Molybdenum Metabolism in Plants

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Abstract: Among the micronutrients essential for plant growth and for microsymbionts, Mo is required in minute amounts. However, since Mo is often sequestered by Fe- or Al-oxihydroxides, especially in acidic soils, the concentration of the water-soluble molybdate anion available for uptake by plants may be limiting for the plant, even when the total Mo content of the soil is sufficient. In contrast to bacteria, no specific molybdenum uptake system is known for plants, but since molybdate and sulfate behave similarly and have similar structure, uptake of molybdate could be mediated unspecifically by one of the sulfate transporters. Transport into the different plant organs proceeds via xylem and phloem. A pterin-bound molybdenum is the cofactor of important plant enzymes involved in redox processes: nitrate reductase, xanthine dehydrogenase, aldehyde oxidase, and probably sulfite oxidase. Biosynthesis of the molybdenum cofactor (Moco) starts with a guanosine-X-phosphate. Subsequently, a sulfur-free pterin is synthesized, sulfur is added, and finally molybdenum is incorporated. In addition to the molybdopterin enzymes, small molybdopterin binding proteins without catalytic function are known and are probably involved in the storage of Moco. In symbiotic systems the nitrogen supply of the host plant is strongly influenced by the availability of Mo in soil, since both bacterial nitrogenase and NADPH-dependent nitrate reductase of mycorrhizal fungi are Mo enzymes.

Key words: Molybdopterin, molybdenum cofactor, precursor Z, nitrate reductase, xanthine dehydrogenase, aldehyde oxidase.

Introduction

For the last 70 years, it has been known that the transition element molybdenum is an essential micronutrient for plants and microorganisms (Bortels, 1930[10]; Arnon and Stout, 1939[5]). However, Mo itself is apparently catalytically inactive in biological systems until it is complexed by a special co-factor. With the exception of the bacterial nitrogenase, Mo is bound to a pterin (molybdopterin) which is the co-factor (Moco) of a series of enzymes in almost all organisms. All of these enzymes catalyze redox reactions, which alter the oxidation state of Mo. Among the 60 enzymes containing Mo which have been described so far for prokaryotes and eukaryotes (Hille, 1996[35]), the known Mo enzymes in plants are (i) nitrate reductase (NR, EC 1.6.6.1) catalyzing the initial step of the assimilatory nitrate reduction; (ii) xanthine dehydrogenase (XDH, EC 1.1.1.204) involved in purine catabolism; (iii) aldehyde oxidases (AO, EC 1.2.3.1) catalyzing the final oxidation in the phytohormone biosynthesis of indoleacetic acid (Koshiba et al., 1996[53]) and abscisic acid; and probably (iv) a sulfite oxidase (SO, EC 1.8.3.1) catalyzing the formation of sulfate. During the last three years detailed structural information has become available for a series of prokaryotic molybdo-enzymes which is applicable to the eukaryotic counterparts. Understanding of the biosynthesis of Moco in prokaryotes, and recently in eukaryotes, is advancing rapidly now that sets of well-defined mutants are available.

Availability of Molybdenum in Soil

Molybdenum is a very rare element with a crustal abundance of about 1.2 mg/kg (Fortescue, 1992[23]). Its oxidation state in soils varies from II to VI. Whereas Mo is predicted to be in the IV oxidation state under anoxic conditions (Brookings, 1987[12]), Mo is found in oxic soils with a pH of 4–8, mainly as Mo(VI) anions and salts ($MgMoO_4$, $CaMoO_4$, MoO_4^{2-}, Reddy and Gloss, 1993[78]). In more acidic soils (pH < 4) protonated Mo(VI) compounds ($HMoO_4^-$, $Mo(OH)_6$, $HMo_2O_7^-$ and H_2MoO_4) occur. Molybdenum can be dissociated in water, adsorbed by soil colloids, held in crystal lattices of minerals, or bound in organic matter. Total Mo concentrations of about 0.8–3.3 mg/kg soil were found in a wide range of different soils (Kubota, 1977[55]). Only the soluble Mo(VI) forms are available for plants. Adsorption on soil particles increases with decreasing pH from 7.75 to 4.45 (Reisenauer et al., 1962[79]). Therefore, more Mo is available for plants at higher soil pH values. Large amounts of Al and Fe oxides enhance soil adsorption and reduce the amount of water-soluble Mo (Karimian and Cox, 1978[45]).

