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REVIEW

Molybdenum cofactor-containing oxidoreductase family in plants

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Abstract

Recent investigations on plant molybdenum-containing enzymes that include xanthine dehydrogenase (EC 1.1.1.204) and xanthine oxidase (EC 1.1.3.22), nitrate reductase (EC 1.7.1.1-3), aldehyde oxidase (EC 1.2.3.1), and sulfite oxidase (EC 1.8.3.1) are reviewed. The enzymes belong to closely related protein family and share common structural features. Special attention is being paid to the recently solved crystal structures their implications for the substrate binding and catalytic mechanism.

Additional key words: aldehyde oxidase, amino acid sequence, xanthine dehydrogenase, xanthine oxidase.

Introduction

The molybdenum cofactor containing enzyme family comprehends more than 30 mostly bacterial enzymes. However, only four molybdenum containing enzymes have been found in plants: nitrate reductase (NR, EC 1.7.1.1-3), aldehyde oxidase (ALO, EC 1.2.3.1), sulfite oxidase (SO, EC 1.8.3.1) and xanthine dehydrogenase (XDH, EC 1.1.1.204). XDH and ALO are very similar in amino acid sequence, which suggests an evolutionary relationship (Turner *et al.* 1995). All these enzymes are homodimeric proteins functioning as a dimer, but not as a monomer. They catalyze important transformations in the global nitrogen, sulfur and carbon metabolic pathways. NR is the key enzyme in inorganic nitrogen assimilation, XDH catalyzes important transformations in degradation of purine bases, ALO is linked with the biosynthesis of indole acetic acid-phytohormone of auxin class and SO catalyzes oxidation of sulfites to sulfates, the important terminal step in the oxidative degradation of cysteine, methionine, and membrane components such as sulfatides. All these enzymes catalyze the transfer of an oxygen atom, ultimately derived from water to a substrate involving coupled one electron/proton transfer processes that are finally linked by the Mo-site to the required two electron redox reaction (Huber *et al.* 1996).

Molybdenum cofactor

Molybdenum cofactor (Moco) was discovered in nitrate reductase deficient mutants of the filamentous fungus *Aspergillus nidulans* in 1964 (Pateman *et al.* 1964). The first biochemical evidence for the existence of a cofactor common to all Mo-containing enzymes was provided by Ketchum *et al.* (1970). Direct analysis of the molybdenum cofactor in native state is impossible due to extreme sensitivity to oxidative degradation. The presence of oxygen is necessary for formation of active Moco, but its absence is essential to prevent inactivation of the free form (Spanning *et al.* 1987). The first indications of the cnemical nature of Moco came from the analysis of blue-fluorescent oxidation products of the cofactor. Johnson *et al.* (1980) revealed the organic

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Abbreviations: ABA - abscisic acid; ALO - aldehyde oxidase (EC 1.2.3.1); IAA - indole-3-acetic acid; Moco - molybdenum cofactor; NR - nitrate reductase (EC 1.7.1.1-3); SO - sulfite oxidase (EC 1.8.3.1); XDH - xanthine dehydrogenase (EC 1.1.1.204); XO - xanthine oxidase (EC 1.1.3.22).

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component of Moco from different Mo proteins to be a unique pterin, which was named molybdopterin. Molybdopterin which has in addition to the pterin ring itself an additional pyran ring that possesses an exocyclic dithiolene moiety, by which the cofactor coordinates the metal and a short phosphorylated side chain (Rajagopalan 1991) (Fig. 1). In prokaryotic system the cofactor is usually found elaborated as the guanine, adenine, cytidine or hypoxanthine dinucleotide (Gremer *et al.* 1996, Rajagopalan *et al.* 1992). The molybdenum is coordinated by one molybdopterin in ALO from *Desulfovibrio gigas* and XDH. There are two molybdopterin cofactors (molybdopterin guanine dinucleotides) in other structurally defined Moco enzymes: DMSO reductase from *Rhodobacter capsulatus* (Schneider *et al.* 1996) and *Rhodobacter sphareoides* (Schindelin *et al.* 1996) and formate dehydrogenase from *Escherichia coli* (Boyington *et al.* 1997). Bacteria may even contain both nucleotide and free Moco forms (Romao *et al.* 1997).

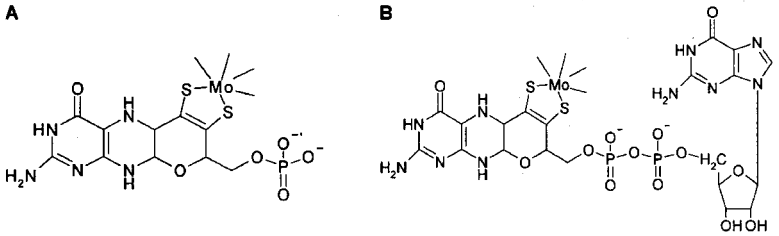

Fig. 1. Structure of molybdopterin and its ene-dithiolate mode of coordination to a metal atom (*A*) and molybdopterin guanine dinucleotide form (*B*) according to McMaster and Enemark (1998) and Rajagopalan and Johnson (1992).

