Pergamon

0031-9422(94)00756-X

Phytochemistry, Vol. 39, No. 1, pp. 1–9, 1995

0031-9422/95 $29.00 + 0.00

REVIEW ARTICLE NUMBER 102

PLANT COPPER-AMINE OXIDASES

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(*Received* 25 *July* 1994)

Key Word Index—Gramineae; Leguminosae; copper-amine oxidase; 6-hydroxydopa.

Abstract—In this review, the widely distributed plant copper-amine oxidases are described. The purification procedures, molecular features, substrate specificities, inhibitors, the stoichiometry of the catalysed reaction, spectroscopic features, the prosthetic groups and reaction mechanisms, are all reviewed.

INTRODUCTION

The amine oxidases are a group of enzymes that catalyse the oxidation of amines, in particular diamines and polyamines, to the corresponding aldehydes. Two main types are known: the copper-containing Cu-amine oxidases (Cu-AOs, EC 1.4.3.6) and the flavin-containing FAD-amine oxidases (FAD-AOs, EC 1.5.3.3). Although their exact physiological role in plants is not yet known, recent years have brought much progress in the understanding of the structure and function of these enzymes.

Werle and co-workers [1] first demonstrated the presence of amine oxidase activity in plant extracts in 1948. Since then amine oxidase activity from the following plant species has been described: *Arachis hypogae* [2], *Avena sativa* [3], *Canavalia ensiformis* [4], *Cicer arietinum* [5], *Cucumis sativus* [6], *Eichornia stricta* [7], *Euphorbia characias* [8], *Glycine max* [9], *Helianthus tuberosus* [10], *Hyoscyamus niger* [11], *Hordeum vulgare* [12, 13], *Lathyrus cicera* [14], *Lathyrus sativus* [15], *Lens esculenta* [16], *Lupinus luteus* [17], *Nicotiana tabacum* [18], *Onobrychis viciifolia* [19], *Oryza sativa* [20], *Phaseolus vulgaris* [14], *Pisum sativum* [21], *Setaria italica* [22], *Thea sinensis* [23], *Trifolium subterraneum* [24], *Triticum aestivum* [25], *Vicia faba* [26] and *Zea mays* [27, 28].

Many enzymes with amine oxidase activity have been purified to homogeneity and characterized. Those from the Leguminosae are from *Cicer arietinum* (seedlings) [5], *Glycine max* (seedlings) [29, 30], *Lathyrus cicera* (seedlings) [14], *Lathyrus sativus* (seedlings) [15], *Lens esculenta* (seedlings) [16], *Lupinus luteus* (seedlings) [17], *Onobrychis viciifolia* (leaves) [19], *Phaseolus vulgaris* (seedlings) [14], *Pisum sativum* (seedlings) [21, 31, 32], (cotyledons and embryos) [33], *Trifolium subterraneum* (leaves) [24], and *Vicia faba* (seedlings) [26] and (leaves) [34]. Two have been characterized in the Gramineae from *Hordeum vulgare* (seedlings) [12], and *Zea mays* (seedlings) [27]. Two species from other families have also been examined in detail: *Euphorbia characias* (latex) [8], and *Thea sinensis* (leaves) [23]. All these enzymes oxidize diamines and polyamines, with the exception of the *Thea* enzyme that oxidizes only monoamines and the *Euphorbia* enzyme that oxidizes only aliphatic diamines. All contain Cu(II) and as second prosthetic group an organic cofactor, probably 6-hydroxydopa. With regard to the enzymes from *Avena sativa* (leaves) [3, 35], *Hordeum vulgare* (leaves) [13, 36], *Oryza sativa* (embryos) [20] and *Zea mays* (shoots and seedlings) [28, 37] these contain FAD as prosthetic group and oxidize only polyamines. Exceptionally, the *Oryza* enzyme oxidizes putrescine and cadaverine much more efficiently that spermidine and spermine (in the order 100, 60, 20 and 12%). It is interesting to note that *Hordeum vulgare* and *Zea mays* contain both Cu-amine oxidase [12, 27] and FAD-amine oxidase [13, 28].

This review deals only with Cu-AOs. The molecular and functional properties of various Cu-AOs will be described and compared. In particular, the enzymes from *Lens*, *Pisum* and *Euphorbia*, the best known and studied, will be examined.

Localization

The *Lens* amine oxidase, like the *Pisum* enzyme, is absent in ungerminated seeds and appears during the early period of germination. The enzymic activity is greatest in the growing parts of the plant and decreases with maturity and senescence. The amine oxidase has been localized in *Lens* seedlings by an immunohistochemical method [38] based on fluorescein-labelled antibodies

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raised against the *Lens* enzyme. This oxidase is present mainly in association with membranes and cell walls of the leaves. In roots the enzyme shows a strong association with the xylem in the differentiated zone and, in differentiating tissue, with cell walls and nuclei. In the cotyledons the amine oxidase is found on the plasma membranes, on the membranes of protein bodies and on the vacuolar surface.