Plant Mo Requirements

Among the micronutrients essential for plant growth, the amount of molybdenum required by plants is lowest. For example, an average concentration of 0.23 mg Mo per kg normal growing alfalfa has been reported (Gupta, 1991[27]). Toxicity of

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molybdenum to plants under field conditions seldom occurs, but can be induced under extreme experimental conditions (Johnson, 1966[38]; Brune and Diez, 1995[13]). Molybdenum deficiency has been reported worldwide for many plant species including herbs, crops and trees (Adriano, 1986[1]; Saco et al., 1995[82]; Weidner et al., 1996[100]; Gupta, 1997[28]) and is mainly due to a lack of nitrate reductase catalyzing the initial step in nitrate assimilation (Hewitt, 1983[33]). Such molybdenum deficiency delays flowering, inhibits tasseling, anthesis and the development of sporogenous tissues (Agarwala et al., 1978[2]; Martin et al., 1995[59]) which finally result in poor crop yield. Molybdenum deficiency in wheat results in lower dormancy levels (Cairns and Kritzinger, 1992[14]) which is probably due to reduced abscisic acid levels in seeds (Modi and Cairns, 1994[63]); this is possibly caused by suboptimal activity of the ABA aldehyde oxidase which is a Mo enzyme (see below). Winter wheat grown in acidic soil displays frost-induced decline of nitrate reductase activity, reduced content of digalactosyl diacylglycerol, and enhanced phospholipid degradation (which could be prevented by supplying Mo), and is probably the basis of the observed cryoprotective effect of Mo on winter wheat (Yaneva et al., 1995[103], 1996[102]). Visible symptoms of molybdenum deficiency vary according to plant species and most often result in chlorosis or a yellowing of the leaves (Gupta, 1997[28]). Mo deficiency in plants can occur in soils (i) with low total Mo, (ii) where Mo is sequestered by oxihydroxides, (iii) in extensively weathered soils, (iv) with pH values below 6, or (v) in sandy well drained soils (Severson and Shacklette, 1988[88]). There are many reports that crop yield can be increased by Mo fertilization (e.g. Silveira et al., 1996[90]; Dwivedi et al., 1996[22]), but in most cases this Mo deficiency can also be corrected by increasing soil pH through addition of lime (Gupta and Lipsett, 1981[29]). However, in the case of extreme Mo exhaustion in acid soils an increase in crop yield could only be obtained by a combination of lime addition and molybdate fertilization (Giddens and Perkins, 1972[25]). Addition of phosphate, which has a higher affinity to Fe oxihydroxides, also leads to release of adsorbed Mo and to an increase in the water-soluble molybdate concentration of the soil (Balistrieri and Chao, 1990[6]). When the nitrogen source was nitrate, it was observed that the Mo deficiency symptoms could be reduced or almost abolished when ammonium was supplied (Chiallou et al., 1986[15]; Hewitt and Gundry 1970[34]). Therefore, a major portion of the Mo deficiency is due to insufficient nitrogen supply via assimilatory nitrate reductase.

Uptake and Transport of Molybdenum in Plants

Since a molybdenum transporter is not yet known in plants, it is thought that molybdate is transported unspecifically by another anion transporter. The observation that phosphorus deficiency enhances uptake of radiolabeled molybdate up to five times in tomato plants, led to the assumption that the uptake of molybdate occurs via phosphate binding/transporting sites at the plasma membrane of root cells (Heuwinkel et al., 1992[31]).

However, the molybdate anion (MoO_4^{2-}) behaves more like the sulfate anion (SO_4^{2-}) than the phosphate anion (HPO_4^{2-}) in soil and uptake of molybdate by plants is known to be decreased in the presence of large amounts of sulfate (e.g., Sims et al., 1979[91]), probably because both anions use the same transport system (Marschner, 1995[58]). High affinity and low affinity sulfate transporter genes from the tropical forage legume *Stylosanthes hamata,* from barley, and from *Arabidopsis thaliana,* have been isolated (Smith et al., 1995[92], 1997[93]; Takahashi et al., 1997[94]), but it is still not clear whether the unspecific transport activity of these transporters is sufficient for molybdate uptake or whether an additional molybdenum specific transporter, as found in bacteria, (Rajagopalan, 1996[76]; Grunden and Shanmugam, 1997[26]) exists, and which requires about three orders of magnitude higher sulfate concentrations than Mo concentrations necessary to inhibit Mo uptake by the specific transporter (Cole et al., 1993[17]). One argument for separate MoO_4^{2-} and SO_4^{2-} uptake systems in plants is the fact that, in contrast to inhibition of molybdate uptake by large amounts of sulfate, molybdate supply does not affect sulfate uptake (Purakayastha and Nad, 1996[75]). Another argument for the existence of an active Mo transporter, at least in the fern *Ophioglossum,* is the observation that in this species Mo accumulates in the whole plant against a concentration gradient and reaches a final concentration of the element which is much higher than in other plants and higher than the concentration of soluble Mo in the soil (Khandelwas, 1988[47]).