Moco deficiency leads to the loss of essential metabolic functions of the plant and can cause its death. In such a case, nitrate reductase shows remarkable decrease in activity, which is more dramatic for the plant than the lack of aldehyde oxidase or xanthine dehydrogenase activity (Mendel *et al.* 1999).

The greater part of knowledge on Moco biosynthesis has been obtained from studies of Moco mutants of *Escherichia coli* (Nichols and Rajagopalan 2002). The core structure of Moco is conserved in all organisms, which suggests a similar biosynthetic pathway. Moco biosynthesis consists of three stages in *E. coli,* a sulfur-free compound development, sulfur transfer and the conversion to molybdopterin and finally the transfer of the Mo atom to molybdopterin. In the first step a guanosine derivative is converted to an unknown precursor. During the second stage, sulfur has to be attached to the precursor and the precursor is converted to molybdopterin. This reaction is catalyzed by molybdopterin synthase, which is composed of two subunits. The third stage comprises the transfer of Mo atom to molybdopterin in order to form active Moco. In eukaryotes, nothing is known about the way in which Mo is taken up into cell.

Principles of the Moco catalysis: Hydroxylation as well as the abstraction of two electrons from the substrate are affected by the molybdenum center. The hydroxyl group is a ligand to the metal in the form of a μ-oxo group. In molybdenum hydroxylases, rather water than molecular oxygen is the source of the oxygen incorporated into the product and reducing equivalents are generated rather than consumed in the course of the reaction (Huber *et al.* 1996).

In the case of XDH about half a dozen mechanism have been proposed. In some of these the molybdenum

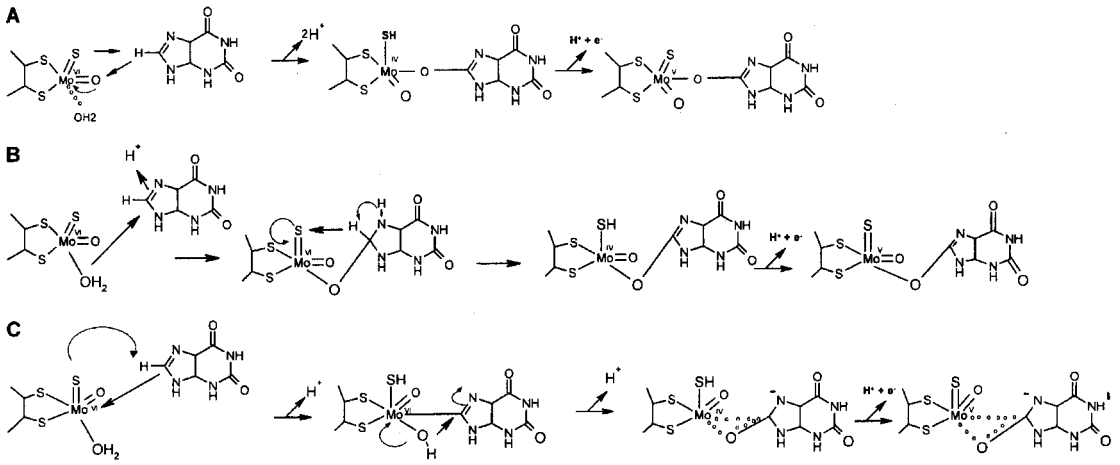

Fig. 2. Hypothetical initials steps of XDH reaction according to *A*) Hille (1996a,b, 1997), *B*) Huber *et al.* (1996), and *C*) Bray *et al.* (1996).

bound H_2O (or OH group) is transferred as a nucleophile, in others the oxygen is transferred from Mo=O species as an electrophile and, again in others, first a molybdenum-carbon bond is formed with the substrate, followed by the addition of molybdenum-coordinated water, Fig. 2 (Hille *et al.* 1999).

Role of the molybdenum cofactor: *1.* The pterin-pyran dithiolene system of Moco can embody several functions in molybdenum containing enzymes (Mendel 1997). *2.* The molybdenum center is anchored by pterin-pyran dithiolene system in the protein matrix of the enzyme. *3.* The electron flow into and out of the molybdenum center follows an electron transfer route that is facilitated by relative proximity of the Fe-S centers. Electrons pass from the molybdenum site to Fe-S II center and further to the exposed Fe-S I center. *4.* The cofactor modulates the redox potential of the molybdenum center via its dithiolene group.

