J. Enzyme Inhibition, 1996, Vol. 10, pp. 251–262
Reprints available directly from the publisher
Photocopying permitted by license only

COMPARISON OF KINETIC PROPERTIES BETWEEN PLANT AND FUNGAL AMINE OXIDASES

LENKA LUHOVÁ,[1] LUDĚK SLAVÍK,[1] IVO FRÉBORT,[2,*]
MAREK ŠEBELA,[1] LUDMILA ZAJONCOVÁ[1] and PAVEL PEČ[1]

[1]*Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 11, 772 36 Olomouc, Czech Republic*

[2]*Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, 1677–1 Yoshida, 753 Yamaguchi, Japan*

(Received 30 September 1995; in final form 18 November 1995)

Kinetic properties of novel amine oxidases isolated from a mold *Aspergillus niger* AKU 3302 were compared to those of typical plant amine oxidase from pea seedling (EC 1.4.3.6). Pea amine oxidase showed highest affinity with diamines, such as putrescine and cadaverine, while fungal enzymes oxidized preferably *n*-hexylamine and tyramine. All enzymes were inhibited by carbonyl reagents, copper chelating agents, some substrate analogs and alkaloids, but there were quite significant differences in the sensitivity and inhibition modes. Aminoguanidine, which strongly inhibited pea amine oxidases showed only little effect on fungal enzymes. Substrate analogs such as 1,5-diamino-3-pentanone and 1-amino-3-phenyl-3-propanone, which were potent competitive inhibitors of pea amine oxidases, inhibited fungal enzymes much more weakly and non competitively. Also various alkaloids behaving as competitive inhibitors of pea amine oxidases inhibited the fungal enzymes non competitively. Very surprising was the potent inhibition of fungal enzymes by artificial substrates of pea amine oxidases, *E*- and *Z*-1,4-diamino-2-butene. The relationships between the different inhibition modes and possible binding at the active site are discussed.

KEY WORDS: Amine oxidase, *Pisum sativum*, *Aspergillus niger*, inhibition

INTRODUCTION

Copper-containing amine oxidases (EC 1.4.3.6) from various sources have been shown to contain topa quinone as a cofactor, bound in the consensus sequences Asn-topa-Asp/Glu.[1,2] Mostly, these amine oxidases are composed of two identical subunits, each containing copper and topa quinone serving as the mediator in amine oxidation.

Pea seedling amine oxidase (P-AO) (dimer of 2×75 kDa) is one of the most studied amine oxidases.[3] Its substrate specificity and sensitivity to inhibitors have been comprehensively reviewed.[4] Similar to other plant amine oxidases, it shows the highest affinity with diamines such as putrescine and cadaverine and it is inhibited by carbonyl reagents, copper chelating agents and some alkaloids. Topa quinone has been confirmed as the cofactor[2] and recently

*Correspondence.

complete amino acid sequence has been obtained.[5] The enzyme has been crystallized,[6] but its structural analysis has not yet been reported.

Amine oxidase of *Aspergillus niger* was discovered in the mid 1960s, purified and crystallized.[7,8] Copper and an undetermined carbonyl cofactor have been detected which function in the catalytic cycle. Benzylamine, phenethylamine, histamine and aliphatic *n*-monoamines (*n*-butylamine, *n*-amylamine and *n*-hexylamine)[9] have been found to be the best substrates. The enzyme has been inhibited by copper chelating agents diethyldithiocarbamate, cuprizone, *o*-phenanthroline, 2,2′-bipyridyl, 8-hydroxyquinoline,[10,11] by carbonyl reagents semicarbazide, guanidine, hydroxylamine, phenylhydrazine, izoniazide and iproniazide,[11] by ethylamine[11] and ethylenediamine,[12] heavy metal ions and sulfhydryl group reagents,[13] and by a mechanism-based inhibitor β-bromoethylamine.[14] However, interpretation of these results was affected by a poor determination of molecular mass of the enzyme and presumption of pyridoxal phosphate as a cofactor. Recently, we have found two distinct quinoprotein amine oxidases, A-AO-I and A-AO-II, in *Aspergillus niger* AKU 3302 mycelia grown on *n*-butylamine medium.[15] A-AO-I is the enzyme previously reported, consisting of two 75 kDa subunits, while A-AO-II is a novel one of 80 kDa. Topa quinone has been confirmed as the cofactor for both amine oxidases.[16,17] The interaction of A-AO-I and A-AO-II with active site covalent modifiers has been studied.[18] The mode of inhibition by 1,4-diamino-2-butyne has been determined as mechanism-based due to formation of pyrrole covalently bound to the lysyl residue in the active site of A-AO-I. Benzophenanthridine alkaloids, which do not inhibit plant amine oxidases, have been found to be weak non-competitive inhibitors reacting with a sulfhydryl group.[19] Recently the cDNA for A-AO-I has been cloned[20] and its complete amino acid sequence determined.[21]

In this work, we compare fundamental kinetic properties of amine oxidases from *A. niger* to those of pea seedling amine oxidase.

