Review

FAD-containing polyamine oxidases: a timely challenge for researchers in biochemistry and physiology of plants

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Abstract

Recent investigations on plant polyamine oxidase (PAO) are reviewed. The enzyme belongs to a new class of flavoenzymes with similar structural features including, among others, monoamine oxidase. Plant PAOs catalyse the oxidation of the polyamine substrates spermidine and spermine. The reaction products are propane-1,3-diamine and 1-pyrroline or 1-(3-aminopropyl)pyrrolinium, respectively, along with hydrogen peroxide. Plant PAOs are predominantly localised in the cell wall. Purification procedures and molecular properties of several plant PAOs are compared. A special attention is being paid to the recently solved crystal structure of the maize enzyme and its implications for the substrate binding and catalytic mechanism. Substrate specificity and inhibitors of plant PAOs are also described. The potential roles for PAO-generated H₂O₂ in lignin biosynthesis and cell wall cross-linking reactions, which may regulate growth and contribute to cell defence, are discussed. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Polyamine oxidase; Flavoprotein; FAD; Hydrogen peroxide; Spermine; Spermidine

1. Introduction

The aliphatic diamines and polyamines show ubiquitous occurrence in prokaryotic and eukaryotic organisms [1]. Their implication in growth and key developmental processes has engaged the interest of an increasing number of researchers during the last three decades. Polyamines have been tentatively proposed as a new class of plant regulatory substances since they are involved in a variety of biological activities [2,3]. During recent years, interest has been growing in naturally occurring polyamines. The polyamines spermidine and spermine and the related diamine putrescine appear to be essential for processes of growth, cell division and differentiation. A role for these compounds has been also postulated in macromolecular synthesis, stress responses and senescence [4,5]. It is well known that polyamines bind DNA. However, there is evidence that some proteins possess a specific polyamine-binding site, possibly having a regulatory function [6]. Polyamine levels are finely controlled through a network regulatory system that includes pathways for polyamine biosynthesis and degradation as well as for their transport across the cell membrane [4,5].

In addition to a suggested role in the regulation of cellular polyamine levels, growing evidence arises indicating that polyamine catabolism in plants is associated with physiological events such as lignification, cell wall stiffening and cellular defence [1,7]. The enzymes concerned with
catabolism of these substances, which are widely distributed in living organisms, have been studied particularly in plants [1,2,8]. In comparison with large and detailed investigations focused on copper amine oxidases in last several years, only little attention has been devoted to study of flavoprotein polyamine oxidases (PAOs), which in addition to different substrate specificity also exhibit distinct pattern of substrate degradation [1,8]. The discovery that some polyamine analogues have strong anticancer activity indicates that the enzymes involved in polyamine metabolism may represent attractive targets for new antineoplastic drugs [9,10].

Recent investigations on plant PAOs are reviewed in the present paper. New findings on the crystal structure, reaction mechanism and physiological role of the enzymes, which appeared within last few years, promise further and intense research work in future.

2. Reaction catalysed by flavin-containing polyamine oxidases

Polyamines (including diamines) are oxidatively deaminated by amine oxidases (AOs) that are widespread in bacteria, fungi, higher plants and animals [1]. These enzymes are formally classified as copper-containing amine oxidases (CuAOs, E.C. 1.4.3.6) and flavin-containing polyamine oxidases (PAOs, E.C. 1.5.3.-). An alternative grouping divides AOs into those that act on the primary amino group of di- and polyamines (diamine oxidases, DAOs) and those that act on the secondary amino group of polyamines (PAOs) [1,8,11]. As suggested by Morgan, the latter should be further subdivided according to whether propane-1,3-diamine or 3-aminopropanal are the reaction products [12].

The exact nature of the reaction products depends on the enzyme source [1,8,11]. Mammalian PAOs transform spermidine and spermine into putrescine and spermidine, respectively, and 3-aminopropanal. On the contrary, plant and bacterial PAOs catalyse the conversion of spermidine and spermine to 4-aminobutanal and 1-(3-aminopropyl)-4-aminobutanal, respectively, and propane-1,3-diamine (Fig. 1). The aminoaldehydes produced in the reaction spontaneously cyclise to 1-pyrroline and 1-(3-aminopropyl)pyrrolinium, respectively [1,12,13], the latter compound occurring mainly in the bicyclic form of 1,5-diazabicyclo[4.3.0]nonane (Fig. 1) in leaves of various cereals [13].