Xanthine dehydrogenase

Purine metabolism in plants: Kalberer (1965) has revealed that coffee plants can catabolize caffeine to xanthine, which is further metabolized to allantoin, then to allantoic acid and finally to urea and CO_2. In 1969, the experiments were done with pea seedlings, which after infiltrated with hypoxanthine were able to catabolize hypoxanthine to xanthine (Silver *et al.* 1969). It was the first proof of a xanthine dehydrogenase activity in higher plant tissues. Further, it was found that isotopically labeled compounds were rapidly converted into urea, allantoin and allantoic acid (Suzuki *et al.* 1975). These studies suggested that both coffee and tea plants have the ability to metabolize xanthine by the same pathway of purine catabolism observed in animals. The synthesis of purine nucleotides runs via purine salvage, even though they could be synthesized *de novo*. The utilization of hypoxanthine for the synthesis of nucleotides is much greater than that of xanthine. The conversion of hypoxanthine into IMP and xanthine into XMP is catalyzed by the same enzyme: hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8) (Murray 1971).

Ogutuga and Northcote (1970) have demonstrated that the purine ring of caffeine is derived from the purine resulting breakdown of nucleic acids and that hypoxanthine can serve as a precursor for caffeine biosynthesis more effectively than xanthine (Ogutuga and Nortcote 1970). This also suggests that caffeine arises from the breakdown of some nucleotides rather than directly from xanthine.

Occurrence of xanthine dehydrogenase: Xanthine oxidoreductase (XDH, EC 1.1.1.204) is an ubiquitous member of the molybdenum cofactor-containing hydroxylases that is involved in the first two steps of purine metabolism. XDH and xanthine oxidase (XO, EC 1.1.3.22) catalyze oxidative hydroxylation of hypoxanthine to xanthine and further to uric acid. Oxidation of hypoxanthine and xanthine is linked with a concomitant reduction of NAD^+ (in the case of XDH) or molecular oxygen (in the XO form). The XDH form is characterized by high xanthine/NAD^+ activity and low xanthine/oxygen activity, whereas the XO form by high xanthine/oxygen activity and a negligible xanthine/NAD^+ activity (Nishino 1994). Primary form of oxidoreductases occurring in plant tissues is XDH, which can be converted to XO in some species. This conversion can be caused by a tryptic digestion, sulfhydryl oxidants or by enzyme-thiol transhydrogenase (EC 1.8.4.7) (Battelli *et al.* 1982).

XDH and XO catalyze turnover of hypoxanthine to xanthine and a consequent transformation of xanthine to uric acid. These enzymes are quite well explored in animals and some microorganisms (Woolfolk *et al.* 1977), but knowledge about their occurrence in plants is not so clear. The most extensively investigated of all Moco enzymes is milk XO (Hille 1995). XDH activity was detected in many higher plants such as *Pharbitis nil*, *Synapis alba*, *Gossypium hirsutum*, *Secale cereale*, *Portulaca oleracea*, *Sorghum sudanense*, and *Nicotiana tabacum* (Nguyen 1980), *Symphytum officinale* (Butler *et al.* 1961), *Glycine max* (Tajima *et al.* 1975), *Pisum sativum* (Silver and Gilmore 1969), *Triticum aestivum* (Montalbini 1998), *Thea sinensis* (Suzuki and Takahachi 1975), *Coffea arabica* (Vitoria *et al.* 1999), *Lens esculenta* (Kumar and Tanja 1975), *Cicer arietinum*, *Lupinus albus*, *Pisum sativum*, *Triticum aestivum*, *Phaseolus vulgaris*, *Trifolium repens*, *Trifolium pratense*, *Vicia faba*, *Vicia sativa*, *Vigna unguiculata* (Montalbini 2000), *Zea mays* (Barabas *et al.* 2000), *Lycopersicum esculentum* (Sagi *et al.* 1999), and *Arabidopsis thaliana* (Hoj *et al.* 1998). Electrophoretically homogeneous proteins were prepared from *Vicia faba*, *Lens esculenta*, *Cicer arietinum*, *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum* (Sauer *et al.* 2002), and *Triticum aestivum*.

Molecular properties of XDH: Xanthine oxidoreductase is a homodimeric protein of molecular mass about 300 kDa that contains three different prosthetic groups. These groups are located in three distinct domains of each 150 kDa peptide chain. Two non-identical iron-sulfur centers are positioned in the 20 kDa domain near the N-terminus, the FAD is in the central 40 kDa domain and molybdopterin cofactor is in the 85 kDa domain consisting of two subdomains near the C-terminus (Nishino 1994). XDH (like ALO) possesses a terminal inorganic sulfide at its Mo-site.

