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Review

Mechanism-based inactivators of plant copper/quinone containing amine oxidases

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Abstract

Copper/quinone amine oxidases contain Cu^{II} and the quinone of 2,4,5-trihydroxyphenylalanine (topaquinone; TPQ) as cofactors. TPQ is derived by post-translational modification of a conserved tyrosine residue in the protein chain. Major advances have been made during the last decade toward understanding the structure/function relationships of the active site in Cu/TPQ amine oxidases using specific inhibitors. Mechanism-based inactivators are substrate analogues that bind to the active site of an enzyme being accepted and processed by the normal catalytic mechanism of the enzyme. During the reaction a covalent modification of the enzyme occurs leading to irreversible inactivation. In this review mechanism-based inactivators of plant Cu/TPQ amine oxidases from the pulses lentil (*Lens esculenta*), pea (*Pisum sativum*), grass pea (*Lathyrus sativus*) and sainfoin (*Onobrychis viciifolia*,) are described. Substrates forming, in aerobiotic and in anaerobiotic conditions, killer products that covalently bound to the quinone cofactor or to a specific amino acid residue of the target enzyme are all reviewed.

Keywords: *Lathyrus sativus*; *Lens esculenta*; *Onobrychis viciifolia*; *Pisum sativum*; Amine oxidase; Mechanism-based inactivation

Contents

Abbreviations: TPQ, topaquinone, 6-hydroxydopa quinone; LSAO, lentil seedling amine oxidase; PSAO, pea seedling amine oxidase; GPAO, grass pea amine oxidase; OVAO, sainfoin amine oxidase; Cu/TPQ-AO, copper/quinone containing amine oxidase; DABI, 1,4-diamino-2-butyne; DAPY, 1,5-diamino-2-pentyne.

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1. Introduction

Amine oxidases belong to the heterogeneous superfamily of enzymes catalyzing the oxidative deamination of biogenic amines, including mono-, di- and polyamines, to the corresponding aldehyde, ammonia and hydrogen peroxide. These enzymes, isolated from numerous organisms ranging from bacteria, yeasts, plants and mammals, show not only heterogeneity in structure, but also differences in substrate and inhibitor specificity, mechanism of oxidation and different subcellular localization. Plant amine oxidases [amine:oxygen oxidoreductase (deaminating) (copper/quinone containing); EC 1.4.3.6] (Cu/TPQ-AOs) are soluble dimeric enzymes, each monomer containing one copper atom and one organic prosthetic group (TPQ) as cofactors. TPQ is the quinone of 2,4,5-trihydroxyphenylalanine (topaquinone; Janes et al., 1990; Mure, 2004 and references therein) formed from a conserved tyrosine residue in a post-translational event (Cai and Klinmann, 1994; Matsuzaki et al., 1994; Dawkes and Phillips, 2001). The catalytic cycle of plant Cu/TPQ-AOs has now been outlined (Padiglia et al., 2001; Mure et al., 2002). Briefly, an aminotransferase mechanism is operative (Fig. 1) in which the oxidized enzyme (a) reacts with an amine substrate giving a Cu^{II}–quinone ketimine (b), which, owing to proton abstraction, gives rise to a Cu^{II}–quinolaldimine (c). After hydrolysis and release of the aldehyde, an aminoquinol species (d) is formed, and the reduced cofactor is reoxidized by molecular oxygen via the Cu^{I}–semiquinolamine intermediate (e).

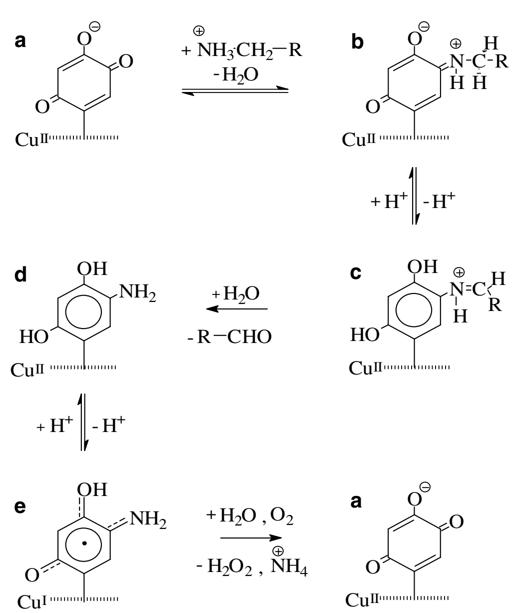


Fig. 1. Catalytic mechanism of lentil amine oxidase: (a) Resting oxidized enzyme; (b) Cu^{II}–quinone ketimine, a substrate Schiff base; (c) Cu^{II}–quinolaldimine, a product Schiff base; (d) Cu^{II}–aminoquinol, (e) Cu^{I}–semiquinolamine radical.