Purification procedure

All the enzymes have been purified to homogeneity by standard methods involving homogenization, salting out and various chromatography techniques including hydrophobic interaction chromatography, recently utilized by Wimmerova *et al.* in the purification of the *Pisum* enzyme [39]. Angelini *et al.* [5] have purified in one step the amine oxidases from *Pisum*, *Lens* and *Cicer*, using immunoaffinity chromatography. It is interesting to note that although no amine oxidase activity was detected in *Hordeum vulgare* and *Phaseolus vulgaris* seedlings until the hydroxylapatite step of the purification procedure, the enzyme could be purified to homogeneity by Cogoni *et al.* [12, 14]. The *Pisum* enzyme has been crystallized using lithium sulphate as precipitant at pH 5.2. The unit cell is orthorhombic. The crystals diffract to 2.5 Å in a synchrotron X-ray beam [40].

Molecular features

Tables 1 and 2 summarize some of the molecular properties of plant Cu-AOs. All the enzymes examined are dimers, formed from apparently identical subunits. All contain one copper ion, Cu(II), and one organic prosthetic group per subunit. M_r and isoelectric point of the amine oxidases from *Pisum*, *Lens*, *Lathyrus sativus* and *Cicer* were determined with great accuracy by Padiglia *et al.* [41]. Isoelectric focusing techniques show a complicated pattern of several bands in the four species. This microheterogeneity may depend on the composition of carbohydrate chains, as suggested by Oratore *et al.* [42]. The carbohydrate content seems to be fairly constant (12–14%); however, it has been reported that the *Lathyrus sativus* enzyme does not contain any sugar [15].

Comparison of the amino acid composition of the enzyme from *Euphorbia*, *Glycine*, *Lens*, *Pisum*, *Trifolium* and *Vicia* shows no similarity (Table 3). Two SH groups per dimer are titratable either in the presence of aminic substrate or in denaturing conditions [43] in the

Table 1. Some properties of plant Cu-AOs from the Leguminosae

Properties	*Cicer arietinum*	*Glycine max*	*Lens esculenta*	*Pisum sativum*
Molecular weight				
Gel chromatography	150000	113000	150000	150000
SDS-PAGE	72000	77000	75000	72000
Cu content				
% (g Cu/g protein)	ND	0.109	0.082	0.085
g atom/mol enzyme	ND	1.94	2	2
Isoelectric points	6.0	7.1	6.1	7.2
	5.8	7.05	5.9	6.8
	5.1	7.0	5.1	6.5
		6.9		
Carbohydrate content % (w/w)	ND	ND	14	14

Ref.: *Cicer* [41], *Glycine* [30], *Lens* [41], *Pisum* [41].

Table 2. Some properties of plant Cu-AOs from other families

Properties	*Hordeum vulgare*	*Zea mays*	*Euphorbia characias*	*Thea sinensis*
Molecular weight				
Gel chromatography	150000	118000	140000	162000
SDS–PAGE	75000	55000	72000	81000
Cu content				
% (g Cu/g protein)	0.082	ND	0.09	ND
g atom/mol enzyme	1.94	ND	2	ND
Isoelectric points	6.8	ND	5.2	5.0
Carbohydrate content % (w/w)	14	ND	12	ND

Ref.: *Hordeum* [12], *Zea* [27], *Euphorbia* [8], *Thea* [23].

Table 3. Amino acid composition of some Cu-AOs

Amino acid	*Euphorbia characias*	*Glycine max*	*Lens esculenta*	*Pisum sativum*	*Trifolium subterraneum*	*Vicia faba*
D	121	70	120	63	41	107
T	67	49	98	37	25	62
S	74	121	100	37	26	65
E	104	42	110	52	34	82
P	80	93	72	13	—	16
G	77	258	70	34	22	67
A	48	237	54	28	14	56
V	87	86	76	55	24	70
M	23	—	12	1	1	1
I	66	48	96	48	24	72
L	76	61	76	42	27	73
Y	43	21	46	21	12	19
F	50	34	60	29	16	53
H	33	26	40	22	24	31
K	56	50	72	33	23	51
R	41	27	42	23	18	33
C	14	—	24	4	—	10
W	17	ND	26	—	—	—
Total	1077	1223	1182	542	331	868

The values under the columns *Euphorbia*, *Glycine* and *Lens* represent the nearest integer per mol of dimer; the values under *Pisum*, *Trifolium* and *Vicia* represent the nearest integer value per mol of methionine.

