The Reductive and Oxidative Half-Reactions and the Role of Copper Ions in Plant and Mammalian Copper−Amine Oxidases

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Keywords: Oxidases / Mammalian amine oxidase / Copper / Cofactors / 6-Hydroxydopa

In this review, the nature and characteristics of the prosthetic group, the intermediates in the catalytic mechanism, and the role of the copper in plant copper–amine oxidases from pea and lentil seedlings are compared with the corresponding features of mammalian amine oxidase from bovine serum. All these enzymes contain the same organic cofactor derived from the modification of a protected tyrosyl residue. Considering the catalytic mechanism, plant amine oxidase may be referred to as a "protein radical enzyme", operating through a free radical located on the cofactor. This is in contrast to bovine serum amine oxidase, which forms the free-radical intermediate only to a minor extent in the course of the catalytic cycle. A scheme for the catalytic mechanism is proposed in order to account for this difference.

I. Introduction

Copper-containing amine oxidases [amine−oxygen oxidoreductase (deaminating) (copper/TPQ-containing); EC 1.4.3.6] are homodimers, in which each subunit (MW 70−90 KDa) contains one tightly bound Cu^{II} ion and one 6-hydroxydopa quinone (TPQ) moiety as prosthetic groups. These enzymes catalyze the oxidative deamination of primary amines by transferring two electrons from the amines to molecular oxygen. The catalytic mechanism can be divided into two half-reactions, namely enzyme reduction by the substrate [Equation (1)] followed by enzyme re-oxidation by molecular oxygen [Equation (2)].

$$E_{ox} + R-CH_2-NH_3^+ \rightarrow E_{red}-NH_2 + R-CHO \quad (1)$$

$$E_{red}-NH_2 + O_2 \rightarrow E_{ox} + NH_3 + H_2O_2 \quad (2)$$

Amine oxidases are ubiquitous enzymes, occurring in microorganisms (fungi and bacteria),[1] plants,[2] and mammals.[3] In the plant kingdom, the presence of amine oxidases was first demonstrated in 1949.[4] Since then, plant amine oxidases from various species of several families have been purified to homogeneity and characterized,[2] the best known and studied being those from seedlings of the pulses lentil[2] (*Lens esculenta*) and pea[5] (*Pisum sativum*) and that from *Euphorbia characias* (a shrub) latex.[6] Mammalian amine oxidases have also been actively investigated for over 40 years, well-studied examples being those from pig kidney,[7−9] human placenta,[10,11] bovine serum,[12−15] pig plasma,[16] and equine plasma.[17] The nature of the covalently bound cofactor and the number of active sites, as well as the role of copper and the reaction mechanism have been also investigated over many years.

Abbreviations used: Cu−AO: copper−amine oxidase; BSAO: bovine serum amine oxidase; LSAO: lentil seedling amine oxidase; PSAO: pea seedling amine oxidase; TPQ: 6-hydroxydopa (2,4,5-trihydroxyphenylethylamine) quinone.

II. The Organic Cofactor and Copper

The presence of an organic cofactor in copper−amine oxidases has been sought for almost 60 years and its nature has been the subject of much debate.[18] Prior to 1984, the most likely candidate was considered to be a covalently bound pyridoxal 5-phosphate[19] (Figure 1, A), but this hypothesis could never be proved. During the 1970s, a new dissociable cofactor was identified in bacterial glucose dehydrogenase and methanol dehydrogenase, which was eventually demonstrated to be pyrrole quinoline quinone by X-ray diffraction analysis[20] (Figure 1, B). This led to the suggestion that pyrrole quinoline quinone might be the active site cofactor of a range of eukaryotic proteins, including copper amine oxidases from bovine serum,[21,22] pig kidney,[23] and pea seedlings.[24] Finally, in 1990, Klinman and co-workers[25] demonstrated that 6-hydroxydopa quinone (TPQ, Figure 1, C), derived from the oxidation of an in

