Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2009; 34 (1) ; 2–8.

Research Article [Araştırma Makalesi]

Yayın tarihi 26 Mart, 2009 © TurkJBiochem.com

[Published online 26 March, 2009]

# The Substrate Specificity of Purified Rat Lung Semicarbazide-Sensitive Amine Oxidase

## [Sıçan Akciğerinden Saflaştırılan Semikarbazid Duyarlı Amin Oksidaz Enziminin Substrat Özgüllüğü]

Samiye Yabanoglu,
Gulberk Ucar

Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, TURKEY.

**Yazışma Adresi**
[Correspondence Address]

Samiye YABANOGLU

Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, TURKEY.
Telephone : +90 (312) 3051499
Fax : +90 (312) 3114777
E-mail: samiye@hacettepe.edu.tr

[Received: 08 August 2008, Accepted: 14 January 2009]

Kayıt tarihi: 08 Ağustos 2008, Kabul tarihi: 14 Ocak 2008

**ABSTRACT**

**Objectives:** The aim of this study was to investigate the substrate specificity of purified rat lung semicarbazide-sensitive amine oxidase.

**Methods:** Substrate specificity and kinetics of purified rat lung semicarbazide sensitive amine oxidase were studied using benzylamine and methylamine as substrates. Substrate competition studies were also performed.

**Results:** Semicarbazide-sensitive amine oxidase was purified from the crude microsomal fractions of rat lung by Cibacron Blue 3GA-agarose and Concanavalin A-Sepharose 4B affinity chromatographies with a specific activity of 5.6 nmol/min/mg protein by using benzylamine as a substrate. Km and Vmax values for benzylamine and methylamine were determined to be 3.7 μM and 5.6 nmol/min/mg protein; and 141.5 μM and 4.2 nmol/min/mg protein, respectively.

**Conclusion:** The velocity of the reaction decreased with increasing substrate concentration in the case of benzylamine indicating that Michaelis-Menten enzyme behaviour was obeyed at only low concentrations for this substrate. Oxidation of methylamine by purified SSAO obeyed to the Michaelis-Menten behaviour. Substrate inhibition was not detected for the oxidation of methylamine by SSAO at high concentrations.

Substrate competition studies showed that methylamine, dopamine, phenylethylamine, kynuramine and serotonin inhibited the oxidation of benzylamine by semicarbazide-sensitive amine oxidase.

**Key Words:** semicarbazide sensitive amine oxidase, rat lung, benzylamine, methylamine

**ÖZET**

**Amaç:** Bu çalışmanın amacı sıçan akciğerinden saflaştırılan semikarbazid duyarlı amin oksidaz enziminin substrat özgüllüğünün belirlenmesidir.

**Yöntem:** Saf sıçan akciğer kaynaklı semikarbazid duyarlı amin oksidaz enziminin substrat özgüllüğü ve kinetiği substrat olarak benzilamin ve metilamin kullanılarak incelendi. Buna ek olarak yarışmalı substrat çalışmaları da gerçekleştirildi.

**Bulgular:** Semikarbazid duyarlı amin oksidaz sıçan akciğerinin mikrozomal fraksiyonlarından Cibacron Blue 3GA-agaroz ve Concanavalin A-Sepharose 4B affinite kromotografileri kullanılarak benzilaminin substrat olarak kullanıldığı durumda 5.6 nmol/min/mg protein spesifik aktivite ile saflaştırıldı.

Benzilamin ve metilamin için $K_m$ ve $V_{max}$ değerleri sırasıyla 3.7 μM ve 5.6 nmol/min/mg protein; 141.5 μM ve 4.2 nmol/min/mg protein olarak bulundu.

**Sonuç:** Benzilaminin kullanıldığı koşullarda reaksiyonun hızı, artan substrat konsantrasyonları ile azaldı ve bu bulgu sözkonusu substratın sadece düşük konsantrasyonlarda Michaelis-Menten enzim davranışına uyduğunu gösterdi. Metilaminin saflaştırılan SSAO ile oksidasyonu ise Michaelis-Menten enzim davranışına uyduğu saptandı. Yarışmalı substrat çalışmaları metilamin, dopamin, feniletilamin, kinuramin ve serotoninin semikarbazid duyarlı amin oksidazın katalizlediği benzilamin oksidasyonunu inhibe ettiğini gösterdi.