After its uptake, the soluble molybdate anion, which is the predominant aqueous species at pH values above pH 4.0, is found in phloem as well as in xylem and is assumed to be the major transport form in these two long-distance transport systems (Marschner, 1995[58]).

Mo Enzymes in Plants

The three Mo enzymes described in plants (NR, XDH and AO) are homodimeric proteins functioning only as a dimer (for a recent review see Mendel and Schwarz, 1999[62]). They are involved in redox processes and harbour an electron transport chain involving different prosthetic groups (FAD, heme or Fe-S, Moco) that are bound to separate domains identified on the enzyme's monomer. Fig. **1** shows that the depicted Mo enzymes fall into two classes: one group is formed by NR and sulfite oxidase (SO), and another group by XDH and AO, both classes sharing similar domains. We included animal SO in this comparison because this enzyme shows strong similarities to NR and also because there are indications that SO might occur in plants. NR and SO have a dioxo Mo centre while AO and XDH form a group of monooxo-Mo hydroxylases with a terminal inorganic sulfide ligand attached to the Mo site. AO and XDH have very similar amino acid sequences so that it is suggested that they evolved from a common ancestor (Turner et al., 1995[97]). Nothing is known about how and when FAD and heme became bound to proteins. For Moco, however, the first crystallographic analyses of Mo enzymes made it evident that the co-factor is deeply buried within the holo-enzyme so that Moco could only have been incorporated prior to or during completion of folding and dimerization of the apoprotein monomers (Kisker et al., 1997 a[50]).

Nitrate Reductase

NR catalyzes the first step in nitrate assimilation, a pathway that is of key importance for plant nutrition. It is a cytoplasmically localized enzyme. As can be seen from Fig. **1**, the monomer of plant NR consists of three functional domains: the N-terminal domain associated with Moco, the central heme domain, and the C-terminal FAD domain, each redox-active pros-

