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13 Molybdenum Enzymes: An Unexploited Biotechnological Treasure and a Challenge for Medicine

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Abstract

Living organisms evolved by exploiting molybdenum as a catalyst, incorporating it in the active site of oxidoreductase enzymes that control a diverse array of oxygen, hydrogen and sulfur atom transfer reactions. The chemical versatility of this successful partnership, protein–molybdenum, enables the fulfillment of distinct physiological roles, from bacterial atmospheric dinitrogen and carbon dioxide fixation to human sulfite

detoxification. After several decades of research, our present comprehensive understanding of the structure and function of molybdoenzymes renders this an opportune time to draw attention to the largely disregarded biological molybdenum reactivity and bring to light its biotechnological potential and health-related challenges. In this chapter, we provide a concise overview of the molybdoenzymes catalytic features, followed by an outline of selected biotechnological applications to tackle some of the challenges that our modern society faces in the fields of environment, agriculture, climate and energy, namely, nitrate remediation, dinitrogen fixation and carbon dioxide capture and utilization. A brief account of molybdenum's human health implications is also included to highlight the relevance of these metalloenzymes to medicine and the pharma industry.

Keywords: Bioremediation, Carbon dioxide, CCU, Climate change, Fertilizers usage, Formate dehydrogenase, Gout, Nitrate, Nitrate reductase, Nitrogen fixation, Nitrogenase, Pharma industry, Purine catabolism, Renewable energy, Sulfite, Sulfite oxidase, Uric acid, Xanthine oxidase, Xenobiotics.

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List of Abbreviations

AO	Aldehyde oxidase
CCU	Carbon dioxide capture and utilization
Cyt	Cytochrome
DMSO	Dimethylsulfoxide
DMSOR	Dimethylsulfoxide reductase

mARC	Mitochondrial amidoxime reducing component
NaR	Nitrate reductase
SO	Sulfite oxidase
XO	Xanthine oxidase

I. Introduction

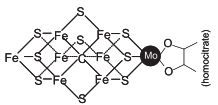
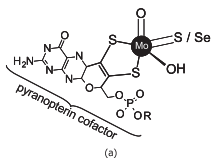
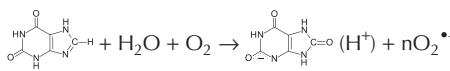
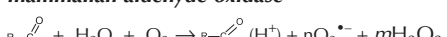
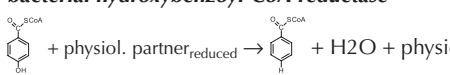
Molybdenum is a heavy metallic element (atomic number 42) that is essential to all forms of life,[1–3] being found in the active site of enzymes that catalyze diverse oxidation–reduction reactions, as exemplified in Table 1.[4–8] Several of these reactions are key steps of the global biogeochemical cycles of carbon, sulfur and nitrogen, where the primordial process of atmospheric dinitrogen fixation (Equation (1)) stands out due to its relevance to life on Earth.[8–14] Other reactions show their relevance at the individual level, as is well illustrated by the human dependency on molybdenum to detoxify (oxidize) sulfite (Equation (6)).[15–19] Noteworthily, many of the molybdenum-dependent biological reactions are highly interesting for tackling some of humankind's problems in the fields of environment, agriculture, climate and energy.

This chapter aims to draw attention to the innovative solutions that the exploitation of molybdoenzymes can provide, namely regarding nitrate remediation, dinitrogen fixation and carbon dioxide capture and utilization (CCU). Hence, this chapter begins with a brief overview of the molybdoenzymes' catalytic features. Currently, more than 60 molybdoenzymes are known, several of which have already been biochemically and structurally characterized,[4–8] and some selected enzymes are herein highlighted (Section II). Subsequently, an outline of selected biotechnological applications is presented to showcase nitrate reductase, nitrogenase and formate dehydrogenase (Section III). The final section (IV) is devoted to framing the molybdoenzymes' relevance to human health and disease and disclosing the challenges these enzymes pose to the pharma industry.