**Anahtar Kelimeler:** semikarbazid duyarlı amin oksidaz, sıçan akciğeri, benzilamin, metilamin

## Introduction

The oxidative deaminations of endogenous and exogenous amines in mammals are catalyzed by a number of oxidases. Monoamine oxidase (MAO), a flavin adenine dinucleotide (FAD) dependent amine oxidase, which plays an essential role in the oxidative deamination of biogenic amines such as serotonin, dopamine, adrenaline and also catalyzes the oxidation of xenobiotic amines has been extensively characterized, whereas, little is known about the structure and function of semicarbazide sensitive amine oxidase [EC 1.4.3.6: amine: oxygen oxidoreductase (deaminating), SSAOs], copper-containing amine oxidase (CAO).

Most SSAOs are dimeric glycoproteins with molecular masses of 140-180 kDa. Subunits are identical and as cofactors, each subunit contains one copper ion [Cu (II)] and topa-quinone (TPQ).

Two forms of SSAOs are present: a membrane bound form and a soluble form found in plasma. Tissue-bound SSAO contains a short intracellular domain, a single transmembrane domain and a long extracellular domain which includes the catalytic site. Plasma SSAO appears to be the result of proteolytic cleavage of membrane bound SSAO [1-3].

Membrane-bound SSAO, like MAO, can readily deaminate aromatic and aliphatic amines. Vascular SSAO may be involved in deaminating circulating amines, from both endogenous and xenobiotic origins. SSAOs catalyze the oxidative conversion of primary amines only, whether the amino group is present in mono-, di- or polyamines [2-4].

The physiological role of SSAO is not yet quite clear, but in recent years indications for the following functions have been described: protection against endogenous/exogenous amines, leukocyte trafficking (VAP-1), role in glucose transport into cells (with GLUT4 transporter), local generation of signalling molecule ($H_2O_2$), role in cell growth and maturation, and cross-linking of proteins (advanced glycation end products=AGEs) [5].

MAO and SSAO are distinct from each other with respect to their substrate specificities and inhibitor sensitivities. However, it is difficult to establish the substrate overlap between MAO and SSAO since tissue-bound SSAO shows wide species differences in specificities and amount of enzyme present. The physiological substrates for SSAO are believed to include aminoacetone, methylamine (MA; it can be ingested from food and drink or inhaled from cigarette smoke), 2-phenylethylamine, histamine, tyramine and dopamine, however, under physiological conditions; the elimination of histamine, tyramine and dopamine is due to MAO activity. SSAO might play a scavenger role in the case of overproduction of these biogenic amines. On the other hand, aminoacetone and MA are not MAO substrates [3, 6, 7].

SSAO catalyzes the oxidative deamination of substrates containing an amine moiety linked to an unsubstituted methylene group. During the reaction, these compounds are converted to considerably more toxic products than the relatively harmless substrates themselves. MA is derived from epinephrine, creatinine (via sarcosine), creatine, choline or nicotine catabolism results in formation of formaldehyde, whereas aminoacetone is a product of glycine and threonin metabolism results in formation of methylglyoxal. Allylamine is a compound used in the organic synthesis of many commercial products, including pharmaceuticals and results in formation of acrolein. These resulting aldehydes are much more toxic than the parent compounds [2, 3].

There are large species differences in the specificities of SSAO, but the nonphysiological amine benzylamine (BA) is a good substrate for the mammalian enzymes. Indeed, plasma SSAO has sometimes been referred to as BA oxidase [4, 6]. In the present study the substrate specificity of the partially purified tissue-bound SSAO from rat lung was evaluated.

## Materials and Methods

### *Chemicals*

Chemicals, except potassium dihydrogen phosphate which was obtained from E.Merck (Darmstadt, Germany), were from Sigma-Aldrich, Co. (Germany).

### *Purification of solubilized rat lung microsomes*

SSAO was purified from Sprague Dawley rat lung (Ethics Committee of Laboratory Animals in Hacettepe University, Turkey, 2004/36, 1082). The solubilized enzyme was purified as previously described [8]. Briefly, rat lung microsomes were obtained according to the method described by Lizcano et al. [9].The final pellets were resuspended in 20 mM potassium phosphate buffer, pH 7.2. This crude microsomal fraction was mixed with an equal volume of 1% (w/v) Triton X-100 in 20 mM potassium phosphate buffer, pH 7.2, and the mixture was stirred for 30 min. at $4^0$C. The solubilized enzyme was obtained by decanting the supernatant after centrifugation at 105,000 x g for 1 h. Solubilized enzyme was loaded onto a Cibacron Blue 3GA-agarose column and the dialyzed output was applied to the Concanavalin A-Sepharose 4B affinity column.