Under standard conditions the velocity (v) of the amino oxidase catalyzed reaction can be defined as: $v = -\mathrm{d}[\mathrm{R{-}CH_2{-}NH_3^+}]/\mathrm{d}t = -\mathrm{d}[\mathrm{O_2}]/\mathrm{d}t = \mathrm{d}[\mathrm{R{-}CHO}]/\mathrm{d}t = \mathrm{d}[\mathrm{NH_4^+}]/\mathrm{d}t = \mathrm{d}[\mathrm{H_2O_2}]/\mathrm{d}t$, where v can be altered by the presence of modifier substances classified as follows.

1.1. Reversible inhibitors

Reversible inhibitor binds the enzyme non-covalently and enzyme activity can be restored when the inhibitor is removed. Two main types of reversible inhibitors can be recognized, competitive and non-competitive.

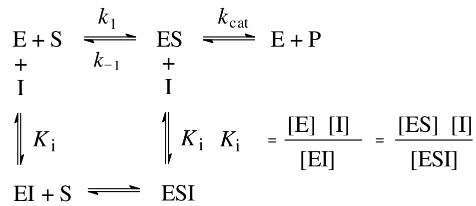
Substances binding the enzyme at the same site as the substrate produce the competitive type of inhibition forming either an enzyme–substrate complex (ES) or an enzyme–inhibitor complex (EI). The system may be represented by the following Scheme 1.

Competitive inhibitors of plant AOs were found to be diamino- and monoaminino-ketonic compounds, some alkaloids, bivalent transition metal complexes with diamines, and the monoamine cysteamine (Padiglia et al., 1998 and references therein).

Substances binding the enzyme at a different site from that for the substrate produce the non-competitive type of inhibition. The non-competitive inhibitor can bind either to the enzyme giving an inactive complex enzyme–inhibitor (EI), or to the ES complex giving inactive ternary complex ESI. The system may be represented by the following Scheme 2.

$$\begin{array}{l} \mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_{cat}}{\rightleftharpoons} \mathrm{E + P} \\ + \\ \mathrm{I} \\ \big\updownarrow K_i \quad K_i = \dfrac{[\mathrm{E}]\,[\mathrm{I}]}{[\mathrm{EI}]} \\ \mathrm{EI} \end{array}$$

Scheme 1.



Scheme 2.

Good examples of non-competitive inhibitors are the copper-chelating agents cyanide, azide, diethyldithiocarbamate, 1,10-phenantroline and 2,2-bipyridyl (Padiglia et al., 1998 and references therein).

1.2. Irreversible inhibitors

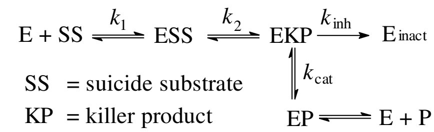
Irreversible inhibitors are represented by substances making a progressive decrease in enzymatic activity with time that becomes complete when all the enzyme is combined with the inhibitor. The most effective irreversible inhibitors of plant AOs are carbonyl group reagents, like substituted hydrazines, interacting with TPQ cofactor.

Several reversible and irreversible inhibitors have been found and used to probe the structure–function relationships of plant amine oxidases (Padiglia et al., 1998; Šebela et al., 2003).

1.3. Mechanism-based inactivators

Mechanism-based enzyme inactivators were initially described by Helmkamp et al. (1968). This exciting type of inactivator consists of a substrate analogue that is able to bind to the active site of an enzyme being accepted and processed by the normal catalytic mechanism of this one. However the substrate analogue contains a chemically unreactive functional group that, during the reaction, is transformed into a highly reactive intermediate which covalently binds to a specific amino acid residue or to a cofactor. Equilibrium is formed between normal turnover and covalent modification of the enzyme leading to irreversible inactivation of the catalytic activity of the target protein. The substrate analogue has also been termed suicide substrate because it induces the target enzyme to commit suicide during the catalytic cycle. The system may be represented by the following Scheme 3.