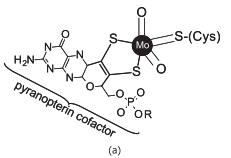
II. Molybdenum in Biology

Living organisms use molybdenum as a catalyst, incorporating it in the active site of diverse enzymes in association with two types of cofactors

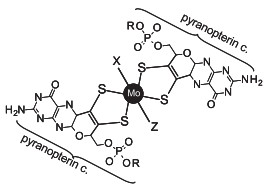
Table 1. Molybdoenzymes families.

Family active site structure	Type of reaction	Representative enzymes reaction equation	
nitrogenase family 	N–N cleavage	***bacterial nitrogenase*** $N{\equiv}N + 8H^+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i$	(1)
xanthine oxidase family 	oxygen and hydrogen atom transfer	***mammalian xanthine oxidase*** 	(2)
		mammalian aldehyde oxidase 	(3)
		bacterial aldehyde oxidoreductase 	(4)
		bacterial hydroxybenzoyl-CoA reductase 	(5)

sulfite oxidase family



dimethylsulfoxide reductase family



dimethylsulfoxide reductases: X, Z = O, Ser
"respiratory" nitrate reductase: X, Z = O, Asp
perip./assimil. nitrate reductases: X, Z = S, Cys
arsenite oxidases: X, Z = O, OH
formate dehydrogenase: X, Z = S, Cys or SeCys
polysulfide reductase: X, Z = O, Cys

oxygen atom transfer

vertebrate sulfite oxidase

$$SO_3^{2-} + H_2O + 2\ \text{cyt. } c\ (Fe^{3+}) \rightarrow OSO_3^{2-} + 2\ \text{cyt. } c\ (Fe^{2+}) + 2H^+ \quad (6)$$

plant sulfite oxidase

$$SO_3^{2-} + H_2O + O_2 \rightarrow OSO_3^{2-} + 2\ O_2^{\cdot-} + 2H^+ \quad (7)$$

bacterial sulfite dehydrogenases

$$SO_3^{2-} + H_2O + \text{physiol partner}_{\text{oxidized}} \rightarrow OSO_3^{2-} + \text{physiol. partner}_{\text{reduced}} \quad (8)$$

plant nitrate reductase

$$ONO_2^- + NAD(P)H + H^+ \rightarrow NO_2^- + H_2O.. + NAD(P)^+ \quad (9)$$

oxygen atom transfer

bacterial dimethylsulfoxide reductases

$$(H_3C)_2\text{-S=O} + \text{physiol. partner}_{\text{red}} \rightarrow (H_3C)_2\text{-S-H} + H_2O + \text{physiol. partner}_{\text{oxid}} \quad (10)$$

bacterial nitrate reductases *("respiratory", periplasmatic and assimilatory enzymes)*

$$ONO_2^- + \text{physiol. partner}_{\text{reduced}} \rightarrow NO_2^- + H_2O + \text{physiol. partner}_{\text{oxidized}} \quad (11)$$

bacterial arsenite oxidases

$$AsO_3^{3-} + H_2O + \text{physiol. partner}_{\text{oxidized}} \rightarrow OAsO_3^{3-} + \text{physiol. partner}_{\text{reduced}} \quad (12)$$

hydrogen atom transfer

bacterial formate dehydrogenases

$$HCOO^- + \text{physiol. partner}_{\text{oxidized}} \rightleftharpoons CO_2 + \text{physiol. partner}_{\text{reduced}} \quad (13)$$

sulfur atom transfer

bacterial polysulfide redutases

$$(S_n)^{2-} + \text{physiol. partner}_{\text{reduced}} \rightarrow (2H^+)\ S^{2-} + (S_{n-1})^{2-} + \text{physiol. partner}_{\text{oxidized}} \quad (14)$$

Note: (a) In eukaryotes, the pyranopterin cofactor is found in the simplest monophosphate form (R = H), while in prokaryotes it is most often found esterified with different nucleotides (R = cytidine monophosphate, guanosine monophosphate or adenosine monophosphate).

