CellPress

Metalloprotein mimics – old tools in a new light

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Metalloproteins utilize metal cofactors to catalyze essential reactions in all organisms. They carry out thermodynamically challenging substrate conversions such as the oxidation of water or hydrocarbons, the reduction of nitrogen to ammonium, and generation of molecular hydrogen. Besides their fundamental role in nature, metalloenzymes have promising biotechnological applications that aim to generate high-value chemicals, drugs, nutrients, biofuels, or electricity. Recent reports that a chemically synthesized compound is able to reconstitute [2Fe]-hydrogenases, harboring an especially elaborate and highly efficient metal cofactor, promise to pave the way for gaining much deeper insight into the function of even complex metal enzymes. What is more, synthetic biology approaches such as the chemical synthesis of artificial hydrogenases seem to be in reach.

Metalloenzymes and their cofactors

Metalloproteins utilize metal cofactors to catalyze essential reactions; several of which are chemically and/or thermodynamically extremely challenging [1]. Examples are the oxidation of water, performed by photosystem (PS)2 during oxygenic photosynthesis [2], or the reduction of dinitrogen (N_2) to ammonium (NH_4^+) by nitrogenase [3]. In terms of genetic and energy investment, some of the responsible protein systems are demanding for the host cell, but in contrast to industrial and chemical processes, enzymes perform their tasks at ambient temperatures and pressures. Therefore, various applied research fields aim to exploit metalloenzymes for challenging drug-generating reactions (e.g., cytochrome P450 enzymes [4]) or to replace energy-demanding and fossil-fuel-based processes (nitrogenase [5] and hydrogenase [6]). Establishing an economy that consumes less energy and natural resources than our society does today is one of the biggest challenges of the present, and there are already many examples where enzymes are used in industrial processes [7].

The cofactors used by metalloenzymes range from single metal atoms coordinated by side chains of the polypeptide to very complex metallo-organic prosthetic groups. Mono-Fe sites in 2-his-1-carboxylate facial triad motif enzymes, for example, are coordinated only by amino acids [1] (Figure 1A). In more extensive arrangements, metal ions are bound both by the polypeptide and non-metal bridges, such as inorganic sulfide in [FeS]-clusters (Figure 1B) or oxo-groups in the Mn-containing O_2-evolving complex of PS2 [8] (Figure 1C). Many enzymes contain metal ions as parts of elaborate organic groups such as tricyclic pterins in Mo enzymes (Figure 1D) [9] or tetrapyrroles in hemoproteins [10] (Figure 1E).

A valuable tool used to understand and copy the reaction mechanisms of metalloenzymes is biomimetic chemistry, whereby both functional and structural analogs are utilized [10,11]. Functional analogs are supposed to mimic the catalytic process in the absence of the protein and can differ substantially from a natural cofactor. Structural analogs are exact chemical reproductions of the native cofactor and can be used to reconstitute the activity of an isolated enzyme. Additionally, chemically generated derivatives of native cofactors can provide substantial insights into essential groups and the interacting protein surrounding, respectively [10]. For example, using apomyoglobin and heme- or corrole derivatives, functional studies on the heme cofactor were conducted and hemoproteins with altered or new functions created [10].

Remarkably, reconstitution is also successful in case of Mo-nitrogenase [5], and, as demonstrated very recently, for hydrogenases of the [2Fe] type [12,13].

Mo-nitrogenases and [2Fe]-hydrogenases – what they have in common, and what not

Nitrogenases are the only enzymes capable of breaking the stable triple bond of the N_2 molecule under ambient conditions [5]. They exist as Mo-, V- or Fe-only nitrogenases, although Mo-nitrogenase is the most common and best studied [5]. Industrial N_2 fixation by the Haber–Bosch process needs high temperatures and pressures, and furthermore, molecular hydrogen (H_2) – nowadays mainly produced from fossil fuels in likewise energy-demanding processes. Renewably generating hydrogen for H_2-dependent syntheses and as energy carrier is therefore a further challenge. Nature's 'H_2 economy' makes use of hydrogenases, which fulfill essential reactions in the energy and redox metabolism in almost every prokaryotic and several eukaryotic groups [6]. [NiFe]- and [2Fe]-hydrogenases,

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Keywords: biocatalysts; biomimetics; hydrogenase; metalloenzymes; reconstitution; white biotechnology.