A mechanism-based inactivator can give a lot of information about the action mechanism of the target enzyme and must follow these criteria (Walsh, 1984): (i) time-dependent loss of enzyme activity with pseudo-first order process; (ii) the inactivation should show saturation kinetics; (iii) kinetic protection by a common substrate; (iv) the inactivation should result in a covalent attachment of the killer product to the enzyme active site making, as a consequence, (v) an irreversible inactivation of the target enzyme.



Scheme 3.

Mechanism-based plant Cu/TPQ-AO inactivators can be distinguished on the basis of their interaction with the enzyme, and this review deals with suicide substrates of amine oxidases from the pulses lentil, pea, grass pea and sainfoin.

2. Compounds causing enzyme inactivation by the formation of a covalent adduct between killer products and TPQ

2.1. Alkylamines

The alkylamines 2-bromoethylamine (Fig. 2(a)) and 2-chloroethylamine, and the short diamine 1,2-diaminoethane are both poor substrates and irreversible inactivators of LSAO (Medda et al., 1997a). The stoichiometry of the products obtained after oxidation of 2-Br-ethylamine with an excess of LSAO shows that a total of 1 mol of ammonia and 1 mol of aldehyde are released. Furthermore, the formation of 1 mol of hydrogen peroxide is demonstrated. During the oxidation of these suicide substrates, the reversible formation of an enzyme–killer product complex [EKP] occurs followed by an irreversible inactivation of the enzyme [E_{inact}], typical of mechanism-based inactivation.

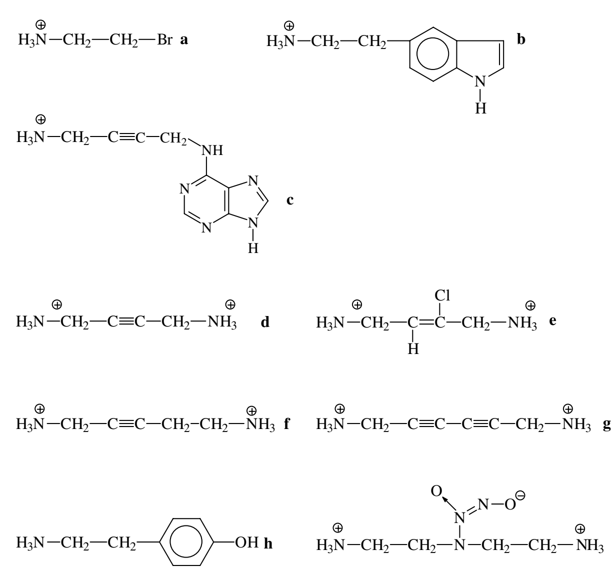


Fig. 2. Structures of suicide substrates: (a) Br-ethylamine; (b) Tryptamine; (c) ABYAD, N^6-(4-aminobut-2-ynyl)adenine; (d) DABI, 1,4-diamino-2-butyne; (e) 1,4-diamino-2-chloro-2-butene; (f) DAPY, 1,5-diamino-2-pentyne; (g) 1,6-diamino-2,4-hexadiyne; (h) Tyramine; (i) DETA-NONOate, 3,3-bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene.

Table 1
Kinetics parameters of Cu/TPQ amine oxidases and suicide substrates reported in Fig. 2

Compound	K_M (mM)	[h]k_{cat} (s^{-1})	[i]k_{inh} (min^{-1})	[j]K_i (mM)
[a]Br-ethylamine	ND	ND	0.8	0.054
[b]Tryptamine	0.5	2.7	ND	ND
[c]DABI	1	ND	4.9	0.32
[d]DAPY	ND	ND	0.13	0.01
[e]DAPY	ND	ND	0.31	0.05
[f]Tyramine	0.71	12.8	ND	ND
[g]DETA-NONOate	ND	ND	0.018	0.28

[a] Enzyme LSAO (Medda et al., 1997a).
[b] Enzyme LSAO (Medda et al., 1997b).
[c] Enzyme PSAO (Peč and Frébort, 1992).
[d] Enzyme OVAO (Lamplot et al., 2004).
[e] Enzyme GPAO (Lamplot et al., 2004).
[f] Enzyme LSAO (Padiglia et al., 2004).
[g] Enzyme LSAO (Longu et al., 2005).
[h] k_{cat}: nmol substrate (nmol enzyme active sites)$^{-1}$ s^{-1}.
[i] k_{inh}: constant for inactivation.
[j] K_i: apparent inhibition constant (half-maximal inhibition).