Fig. 1. Scheme of the reaction catalysed by PAOs from mammalian (M) and plant (P) sources. Small arrows indicate the sites where the respective enzymes attack the polyamine substrates spermidine and spermine [12,13].
3. Purification and molecular features of plant polyamine oxidases

Flavin-containing PAOs have been isolated particularly from plants belonging to the Gramineae. The enzyme was found in oat (*Avena sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*) and rye (*Secale cereale*) [1]. PAOs from oat [21,22], maize [23,24] and barley [25] have been purified to homogeneity and thoroughly studied. The enzyme from millet (*Setaria italica*) has been only partially purified [26]. Only PAOs from water hyacinth (*Eichhornia crassipes*) [27] and lily (*Lilium longiflorum*) [28] have been purified and characterised outside the Gramineae in the monocotyledonous plants. In the dicotyledonous plants, putative PAO activity has been detected in alfalfa (*Medicago sativa*), however, detailed properties of the enzyme remain unclear [29].

Purification procedures, which have been used for the isolation of plant PAO, included an extraction of the enzyme from a crude plant or cell wall extract and its further purification using low pressure chromatography (LPC) techniques [21–28] or fast protein liquid chromatography (FPLC) [25]. For the extraction, acetone [22,23,26–28] or high ionic strength buffers [24,25] have been used. The LPC procedures utilised cation exchangers (CM-cellulose, CM-Sephadex, SP-Sepharose), hydroxyapatite and Sephadex gel matrices. Affinity-interaction sorbents for efficient PAO purifications have been also reported, made on the basis of long-chain diamines (C6, C8) [27,28] or specific antibodies coupled to Sepharose 4B matrix [30].

Molecular properties of several plant PAOs are summarised in Table 1. All the enzymes are monomeric, some of them have been shown to be glycoproteins. Chromatographic properties of plant PAOs on cation exchangers indicate that their pI values fall into the acidic region. The enzymes are retained on CM-cellulose or SP-Sepharose at pH 5.5 and then usually eluted by high ionic strength buffers [22,24,25,27,28]. PAOs purified to homogeneity show a typical absorption spectrum of oxidised flavoprotein with absorption maxima at 280, 380 and 450 to 460 nm [1]. The flavin cofactor confers a yellow colour to purified PAO preparations. It has been shown, that plant PAOs contain flavin adenine dinucleotide as the
cofactor in a ratio 1 mol of FAD per mol of the enzyme [23,25,28]. The addition in anaerobiosis of equimolar amounts of spermidine to oat or maize PAO results in the cofactor reduction as shown by the decrease in absorption at 380 and 450 nm. Reoxygenation then restores the spectrum [22,24].

The N-terminal amino acid sequences of maize [31,32], barley [25] and oat PAO (A. Radová, M. Šebela, S. Jacobsen, unpublished results) are presented in Table 2, where homologous amino acid sequences of four other flavoenzymes: yeast acetylspermidine oxidase [33], bacterial tyramine oxidase [34] and tryptophan 2-monoxygenase [35] and human type B monoamine oxidase [36], are also given. The first complete amino acid sequence of a flavin-containing PAO has been reported for the maize enzyme [32]. The nucleotide sequence of maize PAO cDNA is 1737 bp in length. It shows a single open reading frame encoding a polypeptide (500 amino acids) starting with a 28 amino acid sequence showing the typical features of a secretion signal peptide [32]. This is in agreement with the apoplastic localisation of maize PAO as deduced on the basis of available cytochemical and immunocytochemical evidence [37,38]. Maize PAO matches the signature motif of a new family of FAD-dependent oxidases, identified by sequence alignment. This family includes oxidoreductases catalysing different reactions such as phytoene desaturase, protoporphyrinogen oxidase and monoamine oxidase, all possessing similar ADP-ribityl binding sites [32,39]. A significant structural homology has been detected between maize PAO and vertebrate flavin-containing monoamine oxidases (MAO), even beyond the ADP-ribityl binding motif [32,36]. PAO and MAO share the same overall folding topology and FAD-binding site, although this similarity does not extend, as expected, to the substrate binding site [15]. Furthermore, the Cys residue involved in the covalent binding of FAD in vertebrate MAOs is replaced by a Thr residue in maize PAO [32,36] suggesting a non-covalent interaction of the FAD in plant PAOs [15,39].