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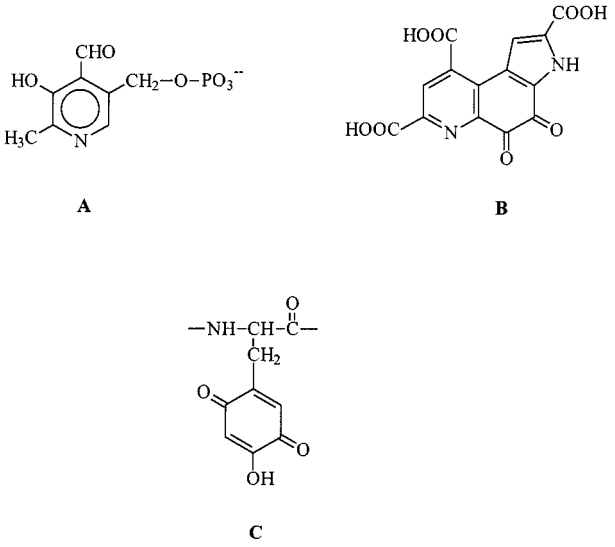

Figure 1. Structural formulae of some cofactors: **A**: pyridoxal 5-phosphate; **B**: pyrrole quinoline quinone; **C**: 6-hydroxydopa quinone

trinsic tyrosine in the amino acid sequence in a post-translational event, is in fact the redox cofactor of eukaryotic amine oxidases.[26] A sequence Asn−TPQ−Asp/Glu was identified in these enzymes,[27] and the cDNA-derived amino acid sequence of *Lens esculenta* (EMB Data Bank accession number X64201),[28] *Hansenula polymorpha*,[26] bovine serum (EMBL Data Bank accession number Q29437),[29] *Pisum sativum* (GB Data Bank accession number L39931),[30] *Cicer arietinum* (EMB Data Bank accession number AJ009825),[31] and *Euphorbia characias* (GB Data Bank accession number AF171698)[6] amine oxidases revealed that a tyrosine codon corresponds to the TPQ cofactor in the mature protein.

Besides TPQ, amine oxidases also contain copper in a 1:1 stoichiometric ratio with the organic cofactor. The crystal structure of PSAO[5] shows that the copper atom is coordinated by three histidine side chains and two water molecules (Figure 2), with the shortest distance between TPQ and the copper atom being ca. 6 Å. Extended X-ray absorption fine-structure (EXAFS) analysis has been used to define the

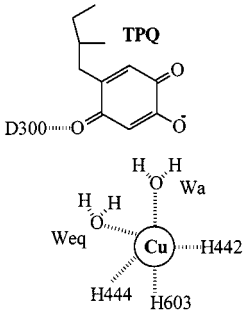

Figure 2. Structural arrangement of the copper center and the organic cofactor in the active site of pea amine oxidase; the copper ion is coordinated by three protected His residues (H442, H603, and H444) and two water molecules (Wa = axial water; We = equatorial water);[5] D300 has been identified as a base required in the active site for the catalytic mechanism

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structure of the copper site in both the oxidized and dithionite-reduced states of some amine oxidases.[32]

III. The Number of Actives Sites

Since all the amine oxidases described so far are homodimers, a problem arose concerning the number of active sites present in the molecule. This problem has mainly been tackled by titrating the enzyme with irreversible inhibitors. The conflicting results obtained on titrating amine oxidases with carbonyl reagents have been rationalized for LSAO and PSAO.[33,34] The titration of highly purified samples extrapolates to 1 mol of inhibitor per enzyme subunit, indicating the presence of two active quinones moieties per protein dimer. A similar result was reported for the titration of highly purified samples of BSAO with phenylhydrazine,[35] but Morpurgo et al.[14] subsequently found that only one enzyme subunit actually reacts with this inhibitor. A somewhat higher value, 1.1−1.3 mol/dimer, was obtained with other reagents, e.g. semicarbazide and 2-hydrazinopyridine.[36,37] In these cases, the reaction of one mol of inhibitor/dimer was faster than the subsequent reaction, which led to the generation of a spectroscopically different adduct.

IV. Optical and EPR Spectroscopy

In addition to the protein absorbance maximum at ca. 280 nm, oxidized Cu−AOs show an absorption band in the visible region associated with their distinctive pink color. The visible spectra of TPQ cofactor models show maxima at 480−484 nm,[38,39] consistent with theoretical calculations. Indeed, the maximum absorbance observed for most mammalian Cu−AOs is very close to 480 nm. In the plant enzymes, this band is red-shifted to 498 nm, probably because of specific interactions with amino acid residues in the active site. Removal of copper from the amino oxidases from both LSAO[40] and *Euphorbia* latex[6] under non-reducing conditions shifts the band to 480 nm. Reconstitution of the metal-depleted protein with cobalt does not restore the native spectrum of LSAO and the absorption maximum remains at 480 nm.[41] In the case of BSAO, the visible band maximum is unaffected by metal depletion and Co^{2+} substitution.[42] The extinction coefficients for some Cu−AOs are listed in Table 1.