The rate constant for inactivation (k_{inh}) is 0.8 min^{-1} for 2-Br-ethylamine (Table 1), 0.3 min^{-1} for 2-Cl-ethylamine and 0.13 min^{-1} for 1,2-diaminoethane, while the half-maximal inhibition constants (K_i) are 5.4×10^{-5}, 2.6×10^{-4} and 1.1×10^{-3} M, respectively. Under these conditions, each enzyme active site turns over about 100 times with 2-Br-ethylamine, 420 times with 2-Cl-ethylamine and 580 times with 1,2-diaminoethane before becoming inactive.

Spectrophotometric features obtained from experiments done with native and copper-free enzyme, under aerobic and anaerobic conditions, in the presence of 2-Br-ethylamine, provide a key to understanding the inhibition mechanism. During the reaction between LSAO and a common substrate such as putrescine, the absorption band at 498 nm of the native enzyme disappears and new sharp bands at 434 and 464 nm appear in the spectrum (Fig. 3). These bands are diagnostic of the free radical intermediate which accumulates in the absence of oxygen (Finazzi Agrò et al., 1984; Dooley et al., 1987; Medda et al., 1999). The radical species generated in the absence of air remains stable for many hours. In contrast, with 2-Br-ethylamine the radical decays with $t_{1/2} = 10$ min. After complete disappearance of the bands at 434 and 464 nm, the enzyme is found to be irreversibly inhibited. In anaerobiosis the enzyme is trapped either in the aminoquinol or in the free radical form (Fig. 1(d), (e)), after having released one molecule of aldehyde per active site (Medda et al., 1995), and the attack of the aldehyde product must take place on one of these two intermediates. The copper-free enzyme, also able to release the aldehyde, does not proceed to the free radical species and exists entirely in the aminoquinol form (Medda et al., 1995). Besides, the apoenzyme is not inactivated by 2-Br-ethylamine indicating that the aldehyde only reacts with the free radical to form a

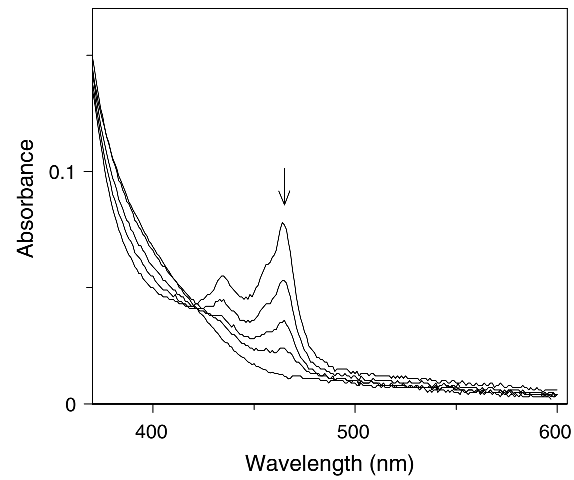


Fig. 3. Reaction of 18 μM LSAO active sites, under anaerobic conditions, with 2 mM Br-ethylamine in potassium phosphate buffer, pH 7.0. Different spectra are recorded in a Thunberg-type spectrophotometer cuvette immediately after addition of Br-ethylamine from 4 min to 20 min with intervals of 5 min.

covalently modified enzyme. Given that the inactivated enzyme does not contain bromide the irreversible inhibition mechanism is certainly caused by the aldehyde product reacting with the highly reactive species of the TPQ-derived free radical catalytic intermediate that, after β-elimination of the halogen, forms a stable 6-membered ring (Fig. 4(a)).

2.2. Indoleamines

It has long been recognized that plant amine oxidases are involved in the tryptophan metabolic pathway leading to the formation of the plant hormone 3-indoleacetic acid, but the exact mechanism has not yet been described (Clarke and Mann, 1957; Percival and Purves, 1974). In this context tryptamine and its derivatives should play an important physiological role in plants. LSAO is able to catalyze the oxidative deamination of some indoleamines, such as tryptamine (Fig. 2(b)), 5-hydroxytryptamine, and 5-methoxytryptamine (Medda et al., 1997b), with K_M values nearly the same as for the normal substrate putrescine (Table 1). However, the oxidation of indoleamines leads to irreversible loss of the enzymatic activity only in the absence of oxygen.