MATERIALS AND METHODS

Pea seedling amine oxidase

Pea amine oxidase was isolated from ten days old etiolated pea (*Pisum sativum*) seedlings by an established procedure.[22] The specific activity was 254 nkat·mg protein^{-1} with putrescine as a substrate, in 0.1 mol·l^{-1} phosphate buffer, pH 7.0, at 30°C, when assayed by monitoring the production of hydrogen peroxide at 436 nm in a coupled reaction with horseradish peroxidase and guaiacol.[23] Protein content was determined by Bradford's method[24] with bovine serum albumin as a standard.

Amine oxidases from the Aspergillus niger AKU 3302

Cultivation of *Aspergillus niger* AKU 3302 mycelia in nitrate medium and induction of amine oxidase by *n*-butylamine was performed using a routine method[7,8] and the amine oxidases were isolated as described previously.[15] Specific activities of AO-I and AO-II assayed by the guaiacol method were 128 and 29 nkat·mg protein^{-1}, respectively, with *n*-hexylamine as a substrate in 0.1 mol·l^{-1} potassium phosphate buffer, pH 7.5, at 30°C.

Other enzymes and chemicals

Peroxidase from horseradish was a salt-free lyophilisate with the specific activity of 1850 $nkat \cdot mg^{-1}$ purchased from Boehringer (Manheim, Germany). Hexylamine hydrochloride was obtained from Fluka (Basel, Switzerland). Other substrates and inhibitors studied were commercially available from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI), except for the hydrochloride of l-amino-3-phenyl-3-propanone[25], dihydrochlorides of 1,5-diamino-3-pentanone[26] and *E*- and *Z*-1,4-diamino-2-butenes[27,28] which were synthesized.

Electrophoresis of native enzymes

The electrophoresis was performed on a slab polyacrylamide gel (7%) with 0.5 $mol \cdot l^{-1}$ Tris-0.38 $mol \cdot l^{-1}$ glycine buffer, pH 8.3, as a mobile phase, at 4°C. The gels were stained for the amine oxidase activity with the substrate (10 $mol \cdot l^{-1}$ *n*-hexylamine for A-AO-I and A-AO-II, 10 $mol \cdot l^{-1}$ cadaverine for P-AO), 0.01% horseradish peroxidase and 0.3 $mg \cdot ml^{-1}$ 4-chloro-3-naphthol in 0.1 $mol \cdot l^{-1}$ phosphate buffer (pH 7.5 for A-AO-I and A-AO-II, pH 7.0 for P-AO).

Amine oxidase activity assay

Spectrophotometric assay of the enzyme activity[23] was performed in a 2 cm cell placed in a light-tight chamber of an EK 5 adapter of a Carl Zeiss Specol 10 spectrophotometer (Jena, Germany), thermostated to 30°C. The reaction mixture (3.45 ml) contained 0.1 $mol \cdot l^{-1}$ potassium phosphate buffer (pH 7.0 or 7.5), guaiacol in final concentration of 0.5 $mmol \cdot l^{-1}$, peroxidase 20 nkat, amine oxidase 2–5 nkat and the inhibitor. The enzyme-inhibitor mixture was preincubated for 10 min and the reaction was started by injecting 0.05 ml of the substrate (final concentration: putrescine 1 $mmol \cdot l^{-1}$ and *n*-hexylamine 2.5 $mmol \cdot l^{-1}$ for saturation, both 0.1–0.5 $mmol \cdot l^{-1}$ for kinetics) directly into the cell, in which the reaction mixture was bubbled for a short time with air. Time-dependent increase in absorption at 436 nm was recorded on-line by a computer[29] for a period of 3 min.

Activity assays of A-AO-I and A-AO-II with putrescine, tyramine and histamine were performed by measuring oxygen consumption[30] because of interferences observed with the spectrophotometric assay.

RESULTS

The molecular mass of the amine oxidases used in this study is 150 kDa for both dimeric P-AO[5] and A-AO-I[15] and 80 kDa for A-AO-II.[15] Since the dimeric enzymes are dissociated on SDS-PAGE showing a band corresponding to 75 kDa, it is virtually impossible to distinguish between them. However, the enzymes have a differential mobility when applied onto a non-denaturing gel. Figure 1 shows native enzyme electrophoresis of crude extracts of pea seedling and *Aspergillus niger* stained for amine oxidase activity. Of the enzymes studied, P-AO showed the highest mobility and A-AO-II the lowest, the activity bands being clearly distinguished.

Comparing the substrate specificity of the amine oxidases studied, P-AO showed the highest affinity with diamines, such as putrescine and cadaverine, having only very low

