electron flow can be controlled and directed. In their capacity to function as a Lewis acid, [Fe-S] clusters facilitate activation of a variety of electron-rich organic substrates. The dehydratase reaction within the tricarboxylic acid cycle, catalyzed by aconitase, provides one example of this type of [Fe-S] cluster-assisted metabolic transformation. For radical-mediated catalysis, [Fe-S] clusters interact with *S*-adenosylmethionine to initiate formation of an activated adenosyl radical, which is subsequently used for generation of protein- or substrate-associated radical intermediates. Members of this class of enzyme include anaerobic ribonucleotide reductase, biotin synthase, and lipoate synthase. Some members of the *S*-adenosylmethionine family of [Fe-S] proteins also appear to use an additional “sacrificial” [Fe-S] cluster as the source of S necessary for the assembly of certain cofactors such as biotin and lipoic acid. The facile assembly, disassembly, and rearrangement of [Fe-S] clusters in response to Fe or oxygen availability, or when exposed to superoxide, has resulted in their ability to serve as environmental sensors. For example, the activity of regulatory proteins that contain [Fe-S] clusters is frequently controlled through conformational changes affected by the redox state or nuclearity of their cognate [Fe-S] clusters. In this issue of PNAS, Luis Rubio, David Britt, and their colleagues (2) propose yet another function for an [Fe-S] cluster. In this case, the cluster is involved in the sequestration and delivery of Mo required for the assembly of FeMo-cofactor, the complex metallocluster that provides the active site for N₂ reduction within the nitrogenase MoFe protein.

The function and formation of FeMo-cofactor (MoFe₇S₉X-homocitrate) (Fig.

Fig. 1. Schematic of the proposed FeMo-cofactor assembly process. NifU, NifB, and NifEN assembly scaffolds are indicated as U, B, and EN. The nitrogenase MoFe protein is indicated by DK. Metalloclusters that have been identified or have been proposed to be attached to these proteins are indicated below the designated proteins. The MoFe₃S₄ cluster, which is proposed to supply Mo required for FeMo-cofactor assembly is indicated below the designation for NifQ. The biological mechanism for insertion of Mo into the Fe₃S₄ cluster is not known. Although not shown here, reducing equivalents, Fe protein and MgATP, are also required for completion of FeMo-cofactor assembly. Cluster carrier proteins are designated as X and Y. V catalyzes the formation of R-homocitrate by using α -ketoglutarate (α -KG) and acetyl-CoA as substrates. Iron, green; sulfur, yellow; oxygen, red; carbon, gray; molybdenum, magenta; unknown light atom, blue.

1) (3) has long captured the interest and imagination of chemists because of its pivotal role in substrate activation during biological nitrogen fixation. This complex metallocluster contains Fe₄S₃ and MoFe₃S₃ cores that are connected by three bridging S²⁻ and an interstitial light atom of unknown identity (Fig. 1). FeMo-cofactor also has an associated organic constituent, homocitrate, which is attached to the Mo atom through its 2-hydroxyl and 2-carboxylate groups. Given the complexity of FeMo-cofactor, understanding its assembly has presented an enormous challenge to both chemists and biochemists. It was established many years ago that FeMo-cofactor is synthesized separately and then inserted into an apo-form of MoFe protein (4). This observation led to the development of the so-called scaffold hypothesis for the assembly of complex metalloclusters (5), a concept that was later demonstrated to also occur in the assembly of simple [Fe-S] clusters (6). A series of remarkable studies from the laboratories of Markus Ribbe and Luis Rubio have now significantly advanced our understanding of how FeMo-cofactor is assembled, and this topic has been described in two recent reviews (7, 8). Although many details remain to be clarified, and some aspects of the process continue to be contentious, a consensus view of some of the key features has emerged: (i) FeMo-cofactor is sequentially assembled *in vivo* on a series

of assembly scaffold proteins designated NifU, NifB, and NifEN (Fig. 1); (ii) a family of small carrier proteins, designated NifX, NifY, and NafY, serve to shuttle FeMo-cofactor or its biosynthetic intermediates to or from the assembly sites; (iii) Fe protein, the agent of nucleotide hydrolysis and electron delivery during the catalytic reduction of N₂, is also required for the nucleotide-dependent completion of FeMo-cofactor assembly, including some aspect of Mo insertion.