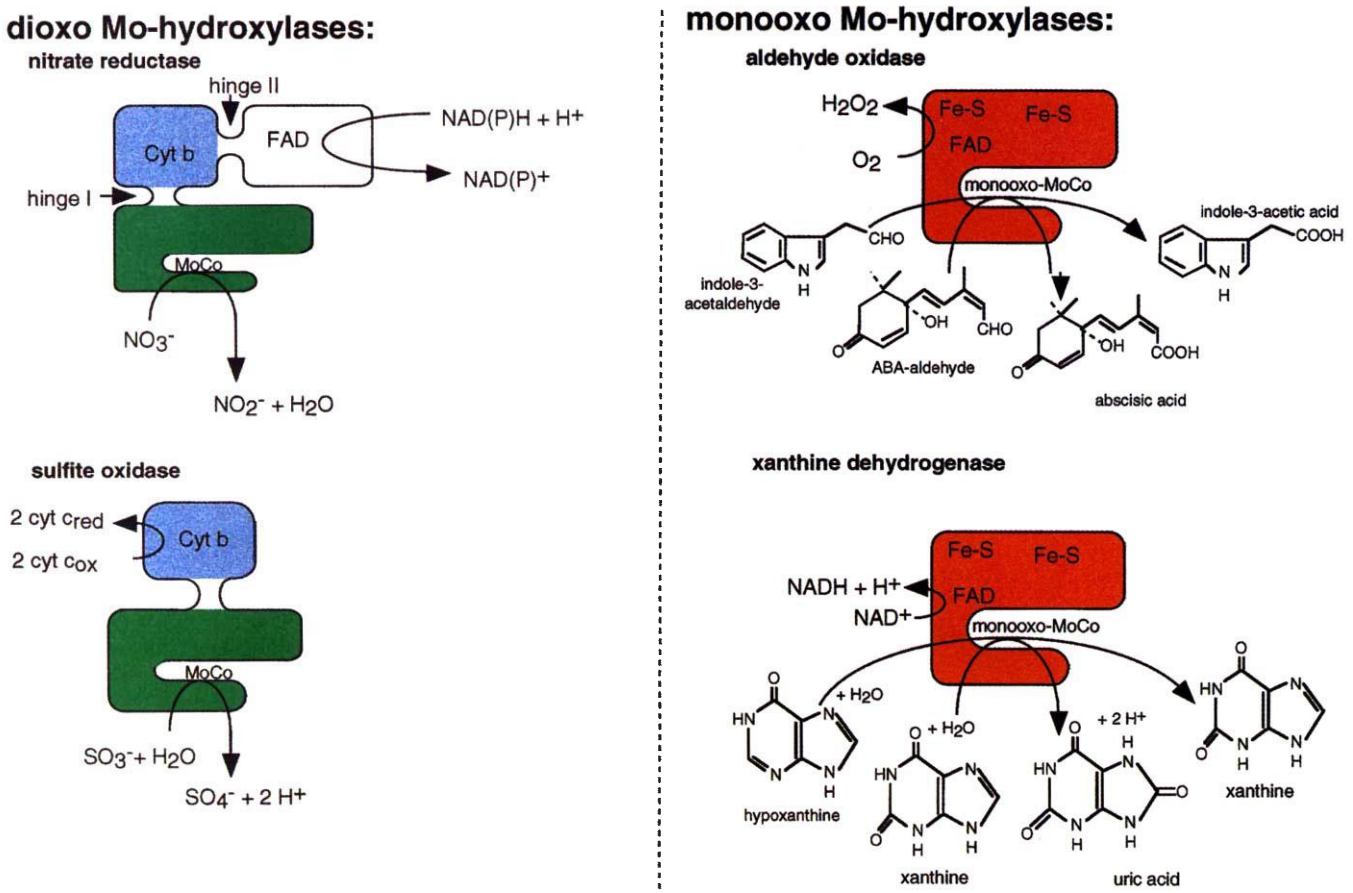

Fig. 1 Reactions catalyzed by eukaryotic Mo enzymes. In the active state, all enzymes are homodimeric. Only one monomer of each enzyme is shown in the figure. Hinge I and II are the sites where the nitrate reductase can be enzymatically digested, obtaining partially functioning domains. The molybdenum binding domain (green) of nitrate reductase and sulfite oxidase are similar in size and amino acid sequence. These enzymes belong to the group of dioxo Mo hydroxylases. The plant aldehyde oxidase and the eukaryotic xanthine dehydrogenase (orange) show significant similarities in their total amino acid sequence and possess an identical set of co-factors. These latter two enzymes belong to the group of monooxo Mo hydroxylases, since one of the oxygen atoms bound to Mo has been exchanged for sulfur (see Fig. **2**).

thetic group is bound to the monomer in a ratio of 1 : 1 : 1. These domains are connected by protease-sensitive hinge regions. The three domains form three redox centres catalyzing the transfer of electrons from the reductant NAD(P)H via FAD, heme, and Moco to nitrate.

Regulation of nitrate assimilation is part of a complex regulatory network responding to diverse environmental and internal signals such as nitrate, light, CO_2, phytohormones, and metabolites of carbon and nitrogen metabolism in order to coordinate nitrate assimilation with other key metabolic processes (for review see Hoff et al., 1994[36]; Kaiser and Huber, 1994[46]; Crawford, 1995[20]). The regulation of NR involves both transcriptional and post-translational mechanisms regulating the amount, as well as the activity, of NR protein. Of special interest is the regulative phosphorylation of the NR protein. In the dark, the NR protein is phosphorylated thereby allowing the stoichiometric binding of the so-called NR inhibitor protein, NIP, which belongs to the class of 14-3-3 proteins (for review see Kaiser and Huber, 1994[46]; Huber et al., 1996[37]). On illumination of leaves, NR is rapidly reactivated by dissociation of NIP and dephosphorylation involving a type 2A phosphatase. NIP cannot inhibit dephosphorylated NR.

The signal transduction cascade linking availability of nitrate to the induction of transcription is still enigmatic but has received a lot of attention in research recently. There are indications that nitrate serves not only as a substrate for assimilation, but also as a regulatory signal for coordinating nitrogen and carbon metabolism (Crawford, 1988[20]; Scheible et al., 1997[83]).