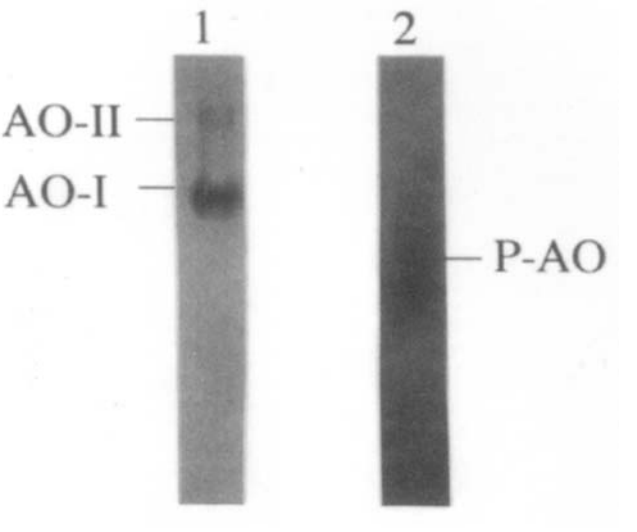

FIGURE 1 Native enzyme electrophoresis of crude extracts of *Aspergillus niger* AKU 3302 (1) and pea seedling (2). The electrophoresis was performed on a slab polyacrylamide gel (7%) with 0.5 mol·l^{-1} Tris-0.38 mol·l^{-1} glycine buffer, pH 8.3, as a mobile phase, at 4°C. The gels were stained wih 10 mol·l^{-1} *n*-hexylamine (1) and cadaverine (2), horseradish peroxidase and 4-chloro-3-naphthol for the activity.

activity with histamine, tyramine, hexylamine and spermine, but considerably higher activity with spermidine (20% of that with putrescine). In contrast, fungal enzymes, A-AO-I and A-AO-II, did not oxidize spermidine at all. They oxidized preferably *n*-hexylamine, tyramine, benzylamine and histamine, having low affinity toward putrescine and cadaverine. *E*- and *Z*-1,4-Diamino-2-butene were not oxidized by fungal enzymes at all. The results are summarized in Table 1.

All the enzymes studied were inhibited by carbonyl reagents, copper chelating agents, some substrate analogs and alkaloids, but significant differences in the sensitivity and character of inhibition were observed. The substrate analog, 1,5-diamino-3-pentanone, which is very potent inhibitor of pea amine oxidase[26] only weakly inhibited A-AO-I and A-AO-II. Also the copper chelators *o*-phenanthroline, 2,2′-bipyridyl and diethylenetetramine had a quite lower inhibition potency with fungal enzymes compared to that for P-AO. On the other hand, artificial substrates of P-AO, *E*- and *Z*-1,4-diamino-2-butene[27,28] acted as potent inhibitors of fungal enzymes. Phenylhydrazine showed potent irreversible inhibition of both classes of amine oxidases reacting with the carbonyl group of the quinone cofactor as already described elsewhere. Surprisingly another carbonyl reagent, aminoguanidine, which strongly inhibited P-AO had only little effect on fungal enzymes. Quinine and lobelia alkaloids showed weak inhibition of both plant and fungal amine oxidases. Also interesting is the weak inhibition noted by pargyline, a typical inhibitor of flavin-containing amine oxidases, e.g., bovine kidney mitochondrial monoamine oxidase[31] (EC 1.4.3.4), where

TABLE 1
Comparison of substrate specificity of pea (P-AO) and *Aspergillus niger* (A-AO-I, A-AO-II) amine oxidases. Velocities were determined as production of hydrogen peroxide measured by coupled reaction with horseradish peroxidase and guaiacol in 0.1 mol·l^{-1} potassium phosphate buffer pH 7.0 for P-AO and pH 7.5 for A-AO-I and A-AO-II with 1.0 mmol·l^{-1} substrates, at 30°C.

Substrate	Relative velocity		
	P-AO	A-AO-I	A-AO-II
putrescine	100	6[a]	10[a]
cadaverine	106	4	5
E-1,4-diamino-2-butene	15	inhibitor	inhibitor
Z-1,4-diamino-2-butene	17	inhibitor	inhibitor
p-dimethylaminomethylbenzylamine	14	5[a]	10[a]
tyramine	7	109[a]	92[a]
histamine	5	42[a]	53[a]
ethylenediamine	3	3	4
benzylamine	1	36	41
hexylamine	2	100	100
spermine	3	2	0
spermidine	20	0	0

[a]measured as oxygen consumption because of discrepancies observed with guaiacol method.

pargyline raises a new absorption maximum at 410 nm due to the reaction with flavin. With the enzymes used, such a spectral change was not observed and the potency of the inhibition was much weaker, probably involving a different inhibition mode as the enzymes studied do not contain flavin.

For reversible inhibitors, the character of the inhibition was examined by the Lineweaver-Burk method and inhibition constants were determined. The results are presented in the Table 2. Substrate analogs such as 1,5-diamino-3-pentanone and 1-amino-3-phenyl-3-propanone, which were potent competitive inhibitors of P-AO,[33] inhibited fungal enzymes much more weakly and non competitively. Double reciprocal plots of non competitive inhibition of A-AO-I and A-AO-II by 1,5-diamino-3-pentanone are shown in the Figure 2. *E*-and *Z*-1,4-Diamino-2-butene, substrates of P-AO, strongly inhibited fungal enzymes non competitively with micromolar inhibition constants. Copper chelators inhibited all enzymes non competitively showing inhibition constants which on the average were two orders of magnitude higher for the fungal enzymes. Plots of the non competitive inhibition of A-AO-I and A-AO-II by *o*-phenanthroline are presented in the Figure 3. Various alkaloids behaving as competitive inhibitors of pea amine oxidase inhibited the fungal enzymes non competitively with lower potency. This includes the quinine alkaloids quinine, quinidine and cinchonine, L-lobeline and pargyline. P-AO was not inhibited by *N*-deacetylcolchicine, which showed weak non competitive inhibition of fungal enzymes. Sodium azide weakly inhibited enzymes of both classes in an uncompetitive manner as shown in Figure 4.