Table 1. Extinction coefficients ($mM^{-1}\ cm^{-1}$) of the amine oxidases from pea and lentil seedlings and from bovine serum at pH = 7; wavelengths are given in italics

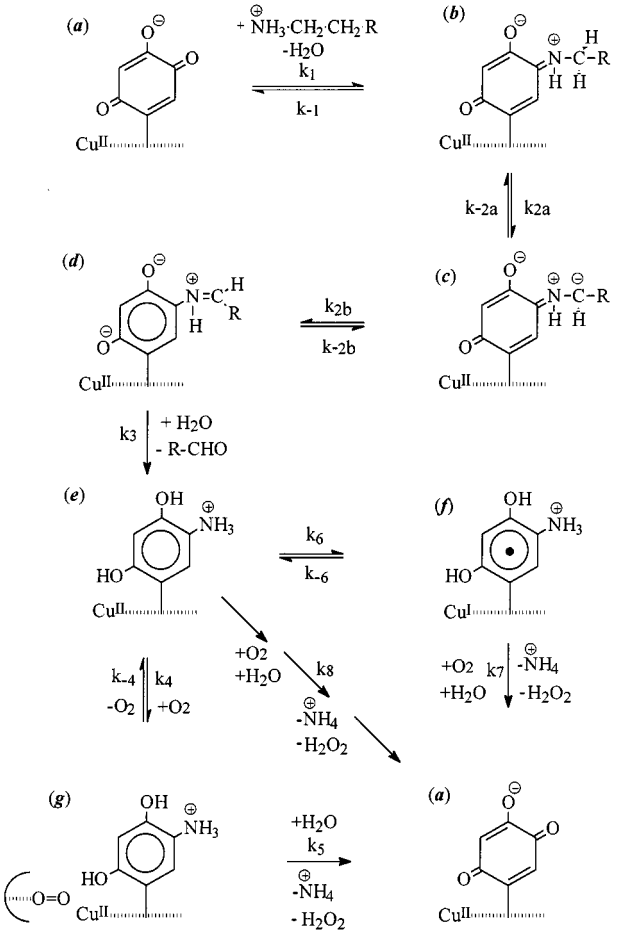
Enzyme	Oxidized form	Reduced form
PSAO	4.9 (*498*), 300 (*278*)[34]	–
LSAO	4.5 (*498*), 245 (*278*)[33]	7.1 (*464*) 4.6 (*434*)[47]
BSAO	3.8 (*480*), 310 (*280*)[74]	–

The copper ion in Cu−AOs is EPR-detectable and its spectrum falls in the category of so-called type-2 Cu EPR spectra.[43] Native LSAO exhibits a typical type-2 copper spectrum in a tetragonal field, characterized by $g_{\|} = 2.32$ and $A_{\perp} = 153$ G, and by the absence of superhyperfine structure.[44] The same applies to BSAO, for which the EPR parameters are $g_{\|} = 2.30$ and $A_{\perp} = 155$ G.[45]

V. The Reaction Mechanism

A. The Reductive Half-Reaction

A plausible kinetic scheme for the catalytic mechanism of Cu−AOs is presented in Scheme 1. It might be somewhat redundant since plant and mammalian enzymes behave differently and some of them may fail to populate all the intermediates, at least under the usual experimental conditions, as detailed below.



Scheme 1. Catalytic mechanism of Cu−AOs: (***a***) resting oxidized enzyme; (***b***) Cu^{II}−quinone ketimine, the substrate Schiff base; (***c***) Cu^{II}−carbanion species; (***d***) Cu^{II}−quinolaldimine, the product Schiff base; (***e***) Cu^{II}−aminoresorcinol; (***f***) Cu^{I}−semiquinolamine radical; (***g***) Cu^{II}−aminoresorcinol−oxygen complex

Step 1: Cu^{II}−Quinone Ketimine

The amine substrate binds to the carbonyl function of TPQ in the resting oxidized enzyme [Cu^{II}−TPQ (***a***)] to form a substrate Schiff base [Cu^{II}−quinone ketimine (***b***)]. In the case of BSAO, Hartmann and co-workers[46] demon-

strated that the quinone and quinone ketimine oxidized forms of the enzyme, both of which absorb at 480 nm, are spectroscopically distinguishable since only ***b*** shows a shoulder at 340 nm. In order to maximize the population of the quinone ketimine species in the catalytic cycle of LSAO, Floris and co-workers used different substrates. With γ-aminobutanoic acid as the substrate,[47] a marked delay (4 min) was observed before bleaching of the enzyme, indicative of the formation of the quinone ketimine intermediate. The authors were unable to distinguish the "pink" spectrum of the latter from that of the quinone species.