### *Determination of SSAO activity*

SSAO activity was measured according to the method of Tabor [10] with some modifications when BA was used as substrate. The reaction mixture contained 20 mM potassium phosphate buffer, pH 7.2, and purified enzyme (100 µl) in a final volume of 1 ml. Mixture was preincubated with l-deprenyl (1 µM) at $37^0$C for one hour to inactivate any monoamine oxidase (MAO) possibly present in the supernatant. The reaction was initiated by the addition of the substrate BA (10-750 µM) and the absorbance change was monitored at 250 nm at $37^0$C. The molar extinction coefficient of benzaldehyde was taken as 11,800 $M^{-1}$ $cm^{-1}$. SSAO activity was expressed as nmol of benzaldehyde formed per hour per

mg. Time course assays were used to ensure that initial rates of reaction were determined and proportionality to enzyme concentration was also tested in each set. Each point presented in all figures and tables is the average of 3 determinations. Standard deviations are within 20% of the mean.

### *Substrate specificity of purified SSAO*

The rat lung SSAO activity toward BA was assayed directly by the method described in the section 2.3 at a concentration range of 10-750 μM BA.

Oxidation of MA by the purified rat lung SSAO was determined according a spectrophotometric coupled assay described earlier [11]. In the coupled assay, the activity of SSAO toward MA was determined by coupling the formation of formaldehyde to the reduction of $NAD^+$ in the presence of formaldehyde dehydrogenase (FDH). The reaction medium contained the SSAO sample, 33 μL $NAD^+$ (10 $mg.ml^{-1}$), 10 μL FDH (from *Candida boidinii*, 10 $mU.\ ml^{-1}$), and 50 mM potassium phosphate buffer, pH 7.2, in a final volume of 900 μL. Following incubation at $37^0$C for 5 min, the reaction was started by the addition of 100 μL of MA, and the NADH generated was monitored at 340 nm. The molar absorbance coefficient of NADH was taken to be 6220 $M^{-1}\ cm^{-1}$. One unit of enzyme activity is defined as the amount catalyzing the formation of 1 μmol product in one minute.

The rat lung SSAO activity toward MA was assayed by the method described above at a concentration range of 25-1000 μM MA.

The kinetic parameters such as corresponding $V_{max}$ and $K_m$ values for deamination of substrates with SSAO were calculated by use of non-linear regression analysis (Systat version 5.0 package) and also by using the Lineweaver-Burk double reciprocal plot, by plotting 1/v vs 1/S analysed over a range of substrate concentrations [12].

In order to examine the ability of some biogenic amines (MA, dopamine, tyramine, phenylethylamine, kynuramine (KA) and serotonin) to interfere with the metabolism of BA by SSAO, competition studies were performed. The concentration ranges of amines tested were 5-1000 μM. Amines and BA (10-100 μM) were incubated together for 15 min. in the assay medium at $37^0$C and the reaction was started by addition of the enzyme which was preincubated by l-deprenyl (1μM) for 60 min.

### *Protein determination*

Protein contents of the samples were determined according to the method of Bradford (13) with bovine serum albumin used as standard.

## Results

### *Purification of SSAO from rat lung microsomes*

SSAO was purified as described in Materials and Methods. Two chromatographic steps were used to obtain the partially pure enzyme as previously described [8].

### *Substrate specificity of rat lung SSAO*

The rat lung SSAO activity was determined toward BA and MA by the methods described in the sections of 2.3 and 2.4 at the concentration ranges of the substrates as 10-750 μM for BA and 25-1000 μM for MA. The measurements of kinetic parameters for these two substrates by the purified SSAO were determined in the presence of 1 mM l-deprenyl to inhibit any MAO activity.

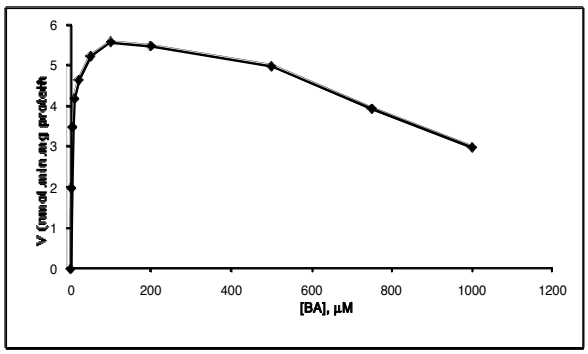



**Figure 1**. Michaelis-Menten plot of BA oxidation by SSAO. BA was used as substrate in the concentration range of 0-1000 mM. Each point is the average of 3 determinations. Standard deviations are within 20% of the mean.

