Time-dependent activation of the semicarbazide-sensitive amine oxidase (SSAO) from ox lung microsomes

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The activity of ox lung microsomal semicarbazide-sensitive amine oxidase (EC 1.4.3.6; SSAO) towards benzylamine increased 20-fold during incubation at 37 °C. After an initial lag-period, activation was first-order with time and complete after approx. 20 h. No significant changes in activity towards methylamine, histamine or 2-phenylethylamine were observed, although mixed-substrate experiments were consistent with the same enzyme being involved in the oxidation of all these substrates, both before and after time-dependent activation. The enzyme-tryptophan fluorescence increased on incubation at 37 °C in parallel with the increase in activity towards benzylamine. Treatment of the activated-enzyme preparation with 6 M guanidinium chloride followed by dialysis, caused both the activity towards benzylamine and the fluorescence to fall to that occurring before activation. However, incubation of this preparation at 37 °C resulted in increases in fluorescence and activity similar to those

seen with the unactivated enzyme. Benzylamine oxidation was inhibited, uncompetitively with respect to oxygen, by high substrate concentrations but no such inhibition was observed with the other amines. Activation resulted in an increase in $V_{\max}$ for benzylamine oxidation, with no significant alterations in the K_m or the K_{si} for high-substrate inhibition. Kinetic studies were consistent with sequential mechanisms being followed for the oxidation of both benzylamine and methylamine but the dependence on oxygen concentration was complex. These results might indicate that benzylamine follows a different reaction pathway from the other substrates, with substrate-specific activation involving a reaction step that is rate-limiting for benzylamine oxidation but not for the others.

Key words: benzylamine, methylamine, reversible activation.

INTRODUCTION

The semicarbazide-sensitive amine oxidases [amine:oxygen oxidoreductase (deaminating) (copper-containing), EC 1.4.3.6; SSAOs] may be distinguished from the monoamine oxidases [amine:oxygen oxidoreductase (deaminating) (flavin containing), EC 1.4.3.4] by their sensitivity to inhibition by carbonyl-group reagents, such as semicarbazide (see [1–4]). A soluble form of the enzyme is present in the blood plasma from several species but there is also a form that is tightly bound to the plasma membrane that is associated with vascular tissues [1], adipocytes [2] and lung [3]. Both the soluble and membrane-bound enzymes are characterized by a high activity towards benzylamine and they have sometimes been referred to as benzylamine oxidases, although this amine is not known to occur physiologically. The enzymes are, however, active towards a number of endogenous physiologically-active and xenobiotic amines, including dopamine, aminoacetone, histamine, 2-phenylethylamine and methylamine (for a review see [4]).

The oxidation of aromatic and aliphatic primary amines by SSAO produces the corresponding aldehyde, ammonia and hydrogen peroxide:

The activity of SSAO towards short-chain aliphatic amines, such as methylamine, which are not substrates for monoamine oxidase, and its presence in relatively high activities in lung tissue suggest that one of its functions might be to remove inhaled volatile amines [5,6]. The observation that at least a proportion of the

activity of the plasma-membrane-associated activity is exposed to the exterior of the cell might indicate a role in scavenging circulating toxic amines, without the necessity for an uptake process. However, SSAO appears to have a number of other physiological functions. It has been shown to act as a regulator of the GLUT4 glucose transporter, with which it associates in some tissues, by a mechanism involving H_2O_2 mediation [7]. The enzyme may also have other functions in the control of cellular activity that involves the generation of H_2O_2 as a second messenger [8–10]. A vascular-adhesion protein-1 with identical sequence to that of the SSAO has been described recently [11,12]. As vascular-adhesion protein-1 is involved in lymphocyte migration to lymphoid organs, a new physiological role for SSAO in cellular trafficking has been proposed [13,14].

Although the plasma enzyme was purified and crystallized over 30 years ago [15], highly-purified preparations of the tissue-bound enzyme have only recently been obtained. In our studies, on the purification of SSAO from the microsomal fraction of ox lung, we observed an anomalous activation behaviour towards benzylamine as substrate [16]. This activation occurred upon incubation of crude and partly-purified preparations of the enzyme at temperatures above 25 °C, without resulting in any gross change in the relative molecular mass of the enzyme. The process was not affected by treatment with phospholipases, detergents, serine proteases, metal-chelating agents, reducing agents or proteases inhibitors. However, it affected the activity towards benzylamine but not that towards methylamine, histamine or 2-phenylethylamine [16]. The purpose of the present work was to study the possible mechanisms underlying this activation using purified preparations of the ox lung enzyme.

Abbreviations used: SSAO, semicarbazide-sensitive amine oxidase.