Ref.: *Euphorbia* [8], *Glycine* [30],*Lens* [16],|*Pisum* [32], *Trifolium* [24], *Vicia* [26].

Table 4. Substrate specificity of some plant Cu-AOs

	Relative reaction rate (% of putrescine)					
Substrate	*Euphorbia characias*	*Glycine max*	*Lathyrus sativus*	*Lens esculenta*	*Pisum sativum*	*Vicia faba*
1,3-Diaminopropane	0	0	0	0	0	2
Putrescine	100	100	100	100	100	100
Cadaverine	100	183	81	91	111	90
1,6-Diaminohexane	56	—	21	31	44	—
Agmatine	43	52	19	15	56	64
Histamine	0	4	—	0	30	9
Spermidine	0	81	31	40	56	52
Spermine	0	15	18	20	8	8

Ref.: *Euphorbia* [8], *Glycine* [30], *Lathyrus* [15], *Lens* [16], *Pisum* [76], *Vicia* [26].

Euphorbia enzyme. No other example of reactive thiols has been reported.

The nucleotide sequence of a 2111 bp clone of the *Lens* amine oxidase cDNAs has been determined [44]. The deduced protein sequence shows that the mature protein is composed of 569 amino acids per subunit. The protein sequence shows a characteristic hexapeptide where the tyrosine in the third position may be modified into 6-hydroxy dopa. Three conserved histidines may be the ligands of copper bound to the enzyme.

Substrate specificity

Specificity varies widely among the different enzymes. The best substrates for all of them are putrescine and cadaverine. Most of the enzymes act indiscriminately on mono-, di- and polyamines; only a few are able to oxidize histamine and tyramine. A restricted specificity is shown by the *Euphorbia* enzyme, which oxidizes only aliphatic diamines of critical dimensions. The stereospecificity and the regioselectivity on the oxidation of substrate analogues like 2-methyl-1,4-diaminobutane was reported for the enzymes from *Pisum* [45], *Lens* and *Euphorbia* [46]. Studies on different metabolic pathways showed that the enzymes from *Pisum* [47], *Lens* [48] and *Euphorbia* [49] are involved also in the oxidation of cystamine, Se-cystamine [48, 49] and other sulphur or selenium-containing amines [50]. The relative reaction rates with the most significant substrates of the Cu-AOs are shown in Table 4. K_m values for putrescine seem to be of the same order of magnitude for all the enzymes investigated (10^{-4} M). The pH optimum is constantly found around pH 7.0 with putrescine or cadaverine as substrates.

Inhibitors

Metal chelators inhibit all plant Cu-AOs non-competitively. The different enzymes have quite different sensitivities; thus diethyldithiocarbamate is a powerful inhibitor of the enzymes from *Vicia* [26, 34], *Lens* [16] and *Euphorbia* [8] but not for the *Lathyrus sativus* [15] enzyme ($K_i = 10^{-2}$ M).

Carbonyl group reagents irreversibly form adducts with all the Cu-AOs with concomitant loss of catalytic activity. The most effective agents for the *Lens* enzyme are hydrazines (phenylhydrazine and benzylhydrazine) and hydrazides (semicarbazide, thiosemicarbazide and phenylsemicarbazide) [51]. The *Pisum* enzyme is inhibited in a time-dependent manner by the hydrazides of acetic, benzoic, nicotinic, picolinic and other acids [52]. The *Lens* enzyme is also inhibited *in vitro* by various complexes between bivalent transition metals and some diamines and polyamines with different metal–ligand ratios [53]. A series of reversible inhibitors of the *Pisum* amine oxidase has been described. Imidazole derivatives such as carnosine, anserine and impromidine appear to behave as non-competitive inhibitors [54]. Methylglyoxalbis-(guanylhydrazone) is an inhibitor for the *Pisum* enzyme and it was used in affinity chromatography to purify this amine oxidase [32]. Pec *et al.* [55] reported that 1,4-diamino-2-butyne is a mechanism-based *Pisum* amine oxidase inhibitor.

In contrast to the other plant enzymes, the *Euphorbia* amine oxidase has been found to be sensitive to thiol reagents [43]. Half-site reactivity is found towards *n*-ethyl-maleimide and iodoacetate; both reagents produce 50% inactivation and modification of only one thiol per dimer. 100% inactivation and modification of both reactive thiols have been obtained with 4,4′-dithiodipyridine. Hydrogen peroxide, which is one of the products of the enzymic reaction, is an inactivating agent for all Cu-AOs but nothing is known about the mechanism of inhibition. However, in the *Euphorbia* enzyme, inactivation by hydrogen peroxide is concomitant with the modification of the reactive thiols [43].