Aldehyde Oxidase

AO has been extensively characterized in animals, where it catalyzes the oxidation of a variety of aldehydes and N heterocyclic compounds in the presence of O_2 or redox dyes (Krenitsky et al., 1974[54]; Hall and Krenitsky, 1986[30]). The domain arrangement of AO (Fig. **1**) shows that redox-active iron is not incorporated, as in heme, but in the form of Fe-S centres localized on the N-terminal two domains. The protein monomer of the homodimeric enzyme binds Fe-S, FAD, and Moco in a stoichiometric ratio of 4 : 1 : 1. In plants, however, much less is

known about AO activities. Recently Koshiba et al. (1996[53]) reported properties of a purified plant AO for the first time. This cytoplasmic enzyme from maize coleoptiles is a homodimer with an apparent molecular mass of 300 kD, containing FAD, iron and Mo as prosthetic groups. The enzyme has a relatively broad substrate specificity with high affinities for indole-3-aldehyde, indole-3-acetaldehyde and benzaldehyde. Two isoforms have been described. From the substrate specificity, mutant analysis, and tissue distribution, it can be concluded that maize AO1 catalyzes the conversion of indole-3-acetaldehyde to IAA, the final step in IAA biosynthesis from tryptophan. This conclusion is supported by the observation that AO1 activity is five times higher in the IAA overproducing mutant *sur1* as compared to the *Arabidopsis* wild type (Seo et al., 1998[87]).

A different line of experimental approaches linked AO activity to the biosynthesis of the plant hormone abscisic acid. It has been shown that a certain class of ABA-deficient tomato mutants *(flacca* and *sitiens)* had impaired oxidation of ABA aldehyde to ABA (Taylor et al., 1988[95]) and, therefore, should lack ABA aldehyde-specific AO activity. In barley, Walker-Simmons et al. (1989[99]) analyzed the Moco-deficient *nar2a* mutant and found that it had strongly reduced levels of ABA, suggesting that this mutant is impaired in converting ABA aldehyde to ABA. In *Arabidopsis,* four AO cDNAs were found and physically mapped to different chromosomes (Sekimoto et al., 1998[86]). Obviously plant AOs form a multigene family whose members catalyze the final step in biosynthesis of the phytohormones IAA and ABA. These two functions are sufficient to assign an important role in plant development and adaptation to environmental stresses to AOs, although there are shunt pathways for the synthesis of both hormones (Normanly et al., 1995[70]; Rock et al., 1991[81]). The broad substrate specificity of AO makes it likely that AOs are involved in metabolic reactions in addition to phytohormone synthesis. Detoxification reactions and pathogen response may be good candidates for these additional functions.

Xanthine Dehydrogenase

In plants, exclusively XDH, but not the oxidase form, has been identified in a variety of organisms and tissues (as reviewed by Nguyen, 1986[68]). The enzyme was purified from nodules of bean (Boland, 1981[8]) and soybean (Triplett et al., 1982[96]; Boland et al., 1983[9]) as well as from the green alga *Chlamydomonas reinhardtii* (Perez-Vincente et al., 1988[74], 1992[73]), and very recently from wheat leaves (Montalbini, 1998[66]). Plant XDH shows highest affinities for xanthine and hypoxanthine as substrate but also accepts purines and pterines at much lower rates (Nguyen, 1986[68]). Like the animal enzyme, plant XDH is a homodimeric enzyme with a molecular mass of around 300 kD (Triplett et al., 1982[96]; Boland et al., 1983[9]; Nguyen and Nato, 1983[69]). Cloning of the plant XDH gene has not yet been reported, however from animals, *Drosophila* and filamentous fungi a wealth of sequence information is available confirming the known biochemical relationship between XO and AO at the molecular level.

XDH is involved in ureide synthesis and purine catabolism. Legumes fall into two classes depending on the transport form of symbiotically fixed nitrogen, the amide type synthesizes glutamine and asparagine, and the ureide producers (e.g., soybean) form allantoin and allantoic acid via the oxidative breakdown of purines (Schubert and Boland, 1990[85]) which requires less energy than in the former class (Triplett et al., 1982[96]). Purines are catabolized via hypoxanthine → xanthine → uric acid → allantoin → allantoic acid, where XDH catalyzes the first two steps. No XDH mutants have been described for higher plants, however, treatment of nodulated soybean plants with the XDH inhibitor, allopurinol, leads to a striking decrease in ureides accompanied by a dramatic increase in the xanthine content in the nodules. Thus XDH is of great importance for at least ureide-forming legumes. Plant XDH is very likely to produce superoxide radicals (Montalbini, 1992 a[64]), and the enzyme was shown to be involved in host–pathogen relationships between phytopathogenic fungi like *Uromyces* (Montalbini, 1992 a[64]) or *Puccinia* (Montalbini, 1992 b[65]) with legumes and cereals, respectively. XDH is also likely to be involved in senescence. Oxidative processes during senescence involve an increase in enzyme activities generating oxygen radicals and superoxide ions. In pea leaves, XDH activity was sharply increased in parallel with superoxide dismutase and other oxygen-related enzymes (Pastori and del Rio, 1997[71]). The question of what role XDH plays in activated oxygen metabolism during senescence remains to be answered.