Step 2: Cu^{II}−Carbanion Species

The oxidation of the amine substrate involves base-catalyzed abstraction of a proton from C1 with the formation of a Cu^{II}−carbanion species (***c***). The formation of the carbanion species was demonstrated in the case of BSAO using *para*-substituted benzylamines.[48] The carbanion species generated in the case of LSAO was later trapped with the electrophilic reagent tetranitromethane.[49] The active site base was identified as Asp300 in PSAO.[5] It is worth noting that the stereospecificity of proton abstraction in the reaction with *Escherichia coli* AO, coupled with the mutagenesis data, shows beyond any doubt that Asp383 is the catalytic base.[50] The protected Asp381 seems to play a similar role in BSAO.[37]

Step 3: Cu^{II}−Quinolaldimine

Transformation of the Cu^{II}−carbanion (***c***) into the product Schiff base, the Cu^{II}−quinolaldimine (***d***), is associated with the bleaching of the 498 nm absorption band. This intermediate is the most elusive species involved in the reaction mechanism of AOs. The tautomeric form of the quinolaldimine (a quino-imine) was identified in both LSAO[40] and BSAO[46] using the poor substrate *p*-(dimethylamino)benzylamine.

Step 4: Cu^{II}−Aminoresorcinol

Oxidation of the bound substrate (followed by hydrolysis) releases the aldehydic product, leaving the Cu^{II}−aminoresorcinol derivative (***e***) bearing a bound ammonia molecule. This species is still colorless. Hartmann and co-workers[46] demonstrated a complex time evolution of the "colorless" component in BSAO, attributed to the transformation of quinolaldimine into aminoresorcinol, although they were unable to assign a defined difference spectrum to this reaction. That the aldehyde is released after hydrolysis of the imine, leaving the amino group bound to the cofactor, has been demonstrated in both mammalian and plant AOs.[6,35,40]

In conclusion, comparison of the spectroscopic changes described above with Scheme 1 clearly shows that the chemistry of TPQ implies the involvement of more than three different species. Indeed, the "pink" oxidized Cu^{II}−TPQ derivatives and the "colorless" reduced forms have at least been demonstrated to represent "families" of derivatives rather than pure chemical species. The species ***a***, ***b***, and ***c*** in Scheme 1 are believed to be pink, albeit with slightly different spectra,[50] whereas species ***d*** and ***e*** are colorless.

B. The Oxidative Half-Reaction

In contrast to the detailed studies on the reductive half-reaction [Equation (1)], little is known about the oxidative half-reaction in plant enzymes [Equation (2)]. This reaction has, however, been well characterized in the case of BSAO.[51,52]

Cu^{I}−Semiquinolamine Radical

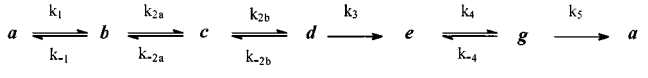
Hill and Mann[19] reported that solutions of PSAO turned from pink to yellow when an appropriate substrate was added under anaerobic conditions. The absorption band of the oxidized enzyme, with a maximum at about 500 nm, disappeared immediately and was replaced by three bands centered at 465, 435, and 350 nm. The authors concluded that the yellow product was the enzyme−substrate complex. Years after this observation, the yellow intermediate was proposed to be a free radical on the basis of its EPR spectrum, which shows a well-resolved hyperfine structure due to coupling with the various protons of an aromatic ring. The presence of copper ions has been shown to be essential for the observation of the free-radical EPR signal.[53] Since the identification of TPQ as the cofactor of several amine oxidases,[25] unequivocal evidence has been obtained for the generation of a Cu^{I}−semiquinone by substrate reduction under anaerobic conditions in plant amine oxidases.[40,54]