The inhibition mechanism is outlined by fluorescence and spectrophotometry. LSAO shows a fluorescence

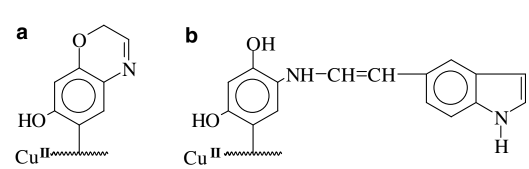


Fig. 4. Structure of TPQ-adducts after the reaction of lentil amine oxidase with: (a) Br-ethylamine; (b) Tryptamine.

emission spectrum with a maximum at 332 nm when excited at 290 nm. The fluorescence emission spectrum of irreversibly inactivated LSAO shows a maximum shifted to 340 nm when excited at 290 nm. The absorption spectrum of the radical formed in anaerobiosis with indoleamines is stable for approximately 15 min. Afterwards the absorptions at 434 and 464 nm begin disappearing with a $t_{1/2}$ of 90 min, and fades away in about 5 h. During this process two isosbestic points at 478 and 424 nm are observed. In the meantime, the absorbance at 300–360 nm and around 245 nm increases reaching its maximum after 5 h. The difference in the fluorescence spectrum of LSAO after treatment with tryptamine and the loss of the absorption bands of the radical species support the assumption that it is due to the formation of a stable adduct between indoleacetaldehyde and TPQ semiquinone as shown in Fig. 4(b).

2.3. Cytokinin analogues

Cytokinins are N^6-derivatives of adenine and have been shown to regulate a wide range of developmental events in plants promoting cell division, seed germination, chloroplast formation and delay of senescence (Miller et al., 1955; Mok et al., 2000). Moreover the hydroxylated cytokinin zeatin is a major constituent in plants and its biochemical pathway is currently under the scope of biomedical research in processes of cell division and proliferation (Veselý et al., 1994; Meijer et al., 1997; Kryštof et al., 2002).

N^6-(4-Aminobut-2-ynyl)adenine (ABYAD; Fig. 2(c)), a combined analogue of cytokinins and a biogenic amine, causes irreversible inhibition of LSAO and GPAO suggesting a likely mechanism-based inactivation (Lamplot et al., 2005). The oxidation of ABYAD is too slow and cannot be registered by kinetic activity assay method. After 120 min incubation with ABYAD, LSAO and GPAO lose their original spectra and gain a yellow brown colour with the formation of a 300-nm absorbing intermediate. The inhibition mechanism can involve either the interaction of the aldehyde product with the TPQ cofactor or with an amino acid residue at the active site.

3. Compounds yielding an irreversible enzyme–inhibitor complex by involvement of a specific amino acid residue at the active site

3.1. 1,4-Diamino-2-butyne, 1,4-diamino-2-chloro-2-butene, 1,5-diamino-2-pentyne and 1,6-diamino-2,4-hexadiyne as analogues of natural substrates of plant amine oxidases

1,4-Diamino-2-butyne (DABI; Fig. 2(d)), a putrescine analogue, is a substrate of pea cotyledon and grass pea amine oxidase and shows saturation kinetics with $K_M = 1$ and 0.8 mM, respectively (Peč and Frébort, 1992; Frébort et al., 2000). The interaction between PSAO or GPAO and DABI leads to an irreversible loss of the enzyme activity. Substrate-saturation kinetic data and the pseudo first-order time-dependent irreversible inactivation of pea enzyme indicate that DABI is a mechanism-based inactivator with characteristic constants $K_i = 0.32$ mM and $k_{inh} = 4.9$ min^{-1} (Table 1). The mechanism of the inactivation involves an intermediate aminoallenic compound, which forms a covalently bound pyrrole in the reaction with a putative Lys_{113} residue that lies in close proximity to the channel for substrate entry to the active site (Fig. 5(a); Frébort et al., 2000). In GPAO the putative amino acid binding site of DABI bound is supposed to be Glu_{113}.

1,4-Diamino-2-chloro-2-butene (Fig. 2(e)), very similar to DABI structure, is a mechanism-based inhibitor for PSAO, a 1.3 equivalent of which inhibits the enzyme by 94% ((Shepard et al., 2002). In this case, the inactivation also involves cyclocondensation onto a nucleophilic residue at the active site channel.