(Table 1, first column)[8]: The heteronuclear molybdenum–iron center, present only in the nitrogen enzyme, and the pyranopterin cofactor, found in all other molybdoenzymes. In addition to the molybdenum-dependent active site, the great majority of these enzymes also hold other redox-active centers, such as hemes, iron–sulfur centers or flavins, which are involved in intramolecular electron transfer and in reactions with co-substrates or intermolecular electron transfer with physiological redox partners (Figure 1).[4–8]

Parallel to heme proteins, in which different modifications of the heme ring and the substrate-binding pocket are combined to achieve different metabolic purposes using a single metal (iron), also in molybdoenzymes, different active site structures have evolved to create diverse chemical reactivities exploiting molybdenum. These different active site architectures are the basis for the classification of these metalloenzymes into four large families, denominated after one benchmark enzyme (Table 1; Figure 1)[4]: sulfite oxidase (SO), xanthine oxidase (XO), dimethylsulfoxide reductase (DMSOR) and nitrogenase families.

II.1. Sulfite Oxidase Family

SO family enzymes are characterized by having an active site that holds a molybdenum ion coordinated in a distorted square-pyramidal geometry, by an apical oxido group (Mo=O) and, in the equatorial plane, by the two sulfur atoms of the *cis*-dithiolene group (–S–C=C–S–) of one pyranopterin cofactor molecule, one oxido group (Mo=O) and one cysteine sulfur atom (Mo-S(Cys)) (Table 1, first column).[20] This family comprises diverse prokaryotic sulfite dehydrogenases,[21–26] among several other enzymes.[27–41] The vertebrate SO (Equation (6); Figure 1),[42–46] vital to detoxify sulfite (see Section IV), and the eukaryotic assimilatory nitrate reductase (NaR; Equation (9)),[10,11,47–54] essential for plants, algae and fungi to assimilate nitrate, also belong to this family.

The enzymes from the SO family typically catalyze oxo-transfer reactions, both the insertion and abstraction of an oxygen atom. During oxygen atom insertion catalysis, as can be exemplified by SO-catalyzed sulfite oxidation to sulfate (Equation (6)), it is the oxidized Mo^{6+}=