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MATERIALS AND METHODS

Materials

Formaldehyde dehydrogenase from *Pseudomonas putida*, trypsin, chymotrypsin, mushroom tyrosinase, phospholipases A2, D, C and C-phosphatidylinositol specific, Triton X-100, sodium cholate, 2-octyl glycoside, non-radioactively labelled substrates and other reagents were obtained from Sigma (Poole, Dorset, U.K.). The radioactive substrates [7-¹⁴C]benzylamine hydrochloride (55 mCi/mmol) and [¹⁴C]methylamine hydrochloride (779 μ Ci/mg) were obtained from Amersham International (Little Chalfont, Bucks., U.K.), and 2-[ethyl-1-¹⁴C]phenylethylamine hydrochloride (50 mCi/mmol) was from New England Nuclear (Boston, U.S.A.). Ox lung microsomes were prepared and SSAO was purified to apparent homogeneity from that source as described previously [3].

Enzyme assays

All enzyme assays were performed at 37 °C. SSAO activity was determined radiochemically using [7-¹⁴C]benzylamine, 2-[ethyl-1-¹⁴C]phenylethylamine [17] or [¹⁴C]methylamine [18] as substrates. A continuous spectrophotometric assay, in which the formaldehyde produced from the oxidation of methylamine was used to reduce NAD⁺ in the presence of formaldehyde dehydrogenase (EC 1.2.1.46), was also used to monitor the oxidation of this substrate [19]. Activity towards benzylamine was also determined spectrophotometrically by continuously monitoring the formation of benzaldehyde at 250 nm [20]. For determining histamine (20 mM) oxidation the fluorimetric method of Crosbie and Callingham [21] was used. All assays were carried out in 50 mM potassium phosphate buffer, pH 7.2. Protein concentration was determined by the method of Markwell et al. [22].

The experiments where the concentration of oxygen was varied, were carried out in 3 ml rubber-stoppered spectrophotometer quartz cuvettes, containing a stainless-steel cannula 'gas-escape' tube in the rubber stopper. The tube supplying the gas mixture to the cuvette could be raised through the cannula tube, so as to eject the gas mixture directly on to the surface of the reaction mixture. O₂ concentrations were varied by passing O₂/N₂ mixtures through the cuvette, and the oxygen concentration was monitored with an O₂ micro-electrode (YSI 5300 Biological Oxygen Monitor; YSI Inc., Yellow Springs, OH, U.S.A.). After equilibration at the desired oxygen concentration, the reaction was started by adding the corresponding substrate through the cannula.

Mixed-substrate experiments involved assaying the activities towards the substrates benzylamine, methylamine and 2-phenylethylamine at their *K_m* concentration (40, 260 and 312 μ M respectively) separately and then as mixtures of each substrate pair at the same concentrations.

Activation experiments involved pre-incubating enzyme samples in 50 mM potassium phosphate buffer, pH 7.2, for different time intervals before starting the reaction with the appropriate substrate.

Kinetic analysis

Kinetic data were analysed according to the Michaelis–Menten relationship, including expressions for high-substrate inhibition, by non-linear regression using the computer program GraphPad Inplot (version 4.03, 1992; GraphPad Software Inc., San Diego, CA, U.S.A.). For analysis of reversible 'dead-end' inhibition, the dependence of the apparent *K_m* and *V_{max}*/*K_m* values upon the inhibitor concentration was used to define the inhibitor type. Data are presented as double-reciprocal plots for illustrative

purposes only. Time-dependent-activation kinetics were analysed using MacCurveFit (Kevin Raner Software, Mt Vernon, Victoria, Australia).

Tryptophan fluorescence

Aliquots of 1 ml of pure ox lung SSAO (0.4 mg/ml) in 50 mM potassium phosphate buffer, pH 7.2, containing 0.1 % 2-octyl glycoside were incubated at 37 °C in a 1 ml water-jacketed quartz cuvette in a spectrophotofluorimeter (Perkin–Elmer Corp., Long Beach, CA, U.S.A.). An experimental blank, comprising 1 ml of the buffer/detergent mixture, was treated in parallel. At the times indicated, the relative fluorescence of tryptophan ($\lambda_{\text{excitation}}$ 280 nm, $\lambda_{\text{emission}}$ 340 nm) was monitored and an aliquot of 2 μ l was taken for determination of the SSAO activity towards 20 μ M benzylamine as the substrate.

RESULTS

Preincubation of either the microsomal fraction or purified SSAO from ox lung at 37 °C in 50 mM potassium phosphate buffer, pH 7.2, resulted, after an initial lag phase, in a time-dependent increase in the activity towards benzylamine which reached a maximum after approx. 20 h. After this time, the specific activity of the enzyme was some 20 times higher than that of the starting activity when benzylamine was used as substrate.

Figure 1 Effects of preincubation at 37 °C on the activity of SSAO

The microsomal enzyme was preincubated at 37 °C in 50 mM potassium phosphate buffer, pH 7.2, for different times before the reaction was started by the addition of substrate. Upper panel: the substrates were 20 μ M [7-¹⁴C]benzylamine ($\blacktriangle$) or 100 μ M [¹⁴C]methylamine ($\bullet$). 100% of SSAO activity is the enzyme activity obtained when the enzyme was preincubated for 5 min in 50 mM potassium phosphate buffer. The behaviour when 100 μ M 2-[ethyl-1-¹⁴C]phenylethylamine was the substrate was not significantly different from that obtained with methylamine. Lower panel: after preincubation of the microsomal enzyme at 37 °C for 1 h, SSAO activity towards 20 μ M [7-¹⁴C]benzylamine was measured with time. The percentage-fractional-activity change (where *A_t* is the activity after any time, *t*, and *A_∞* is the activity of the fully-activated enzyme) is plotted as a function of time. The results were fitted to a first-order kinetic equation as described in the text.