When a good substrate such as putrescine is added to LSAO, the broad absorption band at 498 nm disappears instantaneously, indicative of rapid conversion of the TPQ cofactor to a colorless species, the Cu^{II}−aminoresorcinol (***e***), which is predominant in the steady state. If the amine concentration is high enough to fully consume the available oxygen, the solution turns yellow owing to the emergence of new absorption bands centered at 434 and 464 nm.[40,47,54] These are indicative of the formation of Cu^{I}−semiquinolamine free radical species (***f***), in which the substrate-derived nitrogen is covalently bound to the aromatic ring system.[55−57] The extinction coefficients of substrate-reduced LSAO at pH = 7 and 298 K are reported in Table 1. It may be estimated from EPR measurements that under these experimental conditions the population of the radical species lies in the range 40−50%, the remaining fraction of the enzyme being in the form of Cu^{II}−aminoresorcinol. The yellow radical species ***f*** is in a sense "pure", since it does not share its spectrum with other species. However, it occurs in an equilibrium mixture with species ***e***.[54,58] It proved possible to stabilize the Cu^{I}−aminoresorcinol by treating the radical with dithionite, thus allowing the spectrum of the pure colorless species to be recorded.[47] The addition of substrate to LSAO under anaerobic conditions at room temperature led to a marked decrease in the EPR signal attributed to Cu^{II}, while a new signal appeared at $g \approx 2$, at a field modulation amplitude lower than 1 G. This signal intensified with time, reached a

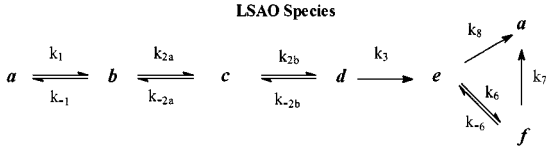
maximum after 5 min of incubation, and thereafter was found to be stable in the frozen sample or in anaerobic liquid solutions. Admission of air led to a gradual disappearance of this signal. These features are indicative of a free radical. In the case of the pea enzyme, Dooley et al.[54] showed that ca. 40% of the Cu^{II} is reduced to Cu^{I} after the addition of substrate to the native enzyme under anaerobiotic conditions, indicating that the Cu^{I}−semiquinolamine and the Cu^{II}−aminoresorcinol are in equilibrium. The interconversion rate constants of these two species were estimated by *T*-jump methods to be $k_{+6} = k_{-6} = 2{\cdot}10^4\ s^{-1}$ [58] (Table 2), taking advantage of the large equilibrium ΔH. Indeed, the Cu^{I}−semiquinolamine EPR signal, monitored as a function of temperature, could not be detected below about 258 K, but was observed to increase on going from 283 to 298 K, the highest temperature investigated.[58][59]

Table 2. Rate constants for the individual steps described in Scheme 1; all data for BSAO are due to Bellelli et al.,[52] whereas those for LSAO are due to Bellelli et al.[65] and Medda et al.,[47] except the values for k_6/k_{-6} for LSAO, which are due to Turowski;[58] the value of k_7 for LSAO has been calculated assuming that the maximum population attained by the radical species is 50%[47]

BSAO Species



LSAO Species



	BSAO	LSAO
k_1 [$M^{-1}s^{-1}$]	$9{\cdot}10^4$	$4{\cdot}10^5$
k_{-1} [s^{-1}]	250	3
$k_{2\ (a\ and\ b)}$ [s^{-1}]	1.2	≥ 200
$k_{-2\ (a\ and\ b)}$ [s^{-1}]	0.01	negligible
k_3 [s^{-1}]	3.3	100
k_4 [$M^{-1}s^{-1}$]	$3.6{\cdot}10^5$	−
k_{-4} [s^{-1}]	33	−
k_5 [s^{-1}]	20	−
k_6 [s^{-1}]	−	$2{\cdot}10^4$
k_{-6} [s^{-1}]	−	$2{\cdot}10^4$
k_7 [$M^{-1}s^{-1}$]	−	$6{\cdot}10^7$
k_8 [$M^{-1}s^{-1}$]	−	$5{\cdot}10^5$

The intensities of the absorbances at 464 and 434 nm of reduced LSAO observed at pH = 7 were found to decrease at both acidic and alkaline pH values, and faded completely at extreme pH values (pH < 5 or > 10).[47] The titration data were fitted to an equilibrium between Cu^{I}−semiquinolamine and the bleached Cu^{II}−aminoresorcinol controlled by two independent ionizable groups with $pK_1 = 5.7$ and $pK_2 = 7.9$: Cu^{I}−semiquinolamine (pH = 7) ⟷ Cu^{II}−aminoresorcinol (pH= 5 or 10)