Stoichiometry of the catalysed reaction

Cu-AOs catalyse the oxidation by molecular oxygen of the primary amino group of di- and polyamines to the corresponding amino aldehydes, hydrogen peroxide and ammonia. The stoichiometry of the reaction is 1 mol of oxygen consumed per mol of substrate and 1 mol of ammonia, 1 mol of hydrogen peroxide and 1 mol of aldehyde formed per mol of substrate. The aminoaldehyde products from putrescine, cadaverine and spermidine spontaneously cyclize to Δ^1-pyrroline, Δ^1-piperidine and 1,5-diazabicyclononane, respectively [56] (Fig. 1). Cogoni *et al.* [57] report that spermine is oxidized by the *Lens* amine oxidase in the NH_3^+ terminal positions with the formation of a dialdehyde. The oxygen uptake is 2 mol per mol of spermine; 2 mol of hydrogen peroxide and 2 mol of ammonia per mol of spermine are formed. The dialdehyde does not occur in aqueous solution in the monocyclic pyrrolinium form but probably reacts with spermine (Schiff bases) or with dialdehydes (aldol condensation) and probably gives rise to a polymer. This compound shows a fluorescence, with emission spectra at 460 and 570 nm when excited at 430 and 520 nm, respectively.

Cystamine and Se-cystamine are oxidatively deaminated by the enzyme from *Pisum* [47], *Lens* [48] and *Euphorbia* [49] to the corresponding aminoaldehydes with a consumption of 1 mol of oxygen per mol of substrate and the formation of 1 mol of hydrogen peroxide and 1 mol of ammonia per mol of substrate. An extensive further degradation of the aminoaldehydes with an extra oxygen consumption occurs for the amine oxidases from *Euphorbia* [49] and *Pisum* [47] and the final products are 2 mol of ammonia and 2 mol of hydrogen peroxide per mol, with the elimination of elemental sulphur or selenium and the formation of C_2 fragment (glycolaldehyde) (Fig. 2). No further degradation of the aminoaldehydes seems to occur using the *Lens* enzyme [48]. Similar results are obtained during the oxidation of cystathionamine and lanthionamine [50].

The stereospecificity and the regioselectivity of the oxidative deamination reaction have been studied using (*R*)- and (*S*)-2-methylputrescine as substrates for the amine oxidases from *Pisum* [45], *Lens* and *Euphorbia* [46]. The substitution of hydrogen at C-2 with a methyl group reduces the activity to a great extent, indicating that the region near the reactive amino group is rather sensitive to steric hindrance. The *Pisum* amine oxidase regioselectively oxidizes only at the less hindered C-4 and shows no stereospecific distinction between (*R*)- and (*S*)-enantiomers. Moreover, the optimum pH is slightly shifted to alkaline values (pH 9.0). No significant stereospecificity is shown by the *Euphorbia* enzyme. The oxidation product at C-4 is clearly predominant and obtained in almost equimolar amounts with both enantiomers. The oxidation product at C-1, however, has higher yields with (*S*)-methylputrescine. The C-4 oxidation is higher for (*S*)-methylputrescine while (*R*)-methylputrescine gives higher yields at C-1 oxidation using the *Lens* enzyme. The amine oxidases from *Euphorbia* and *Lens* have an optimum pH at 7.0 with these substrates. The *Pisum* enzyme shows a lower oxidation rate also with a hydrophilic substitution in the carbon chain between the amino groups of putrescine [58]. Therefore, it is very tempting to postulate that steric hindrance rather than polar interactions is primarily involved in determining lower activity with putrescine analogues.

Spectroscopic features

In addition to the protein absorbance maximum at 278 nm, the visible spectrum of Cu-AOs is characterized by a broad absorption peak centred around 500 nm which confers a typical pink colour to purified preparations of the enzyme (Fig. 3). The colour is stable in the pH range 5–9. The extinction at 500 nm is 4100 $M^{-1}\,cm^{-1}$ and at 278 nm is 246 000 $M^{-1}\,cm^{-1}$ for highly purified *Lens* amine oxidase samples. The value

