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Review

Cell biology of molybdenum in plants and humans ☆

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ABSTRACT

The transition element molybdenum (Mo) needs to be complexed by a special cofactor in order to gain catalytic activity. With the exception of bacterial Mo-nitrogenase, where Mo is a constituent of the FeMo-cofactor, Mo is bound to a pterin, thus forming the molybdenum cofactor Moco, which in different variants is the active compound at the catalytic site of all other Mo-containing enzymes. In eukaryotes, the most prominent Mo-enzymes are nitrate reductase, sulfite oxidase, xanthine dehydrogenase, aldehyde oxidase, and the mitochondrial amidoxime reductase. The biosynthesis of Moco involves the complex interaction of six proteins and is a process of four steps, which also requires iron, ATP and copper. After its synthesis, Moco is distributed to the apoproteins of Mo-enzymes by Moco-carrier/binding proteins. A deficiency in the biosynthesis of Moco has lethal consequences for the respective organisms. In humans, Moco deficiency is a severe inherited inborn error in metabolism resulting in severe neurodegeneration in newborns and causing early childhood death. This article is part of a Special Issue entitled: Cell Biology of Metals.

1. Introduction

Molybdenum (Mo) occurs in a wide range of metalloenzymes in bacteria, archaea, fungi, algae, plants and animals where it forms part of the active sites of these enzymes. However, in order to gain biological activity Mo requires the coordination by a pyranopterin, thus forming a prosthetic group named molybdenum cofactor (Moco). Mo has a versatile redox-chemistry that is used by the enzymes to catalyze diverse redox reactions. This redox-chemistry is controlled both by the different ligands at the Mo atom and the enzyme environment. Mo is very abundant in the oceans in the form of the molybdate anion. In soils, the molybdate anion is the only form of Mo that is available for plants, fungi and bacteria. Mo-containing enzymes are essential for life, since they hold key positions both in the biogeochemical redox cycles of nitrogen, carbon and sulfur on Earth [1] and in the metabolism of the individual organism. Hitherto more than 50 enzymes are known to be Mo-dependent. The vast majority of them are found in bacteria while in eukaryotes only seven have been identified [2] Mo belongs to the group of trace elements, *i.e.* the organism needs it only in minute amounts. Uptake of too high amounts of Mo however results in toxicity symptoms [3]. On the other hand unavailability of Mo is lethal for the organism. However, even if Mo is available for the cell, it is biologically inactive until it becomes complexed to form Moco.

In this article we will give a review on the current understanding of eukaryotic Mo-metabolism, involving Mo uptake, Moco biogenesis, Moco transfer and storage and finally its insertion into apo-metalloenzymes. We will focus on eukaryotes because here our understanding of the cell biology of Mo is much more advanced. The vast majority of this knowledge derives from studies in plants, fungi and humans. Somewhat surprising the commonly used eurkaryotic model organism yeast plays no role in Mo research as *Saccharomyces cerevisiae* does not contain Mo-enzymes nor the Moco biosynthesis pathway. Also *Schizosaccharomyces pombe* does not use Mo. Genome-wide database analyses revealed a significant number of unicellular organisms that do not need Mo. Obviously loss of Mo-utilization is connected to a host-associated life-style that makes Mo-enzymes unnecessary, while all multi-cellular eukaryotes are dependent on Mo [4].

2. Molybdenum uptake

Organisms take up Mo in the form of its oxyanion molybdate [5]. It requires specific uptake systems to scavenge molybdate in the presence of competing anions. These Mo uptake systems were studied in detail in bacteria where high-affinity ABC-type transporters are described consisting of three protein components and requiring ATP-hydrolysis for operation [6]. In higher organisms, only recently first molybdate-transporting proteins have been identified in algae and plants. Two proteins (Mot1 and Mot2) belonging to the large sulfate carrier superfamily were shown to

Abbreviations: AO, aldehyde oxidase; cPMP, cyclic pyranopterin monophosphate; Cu, copper; Fe, iron; Mo, molybdenum; Moco, molybdenum cofactor; MoBP, molybdenum cofactor binding protein; MPT, molybdopterin; NR, nitrate reductase; SO, sulfite oxidase; XDH, xanthine dehydrogenase; XO, xanthine oxidase

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transport molybdate with ultra-high affinity (nanomolar k_M value) across cellular membranes [7–9]. Unexpectedly, none of them was found to reside in the plasma membrane surrounding the cell. Contradictory reports localized Mot1 to the endo-membrane system [7] or to the mitochondrial envelope [9]. A mitochondrial location is questionable as the insertion of Mo into the Moco-backbone takes place in the cytosol. Recently Mot2-GFP analysis indicated a vacuolar location of this carrier protein [10]. Molybdate quantification in isolated vacuoles demonstrated that this organelle serves as an important molybdate storage in *Arabidopsis* cells where Mot2 was shown to be required for vacuolar molybdate export into the cytosol. This result is supported by previous work, analyzing the proteome of the tonoplast where MOT2 was identified to be localized in this cellular compartment [11]. Assuming this export function for Mot2, the endo-membrane (ER) location of Mot1 becomes likely since it may be involved in the transport of molybdate *via* the ER-Golgi route to the vacuole. But how is molybdate imported into the cytosol? Very recently another molybdate transporter has been identified in the alga *Chlamydomonas reinhardtii* that unlike Mot1 and Mot2 is not exclusively found in algae and higher plants but also occurs in humans [12]. Although still not localized it is likely that this transporter serves as the general molybdate importer for the cell. Further, it can be assumed that in addition to a possible high-affinity uptake system molybdate may also enter the cell non-specifically through the sulfate uptake system. Molybdate uptake through a sulfate transporter has recently been described [13], thus supporting this assumption.