Unraveling the specific details of *in vivo* FeMo-cofactor assembly is complicated by the fact that some components are dispensable under certain growth conditions or they are not necessarily required for *in vitro* FeMo-cofactor biosynthesis. For example, the *in vivo* function of NifU can be replaced *in vitro* by the simple addition of S²⁻ and Fe²⁺. In the case of NifQ, physiological manifestation of a defect in the capacity for nitrogen fixation associated with loss of its function can be suppressed by the addition of an excess of either molybdate or cysteine to the growth medium, indicating that a thiol-coordinated form of Mo could be an active species in the as-

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See companion article on page 11679.

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sembly of FeMo-cofactor (9). In the work of Hernandez *et al.* (2), NifQ was purified and found to contain both an [Fe-S] cluster and Mo. Both of these observations were anticipated given the clustering of cysteines within the NifQ sequence and the Mo/cysteine-dependent phenotype that is associated with loss of NifQ function. What was not anticipated, and the exciting aspect of this work, is that Mo is contained within NifQ as part of the associated [Fe-S] cluster as a MoFe₃S₄ species. As described briefly below, this work is an elegant example of how advanced spectroscopy coupled with the precedence of synthetic inorganic chemistry can provide biological insight.

The as-isolated form of NifQ was found to exhibit a narrow, almost radical-like lineshape, electron paramagnetic resonance (EPR) spectrum, indicating the presence of an oxidized Fe₃S₄ cluster, such as found in aconitase (10). Such spectra typically disappear when treated with the reducing agent dithionite. However, in this case, reduction of as-isolated NifQ resulted in the appearance of a new spectrum. Proteins that contain Fe₄S₄ clusters as the active species, such as aconitase, are often isolated in a mixed form that contains both Fe₃S₄ and Fe₄S₄ clusters, owing to the lability of one of the Fe atoms associated with the Fe₄S₄ species. Such species can be spectroscopically differentiated, because oxidized Fe₃S₄ clusters are EPR-active and the reduced form is EPR silent, whereas the opposite

is true for Fe₄S₄ clusters. However, in the current work, the EPR spectrum of dithionite-treated NifQ is very different from what is typically observed for reduced Fe₄S₄ clusters, indicating that this

[Fe-S] clusters facilitate activation of a variety of electron-rich organic substrates.

species is not an Fe₄S₄ cluster. Identification of the composition of this species was guided by spectroscopic comparison to inorganic model compounds previously described by Richard Holm and colleagues (11). Namely, the cluster contained within the reduced NifQ sample was found to exhibit very similar EPR lineshapes and *g* values when compared with MoFe₃S₄ metal clusters prepared synthetically. The initial assignment of the Mo-containing species within NifQ as a MoFe₃S₄ cluster is also supported in the present work by more advanced spectroscopic analyses, metal analysis, and the observation that a portion of the Fe₃S₄ species in as-isolated NifQ can be converted to the proposed MoFe₃S₄ species by the addition of Mo. The MoFe₃S₄-loaded form can be used to complete *in vitro* maturation of the MoFe protein in

the absence of exogenously added molybdate, although this process also requires reducing equivalents, homocitrate, NifEN, Fe protein, and MgATP. These requirements are consistent with the suggested role of NifEN as an assembly scaffold for FeMo-cofactor formation and the proposed nucleotide-dependent participation of Fe protein in the insertion of Mo and attachment during completion of FeMo-cofactor (12).

Exactly how Mo is incorporated and then detached from the MoFe₃S₄ cluster for completion of FeMo-cofactor assembly remains to be determined. For example, it is not clear whether there is a cyclic process involving Mo loading and unloading of the available coordination site within the Fe₃S₄ cluster or whether the MoFe₃S₄ species is a sacrificial cluster that is destroyed during completion of FeMo-cofactor assembly. Furthermore, the redox state(s) of Mo that occur during its mobilization and transfer is not yet understood. Nevertheless, the present work indicates that an [Fe-S] cluster can function in the sequestration and trafficking of Mo, which provides yet another example of the biological versatility of the simple inorganic structures that Beinert *et al.* (1) have described as nature's "modular, multipurpose structures."

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