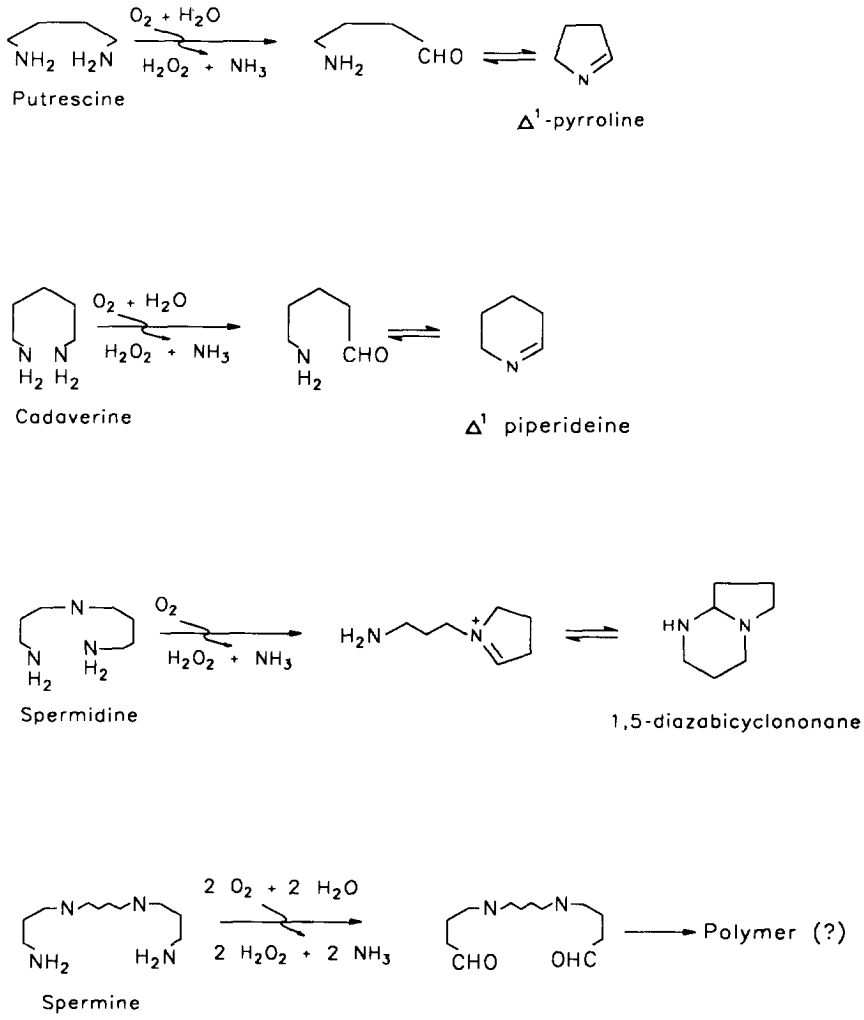

Fig. 1. Oxidative deamination of diamines and polyamines catalysed by Cu-amine oxidases. The aminoaldehyde products from putrescine, cadaverine and spermidine spontaneously cyclize.

A_{500}/A_{410} is $\geqslant 1.6$ [51]. Removal of the copper under non-reducing conditions results in the same visible spectrum which has been ascribed to the carbonyl cofactor [59]. This prosthetic group has more than one electronic transition, since the CD spectrum of the *Lens* enzyme shows a large positive band centred at about 520 nm, clearly resulting from more than one component. The aerobic addition of substrates to the enzyme from *Lens* [59], *Pisum* [60] and *Euphorbia* [61] is followed by a rapid disappearance of the absorption band at 500 nm, which slowly reappears after exhaustion of substrate. The 500 nm band of the copper-free enzyme from the *Lens* enzyme is rapidly bleached by addition of substrate but is not restored even after dialysis [59]. A stable yellow intermediate absorbing at 346, 432 and 462 nm is observable [59–61] in the presence of aminic substrates and in anaerobiosis (Fig. 3). The yellow peaks disappear upon admission of air. These absorption bands are not observed with copper-free enzyme [59]. Shape and position, but not the relative intensities of the absorption bands, are independent of the different substrates used.

The addition of phenylhydrazine to the *Lens* [51] amine oxidase is followed by the formation of a strong absorption band at 445 nm ($\varepsilon = 64\,000\ M^{-1}\,cm^{-1}$) concomitant with the disappearance of the absorption band at 500 nm. The addition of semicarbazide to the *Lens* [51] enzyme is followed by the disappearance of the absorption at 500 nm and the formation of two absorption bands at 492 nm ($\varepsilon = 8\,600\ M^{-1}\,cm^{-1}$) and 360 nm ($\varepsilon = 34\,800\ M^{-1}\,cm^{-1}$). These irreversible reactions of the *Lens* enzyme with hydrazines or hydrazides lead to completely inactive enzyme forms and do not require oxygen. Similar results are obtained with the *Pisum* amine oxidase [60].