Time course of the product formation with BA as substrate was followed at relatively low BA concentrations and at relatively higher BA concentrations. The velocity of the reaction was decreased with increasing substrate concentration indicating that Michaelis-Menten enzyme behaviour was obeyed at only low concentrations of the substrate (Fig. 1), whereas deviation from linearity was observed at higher substrate concentrations. Thus, the apparent $K_m$ was estimated from the linear part of the graph plotted by using BA at the concentration range of 0-100 μM. Rat lung SSAO was found to deaminate BA with a $K_m$ of 3.7 μM and a $V_{max}$ of 5.6 nmol/min/mg protein at this concentration range (Fig. 2). Substrate inhibition which was detected for the oxidation of BA by SSAO at high concentrations was found to be reversible. The apparent $K_m$` estimated from the Dixon plot of reciprocal velocity against substrate concentration was 800 μM (Fig. 3).

In order to test the possibility of product inhibition being responsible, benzaldehyde, benzoic acid and $NH_4^+$, the end products of BA oxidation, were tested as potential inhibitors of BA oxidation. Only $NH_4^+$ showed inhibitory activity on the oxidation of BA by SSAO. Inhibition was found to be competitive in nature with a $K_i$ value of 21.0 ± 1.8 mM (mean ± SEM of three experiments) (data were not shown). However, additional experiments should be carried out to clarify such high substrate inhibition seen with BA.

Oxidation of MA by purified SSAO obeyed to the Michaelis-Menten behaviour (Fig. 4). Rat lung microsomal SSAO was found to deaminate MA with a $K_m$ of 141.5 μM and a $V_{max}$ of 4.2 nmol/min/mg protein (Fig. 5). Substrate inhibition was not detected for the oxidation of

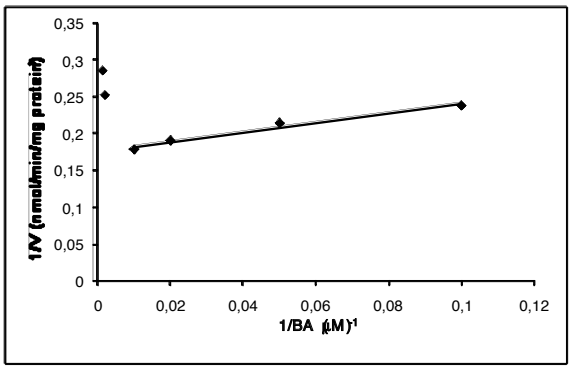



**Figure 2**. Lineweaver-Burk plot of BA oxidation.when BA was used as substrate in the concentration range of 10-750 mM. r2 = 0.9716. Each point is the average of 3 determinations. Standard deviations are within 20% of the mean.

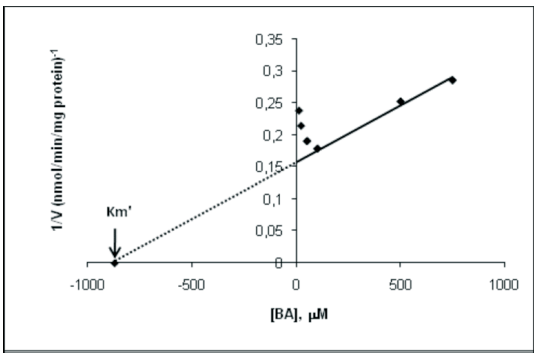



**Figure 3**. . Dixon Plot of BA oxidation when BA was used as substrate in the concentration range of 10-750 µM. r2 = 0,9915. Each point is the average of 3 determinations. Standard deviations are within 20% of the mean.

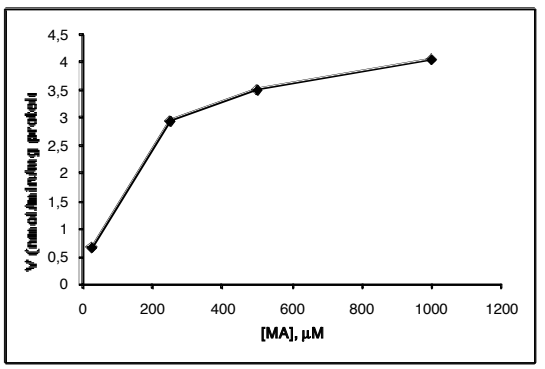



**Figure 4**. Michaelis-Menten plot of MA oxidation by SSAO. MA was used as substrate in the concentration range of 25-1000 mM. Each point is the average of 3 determinations. Standard deviations are within 20% of the mean.

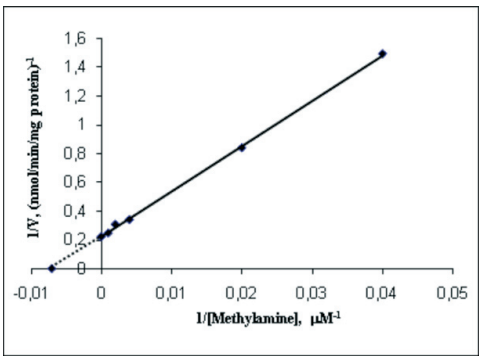



**Figure 5**. Lineweaver-Burk plot of MA oxidation by SSAO. MA was used as substrate in the concentration range of 25-1000 mM. r2 = 0.9994. Each point is the average of 3 determinations. Standard deviations are within 20% of the mean.