3. The molybdenum cofactor

After uptake into the cell, molybdate needs to be coordinated by a unique scaffold in order to become biologically active. This compound is a tricyclic pterin called molybdopterin or metal-containing pterin (MPT). As the result of Mo coordination by MPT, Moco is formed. Moco is one of two Mo-containing cofactors occurring in nature. The other type of Mo-containing cofactor, the so-called iron–molybdenum cofactor, is structurally unrelated to Moco and is exclusively found in a single enzyme, the bacterial nitrogenase [14,15]. In this review, we will focus on the ubiquitously occurring Moco.

Early work with mutants of the filamentous fungus *Aspergillus nidulans* and of the higher plant *Nicotiana tabacum* revealed at that time novel mutant phenotype, namely the simultaneous activity loss of the Mo-enzymes nitrate reductase (NR) and xanthine dehydrogenase (XDH). Mo was the only common link between these two – otherwise highly diverging – enzymes. Therefore it was suggested that both enzymes share a common Mo-related cofactor (Moco). Later Nason et al. [16] provided the first piece of biochemical evidence for a cofactor common to all Mo-enzymes that could be removed as low molecular weight fraction from denatured Mo-enzymes of mammalian, plant and bacterial origin and subsequently incorporated into a cofactor-free apo-NR thus activating the enzyme. The elucidation of the chemical nature of Moco is based on the work of J.L. Johnson and K.V. Rajoagopalan [17]. Since Moco is highly sensitive to oxidation, most of the work was performed using its stable oxidation products FormA and FormB, respectively. Hereby, Moco had originally been identified in mammalian sulfite oxidase to be a pterin derivative comprising a unique four-carbon side chain as C6 substituent [18]. The coordination of Mo *via* an ene-dithiolate group located within the four-carbon side chain of the cofactor was demonstrated by carbamidomethylation of Moco [19].

The nature of Moco structure was finally elucidated upon co-crystallization with a Mo-enzyme aldehyde oxidase (AO) from *Desulfovibro gigas* [20]. Here, a small discrepancy to the originally proposed Moco structure was found, namely the existence of a third ring fused to the pterin backbone. This pyrano ring carries the ene-dithiolate group which is essential for metal coordination (Fig. 1). Crystal structures of several Mo-enzymes from diverse species were meanwhile solved, which in each case confirmed the core structure of Moco. Interestingly, in *Escherichia coli* nitrate reductase the pyrano ring is not closed but remains in the open configuration as four-carbon side chain. Therefore, the recently identified ring-opened dihydro form of MPT in bacterial nitrate reductase seems to occur after cofactor insertion [21].

The fusion of a pterin with a pyrano ring as identified for Moco and its direct precursor, the metal free MPT, is unique in nature and may have been evolved in order to maintain and/or control the special redox properties of Mo. The pyranopterin-part of Moco is necessary for the correct positioning of the catalytic metal (Mo) within the active center of Mo enzymes and in addition to this, may also participate in the electron channeling from or to other prosthetic groups [22].

All analyses of Mo enzymes crystallized so far revealed that Moco is deeply buried within the enzymes rather than being located on the protein surface, whereby a tunnel like structure assures accessibility of the cognate substrates [23,24]. During its life time, the Mo-enzyme does not liberate Moco. *In vitro*, however Moco may be removed from its protein environment either by heat treatment or by acidification. Once Moco is set free, it easily loses the Mo atom and becomes rapidly oxidized, resulting in an irreversible loss of function due to oxidation. The demolybdo-forms of Mo-enzymes are catalytically inactive.

4. Molybdenum cofactor biosynthesis

Mutations in the genes for Moco biosynthesis result in the pleiotropic loss of all Mo-dependent cellular processes. Early work with *A. nidulans* Moco biosynthesis mutants [25] identified six genetic complementation groups which consequently led to the proposal of a multi-step biosynthesis pathway. Congruent with this, also in plants a corresponding number of genetic complementation groups have been identified among Moco deficient mutants. Along with the conserved structure of Moco, these findings provided a basis to propose an evolutionary old multi-step biosynthetic pathway [26]. Already in pre-genomic times, the characterization of Moco mutants contributed significantly to our understanding of Moco biosynthesis in the kingdoms of life, whereby among eukaryotes the biochemical and genetic analysis of Moco mutants was most advanced in higher plants [27]. These results laid the basis to decipher also human Moco biosynthesis [28]. Characterization of both, plant and human Moco biosynthesis documented their strict analogy and therefore in the following will be compared whenever appropriate.

Moco biosynthesis can be divided into four steps, referring to the intermediates cyclic pyranopterin monophosphate (cPMP, previously identified as precursor Z), MPT, adenylated MPT (MPT-AMP) and Moco, respectively (Fig. 1). A total of six involved gene products have been identified in plants [27], fungi [29] and humans [30–32], whereby some but not all of the eukaryotic Moco biosynthesis genes are able to functionally complement the corresponding bacterial mutants, thus documenting the early evolutionary origin of Moco biosynthesis.

Different nomenclatures were introduced for genes and gene products involved in Moco formation. Moco biosynthesis genes and gene products of plants followed to the *cnx* nomenclature (*c*ofactor for *n*itrate reductase and *x*anthine dehydrogenase). Human Moco biosynthesis genes were named differently. Here, the MOCS (*mo*lybdenum *c*ofactor *s*ynthesis) nomenclature was introduced [30]. In the following we will briefly characterize the individual steps of Moco biosynthesis.

4.1. Step 1: Conversion of GTP to cPMP

The initial step of Moco biosynthesis starts with the conversion of GTP to cPMP — the first detectable intermediate of this biosynthetic