The two copper atoms are indistinguishable on the basis of EPR spectra, and the relative spectroscopic parameters, consistent with the presence of Cu(II) in a tetragonal field, are very similar in plant enzymes. Numerous EPR experiments previously failed to detect changes in the copper oxidation state in the presence of substrate amines. Recently Dooley *et al.* [62] presented evidence for the generation of a Cu(I)-semiquinone state

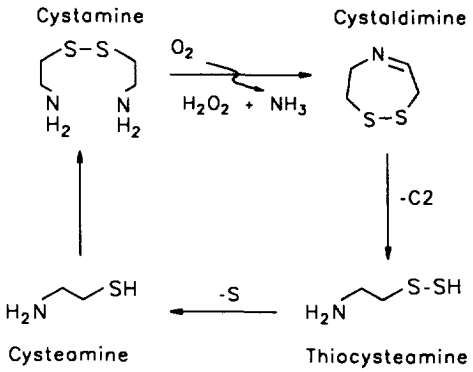

Fig. 2. Cystamine deamination by *Pisum* amine oxidase leads to its complete degradation to ammonia, elemental sulphur and a C_2 fragment (glycolaldehyde).

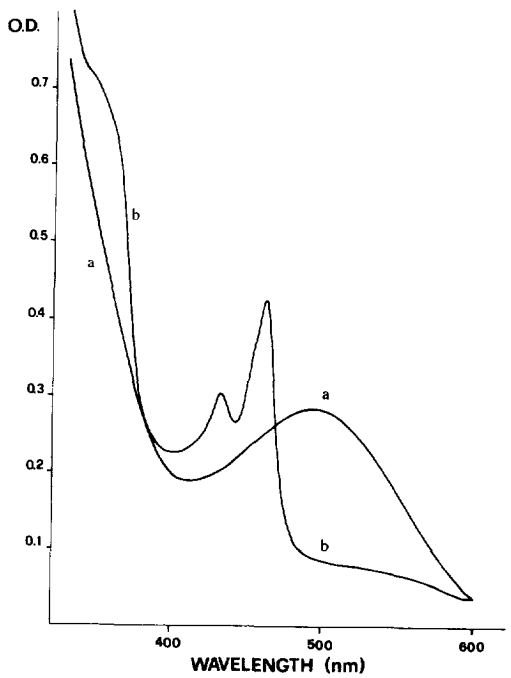

Fig. 3. Reaction of *Lens* amine oxidase with putrescine. Absorption spectra of 6.9×10^{-5} M *Lens* enzyme in 100 mM phosphate buffer, pH 7, in anaerobic conditions. (a) No addition; (b) after addition of putrescine (final concentration 1.7×10^{-2} M).

by substrate reduction of the *Pisum* enzyme under anaerobic conditions and suggested that the Cu(I)-semiquinone may be the catalytic intermediate that reacts directly with oxygen. Copper reduction by substrates is confirmed by CD spectroscopy. Bellelli *et al.* [63] showed that a substrate-reduced species of the *Lens* amine oxidase with absorption bands at 346, 432 and 462 nm is a catalytic intermediate and reacts very rapidly with oxygen. This intermediate can plausibly be assigned as the Cu(I)-semiquinone correlating the room-temperature EPR and absorption spectra.

Prosthetic group

The nature of the covalently bound cofactor has been intensively investigated. Initially the 'active carbonyl' cofactor was tentatively identified as pyridoxal phosphate in the enzyme from *Euphorbia* [8], *Pisum* [60] and *Lathyrus sativus* [15] on the basis of the phenylhydrazine-enzyme adduct, but confirmation of this hypothesis was not obtained. In 1987 Glatz *et al.* [64] suggested that the *Pisum* enzyme contained pyrroloquinoline quinone as cofactor. Citro *et al.* [65] subsequently found that polyclonal antibodies against pyrroloquinoline quinone reacted with the native and denatured *Lens* enzyme as detected by dot-blot and ELISA assays, and Cogoni *et al.* [12] reported that also the *Hordeum* amine oxidase might contain pyrroloquinoline quinone. However, Janes *et al.* [66] in 1990 showed that 6-hydroxydopa was the redox cofactor in the mammalian enzyme and that a product similar to pyrroloquinoline quinone was obtained by cyclization of 6-hydroxydopa derivatives during isolation. Brown *et al.* [67] demonstrated that resonance Raman spectra of phenylhydrazine and *p*-nitrophenylhydrazine derivatives of the *Pisum* amine oxidase were very similar to the spectra of the corresponding derivatives of 6-hydroxydopa quinone. Janes *et al.* [68] have since identified the 6-hydroxydopa quinone and its consensus sequence by Edman degradation in the *Pisum* enzyme. The cDNA derived amino acid sequence of the *Lens* amine oxidase shows that the tyrosine at position 387 may be modified to 6-hydroxydopa in the mature protein [44].