TABLE 2
Character of inhibition and inhibition constants of substrate analogs, copper chelating agents and some alkaloids on pea (P-AO) and *Aspergillus niger* (A-AO-I, A-AO-II) amine oxidases. Inhibitions were measured by guaiacol method in 0.1 mmol·l^{-1} potassium phosphate buffer pH 7.0 for P-AO with putrescine and pH 7.5 for A-AO-I and A-AO-II with *n*-hexylamine as substrates, after 10 min preincubation with the inhibitor, at 30°C.

	Character of inhibition[z], K_i (mmol·l^{-1})		
Inhibitor	P-AO	A-AO-I	A-AO-II
1,5-diamino-3-pentanone	C;0.00021[33]	NC;3.0	NC;2.4
1-amino-3-phenyl-3-propanone	C;0.58[33]	NC;0.0013	NC;0.0047
E-1,4-diamino-2-butene	substrate	NC;0.0033	–
diethylenetriamine	NC;0.0072[34]	very weak	very weak
o-phenanthroline	NC;0.031[34]	NC;2.2	NC;3.1
2,2′-bipyridyl	NC;0.058[34]	NC;1.7	NC;2.5
sodium azide	UC;9.0[35]	UC;39.6	–
quinidine	C;1.1	NC;6.5	–
quinine	C;1.1[36]	NC;5.5	–
cinchonine	C;0.20[36]	NC;10.0	–
L-lobeline	C;0.17[41]	very weak	very weak
pargyline	C;2.5	NC;3.0	–
N-deacetylcolchicine	no inhibition	C;1.6	C;1.0
colchicine	no inhibition	very weak	very weak
quinacrine	very weak	NC;0.7	–

[z] C – competitive; NC – non-competitive; UC – uncompetitive inhibition.

DISCUSSION

During the catalytic cycle of amine oxidases, the substrate is oxidized to the corresponding aldehyde, while topa quinone is reduced to aminocatechol being in equilibrium with the Cu(I)/topa-semiquinone form. Then, in the presence of oxygen, ammonia and hydrogen peroxide are released and the oxidized form of the cofactor is restored.[37] Initial binding of the substrate occurs via its amino group which forms a Schiff base with the carbonyl group of topa quinone. Besides this interaction, it is supposed that carbon chain of aliphatic amines and the aromatic ring of aralkylamines interact with hydrophobic amino acid residues. Moreover, in the case of aliphatic diamines, ionic interaction of a basic residue with their protonated second amino group is expected.[4] Contrary to this, fungal enzymes do not oxidize diamines much therefore such a basic residue probably does not exist in their structure. Aliphatic diaminoketones e.g. 1,5-diamino-3-pentanone and aromatic aminoketones e.g. 1-amino-3-phenyl-3-propanone bind very tightly into the active site of P-AO and are oxidized very slowly so decreasing the oxidation rate of putrescine and cadaverine.[26,33]