Some XDHs can be converted to XO form irreversibly by proteolysis with proteases (trypsin, chymotrypsin) or reversibly by sulfhydryl oxidants (Nishino *et al.* 2000). During conversion from XDH to XO form by a sulfhydryl oxidant, several cysteine residues are oxidized, but possibly not all of them are involved in XDH to XO conversion. The reversible conversion of XDH to XO is caused by modification of Cys 535 and Cys 992. The formation of disulfide bond is one possible mechanism of XDH/XO transformation (Enroth *et al.* 2000). Peroxisomal endoproteases could carry out the partial proteolysis, which results in the irreversible conversion of XDH into the superoxide-generating XO (Distefano *et al.* 1999). Trypsin invokes proteolysis after Lys 551 and pancreatin cleavage after Leu 219 and Lys 569. Both changes result in irreversible transformation to XO (Enroth *et al.* 2000).

By modification of the protein molecule either by proteolysis or disulphide formation, significant conformational changes seem to occur, particularly around the flavin prosthetic group, resulting in changes of reactivity of the flavin as well as the loss of NAD-binding site.

Animal type of XDH and XO shows no difference between iron-sulfur potentials (Hunt *et al.* 1993). The potential for the FAD/$FADH^{\bullet}$ couple was also found to be almost the same as that reported for XO form (-270 mV). The obvious difference between the redox potentials in XO and XDH is in the $FADH^{\bullet}/FADH_2$ potential in XO. This potential is higher than the FAD/$FADH^{\bullet}$ potential in XO (-230 mV). The potential for $FADH^{\bullet}/FADH_2$ in XDH (-410 mV) was found to be 140 mV lower than the $FADH^{\bullet}/FADH_2$ couple in XO.

When XO is converted to XDH, NAD^+ reacts much faster with the enzyme. It suggests conformational changes near the flavin since NAD^+ reacts near the flavin binding site. The midpoint potential of the FAD in XO is not poised to reduce NAD^+. Lowering the FAD midpoint potential in the XDH form of the enzyme places the FAD couple much closer to that of the NAD^+/NADH couple. Redox potential for the molybdopterin cofactor was found the same for XDH and for XO. Content of FMN form in XDH was found bigger than FAD form in plant XDH. Purified XDH from soybean nodules contained 89 % FMN form and 11 % FAD form of total flavin content (Triplett *et al.* 1982).

Spectral characteristics of XDH: The visible absorption spectrum of other Moco oxidoreductases containing molybdenum, flavin and iron-sulfur clusters as prosthetic groups usually shows peak close at 450 nm with a shoulder at 550 nm. Pure enzyme from *Triticum aestivum* has the visible absorption spectrum with a maximum at 329-332 nm, a shoulder at about 392-394 nm and a low absorption peak from 480 to 510 nm (Montalbini 1998).

Crystal structures: Some crystal structures of XDH and XO has been determined (mostly of milk XO and XDH), but no plant XDH structure has been solved yet. Crystal structures of some bacterial enzymes from molybdenum hydroxylases family have also been revealed (aldehyde oxidase from *Desulfovibrio gigas*) (Romao *et al.* 1997). The monomer of bovine milk XDH (see the dimer