1,5-Diamino-2-pentyne (DAPY; Fig. 2(f)), a cadaverine analogue is found to be a weak substrate of grass pea and sainfoin amine oxidases (Lamplot et al., 2004). Prolonged incubation with DAPY, however, results in irreversible inhibition of both enzymes. The rates of inactivation of 0.1–0.3 min^{-1} are determined for GPAO and OVAO, respectively, and the apparent K_i values are in the order of 10^{-5} M (Table 1). The substrate cadaverine significantly prevents irreversible inhibition. Incubations of GPAO or OVAO with DAPY result in the appearance of a yellow-brown chromophore derivative ($\lambda_{max} = 310$–325 nm). Excitation at 310 nm is associated with emitted fluorescence at 445 nm, suggestive of extended conjugation. A lysine modification chemistry responsible for the observed inactivation is proposed as for DABI (Fig. 5(b)).

Finally considerable inactivation is seen for PSAO with 1,6-diamino-2,4-hexadiyne (Fig. 2(g)), with a k_{inact}

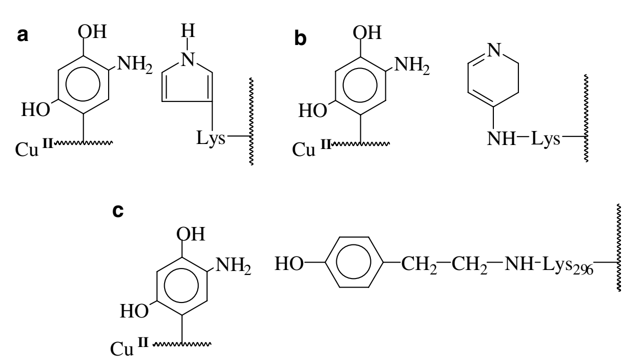


Fig. 5. Structure of amino acid-adducts after the reaction of: (a) pea or grass pea amine oxidase with DABI; (b) grass pea or sainfoin amine oxidase with DAPY; (c) lentil amine oxidase with tyramine.

of 0.46 min^{-1} and K_i of 14.8 μM. The inactivation reflects a covalent modification of an amino acid residue (Shepard et al., 2002).

3.2. Tyramine

Tyramine, an important compound in the plant kingdom, is known to be involved in the biosynthesis of some benzylisoquinoline alkaloids, that have been suggested to function as herbivore deterrents, and in the formation of the hydroxycinnamoyl amines *N*-feruloyltyramine and 4-*N*-coumaroyltyramine (Facchini and DeLuca, 1994; Facchini, 2001). These compounds are considered to be integral components of plant defence responses to pathogens (Facchini et al., 2002; Bonneau et al., 1994).

Tyramine (Fig. 2(h)) turns out to be a good substrate for LSAO (Padiglia et al., 2004) with a K_M value of 0.71 mM and a k_{cat} of 12.8 s^{-1} (Table 1). In the course of tyramine oxidation the enzyme gradually becomes inactivated with the concomitant appearance of a new absorption at 560 nm due to the formation of a stable adduct. Inactivation takes place only in the presence of oxygen and it is due to the reaction of the enzyme with the oxidation product of tyramine, the *p*-hydroxyphenylacetaldehyde (*p*HA) that can bind to one or more lysine residues of LSAO thereby interfering with the normal function of this amino acid in the catalytic process (Fig. 5(c)). The very unstable adduct *p*HA–Lys may rearrange rapidly to give one or more conjugated intermediate(s) with absorbance at 560 nm (Padiglia et al., 2004).

4. Compounds leading to inhibition of the enzyme only in the presence of substrate

Nitric oxide (NO) is a growth regulator and, at low concentrations, it can stimulate the induction of defence reactions whereas, at higher concentrations, it can lead to toxic products. The inorganic forms of oxidized nitrogen are sources of NO formation in plants by enzymatic and non-enzymatic reactions (Stöhr and Ullrich, 2002; Dordas et al., 2003).

3,3-Bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene (DETA-NONOate; Fig. 2(i)) and 3,3′-(hydroxynitrosohydrazino)bis-1-propanamine (DPTA-NONOate) are found to be substrates and irreversible inactivators of lentil enzyme (Longu et al., 2005). For DETA-NONOate an inactivation rate constant k_{inh} of 0.018 min^{-1} and an apparent inhibition constant K_i of 28×10^{-5} M are determined (Table 1). The inhibition is irreversible: neither dialysis against 100 mM potassium phosphate buffer, pH 7, nor filtration through a Sephacryl S-200 column restore the activity of the enzyme. The spectrum of the protein is strongly affected, in the course of reaction, with both substrates, leading to the formation of covalent adduct with a stable band at 334 nm. Since all NONOates spontaneously decompose in solution giving back NO, it really seems to be responsible for LSAO inhibition. The insensitivity of the native enzyme to NO suggests that this compound is unreactive toward both the cofactors, 6-hydroxydopa quinone (TPQ) and Cu^{II}. A model for irreversible inactivation is proposed that involves the Cu^{I}–semiquinolamine radical catalytic intermediate and NO released from NONO-ates.

