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# B – Metal Centres in Supramolecular and Biological Structures

## Design of Artificial Enzymes Using the Metals of the Periodic Table

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# Design of Artificial Enzymes Using the Metals of the Periodic Table

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*Scientific keywords:* Bioinorganic, Biophysics, Biocatalysis, Energy Bioconversion (Hydrogen), Role of metals in Biology (heme and non-heme iron, molybdenum, tungsten, nickel, copper, vanadium and cobalt), Inorganic systems as models for biocatalysis, Spectroscopy (NMR, EPR and Mössbauer), (Bio) Electrochemistry, Protein-Protein interactions.

*Other interests:* Director of the Campus FCT Library and of the Department of Documentation and Culture, promoting Culture/Scientific interfaces, coordinating multidisciplinary curator activities (Art and Science).

http://sites.fct.unl.pt/biologicalchemistryatfctunl

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UNESCO celebrated, in 2019, the "International Year of the Periodic Table of Chemical Elements" and the 150th anniversary of its creation by Dmitry Mendeleev. Metalloproteins and metal-containing enzymes are well known to be essential to life. The elucidation of structural and functional aspects of metal sites in enzymes has been a goal of model studies putting together Inorganic Chemistry and Synthetic Biochemistry. In particular, synthetic peptides and small proteins involving rich sulfur coordination sites have been extensively used, such as Rubredoxins (Rds) and analogues. The four-Cysteine metal coordination motif, available in Rds, has the possibility of coordinating a wide variety of metal ions, with particular interest in aiming to model complex metalloproteins.

## ARTIFICIAL ENZYMES

Enzymes are complex molecules that may or not contain metals at the catalytic site, where chemical transformations occur with amazing selectivity and at high rates. Of the known enzymes, one third contain metals, coordinated by the side chains of amino acids of the polypeptide chain and/or cofactors. In this case, the substrate is activated at the metal site.

Due to the chemical complexity of the system (large molecular masses, multiple sub-unit composition and intricated architectural structures involving metals) the study of model compounds, retaining functional, structural (or both) characteristics has the advantage of working with a smaller size problem, more suitable for biophysical studies enabling an inorganic chemistry approach for revealing the metal active site properties. Metalloenzymes use a wide range of metals in a variety of structural arrangements and geometries, most with parallel with inorganic compounds, but others still a challenge for synthetic chemistry. Iron contained in iron-sulfur centers and in hemes are the most ubiquitous, but several other transition metals have specific roles, such as Ni, Mo, Cu, Zn and others. Modelling efforts also represent an opportunity for further exploring new applications and functionalities.

The chemical design of models for metalloprotein active sites can be based in small inorganic compounds, and now extend to peptides, protein-based synthetic analogues and simple proteins that are used as templates (or scaffolds) [1-4].

In this extended report of the lecture given at Academy of Sciences of Lisbon, at the Periodic Table celebratory sessions, we address metal substituted Rubredoxins as a scaffold for the construction of models of native metalloenzymes.

## WHY RUBREDOXINS?

Rubredoxins (Rds) are small iron containing proteins (approx. 5-6 KDa), which structural and function have been most explored, we could say, by any spectroscopic and structural tool available. Rds provide 4S containing ligands (Cysteines-Cys) in a very well-defined metal coordinating site. Rds are easy to over-express and enable easy amino acid site direct mutagenesis. It is feasible to accomplish the total chemical synthesis of such a small polypeptide chain and quite facile to replace native iron site, by a wide range of metal.

## ARTIFICIAL ENZYMES FOR METAL-SULPHUR RICH COORDINATION SPHERE

Important biochemical functions are performed by metalloproteins active sites with transition metals contained in sulphur-rich environments. Several studies had probed the chemistry of thiolates and transition metals, with very relevant accomplishments [4]. Many examples of nickel, copper, zinc and molybdenum (tungsten) in coordination spheres dominated by sulphur atoms are found in key enzymes.