The first indications that XDH might be associated to microbodies (Nguyen 1986[68]) were recently strengthened by the finding that pea leaf peroxisomes contain XDH which catabolizes xanthine to uric acid inside the organelles (Corpas et al., 1997[18]).

Sulfite Oxidase

Eukaryotic SO is a homodimeric protein consisting of an N-terminal heme domain and a Mo domain (Fig. **1**). Chicken liver SO was the first eukaryotic Mo enzyme whose atomic structure was solved (Kisker et al., 1997 b[51]). In plants, however, the existence of SO is still a matter of controversy. During primary sulfate assimilation in the chloroplasts, sulfate is reduced via sulfite to organic sulfide which is used for cysteine biosynthesis. However, it has also been reported that sulfite can be oxidized back to sulfate, e.g., when plants were subjected to SO_2 gas (as reviewed in Rennenberg and Herschbach, 1996[80]) or when isolated chloroplasts were fed with radioactively labeled sulfite (Dittrich et al., 1992[21]). Sulfite oxidizing activity that also occurred in the dark (Jolivet et al., 1995 a[41]) could be pelleted when isolated chloroplasts were broken (Jolivet et al., 1995 b[42]). The latter activity was purified from spinach chloroplasts and was found to be associated with thylakoid membranes. In comparison, the animal hepatic SO is localized between the inner and the outer membranes of mitochondria (Cohen et al., 1972[16]). Hence, only cloning of the plant SO gene and characterization of the encoded protein will clarify this situation. The first successful steps in this direction have been undertaken by one of the authors (R. Mendel, unpublished).

Molybdenum Co-factor

The genetic analysis of NR-deficient mutants of the filamentous fungus *Aspergillus nidulans* by Pateman et al., (1964[72]) revealed a novel phenotype, namely the simultaneous loss of the two Mo enzymes, NR and XDH. Since Mo was the only common link between these two – otherwise very different –

enzymes, the authors suggested that both enzymes should share a common Mo-related co-factor. Johnson et al. (1980[40]) demonstrated that the organic component of Moco from different Mo proteins is a unique pterin, which was named molybdopterin (MPT), since it differed from all known pterin compounds (Fig. **2**). Later it was shown (Johnson et al., 1990[39]) that an additional form of Moco occurs in prokaryotes where GMP is bound to the C4′ atom of the MPT side chain via a pyrophosphate bond. Other prokaryotic variants of the co-factor containing GMP, CMP, AMP, or IMP linked to the MPT, were also identified (reviewed by Rajagopalan and Johnson, 1992[77]). These dinucleotide forms were only found in prokaryotes. Bacteria may even contain both Moco forms, because it was found that XDH from *Rhodobacter capsulatus* and *Pseudomonas putida* contains the MPT form of the co-factor while the other Mo enzymes (NR, DMSO reductase, chinoline oxidoreductase) contain the dinucleotide form (Leimkühler et al., 1998[56]; Hettrich et al., 1991[32]). The role of the ribonucleotide portion of the bacterial co-factors for the corresponding enzymes has yet to be understood. Nevertheless, the core structure of MPT is identically conserved in all organisms. X-ray crystallographic analyses of Mo enzymes revealed that (1) the co-factor is not on the protein's surface, rather it is buried deeply within the interior of the enzyme and a tunnel-like structure makes it accessible to the appropriate substrates; and (2) the pterin ring system could participate in electron transfer to or from the Mo atom, since the pterin with several possible reduction states as well as structural conformations could play a role in controlling the redox behaviour of the Mo atom (Kisker et al., 1997 a[50], 1997 b[51]).

In summary, Mo itself seems to be biologically inactive; it has to be complexed with MPT in order to become biologically active. The Mo ion in Moco is found coordinated to three types of ligands: (i) sulfur atoms provided by MPT; (ii) non-protein oxygen and/or sulfur species, such as oxo, water, hydroxo or sulfido; and (iii) optionally, amino acid side chains like serine (Schindelin et al., 1996[84]), selenocysteine (Boyington et al., 1997[11]) and cysteine (Kisker et al., 1997 a[50]). Once Moco is liberated from the holoenzyme, it loses Mo and undergoes rapid and irreversible loss of function due to oxidation (Rajagopalan and Johnson, 1992[77]). The demolybdo forms of Mo enzymes are catalytically inactive (Coughlan, 1980[19]). Elevated amounts of the Mo antagonist tungsten (W) were shown to inhibit the activity of Mo enzymes by replacing Mo as ligand of MPT (Wray and Filner, 1970[101]; Coughlan, 1980[19]), whereas in hyperthermophilic archaebacteria, tungsten seems to be the physiological ligand for MPT (Kletzin and Adams, 1996[52]).