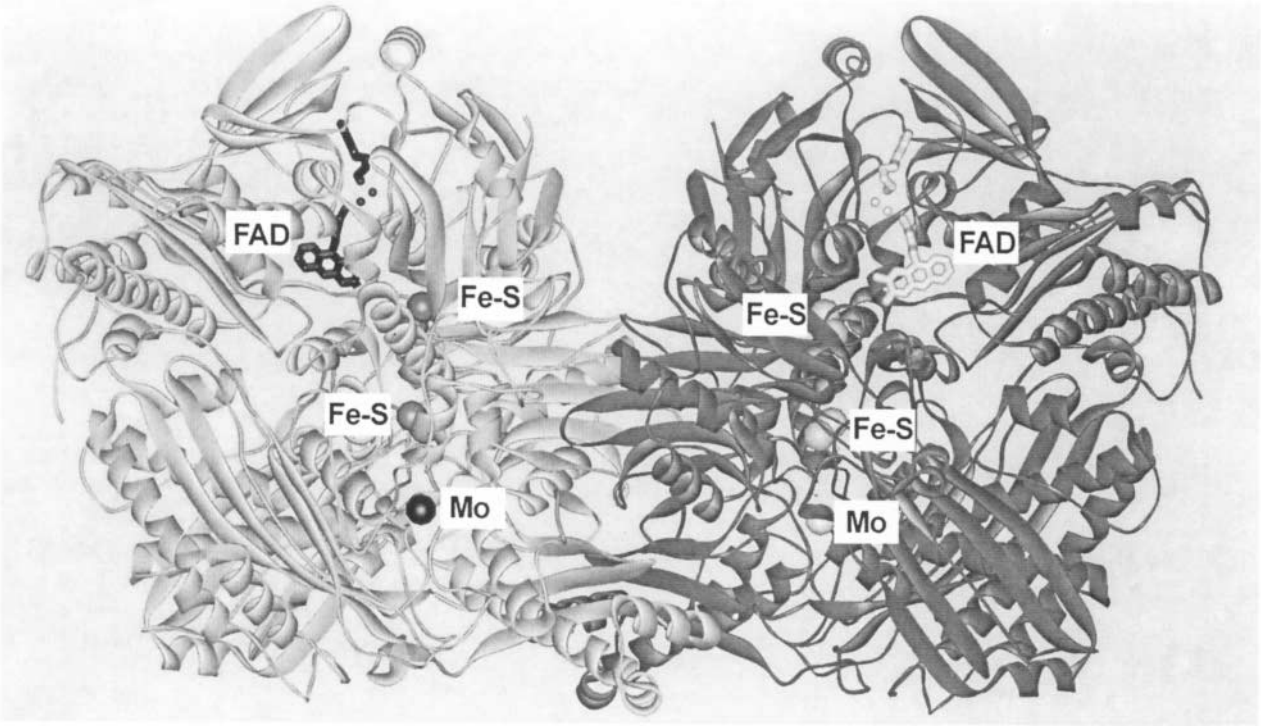

Fig. 3. Structure of bovine XDH according to Enroth (2001).

structure, Fig. 3) can be divided into three domains. N-terminal domain involves residues 1 to 165, contains both Fe-S centers and is connected to the FAD binding domain (residues 226 to 531) by a long segment consisting of residues 166 to 225. The FAD domain is connected to the third domain by a linker segment (residues 532 to 589). The third domain, represented by residues 590 to 1.332, bears Moco close to the interfaces of the Fe-S centers and FAD binding domain (Enroth *et al.* 2000). Mo center lacks double-bonded sulfur atom, a double-bonded oxygen atom, and an oxygen atom with a single bond as a ligand to the Mo ion. The single bound oxygen is resupplied to the Mo-center from solvent water and should be transferred to the substrate during catalysis. The N-terminal domain of XDH, where Fe-S clusters are situated, is in structural aspects very similar to the one found in *Desulfovibrio gigas*. N-terminal subdomain contains 2Fe-2S cluster, which is coordinated to Cys 43, Cys 48, Cys 51 and Cys 73. C-terminal subdomain is bound to Cys 113, Cys 116, Cys 148 and Cys 150.

Comparison of XO and XDH structure: The most obvious difference between XDH and XO form are much longer stretches of polypeptide chain missing in the XO sequence. No significant changes were found in the global folds of XDH and XO and around the two Fe-S centers. The XDH/XO inter-conversion is obvious on the three structure levels: modification of Cys residues, cleavage after either Lys 551 or after Leu 219 and 569 and spatial changes near flavin. One possible mechanism of the reversible oxidative structural transformation of XDH to XO implies the formation of a disulfide bond between Cys 535 and Cys 992. The irreversible change of XDH to XO happens by tryptic proteolysis after Lys 551 or by pancreatin cleavage after Leu 219 and Lys 569. The most different site between XDH and XO structure is the FAD active site. All the amino acids playing a role in the transformation are located on the side opposite to the entrance to the FAD domain active-site cleft. They make a direct influence on the orientation of residues surrounding the FAD binding site. Breakdown in the chain interaction between Phe 549 and Arg 427 by cuts to the peptide chain could trigger the large rearrangement displayed by a highly charged loop (Gln 423-Lys 433) passing opposite to the flavin ring. This transition removes the side chain of Asp 429 from its close contact with atom C6 of the flavin and replaces it with the side chain of Arg 426 as the flavins' closest neighbor. It changes the electrostatic potential of the flavin environment. After the Gln 423-Lys 433 displacement, the access of the NAD^+ to the FAD is blocked.

Catalytic reaction of XDH: The reaction mechanism of xanthine oxidase-related enzymes based on crystallographic data is illustrated in Fig. 4 (Romao *et al.* 1997): *1.* The coordinated water molecule is activated by the neighboring Glu 869, and represents the labile oxygen transferred onto the carbonyl carbon atom of the substrate. *2.* This nucleophilic attack is facilitated by hydrogen-bonding interactions of the carbonyl oxygen with the water ligand, which polarizes the carbonyl double bond. *3.* The resulting carboxylic acid product, generated after hydride transfer to the sulfide group, replaces the coordinated water at the molybdenum. *4.* Finally, the product is released from the reduced molybdenum center, which may be facilitated by the transient binding of the proximal Glu 869 to the metal. A water molecule present in the active site cavity reoccupies the vacant coordination position. The electron transfer within the active site of xanthine oxidase proceeds from the Moco to the FAD and is mediated by the iron-sulfur centers (Hille 1996b).