Active site titration of highly purified *Lens* enzyme samples with the carbonyl reagents extrapolates to 1 per mol of inhibitor/mol of enzyme subunit, indicating the presence of a cofactor in each enzyme subunit [51]. One functional cofactor per enzyme subunit is reported also for the enzymes from *Euphorbia* [8] and *Pisum* [69]. This result is at variance with other reports of only one functional cofactor per enzyme dimer in the amine oxidase from *Pisum* [70] and *Lens* [71]. These controversial results may be a result of variations in the purity of enzyme preparations, different valuations of the number of copper ions, different determinations of the M_r or enzyme concentrations used and the incubation time of the E:I complex, or to a troublesome combination of several of the above factors [51].

Reaction mechanism

The kinetics of reaction between the *Lens* amine oxidase and two amine substrates, putrescine and *p*-dimethylaminomethylbenzylamine, have been studied by the stopped flow technique [72]. Several wavelengths can be monitored allowing the simultaneous measurement of enzyme bleaching and formation of a yellow intermediate. The simple kinetic scheme previously proposed to describe the reaction of the *Lens* enzyme with *p*-dimethylaminomethylbenzylamine (an artificial chromogenic substrate) [63], also describes the physiological reaction mechanism: the resting (oxidized) enzyme reacts with the

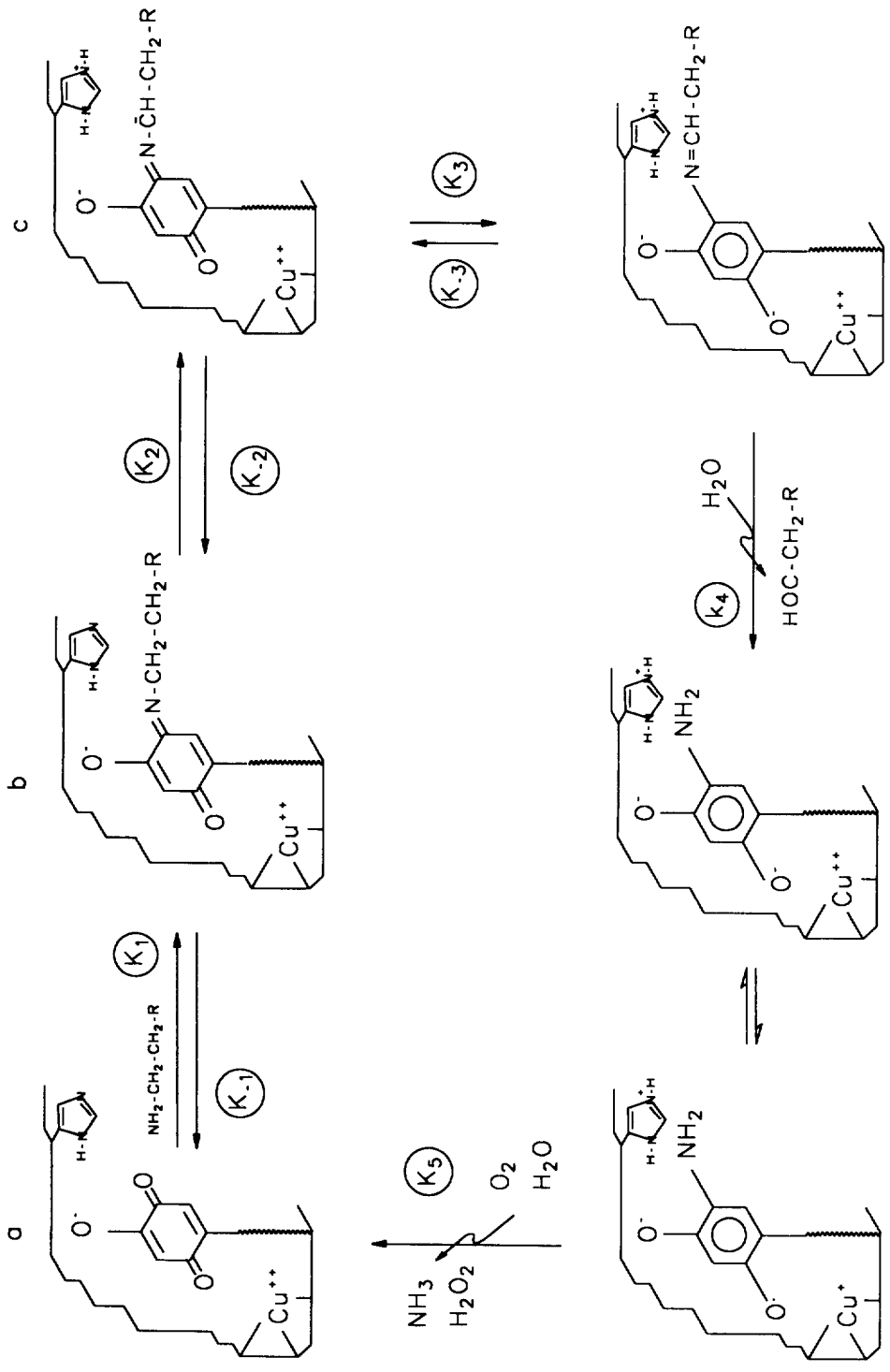

Fig. 4. Reaction mechanism of *Lens* amine oxidase. (a) Oxidized enzyme; (b) quinoketimine; (c) carbanion; (d) quinolaldimine; (e) aminocatechol; (f) semiquinolamine.

amine substrate giving rise to a covalent enzyme–substrate complex. This rapidly converts to a bleached form, which in turn may release the aldehyde product. Thereafter an internal redox reaction, perhaps between the 6-hydroxydopa and copper, produces a radical species, observed by EPR, which can rapidly react with oxygen to restore the oxidized enzyme liberating ammonia and hydrogen peroxide [72]. The oxidation process requires the abstraction of an α-proton and a pair of electrons from the substrate. Medda *et al.* [73] report that in the reaction mechanism of the *Lens* amine oxidase an intermediate enzyme-bound carbanion is formed, reflecting the abstraction of a proton from the substrate. They also indicate a functional role for a histidine residue consistent with that of a general base in proton abstraction [73]. Kluetz *et al.* [74] showed that visible spectra of the *Pisum* enzyme reacting at subzero temperatures provide evidence that an intermediate reduced enzyme form is obtained via several other intermediates and that the environment of the Cu(II) changes dramatically during the course of the reaction. Dooley *et al.