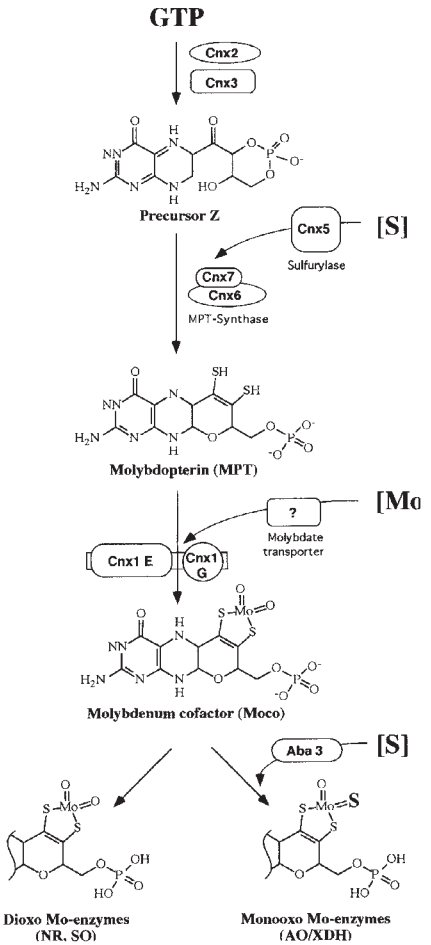

Fig. 2 Model for Moco biosynthesis in plants. In addition to molybdenum, sulfur is also essential for this biosynthetic pathway. The dioxo form (left, lower corner) of Moco has been isolated from plant nitrate reductase. The monooxo form (right, lower corner) is present in aldehyde oxidases and xanthine dehydrogenases. The exchange of oxygen for sulfur occurs after incorporation into the enzyme. Cnx1, Cnx2, Cnx3, Cnx5, Cnx6, Cnx7, Aba3 are involved enzymes named by the mutants lacking such activities.

Molybdenum Co-factor Biosynthesis

For higher plants, a shortage of Mo in the soil or a mutational block of the cellular ability to use Mo, i.e., to synthesize MPT, to take Mo up into the cell, or to bind it to MPT, lead to the loss of essential metabolic functions and can cause the death of the plant. Moco-deficient plant mutants show a pleiotropic loss of the three Mo enzyme activities NR, XDH, and AO. Analysis of *Nicotiana plumbaginifolia* mutants led to the description of six genetic loci (*cnxA–cnxF*) which are involved in the synthesis of active Moco (Gabard et al., 1988[24]). All of the Moco mutants show a similar morphology strongly deviating from that of the wild type and also differing from the habitus of NR apoprotein mutants: chlorosis of leaves and small, narrow and crinkled leaves (Gabard et al., 1988[24]) probably caused by combined impairment of the ability to synthesize abscisic acid and indoleacetic acid due to the loss of AO activities.

The multitude of Moco-specific loci in higher and lower eukaryotes led to the conclusion that Moco biosynthesis is a complex and probably ancient pathway involving several gene products (Müller and Mendel, 1989[67]; Mendel, 1992[60], 1997[61]). Three approaches have been used to clone the genes involved in Moco biosynthesis (functional complementation of *E. coli* Moco mutants, generation of Moco-defective plant mutants by T-DNA tagging, cloning via "expressed sequence tags" by searching for homologies at protein level) resulting in at least seven cloned genes from *Arabidopsis thaliana*.

In summary, the biosynthesis of Moco proceeds as follows (Fig. **2**): during the first stage a guanosine-X-phosphate derivative (probably GTP) is transformed by a hitherto unknown

mechanism into a sulfur-free pterin compound, the precursor Z, which already has the typical Moco four carbon side chain. In the second stage, sulfur is transferred to precursor Z and the precursor is converted to MPT, the organic moiety of the co-factor. Precursor Z and MPT are the only known intermediates of the Moco pathway. In the third stage, Mo has to be transferred to MPT in order to form Moco. Vesicle transport is also involved in Moco synthesis because a membrane protein essential for MPT synthesis seems to be transported via the Golgi pathway to the plasma membrane. There are also early indications that at least part of the Moco biosynthetic pathway is organized as a multienzyme complex fixed to the cytoskeleton. For details see the recent reviews of Mendel (1997[61]) and Mendel and Schwarz (1999[62]).