Polyclonal antibodies raised against PAO from lily did not cross-react with PAOs from maize, oat and water hyacinth. This result suggests that the lily PAO does not share common epitopes with PAOs of the Gramineae [28]. Similarly, immunoprecipitation analysis performed with anti-maize PAO antiserum against oat and barley PAO revealed a high immunological compatibility between the oat and maize enzyme, while the barley PAO shares few antigenic determinants with the latter [30].

4. Kinetic properties of plant polyamine oxidases

All plant PAOs show very restricted substrate specificity oxidising only spermine and spermidine [1]. This observation is in accordance with recently found “U-shaped” structure of the active site [15]. The respective $K_m$ values have been found in the region between $10^{-5}$ M and $10^{-6}$ M. The pH optima for the oxidation of the substrates vary among different species, being equal in the range 5.5 to 6.8 for most of the enzymes [22,24,26–28]. In the case of barley PAO, however, the pH optima 4.8 (spermine) and 8.0 (spermidine) have been reported [25,40]. Is it due to different contact and auxiliary amino acid side chains at the active site? The question has not yet been answered. Plant PAOs are usually equally active with spermine and spermidine [22,24,27]. The only exception is barley PAO again, which oxidises spermine 14 times faster than spermidine at their respective pH optima [25,40]. Maize PAO also catalyses the oxidation of $N^1$-acetylspermidine, $N^1$-acetyllyspermine and $N^8$-acetylspermidine at the same optimal

<table>
<thead>
<tr>
<th>Property</th>
<th>Oat</th>
<th>Maize</th>
<th>Barley</th>
<th>Water hyacinth</th>
<th>Lily</th>
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<td>Molecular weight (SDS-PAGE)</td>
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<td>5.4</td>
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<tr>
<td>FAD content (mol/mol enzyme)</td>
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<td>1</td>
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<td>1</td>
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$^a$ Radová et al. (unpublished result).
pH 6.5 [41]. However, the enzyme is quickly inactivated during the reaction. Maize PAO cleaves $N^1$-acetylspermine and $N^8$-acetylspermidine at the same position as in spermine and spermidine reaction. These findings suggest that cell wall PAO from maize does not affect the interconversion pathway of acetylpolyamines [41]. The activity of oat PAO with spermine was stimulated by 1 M NaCl [42]. Similarly, the activity of lily PAO with spermine and spermidine was increased in the same way [28].

Calorimetric analysis on the maize enzyme revealed a single two-state transition at pH 6.0 with $T_m = 49.8^\circ C$. At pH 5.0, the thermal stability is increased by more than 14$^\circ$C [31]. The pH optimum for the stability of the native enzyme is 5.0, similar to that of barley leaf PAO [31]. Maize and barley PAO do not reduce added electron carriers such as 2,6-dichlorophenolindophenol, phenazine methosulfate, or ferricyanide either in anaerobiosis or in the presence of oxygen [43,44]. However, maize PAO is able to utilise $p$-benzoquinone as electron acceptor in aerobicosis [43]. The coupled reduction of cytochrome $c$ or 2,6-dichlorophenolindophenol could be effected by this system. For barley, maize and oat PAOs, the respective $K_m$ values for oxygen are relatively high [21,24]. The analysis of transient kinetic data revealed that the maize enzyme has a low rate constant for the reaction with oxygen, responsible for the high value 0.2 mM of $K_m$ (O$_2$). Maize PAO is therefore only partially oxidised even under fully aerobic condition and the oxygen concentration may be a relevant rate-limiting factor in vivo [45]. On the other hand, the reaction with spermidine is much more efficient and both the turnover number (90 s$^{-1}$) and the low $K_m$ value (27 $\mu$M) suggest that the transformation proceeds rapidly under physiological conditions [45].