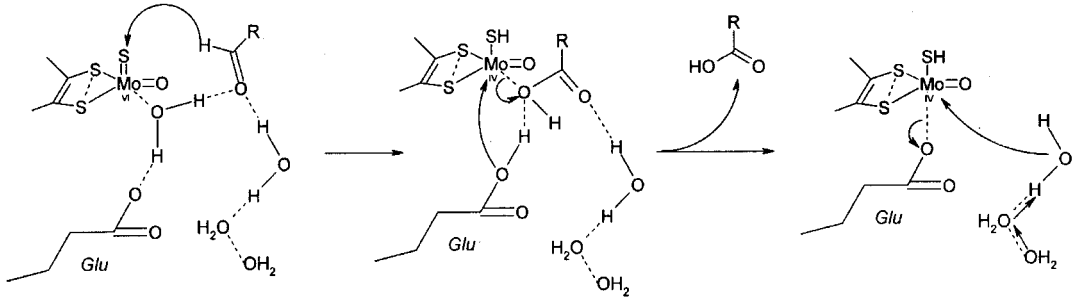

Fig. 4. Proposed mechanism for the reductive half-cycle of the hydroxylation reaction of Moco at the active site of XDH according to Romao *et al.* (1997).

Physiological function of XDH: Biological function of XDH in higher plants still remains unclear. There are no XDH mutants described for higher plants. In the Moco-deficient mutants of plants being characterized by the pleiotropic loss of all Mo enzyme activities, there are no symptoms that could be traced back specifically to the lack of XDH. Obviously, it seems that XDH does not play a vital role in plants, but it is perhaps involved in important physiological processes like ureide synthesis and purine catabolism, pathogen response, cell death and senescence of plant. In all plant organs and tissues, the main physiological role of XDH seems to lie in its

functioning as a part of the general purine catabolism. Purines are catabolized via hypoxanthine-xanthine-uric acid-allantoin-allantoic acid pathway, in which XDH catalyzes the first two steps. Treatment of nodulated soybean plants with a structural analogue inhibitor allopurinol led to a striking decrease in ureides attended by a dramatic increase in the xanthine content in the nodules. Plant XDH is very likely to produce superoxide radicals as does the animal XO (Montalbini 1992a) and was shown to be involved in host-pathogen relationships between phytopathogenic fungi like *Uromyces* (Montalbini 1992a) and *Puccinia* (Montalbini 1992b) with legumes, and cereals respectively. XDH activity during host-pathogen interaction was increased several folds. Oxidative processes during senescence or salinity stress involve the increase of activities of oxygen radical generating enzymes and superoxide ions. XDH activity sharply increased in parallel to an increase in superoxide dismutase activity and other oxygen related enzymes in pea leaves (Del Rio *et al.* 1998, Sagi *et al.* 1998, 1999).

Localization of XDH: The highest activities of XDH were found in legume root nodules with levels 500 to 1000-fold higher than in leaves of legumes and non legume plants (Nguyen *et al.* 1978). Intracellular localization shows that XDH seems to be associated with peroxisome organelles (Corpas *et al.* 1997). Xanthine and uric acid, and their metabolite allantoin were determined in pea peroxisomes (Del Rio *et al.* 1998). On the other hand, XDH was shown to be an extracellular enzyme by using immunocytochemical methods in cowpea root nodules (Datta *et al.* 1991). No labeling was observed in the enlarged peroxisomes, mitochondria or plastids on either cytoplasmic or peribacteroid membrane.

Cytokinins and XDH: The important role of xanthine oxidoreductase in purine degradation initiated speculations about its involvement in degradation pathways of plant regulators cytokinins. However, no solid data have been obtained so far to prove actual participation of XDH in these processes. Cytokinins are chemical compounds promoting cell division and differentiation that affect important events in the plant body, growth of lateral buds, leaf expansion, delay of senescence, promotion of seed germination and chloroplast formation (Mok *et al.* 2001).

NAD(P)H: nitrate reductase

Another member of Moco containing enzymes in plants is NAD(P)H:nitrate reductase (NR, EC 1.7.1.1-3). NR belongs to closely cognate family occurring in plants, fungi and algae, which catalyzes assimilation of nitrate by irreversible reduction of nitrate to nitrite using NAD(P)H cofactor (Campbell 1999). Changes in nitrogen supply affect Mo distribution and NR activity in eggplants (Víllora *et al.* 2002).