The Fe-site in Rd is stabilized by the native folded polypeptide structure that provides four cysteinyl residues as ligands to the metal, in a tetrahedral arrangement. Due to the interplay between side chain amino-acid residues and metals, derivatives can be envisaged in two ways:

– site specific direct mutagenesis of crucial amino acids at the active site (specially coordinating ones)
– substitution of native metal (iron) by a wide range of metals ($^{57}$Fe(II), Co(II), Ni(II), Cu(I), Zn(II), Cd(II), Hg(II), Ga(III), In(III), Ge(IV), Hg(II) and Mo(VI,V,IV)) with different objectives, as detailed above [5-15].

The apo Rd is a scaffold and the replacement of native iron by other metals is simples, following a simple process of metal reconstitution/substitution, a simple chemical procedure.

The design of metal substituted Rds aims two main proposes [3]

– to be used as spectroscopic probes for the elucidate the structure/function aspects, and
– to synthetize simple (bio)models of active site of metalloenzymes.

Under this context, the metal substituted Rds, so far prepared, have precise applications:

– replacements by $^{57}$Fe, Zn, Co, Cd, Ga, In, and Hg originate specific structural probes for unrevealing structural details and properties of the metal site *per se*, and
– metals such Zn, Ni, Cu, as well as Mo (and recently, W) in sulphur-rich coordination sites are promising models for metal centres in metalloenzymes.

Figure 1 compiles examples of three different enzymes that contain metals in a Sulphur rich environment, most relevant in Energy, Health and Agriculture/Environment. The 3D structures are shown as well as detailed structures of the Sulphur rich coordination found around the metals. [15-18]

Hydrogenase is a Ni-Fe enzyme involved in hydrogen consumption and evolution, relevant for the development of projects on clean fuels – Hydrogen). Xanthine oxidase is a member of mononuclear Mo containing enzymes relevant for health-related problems (i.e. Gout) whose structure drive to the design of pharmaceutical drugs. The nitrogen fixation process, a part of the N-cycle is responsible for the production of di-nitrogen in a assimilative form of nitrogen (ammonia) of primordial importance in agriculture, and contains Mo at the active site in a complex Mo-Fe structure.