The activity of plant PAOs is measured either spectrophotometrically by means of a coupled reaction with horseradish peroxidase and guaiacol [21,25] or by measuring oxygen consumption using a Clark type electrode [22,24]. Specific activities of the final PAO preparations from cereals usually reach about 100–1500 nkat per mg with spermidine or spermine substrates [22,24,27,28]. The preparations are stable without a significant loss of the activity for several weeks at 4$^\circ$C [25,27] or frozen at $-15^\circ$C [21].

A loss of enzymatic activity during polyamine degradation has been observed for barley and oat PAO [16,25,46]. As it was shown for the maize enzyme, the inhibition is caused by the aminoaldehydes produced during the reaction. Polyamine oxidation products, 4-aminobutanal and 1-(3-aminopropyl)-4-aminobutanal, behave as competitive inhibitors of maize PAO ($K_i \sim 10^{-4}$ M) [31]. The aminoaldehyde formed by the oxidation of $N^1$-acetylspermine also inhibits in a competitive manner ($K_i \sim 10^{-5}$ M) [41]. PAO from water hyacinth is competitively inhibited by octamethylenediamine ($K_i \sim 10^{-6}$ M) [27]; similarly, the lily enzyme is competitively inhibited by hexamethylenediamine ($K_i \sim 10^{-6}$ M) [28]. Analogues of spermidine and spermine, in which the length of

<table>
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<tr>
<th>Enzyme</th>
<th>Sequence</th>
<th>Reference</th>
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<td>Barley PAO</td>
<td>01 GKGPRVIVGAGMSGISAARLDDAGVVL</td>
<td>[25]</td>
</tr>
<tr>
<td>Oat PAO</td>
<td>01 AAGPRVIVGAGGSGAGK</td>
<td>Radová et al. (unpublished results)</td>
</tr>
<tr>
<td>Maize PAO$^b$</td>
<td>29 ATVGPRVIVVAGMSGISAARLSEAGITDL</td>
<td>[32]</td>
</tr>
<tr>
<td>ASO1$^b$</td>
<td>02 TTVRIDAIVGAGVASKLTKAGVSNI</td>
<td>[33]</td>
</tr>
<tr>
<td>TYO$^b$</td>
<td>01 SNPHVIVVAGFAGLVAARELQMAGVYDVE</td>
<td>[34]</td>
</tr>
<tr>
<td>TR2M$^b$</td>
<td>35 GTPTPRVIAVAGISGLVAAETLRRAGKVD</td>
<td>[35]</td>
</tr>
<tr>
<td>MAO-B$^b$</td>
<td>01 MSNKCDVVVVGGGIGSGMAAAKLLHDSGLNVV</td>
<td>[36]</td>
</tr>
</tbody>
</table>

$^a$ The N-terminal amino acid sequences of barley and oat PAOs were obtained by automated Edman degradation, for the enzyme from maize seedlings the sequence was deduced from cloned cDNA. Translated sequences of homologous flavoenzymes: yeast acetylsermidine oxidase (Candida boidinii, ASO1), bacterial tyramine oxidase (Micrococcus luteus, TYO), bacterial tryptophan 2-monooxygenase (Pseudomonas syringae savastanoi, TR2M) and human type B monoamine oxidase (MAO-B), are given for comparison. The identical residues of the three PAO sequences are in bold. Underlined amino acids in the MAO-B sequence are conserved in all seven sequences.

$^b$ Numbering corresponds to the respective cDNA sequence.
the tetramethylene chain was shortened or lengthened, caused competitive inhibition of polyamine oxidation by barley and oat PAOs \( (K_i \text{ values between } 10^{-6} \text{ and } 10^{-4} \text{ M}) [40,42,44] \). Oat PAO is also inhibited by fungicide guazatine \( (K_i \sim 10^{-8} \text{ M}) \) containing guanidine groups [21]. Methylglyoxal-bis(guanylhydrazone), semicarbazide and \( \beta \)-hydroxyethylhydrazine were shown to be inhibitors of PAO from barley [16,25,44]. PAOs from cereals and water hyacinth are significantly inhibited by acridine compounds, namely quinacrine, at 1 mM concentration due to the presence of flavin cofactor [22–27].