MA by SSAO at high concentrations.

The kinetic parameters for the metabolism of BA and MA by purified rat lung SSAO are shown in Table 1. As seen in the table, the highest affinity and catalytic efficacy were obtained towards the non-physiological substrate bezylamine.

**Table 1.** Kinetic constants for amine metabolism by rat lung SSAO*

| Substrate | Km ($\mu$M) | Vmax (nmol/ min/mg protein) | Vmax/Km |
|---|---|---|---|
| Benzyl-amine | 3,7 ± 0,4 | 5,6 ± 0.5 | 1,5 ± 0,1 |
| Methyl-amine | 141,5 ± 10,2 | 4,2 ± 0.3 | 0.03± 0.002 |

*Values are means ± SEM of three independent experiments

## *Substrate competition*

In order to examine the ability of some biogenic amines (MA, dopamine, tyramine, phenylethylamine, KA and serotonin) to interfere with the metabolism of BA by SSAO, a limited number of competition studies were performed.

All the amines tested, except tyramine, showed an inhibition of BA oxidation by rat lung SSAO (Table 2). KA was the most potent amine assayed in this way with an $IC_{50}$ value of 12.2±1.3 µM. With regard to the rest of the amines tested, the inhibitory potency for rat lung SSAO showed, in decreasing order, MA, dopamine, serotonin and phenylethylamine. Tyramine did not show any inhibitory effect on the oxidation of BA by rat lung SSAO (Table 2).

In order to investigate the interaction of rat lung SSAO with these amines, kinetic parameters versus BA in the presence of the amines at different concentrations were determined. Figure 6a presents the reciprocal plots obtained for rat lung SSAO towards MA. MA behaved as a competitive inhibitor of BA metabolism by SSAO of rat lung with a $K_i$ value of 170.6±16.1 µM (Fig. 6b). Dopamine, serotonin and phenylethylamine appeared also as the competitive-type inhibitors of BA metabolism by rat lung SSAO suggesting that these amines compete with BA for the active site of the enzyme.

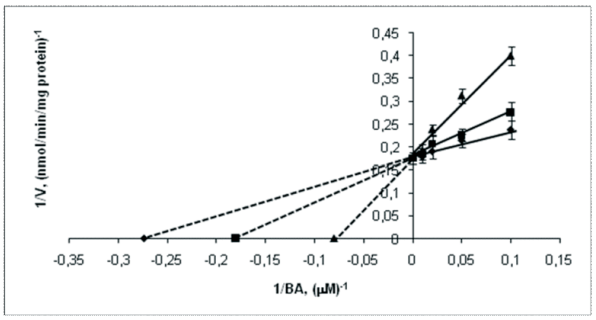
Figure 7a shows the Lineweaver-Burk plot of inhibition of SSAO activity with KA when BA was used as substrate. Since the kinetic behavior of KA as inhibitor of BA metabolism was non-competitive, it was suggested that KA possibly bind to another site different from the active site of the enzyme. $K_i$ value was for the inhibition

**Table 2.** Kinetic parameters corresponding to the inhibition of the purified SSAO by some amines when BA was used as substrate*

| Amines | IC50 value ($\mu$M) | Ki value ($\mu$M) | Inhibition type |
|---|---|---|---|
| Methylamine | 150.2±10.1 | 170.6±16.1 | Competitive |
| Kynuramine | 12.2±1.3 | 19.9±1.8 | Non-competitive |
| Dopamine | 270.7±19.2 | 279.6±18.3 | Competitive |
| Phenylethylamine | 890.2±61.3 | 750.2±68.4 | Competitive |
| Serotonin | 300.3±22.7 | 324.6±27.1 | Competitive |
| Tyramine | NI | NI | NI |

* SSAO was incubated with the amines at 37$^0$C for 60 min.at a concentration rate of 5-1000 µM. Values were expressed as mean±SEM of three separate determinations.