The shape and position, but not the relative intensities, of the absorption bands were found to be independent of the substrate used.[40,47][60]

Mechanism of Proton-Coupled Electron-Transfer to Dioxygen

In an extensive study of the re-oxidation of bovine serum Cu−amine oxidase (one of those enzymes that do not populate the Cu^{I}−semiquinolamine radical), Su and Klinman[51] reported a detailed analysis of the chemical and kinetic mechanisms of dioxygen conversion into hydrogen peroxide not requiring the conversion of Cu^{II} to Cu^{I}. A hydrophobic niche in the vicinity of the oxygen atom at the C2 position of the reduced cofactor has been identified as the putative dioxygen binding site in the X-ray structure of the active form of the eukaryotic yeast *Hansenula polymorpha*.[61] Reaction of dioxygen with the semiquinolamine does not lead to superoxide release, but two proton-transfer steps are probably coupled to the release of bound H_2O_2 after the formation of a metal-bound hydroperoxide.[62] Since in mammalian amine oxidases the radical species is not present to any significant extent (ca. 1%),[15] it is reasonable to assume that both forms of the reduced enzyme (***e*** and ***f***) can react with O_2 to release H_2O_2 and ammonia, thereby regenerating the Cu^{II}−quinone species.[47] This is also supported by kinetic evidence. It is worthy of note that at least in some Cu−AOs a labile complex with O_2 is formed before oxidation takes place.[51] The reaction of Cu^{II}−aminoresorcinol with oxygen should not be considered as surprising, since the model compound (2,4,5-trihydroxybenzyl)hydantoin is rapidly oxidized by atmospheric O_2 to TPQ−hydantoin.[25] A similar reaction has also been proposed for BSAO.[38]

Stopped-Flow Experiments

Since some of the TPQ derivatives encountered in the catalytic cycle are short-lived and elusive, transient spectroscopy (i.e. stopped-flow methodology) has been exploited in an effort to characterize the spectra and reactivities of as many intermediates as possible. In a typical stopped-flow experiment, the three spectroscopic components, i.e. pink, colorless, and yellow are easily resolved, at least in those Cu−AOs that populate the radical species. The spectroscopic information obtainable is, however, insufficient to resolve all the intermediates, given that some of them have very similar spectra, and a three- or four-step scheme is usually sufficient to describe the time courses recorded. For this review, we carried out an extensive re-evaluation of our data on BSAO and LSAO using the same kinetic scheme, in order to simplify the comparison.

When oxidized LSAO is mixed with putrescine, the bleaching of the 498-nm band is a second-order process, but does not reach completion as the steady-state mixture contains significant amounts of the quinone and aminoresorcinol derivatives. The precise amounts of the latter depend on the chemical nature and concentration of the substrate. This result is in contrast to that obtained for BSAO, for which bleaching is minimal and the steady-state mixture contains mostly the quinone and quinone ketimine derivatives. The following step, i.e. the conversion of ketimine into aldimine, is fast with LSAO and slow with BSAO, consistent with the

isotopic effect observed using dideuterated benzylamine.[63][64] Release of the aldehyde product is usually fast in plant Cu−AOs, at least when physiological substrates are used, and slow in BSAO. This step yields the aminoresorcinol derivative of TPQ, a reduced and bleached species that reacts slowly with oxygen. In the case of BSAO, a labile oxygen complex forms[51] (Scheme 1, ***g***), making the re-oxidation non-second-order,[52] while with LSAO the same complex decays immediately to the oxidized enzyme and the time courses recorded conform to second-order kinetics.

In plant Cu−AOs at a neutral pH value, the aminoresorcinol rapidly equilibrates with the semiquinolamine radical, which attains a population of 40−50%.[54,58] The reactivity of the radical towards dioxygen is 20- to 100-fold higher than that of aminoresorcinol, thus the apparent rate constant for re-oxidation is close to half of that of the radical species[65] (Table 2). Because of the high efficiency of the oxidation reaction, the value of K_m for dioxygen is low in most or all Cu−AOs, irrespective of the complexity of the reaction mechanism. From the rate constants reported in Table 2, a complete and consistent kinetic characterization of the catalytic cycle can be put forward, even though the accuracy of the parameters is low (with SDs between 20 and 50%) due to the redundancy of the kinetic mechanism. In our opinion, such a complete description, even if approximate, is desirable since it allows comparison with the steady-state parameters and hence guarantees a much higher degree of confidence than the determination of the rate constants for single steps.