5. Crystal structure of a plant polyamine oxidase

The only PAO that has been crystallised and whose three-dimensional structure has been solved up to date is the enzyme of maize seedlings [15,47]. Maize PAO is a monomeric enzyme [24] consisting of 13\( \alpha \) helices and 19\( \beta \) strands, which fold to form two well-defined domains (Fig. 3). The FAD-binding domain comprises three fragments, whose main structural elements are a central parallel \( \beta \) sheet flanked by a \( \beta \) meander and three \( \alpha \) helices. The substrate-binding domain is composed of two fragments and is characterised by a six-stranded mixed \( \beta \) sheet flanked by five \( \alpha \) helices. The two domains create a tunnel that defines the enzyme active site at their interface. The folding topology of maize PAO resembles that of several other flavoenzymes catalysing dehydrogenation reactions such as glucose oxidase or \( \beta \)-amino acid oxidase [15].

PAO from maize seedlings is a glycoprotein [24]. The glycosylation site has been identified to be Asn77 [15]. The FAD cofactor is non-covalently bound to the protein and is deeply buried within the structure. The isoalloxazine ring of FAD is located at the interface of the two domains [15]. With the exception for the flavin C5a, N5 and C4a atom that line the active site, all FAD atoms are solvent-inaccessible. The conformation of the oxidised flavin is nonplanar — the orientation might be important in precisely aligning the cofactor with respect to the polyamine substrate. The PAO active centre consists of remarkable “U-shaped” tunnel, which passes through the protein structure at the interface between the two above-mentioned domains [15]. The tunnel extends to a length of about 30 Å. The U-shape brings its two openings onto the same side of the protein surface. The turning point, around which the tunnel sharply bends and reverses its direction, represents the core of the catalytic centre, where the flavin ring is located (Fig. 3). There is a marked contrast in the chemical nature of the two arms of the U-shaped catalytic centre [15]. One arm is lined mainly by aromatic residues and it opens to the outside like a funnel with several acidic side-chains (Asp, Glu) on its rim (Fig. 4). In contrast, the other arm contains mostly oxygen atoms on its surface and displays a narrow entrance. In this respect, the ring of Asp and Glu residues seems to be suited to fulfil the role of guiding the polyamine substrate into the tunnel. Thus the substrate might be admitted into its binding site preferentially through only one of the two tunnel openings [15].

6. Substrate binding and its implications for the catalytic mechanism

The precise mechanism by which plant PAOs catalyse the oxidation of the polyamine substrates is still unknown. Overall, the catalytic cycle consists of two steps. During the first step (reductive), FAD is reduced upon polyamine oxidation. The second step (oxidative) represents re-oxidation of
the reduced flavin cofactor by molecular oxygen with the release of H$_2$O$_2$ (Fig. 2). In this respect, the three-dimensional structure of the maize enzyme in complex with substrate analogues reveals several relevant features [15]. The substrate analogue $N,N'$-bis(2,3-butadienyl)-1,4-butanediamine (MDL72527) [48], which competitively inhibits maize PAO ($K_i \sim 10^{-7}$ M) (R. Federico, unpublished results) was used for substrate-binding studies. MDL72527 binds in the central part of the catalytic tunnel, adopting a conformation that closely matches the shape of the tunnel (Fig. 4). The ligand binding does not cause any significant conformational change of the enzyme. The bound inhibitor molecule is completely solvent-inaccessible and it is involved in extensive H-bond and van der Waals contacts with the surrounding protein atoms. Moreover, it interacts with the flavin ring. The flavin C4a-N5-C5 locus becomes shielded from the solvent after the inhibitor binding. Six of the twelve inhibitor carbons are located in close proximity to protein oxygens. These PAO-inhibitor interactions appear to have the stereochemical characteristics of the so-called CH–O hydrogen bonds [15]. These features suggest that the reaction takes place in the solvent-protected environment provided by the catalytic tunnel. The water molecule that is H-bonded to the flavin N5 atom (Fig. 4) in the native enzyme seems to be well positioned to perform the hydrolytic attack on the imino compound resulting from polyamine oxidation to produce the final aldehyde product (Fig. 2). The three-dimensional structure shows that MDL72527 is a non-covalent inhibitor of maize PAO [15]. In this respect the plant PAO differs from the mammalian enzymes. It may be related to the different substrate cleavage displayed by mammalian and plant PAOs. The inhibitor differs from the spermine substrate only in the absence of the primary amino groups. Thus, the substrate primary amino groups are strictly required by the enzyme in order to catalyse substrate oxidation. Primary nitrogen atoms of the substrate spermine possess a positive charge, which may affect $pK_a$ values of the surrounding catalytic residues and those of the secondary substrate amino groups. The primary amino groups are likely to affect the binding mode of the substrate in particular [15].
used for rapid purification of the enzyme. Unlike the oat and maize enzyme, PAO is apparently symplastic in barley primary leaves [52]. The apoplastic nature of maize PAO has been recently confirmed by an electron-microscopic cytochemical method based on the precipitation of H₂O₂ generated by the in situ polyamine oxidation. PAO activity staining was most intense in the middle lamellar region of the wall and in cells exhibiting highly lignified walls [37]. PAO activity is very low in resting seeds and can be detected as soon as germination begins. During the first phases of growth, a continuous increase of enzyme activity is observable in the shoot [53].