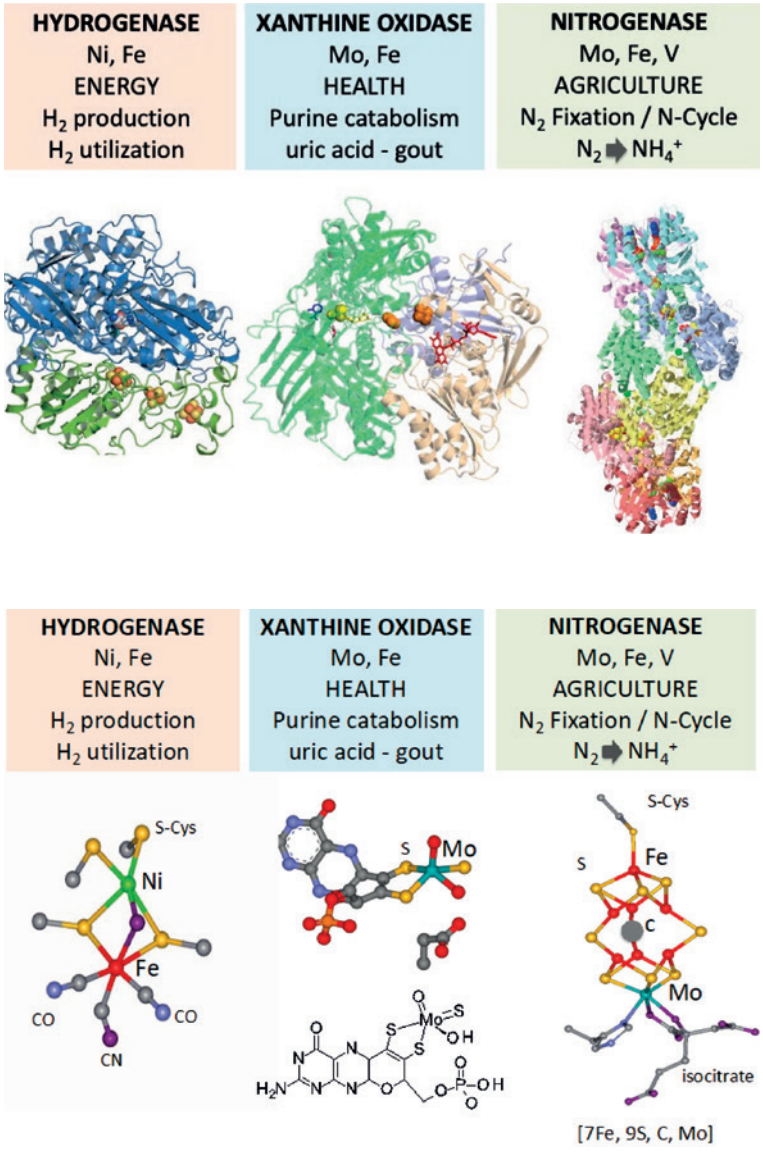


**Figure 1**
PANEL A – Complex enzymes with sulphur-rich-metal active sites. Examples relevant for Biology (Energy, Health and Environment)
PANEL B – Visualization of the metal-sulfur-rich active centers (the polypeptide was removed).

## NI-SUBSTITUTED RUBREDOXIN

There are three classes of bacterial Hydrogenases: [NiFe,] [FeFe] and [Fe only] [18]. The active site of [NiFe] Hydrogenases (as seen in Figure 1) [15, 18,19 ] is rich in sulfur and the Ni moiety is coordinated by four sulfur atoms (two from Cysteine residues and two sulfur atoms from the chemical bridge established between Ni and Fe, in a distorted tetrahedral geometry) [15,18]. The substitution of Fe by Ni in Rds is a plausible structural and functional model of the Ni moiety in the [NiFe] cluster in hydrogenase [21]. The Ni(S4) site in *Desulfovibrio* Rds was probed by different spectroscopic tools including UV-vis, NMR, and low temperature MCD [7,9,10,11,12,20]. The resolution of the 3D structure of Rd-Ni isolated

from *C. pasteurianum* corroborates with four coordinating S-Cysteine binding to Ni(II) in a tetrahedral arrangement, matching the one depicted for the Ni moiety of bacterial hydrogenases [15-18].

Functional aspects were probed with this model in order to mimic the functional aspects of nickel--containing hydrogenases: hydrogen production, deuterium-proton exchange, and inhibition by carbon monoxide [21]. Ni(II) derivatives built in Rds from *D. vulgaris* Hildenborough and P. *furiosus* can be oxidized and are EPR active with spectral parameters very closeto those observed in Ni-C signal of Hydrogenases, assigned to a Ni(III) oxidation state [7,21]. Electro-catalysis (Hydrogen production) was reported to be promote by Ni-Rd [22].

## Mo- SUBSTITUTED RUBREDOXIN

Molybdenum (and tungsten) is found in complex enzymes such as Nitrogenase include in a complex metal site Mo-7Fe-9S-C or in mononuclear Mo(W) enzymes, whose active site is coordinated to one or two pyranopterin molecules, and (or not) to side chain amino acids from the polypeptide chains (Figure 1). In this last case, the pyranopterins and Cysteines provide a rich sulfur environment to the metal Mononuclear Mo(W)-containing enzymes [23,24] comprehends a large group of enzymes classified in different families (Xanthine oxidase, DMSO reductase and Sulfite oxidase) and carry out atom transfer reactions between other Mo(W) gain a special relevance in Bioinorganic Chemistry, being recognized as essential metals to life and the heaviest elements used by biology.

Therefore, Rds replaced by molybdenum, synthesized from the apo form of *D. gigas*, are potential models for the catalytic site of mononuclear Mo-enzymes, where four Cys residues mimics a pyranopterin ligation [xx], and additional ligands containing O or S atoms may complete de coordination environment. The Mo-Rd compound was obtained replacing Fe in Rd isolated from Desulfobrio gigas [25].