0167-7799/$ – see front matter
 http://dx.doi.org/10.1016/j.tibtech.2014.02.004

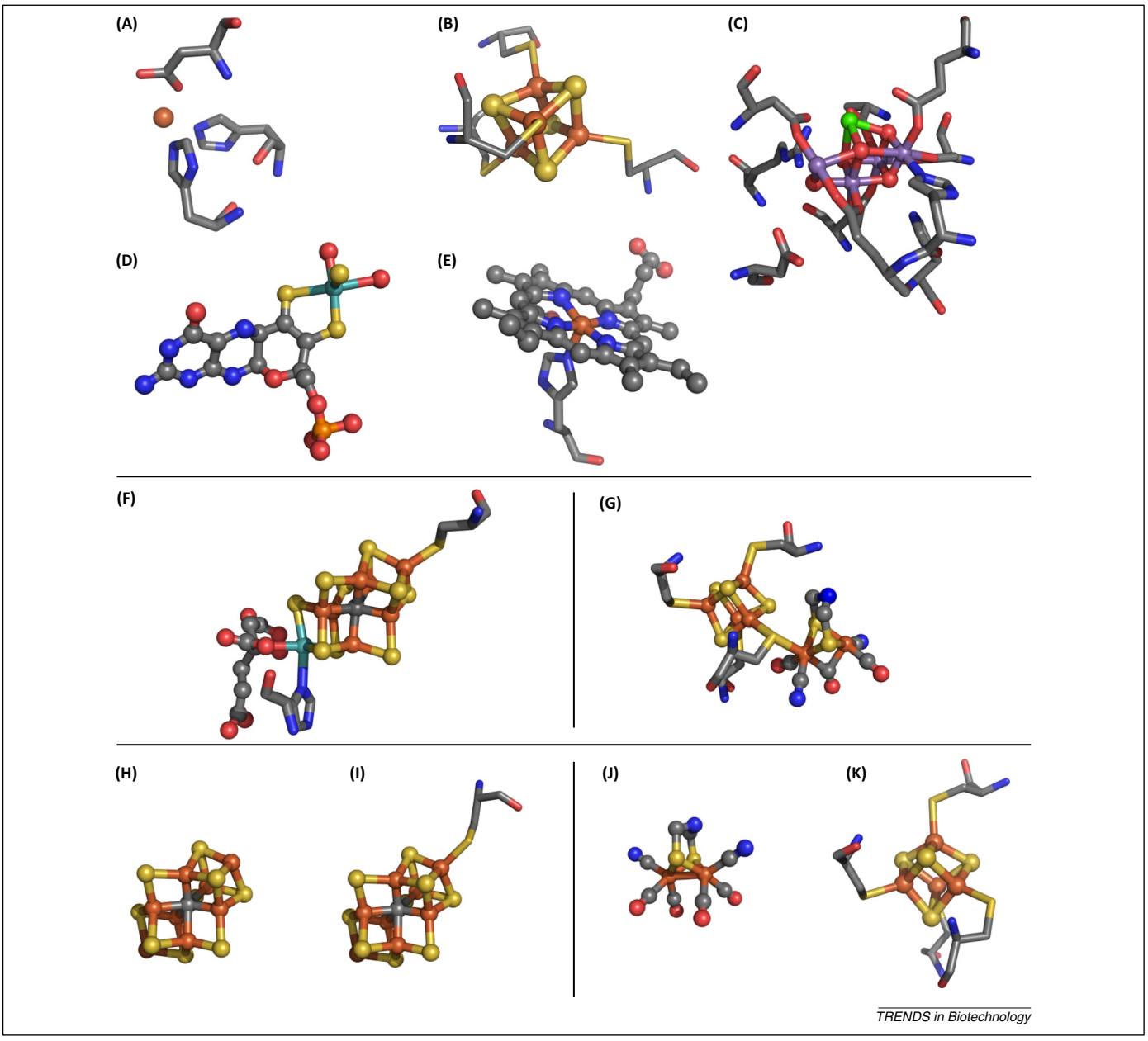

Figure 1. Active sites of metalloenzymes. Structures were modeled in PyMOL using the Protein Data Bank accession numbers indicated in brackets. **(A)** 2-His-1-carboxylate facial triad of deacetoxycephalosporin C synthase (1RXF), **(B)** [Fe_4S_4]-cluster (1ISU), **(C)** water-oxidizing complex from photosystem 2 (3ARC), **(D)** molybdopterin cofactor from xanthine oxidase (1FIQ), **(E)** heme group of myoglobin (1MBO), **(F)** FeMoco (1M1N), **(G)** H-cluster (3C8Y), **(H)** NifB-co shown as [Fe_8S_9C]-cluster as suggested by [18][a], **(I)** L-cluster[a], **(J)** $[2Fe]^{MIM}$ (artificial H-cluster diiron subsite with an azadithiolate bridge), **(K)** [Fe_4S_4]-cluster of apo-HydA (3LX4). Key: C, gray; N, blue; O, red; Fe, brown; S, yellow; Mo, turquoise; P, orange; Ca, green; Mn, purple. Abbreviations: FeMoco, FeMo cofactor; NifB, FeMoco assembly protein; NifB-co, FeMoco precursor on the NifB protein.
[a]NifB-co and L-cluster were modeled using FeMoco geometry (1M1N).

phylogenetically distinct classes with NiFe or 2Fe dinuclear active sites [6], both catalyze the chemical reaction $2e^- + 2H^+ \leftrightarrow H_2$. [NiFe]-hydrogenases have a lower efficiency and tend to oxidize H_2, whereas [2Fe]-hydrogenases are biased towards H_2 generation and can reach specific activities of 9.000 molecules H_2 per enzyme per second [14]. Not surprisingly, biotechnologists avidly work on establishing nitrogenases and [2Fe]-hydrogenases or their cofactors to be utilized economically. However, their complex and air-sensitive metal active sites, whose assembly *in vivo* requires specific maturases [15] (see below) and whose activity is strongly dependent on the surrounding protein sphere [16,17], have so far prevented substantial progress.

Structures of both enzymes have been elucidated [15]. The Mo-nitrogenase binary enzyme system consists of the dinitrogenase reductase (NifH), an α_2 homodimer containing one [Fe_4S_4]-cluster, and the dinitrogenase (NifDK), an $\alpha_2\beta_2$ heterotetramer. Per $\alpha\beta$ heterodimer, NifDK harbors a [Fe_8S_7]-cluster (termed P-cluster) and an FeMo cofactor (FeMoco), a [$MoFe_7S_9C$-homocitrate]-cluster [18] (Figure 1F). A third metal-binding site made of six protein ligands has recently been shown to bind iron, although its function remains unknown [19]. [2Fe]-hydrogenases (HydA) often contain traditional [FeS]-clusters that function as internal electron carriers [6], but catalysis takes place at a unique cofactor termed the H-cluster. It consists of a common cubane [Fe_4S_4]-cluster linked via the thiolate of