NI: No inhibition was observed



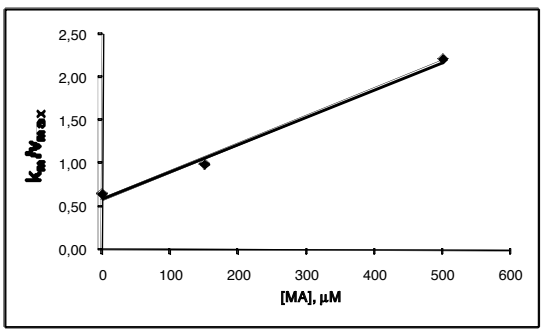
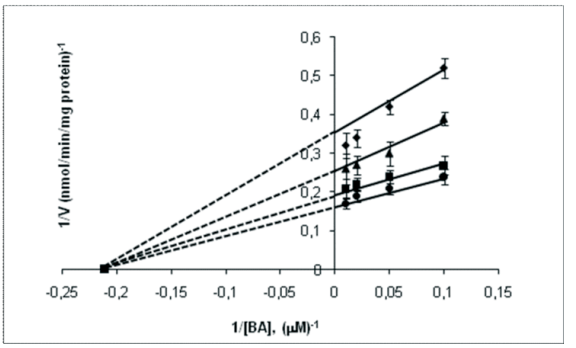




**Figure 6**. Inhibitory effect of MA on BA oxidation by the purified SSAO. a) The 1/V versus 1/[BA] plot in the presence of different concentrations of MA as an inhibitor. b) Replots of data taken from the reciprocal plot.BA was used as substrate in the concentration range 10-100 mM.[MA] = (-_-) 0 mM, (-_-) 375 mM and (-_-) 750 mM. (a) r2 ≥ 0.97. (b) r2 = 0.9928. Each point is the average of 3 determinations. Standard deviations are within 20% of the mean.

of SSAO activity with KA was detected as 19.9±1.8 µM (Fig. 7b).

## Discussion

SSAO is a copper containing amine oxidase which is present in almost all mammalian tissues [14]. The physiological role of SSAO is still far from clear, but some functions such as protection against exogenous amines,



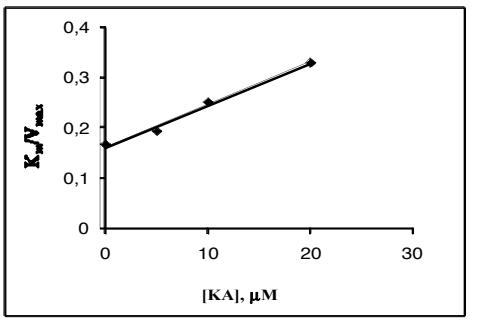




**Figure 7**. Inhibitory effect of KA on BA oxidation by the purified SSAO. a) The 1/V versus 1/[BA] plot in the presence of different concentrations of KA as an inhibitor. b) Replots of data taken from the reciprocal plot. BA was used as substrate in the concentration range 10-100 mM. [KA] = (-_-) 0 mM, (-_-) 5 mM, (-_-) 10 mM and (-_-) 20 mM. (a) (r2 ≥ 0.9615). (b) r2 = 0.9896. Each point is the average of 3 determinations. Standard deviations are within 20% of the mean.

regulator role in glucose transport, supressor role in apoptosis, induction of atherogenesis and cell adhesion, local generation of hydrogen peroxide as signal molecule, cross-linking of proteins and leucocyte trafficking have been recently described [7].

SSAO substrates include aminoacetone, MA, 2-phenylethylamine, tyramine and dopamine whereas BA is reported to be a good non-physiological substrate for the mammalian SSAO [15, 16]. The mammalian SSAO is suggested to be a dimeric protein which contains 1 mol of copper per subunit encoded by two genes, plus a pseudo-gene [17].

SSAO was purified from the crude microsomal fractions of rat lung by Cibacron Blue 3GA-agarose and Concanavalin A-Sepharose 4B affinity chromatographies according to the method previously described [8].

In this study the rat lung SSAO activity was determined toward BA and MA at the concentration ranges of 10-750 µM for BA and 25-1000 µM for MA.

The velocity of the reaction decreased with increasing substrate concentration indicating that Michaelis-Menten enzyme behaviour was obeyed at only low concentrations of BA, thus a substrate inhibition at high substrate concentrations was suggested in the case of BA. Rat lung SSAO was found to deaminate BA with a $K_m$ value of 3.7 µM and a $V_{max}$ of 5.6 nmol/min/mg protein

at the concentration range of 0-100 µM. In order to test the possibility of product inhibition being responsible, benzaldehyde, benzoic acid and $NH_4^+$, the end products of BA oxidation, were tested as potential inhibitors of BA oxidation. Although $NH_4^+$ showed inhibitory activity on the oxidation of BA by SSAO, it was concluded that further kinetic studies with the end-products of BA oxidation under different conditions may clarify the high substrate inhibition seen with BA.