Structure and function: NR is a soluble enzyme composed of ~100 kDa polypeptides which carries equivalents of Moco, heme-Fe and FAD. NR is active only as a homodimer, but has tendencies to further dimerize to a homotetramer. Active sites in NR are joined by an electron transport pathway from FAD via heme-Fe to the Moco (Campbell 2001). Three domains of NR are connected by protease sensitive hinge regions and bind Moco, heme and FAD. There are three conserved amino acid sequence regions and other three highly variable regions in the NR: two hydrophilic inter-domain regions hinge 1 (about 30 amino acids long) and hinge 2 (about 20 amino acids long), and a non conserved N-terminus varying in length from 60 to 100 residues (Mendel *et al.* 1999). Electron transfer runs from natural reductant NAD(P)H via FAD, heme and Moco to nitrate.

Eukaryotic NR differs from prokaryotic NR in the structure of Moco. Prokaryotic Moco contains an additional nucleotide, in some cases two pterins are bound to the Mo atom (Dias *et al.* 1999). Bacterial NR can be membrane bound or may be a soluble enzyme in the periplasmic space involved in denitrification. No eukaryotic NR has been found to have an iron-sulfur redox center built into the same polypeptide with the Mo cofactor. Fe works as a mediator of the electron transfer in the enzyme from the donor and an electron reservoir.

Monomer of NR has 8 distinct sequence region (Campbell 2001): *1.* N-terminal sequence that differs in length for different NR forms and can be important for activity regulation. *2.* Moco domain that possesses sites for nitrate binding and a reducing active site. *3.* Dimer interface domain, which contributes to the nitrate-reducing active site. *4.* Proteolytic site hinge 1 and the site of the regulatory Ser residue in plant NR, which becomes phosporylated. *5.* Cyt *b* domain, which holds heme-Fe. 6. Hinge 2 site different for many NR forms. *7.* FAD binding domain. *8.* NAD(P)H binding domain at the C-terminus joined to the FAD domain by a short linker containing a three-strand β-sheet.

The Moco-domain of NR consists of two separate domains. The larger one binds Moco and contains α and β structure. The C-terminal domain contains mostly β structures. This domain constitutes the dimerization interface of the homodimeric holoenzyme. Moco is deeply buried in the protein structure. Amino acids participating in Moco binding are completely conserved within all NRs (Kisker *et al.* 1997). NR-heme domain is very similar to other known heme domains of different phylogenetic origin. Invariant His residue is essential for binding of the heme group. The similarity between heme

domains is so big, that a chimeric protein where the original heme domain was replaced with a mammalian heme domain (Cyt b_5) is active in plant NR (Quinn *et al.* 1994). FAD domain of NR is about 30 kDa. Like the Moco domain, the FAD domain is built up from two subdomains. One is located on the N-terminus of the domain and constrains FAD and the other one is on C-terminus and binds NADH. The FAD domain is six stranded β-barrel structure with a single α-helix (Lu *et al.* 1994). The α-helix is necessary for FAD binding. Cys residue (maize C242, *Arabidopsis* C889) is conserved among NRs. By mutagenesis of this invariant Cys, the FAD becomes inactive (Dwivedi *et al.* 1994).

NR is an important enzyme of nitrate assimilation, where it catalyzes the first step in nitrate metabolism. The activity of NR could be regulated by posttranslational conversions, that reversibly inhibit the enzyme. This procedure functions as a response to external or internal signals, CO_2 levels, light/dark transitions, changes in cytosolic pH. Regulation of NR activity is caused by phosphorylation (Ser residue) in the hinge 1 region. This phosphorylation has not a direct effect on the activity of NR but is linked with binding of a NR inhibitor protein.

Aldehyde oxidoreductase

Aldehyde oxidoreductase (ALO, E.C. 1.2.3.1) is an enzyme catalyzing degradation of broad variety of aldehydes and N-heterocyclic compounds among bacteria and eukaryotes. The first ALO presence in plants was published in potato (Rothe 1974). There were found two isozymes in cytoplasmic fraction that were able to convert large-scale aldehydes but not xanthine as substrate.

Molecular characteristics of ALO: Molecular mass of plant ALO is about 300 kDa. The first purified ALO from potato has been shown to be 360 kDa (Rothe 1975), while maize ALO has a molecular mass of 300 kDa (Koshiba *et al.* 1996). ALO is a homodimer. Each monomeric protein contains a redox-active iron center in the form of Fe-S located near the N-terminus, FAD, and Moco in the stoichiometry of 4:1:1.