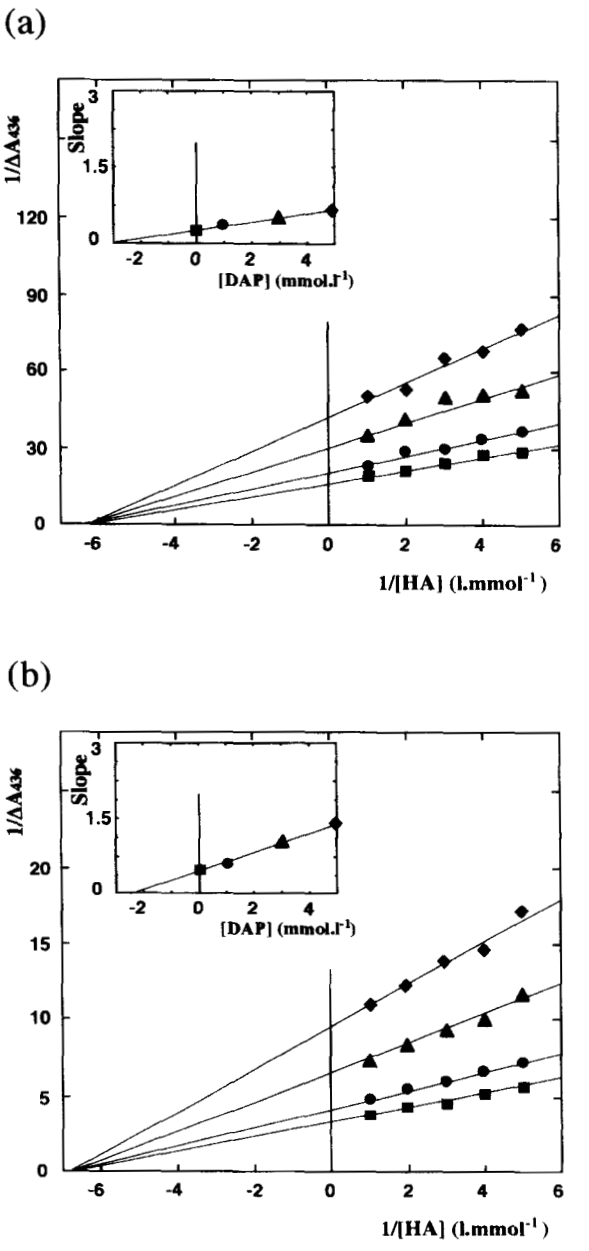

FIGURE 2 Non competitive inhibition of A-AO-I (a) and A-AO-II (b) with *n*-hexylamine (HA) as a substrate by 1,5-diamino-3-pentanone (DAP). The assays were carried out in 0.1 mmol·l^{-1} potassium phosphate buffer, pH 7.5, at 30°C, in the absence (■) and with 1.0 (●) 3.0 (▲) and 5.0 mmol·l^{-1} (♦) inhibitor. The Lineweaver-Burk plots are shown in the main graphs; the inserts show the replots of slopes as the function of the inhibitor concentration. The inhibition constants are 3.0 and 2.4 mmol·l^{-1} for A-AO-I and A-AO-II, respectively.

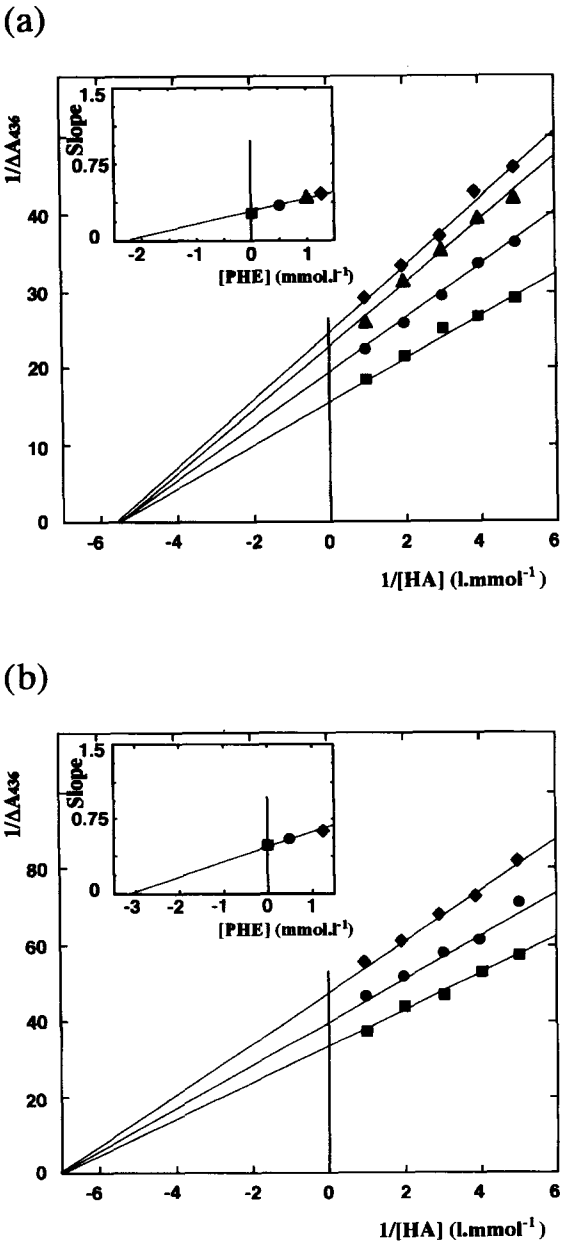

FIGURE 3 Non competitive inhibition of A-AO-I (a) and A-AO-II (b) with *n*-hexylamine (HA) as a substrate by *o*-phenanthroline (PHE). The assays were carried out in 0.1 mmol·l^{-1} potassium phosphate buffer, pH 7.5, at 30°C, in the absence (■) and with 0.5 (●), 1.0 (▲) and 1.25 mmol·l^{-1} (♦) inhibitor. The Lineweaver-Burk plots are shown in the main graphs; the inserts show the replots of slopes as the function of the inhibitor concentration. The inhibition constants are 2.2 and 3.1 mmol·l^{-1} for A-AO-I and A-AO-II, respectively.