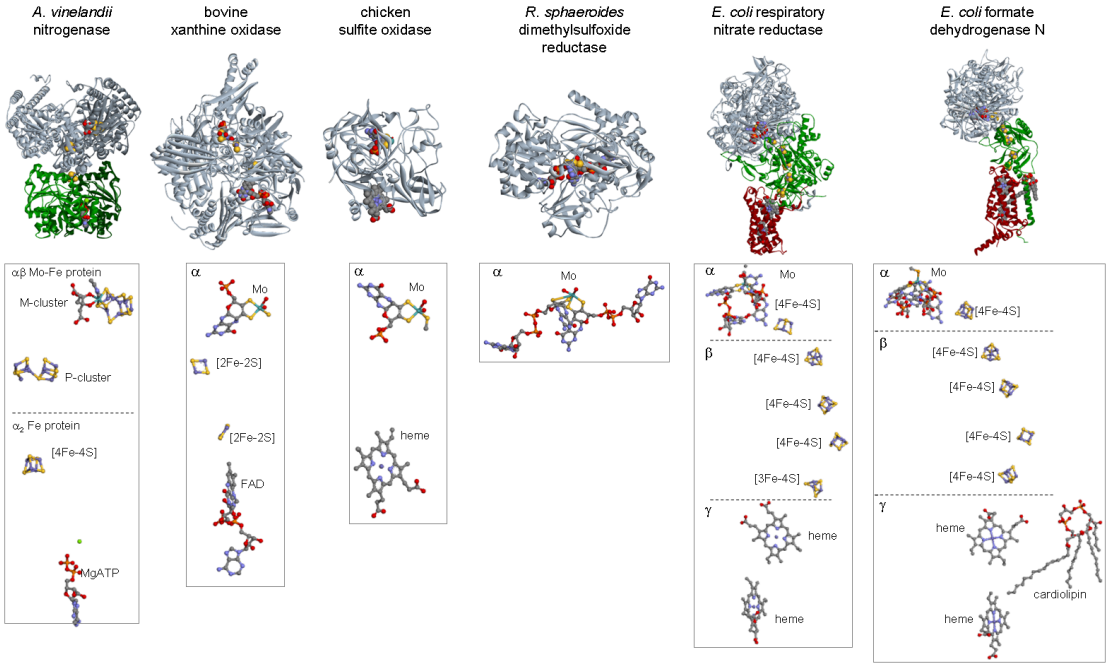


Figure 1. Three-dimensional structure view of representative molybdoenzymes (top) and of the arrangement of their redox cofactors (bottom). Represented are *Azotobacter vinelandii* nitrogenase, bovine xanthine oxidase, chicken sulfite oxidase, *Rhodobacter sphaeroides* dimethylsulfoxide reductase, *E. coli* "respiratory" nitrate reductase and *E. coli* formate dehydrogenase. Regarding nitrogenase, only one $\alpha\beta$ unit of the "molybdenum–iron protein" ($(\alpha\beta)_2$) is represented (gray and light gray); both α subunits of the "iron protein" (α_2) are represented. Regarding xanthine oxidase and sulfite oxidase (α_2), only one subunit is represented. One $\alpha\beta\gamma$ group of nitrate reductase ($(\alpha\beta\gamma)_2$) and of formate dehydrogenase ($(\alpha\beta\gamma)_3$) is represented. Structures shown are based on the PDB files 1N2C (nitrogenase), 1FO4 (xanthine oxidase), 1SOX (sulfite oxidase), 1EU1 (dimethylsulfoxide reductase), 1Q16 (nitrate reductase) and 1KQF (formate dehydrogenase). Cofactors structures: carbon, gray; nitrogen, blue; oxygen, red; sulfur, gold; phosphorus, orange; magnesium, green; iron, dark blue and molybdenum, teal.

$O_{equatorial}$ center that acts as the direct oxygen donor and oxidant (Figure 2(a)).[29,37,43,55–62] Conversely, during the oxygen atom abstraction catalysis, illustrated by NaR-catalyzed nitrate reduction to nitrite (Equation (9)), it is the reduced Mo^{4+} center, whose equatorial oxido group was eliminated in the form of a water molecule, that acts as the direct oxygen acceptor and reducer (Figure 2(b)).[47,51,53,63–66] In both cases, the ultimate oxygen atom donor or acceptor is a water