ALO plays an important role in the biosynthesis of plant hormones such as indole-3-acetic acid (IAA) and abscisic acid (ABA) by catalyzing the final steps in the biosynthesis of these two plant hormones. IAA is the most important member of plant hormones auxins. It plays important roles in a number of plant activities, including phototropism, gravitropism, apical dominance, fruit development, abscission and root initiation. Auxins travel from cell to cell being taken up on one side of the cell by influx transporters in the plasma membrane and moved out on the other side by efflux transporters, where it can then be taken up by the adjacent cell. Auxins have direct effects and effects on gene expression. Direct effects are mediated by changes in movement of ions in and out of the cell through the plasma membrane and by the extension of the cell wall causing elongation of the cell. Many auxin effects are mediated by changes in the transcription of genes. Indole-3-acetaldehyde oxidase activity was found in cucumber seedlings (Bower *et al.* 1978) and pea seedlings (Miyata *et al.* 1981). Deeper investigation was done on maize ALO (Koshiba *et al.* 1996). This enzyme was identified to be Mo-containing with FAD and iron present in the molecule and molecular mass of 300 kDa.

Abscisic acid is a key factor regulating transpiration, stress responses, germination of seeds and embryogenesis. Most effects of ABA seem to be related to water relations – it apparently acts as a signal of reduced soil water availability. ABA conserves water by reducing stomatal opening and in consequence water loss, slowing growth and mediating adaptive responses. It was also shown that a certain class of ABA-deficient tomato mutants (*flacca* and *sitiens*) was impaired in the oxidation of ABA aldehyde to ABA (Taylor *et al.* 1988) and may therefore lack ABA-aldehyde specific ALO activity. However, such ABA-aldehyde ALO has not been purified yet.

Molecular cloning of ALO: ALO is the most extensively studied member of the Moco family at the genetic level. Recently, the molecular cloning of *alo* genes from *Zea mays* (Sekimoto *et al.* 1997) and *Lycopersicon esculentum* (Min *et al.* 2000) have been accomplished. There has been cloned two cDNAs for plant ALO from *Zea mays* (*zmALO*). Predicted proteins have a significant level of similarity with animal ALOs and XDHs from various sources. The overall level of respective identities with zmALO-1 and zmALO-2 are 30.9 and 31.2 % to bovine ALO (Li Calzi *et al.* 1995), 29.9 and 30.1 % to human XDH (Wright *et al.* 1993), 31.2 and 32.1 % to another human XDH (Ichida *et al.* 1993), 31.5 and 30.3 % to *Drosophila pseudoobscura* XDH (Riley 1989), and 30.3 and 30.8 % to *Aspergillus nidulans* XDH (Glatigny *et al.* 1995).

Sulfite oxidase

In plants, the existence of sulfite oxidase has been for a long time unclear, while sulfite reductase as part of sulfur assimilation was well characterized. Recently, the purification of an SO enzyme from the plant *Malva sylvestris* (Ganai *et al.* 1997), cloning of the respective gene from *Arabidopsis thaliana* and a biochemical characterization of the encoded protein (Eilers *et al.* 2001) have been reported.

Molecular characteristics: Animal SO is a homodimeric enzyme consisting of an N-terminal heme domain and a C-terminal molybdenum domain. In contrast to homologous animal enzymes, protein encoded by *Arabidopsis thaliana* gene lacks the heme domain as found by comparing the amino sequences and spectral characteristics (Eilers *et al.* 2001). Using SDS-PAGE electrophoresis, SO was detected as a single band at 45 kDa in *Nicotiana tabacum,* pea, spinach, barley, carrot and poplar trees by using a polyclonal antiserum raised against recombinant SO from *Arabidopsis thaliana.* The enzymes of primary sulfate assimilation were localized in the chloroplasts, but not SO activity from *Nicotiana plumbaginifolia* and *Arabidopsis thaliana.*

Conclusions

Considerable physiological functions of Moco-containing enzyme family show importance of relatively rare transition metal Mo in plants. Moco has essential role for higher plants and defective Moco has lethal consequences for the organism due to the loss of activity of all Mo containing enzymes. The most important role plays nitrate reductase, which is irrecoverable for growth of the plant by control of nitrate assimilation. The loss of NR activity is fatal for the plant. Other enzymes of Moco containing family also play vital roles in plants. ALO is important for growth of plant by controlling of IAA and ABA content and XDH plays a role in nitrogen assimilation through the catabolism of purine compounds. Another role of XDH is probably in responses to stress conditions. Free radicals generated by stresses (salinity, senescence of plant) may be neutralized by uric acid, product of the XDH reaction. Uric acid is an effective scavenger of active oxygen species (Sagi *et al.* 1998). XDH is involved in relationships between phytopathogenic fungi and legumes or cereals (Montalbini 1992 a,b).

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