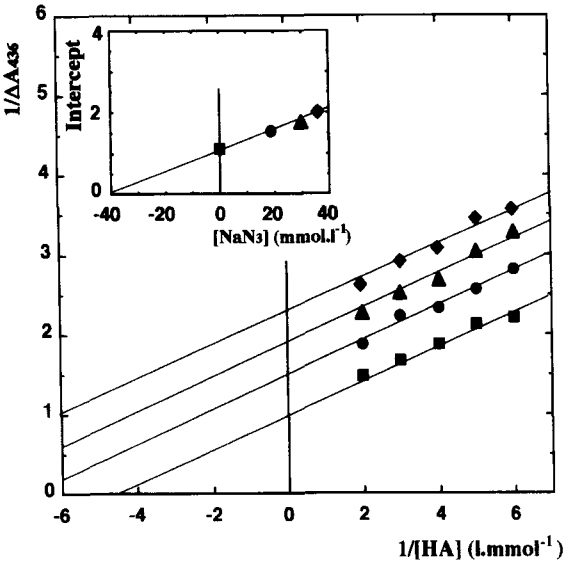

FIGURE 4 Uncompetitive inhibition of A-AO-I by sodium azide with *n*-hexylamine (HA) as the substrate. The assays were carried out in 0.1 mmol·l^{-1} potassium phosphate buffer, pH 7.5, at 30°C, in the absence (■) and with 18.5 (●), 29.6 (▲) and 37.0 mmol·l^{-1} (♦) inhibitor. The Lineweaver-Burk plot is shown in the main graph; the insert shows the replot of intercepts as the function of the inhibitor concentration. The inhibition constant is 39.6 mmol·l^{-1}.

With fungal amine oxidases, these compounds were not oxidized and behaved as non competitive inhibitors. Hence, their molecular interaction must be completely different, possibly being related to the absence of a base binding the second protonated amino group as well as the non competitive inhibition of fungal enzymes by substrates of P-AO, *E*- and *Z*-1,4-diamino-2-butene. Also alkaloids inhibiting P-AO competitively showed non competitive inhibition of fungal enzymes. As reported previously,[4] the interaction of P-AO with these alkaloids, which do not possess a free primary amino group, occurs probably by binding to the hydrophobic site and blocking the cofactor, which is closely located. Also ionic interaction of their secondary amino group with the cofactor may take place. From the results obtained with fungal enzymes, it is very probable that this hydrophobic site is localized somewhat distantly from the cofactor, which accounts for the non competitive inhibition since the cofactor is not blocked effectively.

Amino acid sequences that have been obtained recently for P-AO and A-AO-I along with other kinetic data published previously allow a comparison of their active sites concerning amino acid residues taking part in the catalytic cycle and in interactions with some inhibitors. The P-AO sequence[5] includes 674 residues, with topa quinone at position 412. Similarly to the *Escherichia coli* amine oxidase, of which the crystal structure has been published recently,[38] the subunits of the dimeric enzyme are likely to be linked via H-382 and T-408. Then the copper ligands are topa quinone, a pair of histidyls H-467 and H-469 in