Sub-cellular and tissue distribution of PAO activity has been investigated in maize seedlings. It was observed that from 30 to 90% of maize PAO activity is tightly bound to cell walls of maize seedlings (in the shoot and root, respectively), while the remainder can be eluted with high ionic strength buffers [54]. Despite some apparent differences, the ionically bound and the tightly wall-bound PAOs are probably the same protein [55]. Tissue and organ distribution of PAO in the maize seedling, studied by a histochemical approach, shows an apparent PAO localisation mainly in the cell walls of xylem (coleoptile), xylem and xylem parenchyma (mesocotyl, root), rhizodermis, hypodermis and endodermis (root) and epidermis (coleoptile, mesocotyl) [54]. Apparent histochemical peroxidase activity shows the same tissue and organ distribution indicating the preferential association of the two enzymes with the cell walls of tissues where lignification, suberisation, and wall stiffening occur [54].

Moreover, ultrastructural localisation of PAO has been done by means of immuno-gold experiments with antibodies specific for the polypeptide moiety in different organs of the maize seedling [38]. The antibodies have been raised in order to develop immunocytochemical method clearly distinguishing between CuAO and PAO coexisting in plant tissues. The occurrence of both enzymes has been recently demonstrated in barley [56] and maize seedlings [57]. In the endodermal cells of the primary and lateral roots only the cells with secondary walls were labelled. No labelling was observed in the intercellular spaces, the suberin layer or in the primary wall. All the secondary walls of the xylem cells were labelled. In the etiolated mesocotyl, labelling was limited to the secondary walls of large vessel and vascular parenchyma, while the parenchymal cells were unreactive. The gold particles were limited to the secondary thickenings, in particular in the differentiating cells that were involved in secondary wall deposition. No labelling was found over the primary wall. PAO was detected only rarely over the epidermal cell walls. All these findings indicate that PAO may play a crucial role in cell wall differentiation [38]. The available evidence suggests a considerable similarity for the physiological role of CuAO and PAO in the apoplast with regard to the functional correlation with peroxidase [58]. Indeed, it has been reported that peroxidase-mediated oxidative cross-linking reactions between phenolic residues of matrix polysaccharides, as well as those of extensins may partially account for wall stiffening, and have remarkable consequences for the wall expansion during growth and differentiation [59]. It could indicate that wall stiffening in vivo is controlled by the synthesis of hydrogen peroxide in the cell wall. Unravelling the biochemical systems providing H₂O₂ to peroxidase in the above-mentioned reactions, as well as establishing their functional correlation with light-mediated growth, represents a major unsolved problem. Several lines of evidence suggest that PAO may be functionally correlated with peroxidase and may have a key role in the production of reactive oxygen intermediates in the apoplast [38, 58].