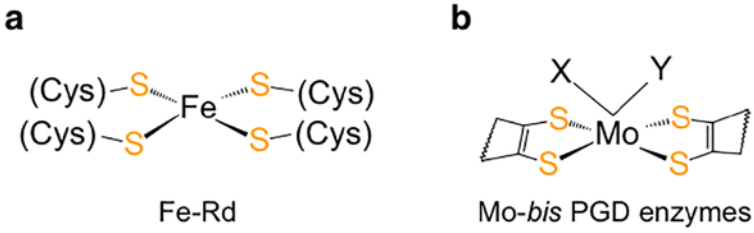


**Figure 2**.
Schematic representations of structures of a) [Fe(S-Cys)$_4$] in Rd and (b) mononuclear molybdenum enzymes (Mo-*bis*-pyranopterin), where X,Y are coordination sites with a large versatility (X,Y=O, S, Se, Asp, Ser, S-Cys, Se-Cys). The Mo-Rd compound was synthesized from the apo form of *D. gigas* Rd [23,25].

Apo-Rd does not provide enough coordination sites to satisfy the higher coordinated numbers required by Molybdenum. The four S-Cysteine residues ligate Mo, being complemented by other exogenous ligands, such as oxygen and thiol, forming a Mo(VI)-(S-Cys)$_4$(O)(X) complex, where X represents –OH or –SR. The Rd-Mo centre is prepared in a Mo(VI) oxidation state, and can attained other oxidation states: Mo(IV) via Mo(V). Mo(V) species (EPR active) observed in reduced reconstituted Mo-Rd, are most relevant for the study of catalytic mechanism (Figure 3).

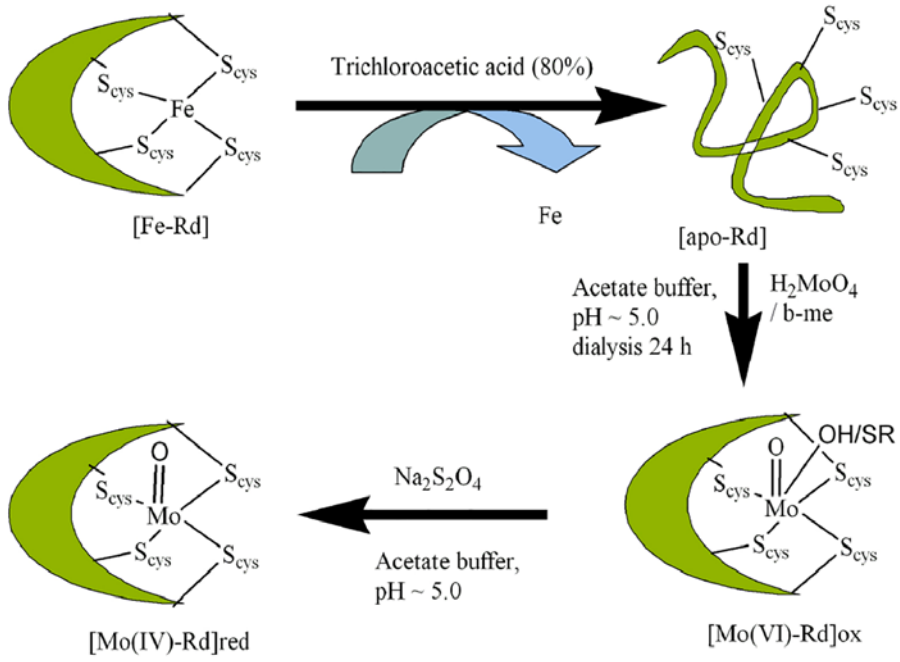


**Figure 3**
Schematic representation of reconstitution apo-Rd with molybdenum. The figure also indicates coordination modes and oxidation states of molybdenum-substituted-Rd (Mo-Rd). (from [3])

Rd-Mo model provides a simple complex for the study of spectroscopic properties of resting and reduced forms for the DMSO family of mononuclear Mo-*bis* pyranopterin-containing enzymes. The molybdenum site built in Rds was shown to be able to promote oxo-transfer reactions, one of the typical reactions performed by mononuclear Molybdenum enzymes [23,24].

An extension to other biological relevant metals can be consulted in an compreensive review article (3), that was a guide line to this lecture.

## ACKNOWLEDGEMENTS:

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