Since some of the earlier reports suggested the presence of two binding sites for the interaction of BA with bovine lung SSAO [8], this possible mechanism was tested by using a general equation (Eq 1) derived from Haldane including $K_m$, the dissociation constant for the substrate in active position, and $K_{m'}$ the apparent dissociation constant for the same substrate as inhibitor. A possible mechanism for such substrate inhibition may be explained by the possibility of binding of more than one substrate molecule at the active site of SSAO with the formation of a relatively inactive substrate-enzyme complex. The general equation for this inhibitory mechanism can be derived as [18]:

$$\frac{V_{max}}{V} = 1 + \frac{K_m}{[S]} + \frac{[S]}{K_m'} \quad \text{Eq.1}$$

with v being the velocity, (S) the BA concentration, $K_m$ the dissociation constant for the substrate , and $K_m$` the apparent dissociation constant for BA as inhibitor. The optimum substrate concentration ($S_0$) then can be expressed as:

$$S_0 = \sqrt{K_m . K_m'}$$

The apparent $K_m$ for BA oxidation obtained from the Lineweaver-Burk plot was 3.7 µM, while the apparent $K_m$` estimated from the Dixon plot of reciprocal velocity against substrate concentration was 800 µM (Fig. 3). So the $S_0$ value calculated from the equation was 54.1 µM, and coincides with the value calculated graphically.

$$S_0 = \sqrt{(3{,}65)(800)} = \sqrt{2920} = 54.1\ \mu M$$

Although $S_0$ value for BA has been found as 54.1 µM, 100 µM of BA was used in the determination of SSAO in kinetic studies since no substrate inhibition has been recorded at this BA concentration in practical.

The observed maximal velocity (6.3 nmol/min/mg protein), was in agreement with the value calculated (6.3 nmol/min/mg protein) from the equation 1:

$$\frac{V_{max}}{5.5} = 1 + \frac{3.6}{100} + \frac{100}{800'} = 6.3$$

Since the experimental data were found to be consistent with the equation, it seems possible that the active site of rat lung SSAO may have more than one site for the interaction with BA as substrate.

Whereas MAO is active toward primary amines as well as some secondary and tertiary amines, the specificity of SSAO appears to be restricted to primary amines. MA, which is not known to be a substrate for MAO, has been suggested to be a physiologically important SSAO substrate [9]. MA can be produced and absorbed as a result of gut bacterial degradation of dietary creatinin, lecithin, and choline and also arise from endogenous metabolic degradation of sarcosin and creatinine, as well as from the MAO-catalyzed oxidative deamination of N-methyl substituted amines, such as adrenaline [19, 20]. The oxidation of MA by SSAO yields formaldehyde, hydrogen peroxide and ammonia. Formaldehyde is suggested to interact with monoamines or amides to form methylene bridges and to produce irreversibly covalently cross-linked complexes with proteins and with DNA. It is extremely cytotoxic and has been considered to be potentially carcinogenic [3, 7, 21, 22].

Oxidation of MA by purified SSAO obeyed to the Michaelis-Menten behaviour. Rat lung microsomal SSAO was found to deaminate MA with a $K_m$ of 141.5 µM and a $V_{max}$ of 4.2 nmol/min/mg protein. No high substrate inhibition was detected for the oxidation of SSAO by MA. The observation of high-substrate inhibition by BA but not by MA may be resulted from a difference in the kinetic mechanisms involved. It has been postulated that high substrate inhibition seen in the case of BA is uncompetitive suggesting that the substrate is capable of binding to an enzyme-substrate or enzyme-product complex in a sequential kinetic mechanism. The kinetic behaviour with MA was also suggested to be consistent with a sequencial reaction mechanism [23]. However, further works need to be done to confirm these hypotheses.

In order to examine the ability of some biogenic amines (MA, dopamine, tyramine, phenylethylamine, KA and serotonin) to interfere with the metabolism of BA by SSAO, competition studies were performed. All the amines tested, except tyramine, inhibited the BA oxidation by rat lung SSAO. KA was found to be the most potent amine with the lowest $IC_{50}$ value, and behaved as a non-competitive inhibitor. This result was in accordance with a previous report suggested that KA acts as a non-competitive inhibitor for the oxidation of serotonin by rat arterial SSAO [24]. On the other hand, MA, dopamine, phenylethylamine and serotonin behaved as competitive inhibitors which may be considered as endogenous substrates of rat lung SSAO.

## ACKNOWLEDGEMENTS

The present study was supported by a grant from the Scientific Research Unit of Hacettepe University (HUBAB 05-D06-301-001).

## References


[1] Ochiai Y, Itoh K, Sakurai E, Tanaka Y. (2005) Molecular cloning and characterization of rat semicarbazide-sensitive amine oxidase. Biol. Pharm. Bull. 28(3) 413-18.