A correlation between the photomodulation of plant growth, cell wall differentiation and stiffening and PAO expression has been recently confirmed [7]. The accumulation of PAO transcript and activity were enhanced by light treatment in cortical and epidermal (outer) tissues of the mesocotyl. Histochemical analysis revealed that this phenomenon is mostly due to the increased level of PAO activity in epidermal and sub-epidermal tissues. The photomodulation of PAO activity upon de-etiolation in outer tissues is mediated by a phytochrome. A close correlation was found between the time course of red-light-elicited increase of PAO activity and that of growth inhibition in the outer tissues of apical, growing zone of the mesocotyl. Light exposure of etiolated, sub-apical mesocotyl segments resulted in a higher production of hydrogen peroxide in the incubation medium as compared to segments incubated in the dark. The latter phenomenon was inhibited by the specific PAO inhibitor guazatine. A short pre-
treatment of mesocotyl and coleoptile segments with 1 mM spermidine, but not propane-1,3-diamine, inhibited elongation growth induced by indoleacetic acid; this phenomenon being reverted by catalase. Similarly, spermidine-grown *Arabidopsis thaliana* plants showed a different morphology (shorter stalks and darker green-leaves) when compared with control ones. In most plant organs, the uptaken spermidine was converted to putrescine. The successive increase in putrescine level suggests the presence of an interconversion pathway mediated by a putative polyamine oxidase [60]. These results indicate that PAO activity is important in producing H$_2$O$_2$ in vivo for wall stiffening reactions and may be involved in the modulation of growth and cell wall differentiation [7]. In this context, it was previously observed that spermidine incubation of intact maize roots resulted in an increase of PAO and peroxidase activity, associated to an earlier differentiation of xylem tissues [61]. Among others, these aspects of polyamine catabolism may shed new light on the context of programmed cell death, host–pathogen interactions and resistance to abiotic stress.

8. Conclusions and future perspectives

Plant PAOs play an important role in several physiological processes including polyamine homeostasis and cell death [1]. The enzymes exhibit many similar properties at the molecular level. However, the situation in higher plants is quite puzzling because the enzymes occur at a high concentration only in some species. Two explanations are possible: some species have alternative mechanisms of polyamine catabolism, or the apparent lack of PAO activity is due to a very low enzyme concentration in plant tissues and/or to its inhibition by still unknown factors [1]. The potential roles for amine oxidase-generated hydrogen peroxide in lignin biosynthesis and cell wall cross-linking reactions that may regulate growth as well as defence and cell death responses have been discussed. In addition to polyamine catabolism, the enzymes and their reaction products may have other important functions in some species. In this light, the study of polyamine catabolism merits a renewed interest and further experimental effort. Several ways for pursuing the research work on plant PAOs may be outlined. The cloning of the maize enzyme opened new horizons for studying PAO gene expression. There are many chemical and physiological factors that can influence such a delicate process. The expression also depends on the stage of plant development, it could be influenced by growth conditions and by stress during pathogen infections. Open topics for further research on plant PAOs also include studies on distribution of the enzyme protein and the activity on conditions of growth, pathogen infections, injuries and wound healing. Moreover, site-directed mutagenesis experiments using overexpressed PAO will contribute to a deeper insight into structure–function relationships and biotechnological applications of this class of enzymes. Applications of PAOs in food industry and agriculture are under development. Just recently, a polyamine oxidase-based biosensor appeared [62]. The aminoaldehyde products of the enzyme reaction show antiprotozoal effects [63] suggesting possible application of PAOs in medicine. Regarding to enzymologic studies, a big attention is being paid to search for new substrates and sensitive and potent inhibitors. Chemical synthesis of highly reactive mechanism-based inhibitors (made on the basis of a natural substrate) has the top priority. Kinetic studies with substrates and inhibitors could bring informations about intermediates of the catalytic cycle of plant PAOs, which still remain unclear. Many questions could be answered by comparative experiments on expression, activity modulation and physiological functions of plant FAD-containing PAOs and copper-containing DAOs. Both types of amine oxidases have been found in barley, which seems to be very suitable subject for such investigations.

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