5. Concluding remarks

The use of mechanism-based inactivators provides substantial information to understand the catalytic mechanism of plant amine oxidases. Inactivation by suicide substrates reported involves:

(i) The formation of a covalent adduct between a killer product and TPQ. In the reductive half-reaction the Schiff base Cu^{II}–quinolaldimine, after hydrolysis, releases the aldehyde leading TPQ in the reduced aminoquinol species which is in equilibrium with Cu^{I}–semiquinolamine intermediate. It has been well demonstrated that aldehydes, released from ethylamine and its halogen derivatives and from indoleamines, are the killer products reacting with the high reactive TPQ-derived free radical catalytic intermediate: the aminoquinol is apparently unreactive. The inactivation of nitric oxide released from NONOates is interesting: as observed NO does not react with TPQ, Cu^{II}, and aminoquinol, but is proposed to form a covalent adduct reacting with the Cu^{I}–semiquinolamine radical catalytic intermediate.

(ii) A covalent attachment of the inactivator to an amino acid residue at the site active. It has been demonstrated that, during the oxidative deamination of 1,4-diamino-2-butyne (DABI), 1,5-diamino-2-pentyne (DAPY) and tyramine, the released aldehydes are able to react to a lysine residue which is certainly involved in the inactivation of AOs. A similar inactivation mechanism is proposed for 1,4-diamino-2-chloro-2-butene and 1,6-diamino-2,4-hexadiyne.

Since the reaction between N^6-(4-aminobut-2-ynyl) adenine (ABYAD) and AOs has not been studied in detail, the inhibition mechanism can involve either the interaction of the aldehyde product with the TPQ cofactor or with an amino acid residue at the active site.

The suicide substrates tryptamine, the analogue of cytokinins N^6-(4-aminobut-2-ynyl)adenine, and tyramine acting as physiological substances, should aid greatly in understanding the mechanism of regulation of amine synthesis and degradation. It is also plausible that the oxidation of natural substances, interfering in some metabolic pathways, can be important in wound healing and in cell growth. The formation of indoleacetaldehyde from tryptamine can make inactivation of plant amine oxidases in conditions of hypoxia leading to or preventing cell damage. Although several enzyme systems are implicated in the regulation of tyramine level (for example tyramine hydroxycinnamoyl transferase), amine oxidase can act as a tyramine scavenger at low concentrations of such amine, while the activity should be suppressed at higher tyramine levels. This complex interplay can be important for the regulation of benzylisoquinoline alkaloids and hydroxycinnamoyl amine synthesis. N^6-(4-aminobut-2-ynyl)adenine, a combined analogue of cytokinins and biogenic amines, can interact not only with amine oxidase, but also with other enzymes involved in the metabolism of biogenic amines. In addition, its structure resembles that of natural purine-derived cytokinins (zeatin, isopentenyl adenine) as plant hormones. Last but not least is the inactivation of amine oxidases by nitric oxide. NO is a highly reactive gas with poisonous nature. Due to its instability, NO cannot be manipulated in the presence of oxygen and several compounds have been used for slow generation of NO in vitro. $[N(O)NO]^-$-derivatized compounds from polyamines are very useful compounds for the investigation of NO effects in biological studies (Hrabie et al., 1993). As demonstrated, amine oxidase activity can be suppressed at low NO levels especially under hypoxia interfering in the metabolism of biogenic amines. Thus, the inactivation of an enzyme by NO indicates that caution should be exerted whenever NONO-polyamines are used in studies of the biology of nitric oxide or for pharmaceutical applications.

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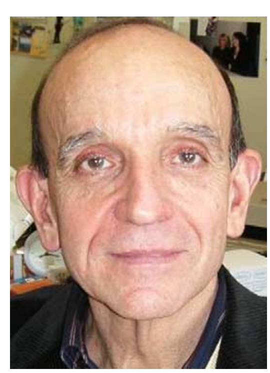

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