Under the same experimental conditions as used for LSAO, BSAO does not show the 460−350-nm spectrum typical of the radical species,[46,52] while a radical giving a low-intensity signal 1−2% of the intensity of the copper signal is detected in the room-temperature EPR spectrum.[51,54] According to Dooley,[54] the low concentration does not preclude a catalytic relevance of this radical, while Su and Klinman[51] proposed that the predominant Cu^{II}−aminoresorcinol is the species that reacts with O_2 forming a Cu^{II}−peroxide intermediate. The latter hypothesis is consistent with much experimental data that show Co^{II}-substituted BSAO to be catalytically competent and thus suggest a metal structural function[42,66,69] and/or a Lewis acid role.[45] The nature of the radical in BSAO has recently been questioned, as it also forms when the enzyme is largely inactivated by reaction with hydrogen peroxide.[70]

The Role of Copper

As mentioned above, besides TPQ, amine oxidases also contain copper ions in a 1:1 stoichiometric ratio with the organic cofactor. Since TPQ undergoes reversible redox chemistry even in the absence of copper,[71,72] the function of the metal center in the catalytic mechanism is controversial. Crucial questions are the role of copper ions in the catalytic mechanism and their valence state during the catalytic cycle. Partial answers are now available for each of these questions. In plant enzymes, copper ions have been shown to be involved in the re-oxidation of the substrate-reduced enzyme in the presence of oxygen. It has been unequivocally demonstrated that copper ion reduction occurs in PSAO and LSAO,[40,47,54][58] and that this is coupled to the appearance of a characteristic radical species of the reduced TPQ (the Cu^{I}−semiquinolamine). Through a detailed analysis of fully Cu-depleted, half and fully Cu-reconstituted, and Co-, Ni-, and Zn-substituted lentil amine oxidase, Floris and co-workers[41] have shown that copper ions are also essential for the faster catalytic rate of plant enzymes as compared with mammalian amine oxidases.

The effects of copper ion removal and Co^{II} substitution have been studied in detail with BSAO. In the Cu-depleted protein, the rate of TPQ reduction by benzylamine was found to decrease by a factor of about 10^3 without a substantial change in the enzyme−substrate affinity, the rate of binding to hydrazine inhibitors decreased substantially, and the re-oxidation process was no longer observed.[42] Co^{II} substitution partly restored the catalytic activity (up to 10−40%), decreased K_m to an extent depending on the amino substrate, restored the reactivity towards hydrazine inhibitors to almost the native level,[69] and led to an almost 50-fold increase in the oxygen K_m.[73] Thus, the Co^{II} derivative represents a distinct enzyme from the native one, with quite different kinetic parameters. This implies that the metal center has no redox role in BSAO,[42,74,75] but it may have a structural role and may act as a Lewis acid by modulating the pK_a value of the water molecule through which it is bonded to TPQ−O_2.[37]

Concluding Remarks

Amine oxidases are extensively studied enzymes owing to their ubiquitous distribution in living systems, even though their role has not yet been unambiguously established. The kinetic mechanism of these copper−carbonyl enzymes has only recently been elucidated, following the discovery of the long-sought organic cofactor. Both, copper ions and TPQ, are essential for the enzyme function. However, while the redox cycling of TPQ has been well documented in every enzyme tested, a change in the valence of the copper ions is found only in plant enzymes, which are much more active than their animal homologues. The reduction of copper ions is accompanied by the appearance of a very characteristic radical species, although this has not been proven to be an essential step in the enzymatic mechanism. Nonetheless, plant amine oxidases are nowadays rightly considered as belonging to the group of so-called radical enzymes. A full understanding of their mode of action will require further experiments involving site-directed mutagenesis, while that of their physiological role might be advantageously applied in the construction of "knock-out" organisms lacking the amine oxidase gene.

Acknowledgments

This study was supported in part by funds from the MURST (60%), from the C.N.R. Target Project on Biotechnology, and from contract nos. 97.03975.CT04, 97.04376.CT14, and 98.0115.CT14.

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Received March 29, 2000
[I00120]