[2] Jalkanen S, Salmi M. (2001) Cell-surface monoamine oxidases: enzymes in search of a function. EMBO J. 20 3893–901.

[3] O'Sullivan J, Unzeta M, Healy J, O'Sullivan MI, Davey G, Tipton KF. (2004) Semicarbazide-sensitive amine oxidases: enzymes with quite a lot to do. Neurotoxicology 25 303-315.

[4] Castillo V, Lizcano JM, Visa J, Unzeta M. (1998) Semicarbazide-sensitive amine oxidase (SSAO) from human and bovine cerebrovascular tissues: biochemical and immunohistological characterization. Neurochem. Int. 33 415-423.

[5] Boomsma F, Bhaggoe UM, van der Houwen AMB, van den Meiracker AH. (2003) Plasma semicarbazide-sensitive amine oxidase in human (patho)physiology. Biochim. Biophys. Acta 1647 48-54.

[6] Magyar K, Mészáros Z, Mátyus P. (2001) Semicarbazide-sensitive amine oxidase. Its physiological significance. Pure Appl. Chem. 73(9) 1393–1400.

[7] Yu PH, Wright S, Fan EH, Lun ZR, Gubisne-Harberle D. (2003) Physiological and pathological implications of semicarbazide-sensitive amine oxidase. Biochim. Biophys. Acta 1647 193–199.

[8] Yabanoglu S, Ucar G, Gokhan N, Salgin U, Yesilada A, Bilgin AA. (2007) Interaction of rat lung SSAO with the novel 1-N-substituted thiocarbamoyl-3-substituted phenyl-5-pyrolyl-2-pyrazoline derivatives. J Neural Transm. 114 769-773.

[9] Lizcano JM, Fernandez de Arriba A, Lyles GA, Unzeta M. (1994) Several aspects on the amine oxidation by semicarbazide-sensitive amine oxidase (SSAO) from bovine lung. J. Neural. Transm. Suppl. 41 415-420.

[10] Tabor CW, Tabor H, Rosenthal SM. (1954) Purification of amine oxidase from beef plasma. J. Biol. Chem. 208 645-661.

[11] Lizcano JM, Unzeta M, Tipton KF. (2000) A spectrophotometric method for determining the oxidative deamination of methylamine by the amine oxidases. Anal. Biochem. 286 75-79.

[12] Segel IH. (1975) Enzyme Kinetics. Wiley-Interscience Publication, New York, 125-135.

[13] Bradford, M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248-254.

[14] Boomsma F, van Dijk J, Bhaggoe UM, Bouhuizen AM, van den Meiracker AH. (2000) Variation in semicarbazide-sensitive amine oxidase activity in plasma and tissues of mammals. Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol. 126 69–78.

[15] Deng Y, Yu PH. (1999) Assessment of the Deamination of Aminoacetone, an Endogenous Substrate for Semicarbazide-Sensitive Amine Oxidase. Anal. Biochem. 270 97–102.

[16] Yu PM, Tipton TF, Boultan AA. (1995) Current neurochemical and pharmacological aspects of biogenic amines. Current Prog. Brain. Res. 106 85- 90.

[17] Zhang X, McIntire WS. (1996) Cloning and sequencing of a copper-containing, topa quinone-containing monoamine oxidase from human placenta. Gene 179 279–286.

[18] Dixon M, Webb EC, Thorne CJR, Tipton KF. (1979) Enzymes, s 126-136, 3rd ed., Longman, London.

[19] Yu PH, Wright S, Fan EH, Lun ZR, Gubisne-Harberle D. (2003) Physiological and pathological implications of semicarbazide-sensitive amine oxidase, Biochim. Biophys. Acta. 1647 193– 199.

[20] Deng Y, Boomsma F, Yu PH. (1998) Deamination of methylamine and aminoacetone increases aldehydes and oxidative stress in rats. Life Sci., 63(23) 2049-2058.

[21] Uçar G. (2004) Semicarbazide-sensitive amine oxidase: biochemical and physiological properties. Turk J Biochem. 29(3) 247-254.

[22] Ekblom J. (1998) Potential therapeutic value of drugs inhibiting semicarbazide-sensitive amine oxidase: vascular cytoprotection in diabetes mellitus. Pharmacol. Res. 37(2) 87-92.

[23] Lizcano JM, Tipton KF, Unzeta M. (1998) Purification and characterization of membrane-bound semicarbazide-sensitive amine oxidase (SSAO) from bovine lung. Biochem. J. 331 69-78.

[24] Elliot J, Callingham BA, Sharman DF. (1989) The influence of amine metabolizing enzymes on the pharmacology of tyramine in the isolated perfused mesenteric arterial bed of the rat. Br. J. Pharmacol. 98 515-522.