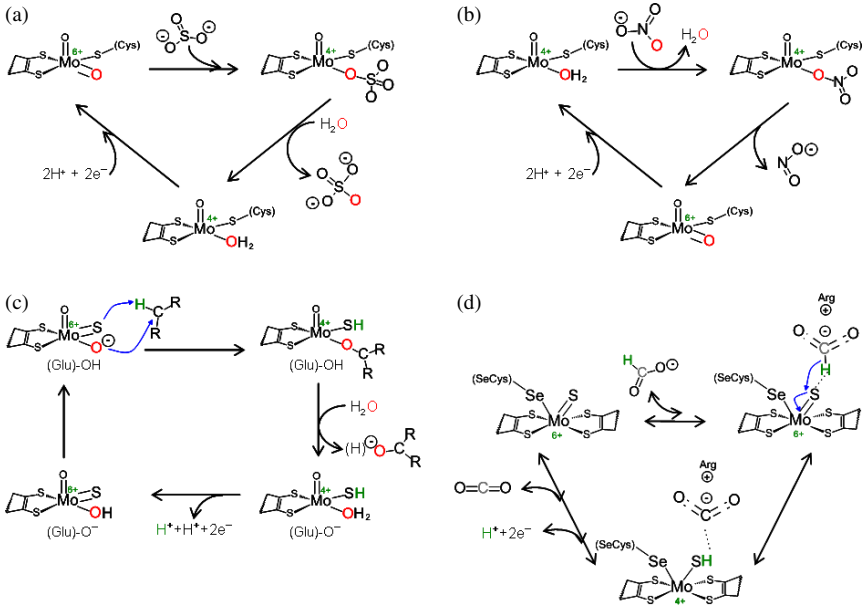


Figure 2. Simplified mechanistic proposals for the reactions catalyzed by selected molybdoenzymes. Reactions were chosen to highlight the key mechanistic features of molybdoenzymes: (a) sulfite oxidase family: oxygen atom insertion: sulfite oxidase-catalyzed sulfite oxidation to sulfate; (b) sulfite oxidase family: oxygen atom abstraction: eukaryotic nitrate reductase-catalyzed nitrate reduction to nitritel; (c) xanthine oxidase family: oxygen atom insertion with hydrogen atom abstraction: xanthine oxidase-catalyzed xanthine hydroxylation to urate. Also represented is the conserved glutamate residue essential to convert the equatorial Mo–OH group into a Mo^{6+}–O^{-} core able to undertake a nucleophilic attack on the carbon atom to be hydroxylated; (d) Dimethylsulfoxide reductase family: hydrogen atom abstraction and insertion: formate dehydrogenase-catalyzed reversible formate oxidation — carbon dioxide reduction. *Note*: The active site of these enzymes can harbor a selenocysteine or a cysteine residue; for simplicity, only the former case is represented. See text for details.

molecule, and the molybdenum ion is re-oxidized or re-reduced via electron transfer to or from the physiological partner.

II.2. Xanthine Oxidase Family

XO family enzymes hold a molybdenum-containing active site that is closely related to one of the SO family, but with the distinctive feature of not having the molybdenum directly coordinated to the protein (Table 1, first column).[7,8,67] These enzymes, instead, harbor one equatorial, catalytically labile –OH group plus one terminal sulfido (Mo=S) or selenido (Mo=Se) group. This family comprises not only the human-health-relevant mammalian XO (Equation (2); Figure 1)[54,67–83] and aldehyde oxidase (AO) (Equation (3))[71,72,75,80,82–106] (see Section IV) but also the closely related bacterial aldehyde oxidoreductase (Equation (4))[107–112] or the distinct hydroxybenzoyl-CoA reductase (Equation (5)).[113–116]

The enzymes from this family typically catalyze the hydroxylation of a C–H bond in aromatic heterocyclic compounds (Equation (2)) and aldehydes (Equations (3) and (4)), as well as, the reverse reaction of dehydroxylation (Equation (5)). During the hydroxylation catalysis, as is well established in XO catalysis (Equation (2)), the terminal sulfido (or selenido) group of the oxidized molybdenum center, Mo^{6+}= $S_{equatorial}$ (or Mo^{6+}= $Se_{equatorial}$) is key to activating the C–H bond to be cleaved, acting as a hydride acceptor, while the equatorial labile oxygen acts as the direct oxygen donor (Figure 2(c)).[54,68,70,71,74,76,79,117–122] In the reverse dehydroxylation reaction (Equation (5)), it is the reduced Mo^{4+}-$SH_{equatorial}$ that acts as the direct hydride donor.[115,116] As described for the SO family, water is the ultimate source of the oxygen atom incorporated into the hydroxylated product, and the molybdenum ion is re-oxidized via electron transfer from the physiological partner.

II.3. Dimethylsulfoxide Reductase Family

The DMSOR family enzymes are characterized by having an active site that holds a molybdenum ion coordinated by four sulfur atoms of two pyranopterin cofactor molecules; the molybdenum coordination sphere is completed by oxygen and/or sulfur and/or selenium atoms in a diversity

of combinations, in a trigonal-prismatic geometry (Table 1, first column).[7,8,123] Most often, the molybdenum ion is directly coordinated to the polypeptide chain, as in the SO family enzymes. Yet, the DMSOR family enzymes display a remarkable diversity of coordinating residue side chains, including not only cysteine but also selenocysteine, aspartate and serine residues. In addition, and similar to the enzymes of the XO and SO families, the active site of these enzymes can also have terminal sulfido and oxido groups. The DMSOR family is the most diverse of the molybdoenzymes, comprising only prokaryotic enzymes, such as the prototype DMSOR (Equation (10); Figure 1),[123–132] arsenite oxidase (Equation (12)),[133–136] polysulfide reductase (Equation (14))[137–141] and many other diverse enzymes.[123,142–145] This family also includes three different types of NaR enzymes (Equation (11); Figure 1), a dissimilatory membrane-bound enzyme, a dissimilatory periplasmatic enzyme and an assimilatory cytoplasmatic enzyme,[146–157] as well as, many forms of formate dehydrogenases (Equation (13); Figure 1).[158–195]

The enzymes from this family catalyze remarkably different reactions of oxygen, hydrogen and sulfur atom transfer. This extraordinarily diverse chemical reactivity is, as can be anticipated, matched by the highly diverse active site architecture displayed by these enzymes.