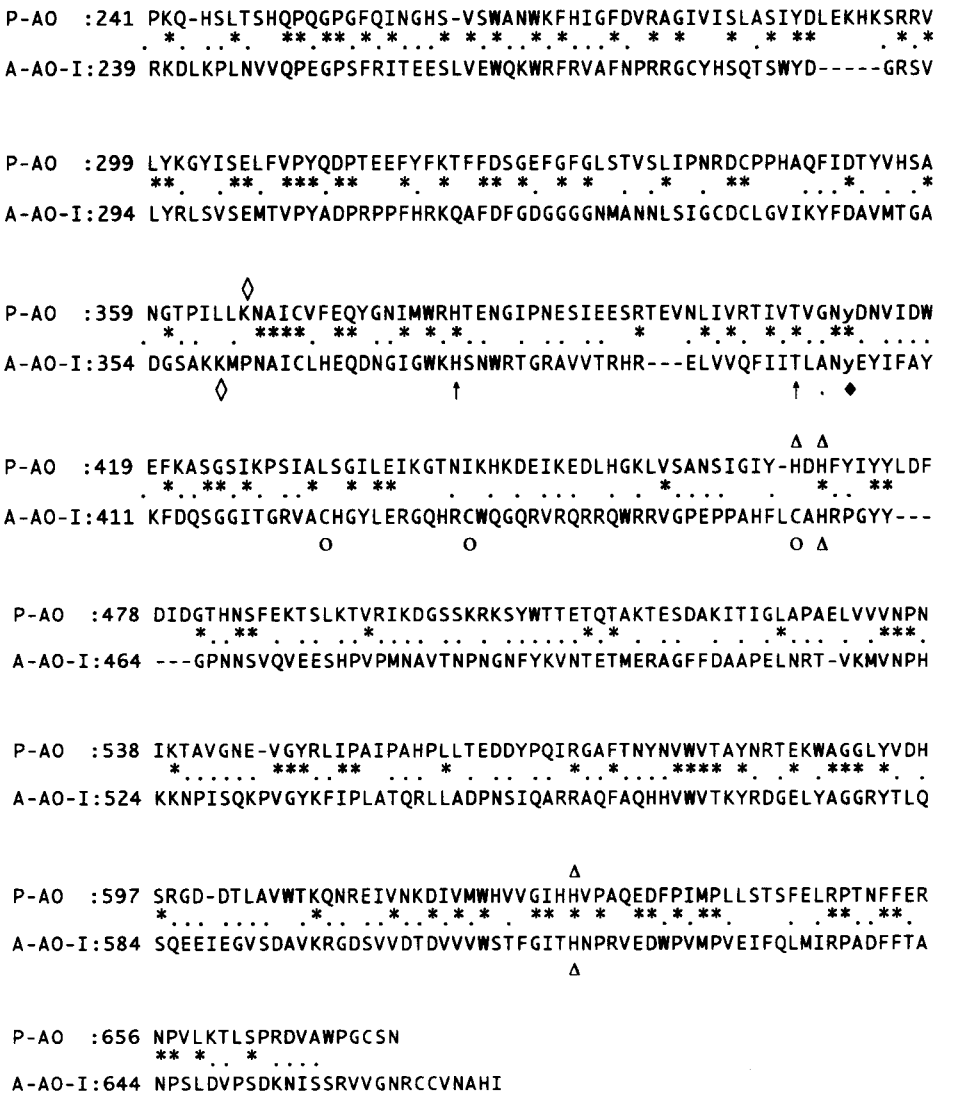

FIGURE 5 Partial computer alignment between A-AO-I[21] and P-AO[5] sequences by GENETYX-MAC 6.2.0. Asterisks mark the positions of conserved residues and partial similarity is indicated by dots. The gaps (hyphens) are inserted to maximize the match. Black diamond shows the position of topa quinone (y), arrows show the subunit crosslink[38] between H and T and open triangles indicate putative histidyl residues serving as the copper ligands.[38,39] The lysyl residue reacting with a mechanism-based inhibitor, 1,4-diamino-2-butyne, is marked by an open diamond and the cysteinyls in the A-AO-I sequence, where inhibition by benzophenanthridine alkaloids and β-bromoethylamine may occur, are marked by open circles.

the typical copper binding motif[39] HDH and H-628 near the C-terminal.[38] The A-AO-I sequence[21] has 670 amino acids, with topa quinone at the position 404, showing 27.8% similarity to the P-AO sequence. The enzymes share only a little sequence similarity near the N-terminal, but much more in the central and C-terminal parts. A subunit link via H-378 and T-400 is expected, but copper ligands are not clear at all. The enzyme contains only 1 mol Cu/dimer and the sequence lacks the HXH copper binding motif (X = D, Q, T) present in all other known amine oxidases[39] which is replaced by CAH. The residue H-616 near the C-terminal is expected to be the copper ligand. Computer alignment of the central and C-terminal part of both sequences is shown in Figure 5.

The A-AO-I sequence contains the peptide KMPNACLHEQ starting with K-359. The lysyl residue of this sequence was confirmed as the binding site of a mechanism-based inhibitor, 1,4-diamino-2-butyne. The inhibitor causes irreversible inactivation forming pyrrole bound to the lysyl residue and raising a new absorption maximum at 310 nm. A similar interaction was previously described also for P-AO, however the reacting residue was not identified.[40] From the alignment presented in Figure 5, it becomes obvious that the binding site of 1,4-diamino-2-butyne in P-AO should be the residue K-366 in the sequence LLKNACVFEQ, since both enzymes share a high degree of sequence similarity in this area.

Another interesting conclusion can be drawn from the positions of the cysteinyl residues in the A-AO-I sequence. The sequence contains 14 cysteinyls, while P-AO contains only five. As reported previously A-AO-I was inhibited by β-bromoethylamine, which reacts with a cysteinyl residue.[14] Moreover, recently the non competitive inhibition of A-AO-I and A-AO-II by benzophenanthridine alkaloids due to the interaction with a sulfhydryl group has been studied.[18] Interestingly, these alkaloids showed no inhibition effect on P-AO. Thus, it is very likely that the cysteinyl residue(s) involved in this interaction are these present only in the structure of A-AO-I, but not in P-AO. If we exclude the cysteinyls in the N-terminal part and near the C-terminal, which are too far from the active site of A-AO-I and those conserved in P-AO, there are three possible candidates for the interaction with the benzophenanthridine alkaloids C-424, C-435 and C-460. The last one deserves special attention, since it is placed in the position usually occupied by the first histidyl in the HXH motif serving as the copper ligand in amine oxidases.

Acknowledgements

The authors thank Osao Adachi from Yamaguchi University, Hidehiko Kumagai, Hisanori Tamaki from Kyoto University and Lumír Macholán from Masaryk University of Brno, for helpful discussion. This work was supported by grant V 303/1994 from the Ministry of Education, Czech Republic.

References

1. Janes, S.M., Mu, D., Wemmer, D., Smith, A.J., Kaur, S., Maltby, D., Burlingame, A.L. and Klinman, J.P. (1990) *Science*, **248**, 981.
2. Janes, S.M., Palcic, M.M., Scaman, C.H., Smith, A.J., Brown, D.E., Dooley, D.M., Mure, M. and Klinman, J.P. (1992) *Biochemistry* **31**, 12147.
3. Mondovi, B. and Riccio P. (1985) In *Structure and Functions of Amine Oxidases* (Mondovi, B., ed.) p. 64. CRC Press, Boca Raton FL.
4. Frébort, I. (1993) *Acta Univ. Palacki. Olomuc., Fac. Rer. Nat.*, **112** (Chemica XXXII), 105.
5. Tipping, A.J. and McPherson, M.J. (1995) *J. Biol. Chem.*, **270**, 16939.