Molybdopterin Binding Proteins

The availability of sufficient amounts of Moco is essential for the cell to meet its changing demand for synthesizing Mo enzymes. In particular, diurnal variation in the amount of NR protein requires flexible regulation of Moco synthesis. Here, the existence of Moco storage proteins would be a good way to buffer supply and demand for Moco. The Mo concentration in seeds of some legumes has been described to be more than 10 times higher than the concentration in the total plant (see Gupta, 1997[28]), which is favorable for germination in nitrate-containing soils with low Mo availability. From pea seeds, a homodimeric molybdenum co-factor containing protein (monomers of 150 kDa) could be isolated which lacks a prosthetic group such as FAD, Cyt of FeS in addition to Moco (Kildibekov et al., 1996[48]). Therefore, it was concluded that the protein does not have any enzymatic activity but stores Moco in the seeds for germination. From seeds of *Vicia faba*, a small, molybdenum co-factor carrier protein of 70 kDa was purified which seems to have no enzymatic function, but released Moco efficiently when the protein comes in contact with the apo-nitrate reductase (Kalakoutskii and Fernandez, 1997[43]). The size of the protein is similar to that of the 60 kDa protein previously isolated from winter wheat and barley seeds, which actively bound Moco (Alikulov and Schiemann, 1985[4]; Vunkova-Radeva et al., 1988[98]). For *Chlamydomonas reinhardtii* a Moco binding protein has also been described (Aguilar et al., 1992[3]).

Molybdenum in Plant Symbiosis

Several plant species live in symbiosis with bacteria capable of fixing molecular nitrogen. The microsymbionts are able to supply the host plant with reduced nitrogen. For over 70 years it has been known that Mo is essential for biological nitrogen fixation (Bortels, 1930[10]). The catalyzing enzyme is the molybdenum-containing nitrogenase which only exists in prokaryotes. In contrast to all other molybdenum enzymes, the Mo in the nitrogenase is not bound to a pterin but is part of a Fe–Mo co-factor in which Mo is not directly involved in the reduction of dinitrogen but has a structural function in the complex (Kim and Rees, 1992[49]). Thus, several bacteria have nitrogenases which do not contain Mo in case of molybdenum deficiency, the most active nitrogen fixing nitrogenase is the Mo enzyme (Bishop et al., 1988[7]), and therefore the effectiveness of these symbioses depends on molybdenum availability in soil. For the *Rhizobium* legume symbiosis, the number of nodules and the crop yield increased with increasing Mo application (e.g., shown for peanuts and soybeans, Shivashankar and Hagstrom, 1991[89]).

In the common plant–microbe symbiosis, between plants and arbuscular mycorrhizal fungi, the fungal NR is apparently essentially involved in the nitrogen exchange between plant and microsymbiont. In the interaction between maize and the mycorrhizal fungus, *Glomus intraradices*, the fungus highly expresses an NADPH-dependent nitrate reductase (EC 1.6.6.3) in the fungal arbuscular structures of the root cells, whereas expression of the NADH-dependent nitrate reductase of maize in the leaves or roots of the host plant was found to be low (Kaldorf et al., 1998[44]). In controls grown without the symbiotic fungus, the level of nitrate reductase expression in leaves and roots of the plant was much higher. Therefore, the fungus has apparently taken over the function of nitrate reduction in the symbiosis, but it must still be determined if the microsymbiont requires more Mo than the symbiotic plant. For *Vicia faba*, absorption of Mo was described to be promoted in plants which were infected by arbuscular mycorrhizal fungi (Liu and Lei, 1992[57]), indicating that a transfer of molybdenum to the plant occurred in this symbiosis.

Outlook

Investigation of the role and the function of Mo in plants is currently progressing rapidly. Work is concentrated on the detailed elucidation of the Mo pterin biosynthesis. The clearcut identification of sulfite oxidase as an enzyme existing in plants is also being intensively investigated. As the crystallization of the Mo pterin-containing domain of the plant nitrate reductase is imminent the structural analyses will also soon be available. Fields, which are still open include the cloning of XDH, the search for further metabolic reactions catalyzed by the AOs, and the determination of whether a specific Mo uptake system exists.

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