Arsenite oxidase and DMSOR reductase, enzymes that catalyze oxygen atom insertion (Equation (12)) and abstraction (Equation (10)), respectively, hold one terminal oxido group. During arsenite oxidase-catalyzed oxygen insertion, it is the labile oxido group of the oxidized center, Mo^{6+}=O, that acts as the direct oxygen donor,[133–136] in a similar way to SO catalysis (Figure 2(a)). Conversely, during DMSOR-catalyzed oxygen abstraction, it is the reduced Mo^{4+} center, whose catalytically labile oxido group was eliminated in the form of a water molecule, that acts as the direct oxygen acceptor,[196–199] in parallel to NaR catalysis (Figure 2(b)). As in the SO and XO families of enzymes, the ultimate oxygen atom donor or acceptor in these two DMSOR family enzymes is a water molecule, and the molybdenum ion is re-oxidized or re-reduced, respectively, via electron transfer from the physiological partner. A similar mechanistic strategy is proposed for sulfur atom abstraction catalyzed by polysulfide reductase (Equation (14)), with the reduced Mo^{4+} center acting as the sulfur atom acceptor.[137,138,141]

In contrast, FDH, which catalyzes the reversible hydrogen atom transfer (Equation (13)), holds one terminal sulfido group. During catalysis, it is the terminal sulfido group of the FDH-oxidized molybdenum center, Mo^{6+}=S, that acts as hydride acceptor, while the reduced center, Mo^{4+}-SH, acts as a hydride donor (Figure 2(d)),[175–182] in a mechanistic strategy comparable to the XO family enzymes.

II.4. Nitrogenase Family

This family comprises only the molybdenum/iron-dependent nitrogenase. The nitrogenase active site holds a complex, high-nuclearity center, comprising one molybdenum and seven iron ions (Table 1, first column; Figure 1).[8,200–216] This unique center, present only in the nitrogenase, can be viewed as two cubes with a common corner made of a carbide ion (C^{-4}), ([$Fe_4S_3C_{common}$] and [$MoFe_3S_3C_{common}$]), and bridged by three additional sulfur ions; in addition to the three sulfur ions from the "cube", the molybdenum ion is further coordinated by one homocitrate ion.

Nitrogenase enzymes have been known for long, with the first mention of biological dinitrogen fixation occurring in the middle of the 19th century. These bacterial enzymes catalyze the remarkable reaction of dinitrogen reduction (fixation) to ammonium, with the cleavage of the exceptionally stable N≡N triple bond (Equation (1)), a key step in the global biogeochemical cycle of nitrogen.

Unraveling the mechanism by which the triple bond of dinitrogen is cleaved by nitrogenase has been a highly challenging task. While the structure of the nitrogenase proteins and of their metal centers is presently well known, there are still many uncertainties regarding the reaction mechanism. The most recent mechanistic proposals suggest that the activation and reduction of dinitrogen depend on the prior reductive elimination of iron-based hydrides in the form of dihydrogen.[208,211–216] This proposal is based on the observation that the formation of dihydrogen is mandatory for dinitrogen reduction (Equation (1)) and this mechanistic strategy would allow the enzyme to store the necessary high reducing power to cleave the dinitrogen bond. The molybdenum ion, suggested to be present in an unprecedented 3+ oxidation state (Mo^{3+}), is considered

essential for tuning the central electronic structure to modulate its reduction potential and/or to facilitate substrate binding or protonation.