6. Vignevich, V., Dooley, D.M., Guss, J.M., Harvey, I., McGuirl, M.A. and Freeman, H.C. (1993) *J. Mol. Biol.*, **229**, 243.
7. Yamada, H., Adachi, O. and Ogata, K. (1965) *Agric. Biol. Chem.*, **29**, 649.
8. Adachi, O. and Yamada, H. (1969) *Agric. Biol. Chem.*, **33**, 1707.
9. Yamada, H., Adachi, O. and Ogata, K. (1965) *Agric. Biol. Chem.*, **29**, 864.
10. Suzuki, H. and Ogura, Y. (1972) *J. Biochem. (Tokyo)*, **72**, 833.
11. Yamada, H., Adachi, O. and Ogata, K. (1965) *Agric. Biol. Chem.*, **29**, 912.
12. Suzuki, H., Ogura, Y. and Yamada, H. (1971) *J. Biochem. (Tokyo)*, **69**, 1065.
13. Suzuki, H., Ogura, Y., Yamada, H. and Arima, K. (1975) *Biochim. Biophys. Acta*, **403**, 23.
14. Kumagai, H., Uchida, H. and Yamada, H. (1979) *J. Biol. Chem.*, **254**, 10913.
15. Frébort, I., Yanagisawa, K. and Adachi, O. (1994) *Acta Univ. Palacki. Olomuc., Fac. Rer. Nat.*, **117** (Chemica XXXIII), 79.
16. Frébort, I., Toyama, H., Matsushita, K., Kumagai, H., Peč, P., Luhová, L. and Adachi, O. (1994) In *Biochemistry of Vitamin B6 and PQQ* (Marino G., Sannia G., Bossa F., eds.) p. 241. Birkhäuser Verlag Basel/Switzerland.
17. Frébort, I., Toyama, H., Matsushita, K. and Adachi O. (1995) *BioFactors*, **5**, 56.
18. Frébort, I., Peč, P., Luhová, L., Matsushita, K., Toyama, H. and Adachi, O. (1994) *Eur. J. Biochem.*, **225**, 959.
19. Luhová, L., Frébort, I., Ulrichová, J., Adachi, O., Šimánek, V. and Peč, P. (1995) *J. Enz. Inhib.*, **9**, 295.
20. Tamaki, T., Ishida, H., Tsuno, Y., Yamamoto, K., Adachi, O. and Kumagai, H. (1995) *BioFactors*, **5**, 45.
21. Frébort, I., Tamaki, H., Ishida, H., Peč, P., Luhová, L., Tsuno, H., Halata, M., Asano, Y., Kato, Y., Matsushita, K., Toyama, H., Kumagai, H. and Adachi, O. (1996) *Eur. J. Biochem.*, in press; GenBank, Seq ID U31869.
22. Macholán, L. and Haubrová, J. (1976) *Coll. Czech. Chem. Commun.* **41**, 2987.
23. Frébort, I., Haviger, A. and Peč, P. (1989) *Biológia (Bratislava)*, **44**, 729.
24. Bradford, M.M. (1976) *Anal. Biochem.*, **72**, 248.
25. Gabriel, S. (1908) *Ber. Deut. Chem. Ges.*, **41**, 242.
26. Macholán, L. (1974) *Coll. Czech. Chem. Commun.*, **39**, 653.
27. Macholán, L., Hubálek, F. and Šubová, H. (1975) *Coll. Czech. Chem. Commun.*, **40**, 1247.
28. Peč, P., Chudý, J. and Macholán, L. (1991) *Biológia (Bratislava)*, **46**, 665.
29. Frébort, I. (1991) *Chem. Listy*, **85**, 423.
30. Macholán, L. (1968) 62, *Chem. Listy*, **62**, 1256.
31. Peč, P. and Frébort, I. (1991) *Biochem. Int.*, **24**, 633.
32. Patek, D.R. and Hellerman, L. (1974) *J. Biol. Chem.*, **249**, 2373.
33. Peč, P. and Frébort, I. (1991) *J. Enz. Inhib.*, **4**, 327.
34. Peč, P. and Frébort, I. (1992) *J. Enz. Inhib.*, **6**, 243.
35. Peč, P. and Haviger, A. (1988) *Acta Univ. Palacki. Olomuc., Fac. Rer. Nat.*, **91** (Chemica XXVII), 245.
36. Peč, P. and Macholán, L. (1976) *Coll. Czech. Chem. Commun.*, **41**, 3474.
37. Turowski, P.N., McGuirl, M.A. and Dooley, D.M. (1993) *J. Biol. Chem.*, **268**, 17680.
38. Parsons, M.R., Convery, M.A., Wilmot, G.M., Yadav, K.D.S., Blakeley, V., Corner, A.S., Phillips, S.E.V., McPherson, M.J. and Knowles, P.F. (1995) *Structure*, **3**, 1171.
39. Mu, D., Medzihradszky, K.F., Adams, G.W., Mayer, P., Hines, W.M., Burlingame, A.L., Smith, A.J., Cai, D. and Klinman, J.P. (1994) *J. Biol. Chem.*, **269**, 9926.
40. Peč, P. and Frébort, I. (1992) *Eur. J. Biochem.*, **209**, 661.
41. Peč, P. (1985) *Biológia (Bratislava)*, **40**, 1209.