* [62] demonstrated that the *Pisum* amine oxidase is able to generate a radical in the reaction with the substrate when the copper is reduced to Cu(I) and attributed to this radical the Cu(I)-semiquinone spectrum of the oxygen reacting species, with peaks at 432 and 462 nm. Pedersen *et al.* [75] examined the radical species through studies of the semiquinones of 6-hydroxydopamine, an analogue of the 6-hydroxydopa. They find a striking similarity between the radical formed during the catalytic cycle of the *Lens* enzyme and that of an amino derivative of 6-hydroxydopamine. Turowski *et al.* [69], using visible spectroscopy as a function of temperature, investigated the equilibrium between two substrate-reduced forms of the *Pisum* amine oxidase, one containing Cu(II) and reduced form of 6-hydroxydopaquinone, probably the aminocatechol, and one containing Cu(I) and 6-hydroxydopasemiquinone. No visible absorbance bands are present in the spectrum of Cu(II)-aminocatechol and the intramolecular electron transfer rate between copper and 6-hydroxydopa is extremely rapid.

We have recently examined the interaction of various amino-containing compounds by the *Lens* amine oxidase by optical spectroscopy and by EPR (unpublished results). The amino containing compounds used include effective substrates (putrescine and *p*-dimethylaminomethylbenzylamine), a very poor substrate (*p*-dimethylaminobenzylamine), and inhibitors in which the isomerization of ketoimine leads to a very stable diazo-like compound (phenylhydrazine, 2-methylbenzothiazolone hydrazone). They conclude that six spectroscopic species can be observed in the reaction mechanism of the *Lens* amine oxidase (Fig. 4): the interaction of the amine oxidase (a) with primary amines gives rise to a covalent amino group-quinone compound, a quinoketimine (b); the quinoketimine owing to the abstraction of an α-proton from a substrate by a near histidine, gives rise to a carbanion (c); the carbanion is rapidly converted to a bleached form, the quinoaldimine (d); the quinolaldimine releases the aldehyde product and form a species containing Cu(II) and a reduced form of 6-hydroxydopaquinone, the aminocatechol (e) which coexists with the radical species containing Cu(I) and 6-hydroxydopasemiquinone, the semiquinolamine (f); the semiquinolamine reacts with oxygen and restores the oxidized enzyme (a) liberating ammonia and hydrogen peroxide.

The model outlined in Fig. 4 is based on the sum of our current knowledge of the copper amine oxidase reaction mechanism. In the years to come it can be expected that the determination of the enzyme structure, and in particular the application of molecular biology techniques, will contribute to complete our understanding of these interesting enzymes.

Acknowledgements—We thank Dott. Jens Z. Pedersen (Dipartimento di Biologia, Università di Roma "Tor Vergata", Roma, Italy) for valuable discussions.

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