III. Landscapes for Molybdoenzymes' Biotechnological Applications

III.1. Environment and Food: A Global Problem

The exponential growth of the human population and the consequent need for food have led to a global increase in intensive agriculture, with the massive use of nitrogen-based fertilizers.[217] Overloading soils with nitrate and ammonium contaminates not only the soils, land, and rocks themselves but also aerial and underground freshwater and marine waters, causing a cascade of environmental and human health problems. Nitrate, which is very soluble, pollutes the aquifers that supply water for human consumption and contaminates the entire food chain, culminating in the fish we consume. Together with phosphorus compounds (also present in fertilizers), nitrate is responsible for the increasingly frequent phenomena of eutrophication of aquatic ecosystems and their concomitant destruction.[218] Furthermore, the high solubility of these compounds means that they are transported over great distances, taking environmental and human health problems to places far away from their origins. In this context, it is urgent to implement measures to reduce the use of fertilizers and solutions to mitigate the current nitrate contamination. The introduction of nitrate removal steps in water treatment plants is a strategy already implemented in several places using organisms capable of metabolizing and/or converting it, for example, into inert dinitrogen.[9–14] Here, the role of molybdoenzymes responsible for reducing nitrate (NaR enzymes; Equations (9) and (11); Figure 1) is crucial.

Another dimension of this problem that needs to be rethought concerns the production of fertilizers. In our modern society, the factor that most often limits agricultural production is the bioavailability of nitrogen in soils. The scale of this problem is evidenced in studies that show that approximately half of the human population could not be fed without the use of industrially produced fertilizers.[9] The industrial production of ammonia is achieved through the Haber–Bosch process, a heterogeneous catalysis

process that operates at very high temperatures and pressures to reduce gaseous dinitrogen to ammonia.[219,220] This industrial process is neither energy efficient nor environmentally responsible. Its inefficiency is even more noticeable when compared to the mild and "green" biological process carried out by nitrogenase (Equation (1); Figure 1) at ambient temperatures and pressures, pH values close to neutral and in water.

The awareness of environmental problems associated with the need to feed the human population has motivated numerous efforts to better understand the biological reduction of nitrogen. The knowledge gathered should lead to the development of more efficient, sustainable, and environment-friendly ("green") catalysts; it could also lead to the development of genetically modified organisms capable of producing "their own" nitrogen (although this solution is ecologically questionable).

III.2. Carbon Footprint and Atmospheric Carbon Dioxide Utilization

The global need for energy and our current dependence on fossil fuels have caused (and will continue to cause in the forthcoming years) the atmospheric carbon dioxide to rise to the highest levels since records are available. The increase in carbon dioxide, due to its significant greenhouse effect, is responsible for enormous and unpredictable impacts on the Earth's climate, in addition to being responsible for ocean acidification (the largest reservoir of carbon dioxide).[192,221–224] While these changes are no longer reversible, carbon dioxide emissions must be immediately and significantly reduced, and the future (present) energy sources must be "carbon neutral" and based on renewable energies, such as solar, wind or geothermal energy.

In recent years, there has been an increasing interest in the development of strategies to efficiently capture and convert atmospheric carbon dioxide (CCU) into value-added compounds using renewable energy sources. However, the kinetic and thermodynamic stability of the carbon dioxide molecule makes its activation at the industrial scale a highly challenging task. In contrast, living organisms have developed a wide range of strategies to activate and use carbon dioxide to their advantage, exploring different chemical approaches with a variety of enzymes. Hence, the

exploitation of enzymes, or inorganic catalysts inspired by their active sites, is very promising for the development of new (bio)catalysts for the efficient conversion of carbon dioxide.[192]

In this context, the exploitation of formate dehydrogenases (Equation (13); Figure 1), which reduce carbon dioxide to formate, presents the following key advantages: (i) Formate is the first stable intermediate in the reduction of carbon dioxide to methanol or methane; (ii) it can be used as a "building block" in different chemical industries; (iii) it is a viable source of energy, easier to store and transport than dihydrogen (both are oxidized at similar potentials); (iv) different "fuel cells" that use formate are already being developed.[192] The specificity, selectivity and efficiency characteristics of an enzyme are also central features for a catalytic system: (i) Only formate will be formed, and no additional purification processes are needed; (ii) the reaction will proceed under environment-friendly ("green") conditions of ambient temperature and pressure, pH values close to neutral and in water.

IV. Molybdoenzymes and Human Health Diseases

Molybdenum plays several physiological roles in humans, a remarkable fact when it is noted that we have only four molybdoenzymes: XO, AO, SO and mARC (Table 1).

XO (Equation (2); Figure 1) is responsible for the formation of urate, which, in humans, is the terminal product of purine catabolism.[225,226] An elevated concentration of urate in the blood is a risk factor for developing gout (a condition caused by the deposition of uric acid crystals in the joints, tendons and surrounding tissues),[227] kidney disease,[228] endothelial dysfunction and other related diseases.[229–233] To control the concentration of urate, different clinical strategies have been applied, including increasing its excretion and/or decreasing its formation through the inhibition of XO.[234] The effectiveness of XO inhibition as a therapeutic strategy against gout is now well established, and the inhibitor allopurinol (an isomer of hypoxanthine) has been successfully used in the clinic for over 40 years. Currently, there is great interest in the identification of new XO inhibitors that do not interfere with other metabolic pathways, and some compounds are already at different stages of the drug development process.

The XO and AO "promiscuity" for substrates (these two enzymes are able to oxidize a wide range of xenobiotic heterocyclic compounds) has also attracted the attention of the pharmaceutical industry.[105,235] The toxicological and pharmacological importance of these enzymes can be seen, for example, in the following: (i) conversion of azo compounds used as colorants in foods and cosmetics into harmful compounds ("toxic activation")[236]; (ii) conversion of anticancer[237,238] and antihypertensive[239] drugs into the respective bioactive compounds (beneficial activation); (iii) conversion of bioactive compounds into compounds without biological activity ("deactivation").[86,88,105,240–246] The "promiscuity" of XO, AO and mARC has caused serious problems in the development of new drugs, as compounds designed to resist the cytochrome P_{450}-dependent isoenzyme system are often metabolized by these molybdoenzymes; this results in a reduced bioavailability of the active drug or in its "toxic activation" and, consequently, alters its therapeutic effectiveness, causing many clinical trials to fail (with high financial losses for the pharmaceutical industry). To overcome potential trial failures, the catalytic properties of molybdoenzymes are being intensively studied, which has aroused renewed interest in these enzymes.

SO (Equation 6; Figure 1) provides the most striking role of molybdenum in human health. Sulfite, derived not only from the catabolism of sulfur-containing amino acids but also from xenobiotic sulfur compounds, is highly toxic to cells, and its SO-catalyzed oxidation to sulfate is critical for cell survival. This vital role is well demonstrated by the severe neonatal neurological problems caused by deficiencies in sulfite oxidase, which include impaired brain growth, severe cognitive impairment, seizures and premature death.[15–19] SO deficiency can be caused by an inability to synthesize the pyranopterin cofactor or by mutation(s) in the protein. In the first case, SO deficiency can be treated with continuous administration of a synthetically produced cofactor, which presently constitutes the only life expectancy for individuals born with this condition.

V. Outlook

Only in the second half of the 20th century was molybdenum considered essential for living organisms and, later, particularly relevant for humans.

Writing this brief account in 2022 is highly rewarding due to the very large amount of information available regarding the number of molybdoenzymes identified and biochemically and kinetically characterized, as well as the structural data accumulated and mechanistic atomic details disclosed. The extended knowledge gathered is allowing us to recognize and foresee major biotechnological applications related to the environment (nitrogen and carbon footprints), agriculture, climate and energy. The implications of molybdoenzymes for medicine and the pharma industry are also increasingly recognized.

Overall, these biotechnological applications reflect the involvement of molybdoenzymes in key steps of the global biogeochemical cycles of nitrogen, carbon and sulfur. Most impressively, the molybdenum participation in the nitrogen cycle is of major relevance: Nitrogen fixation and photosynthesis are two of the most important processes for life on Earth and dinitrogen reduction is highly dependent on molybdenum. Life without molybdenum would not be possible.

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