Table 1 Changes in the kinetic parameters for benzylamine oxidation by SSAO after different times of preincubation of the enzyme at 37 °C

SSAO from crude microsomal membranes was preincubated at different times at 37 °C and then assayed using benzylamine at different concentrations (25–4000 μM). Value are the means $\pm$ S.D. for three separate determinations. K_{si} is the constant for high-substrate inhibition by benzylamine.

Preincubation (h)	K_m (μM)	K_{si} (μM)	V_{max} ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)
0	40 ± 5	1310 ± 75	2.09 ± 0.15
1	43 ± 4	1289 ± 63	7.89 ± 0.45
3	46 ± 5	1300 ± 37	25.56 ± 0.98
24	39 ± 3	1388 ± 107	37.88 ± 1.45

Figure 2 Changes in the tryptophan fluorescence (○) and activity of SSAO (●) during activation and after denaturation

Samples of 1 ml of pure SSAO were preincubated at 37 °C for different times, before measuring both the relative tryptophan fluorescence and the enzyme activity towards 20 μM [7- ^{14}C]benzylamine. After 28 h, the enzyme was denatured by the addition of 6 M guanidinium hydrochloride, renatured by dialysis at 4 °C against 2 litres of 50 mM potassium phosphate buffer, pH 7.2, containing 0.1 % 2-octyl glycoside (for 6 h with four changes), and assayed again. The relative tryptophan fluorescence and the specific SSAO activity ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) are shown. The dialysate was then incubated at 37 °C for different times, after which the tryptophan fluorescence and enzyme activities were again determined.

This behaviour was not observed when 2-phenylethylamine, methylamine or histamine were used (Figure 1, upper panel). After the initial lag phase, activation obeyed apparently first-order kinetics with respect to time, described by a first-order rate constant of $0.22 \pm 0.03 \text{ h}^{-1}$, corresponding to a half-life of 3.5 h (Figure 1, lower panel). The initial lag period was approx. 15 min for the enzyme in crude microsomal membranes and increased during the enzyme purification process, being almost 3 h for the pure enzyme. Activation was more rapid and the lag period was shorter when the incubation temperature was raised to 50 °C (results not shown). Determination of the steady-state kinetic parameters with benzylamine as substrate indicated that the activation was due solely to an increase in V_{max} , with the K_m not being significantly affected (Table 1).

This activation phenomenon was independent of the pH in the range 6–10, ionic strength (50–400 mM potassium phosphate buffer, pH 7.2) and the nature of the buffer utilized (Tris/HCl, Hepes, or phosphate; results not shown). Since the elution profile on gel filtration through Superdex 200HR was not significantly affected by the enzyme activation [16], neither an aggregation process nor extensive degradation of the enzyme were involved. The absence of changes in the SDS/PAGE

Figure 3 Substrate competition for oxidation by microsomal SSAO

Upper panel: inhibition of methylamine oxidation by benzylamine. The initial velocities of [^{14}C]methylamine (0.1–5 mM) oxidation by SSAO were assayed in the presence of different concentrations of benzylamine: 0 μM (■), 10 μM (●), 25 μM (▲) and 50 μM (◆). Lower panel: inhibition of benzylamine oxidation by methylamine. The initial velocities of [7- ^{14}C]benzylamine (25–250 μM) oxidation by SSAO were assayed in the presence of different concentrations of methylamine: 0 μM (■), 33 μM (●) and 100 μM (▲). Each point represents the mean for three separate determinations.

pattern of the purified enzyme after activation, and the fact that different protease inhibitors (1 mM PMSF, 5 mM EDTA, 5 mM EGTA, 0.1 μM pepstatin and 1 mM *N*-ethylmaleimide) did not affect the activation process (results not shown), is also consistent with the conclusion that it is not a result of proteolytic cleavage. Furthermore, inclusion of either trypsin or chymotrypsin at a ratio of 1:10 (protease to protein) neither affected the enzyme activity nor the activation profile over a 20 h incubation period.

To examine the possibility that the activation might involve changes in the interaction with a membrane-lipid component, the microsomal SSAO was treated with different detergents (0.6 % Triton X-100, 0.75 % sodium cholate, or 0.75 % 2-octyl glycoside) or with phospholipases (30 mg of A2, 3 units of C, 0.5 unit of C-phosphoinositol specific, or 90 units of D). In all cases the activation was similar to that observed in the untreated control.

Mu et al. [23], have reported that the topa-quinone cofactor of some copper-containing amine oxidases is generated by oxidation of a tyrosine residue present in the polypeptide chain. Preincubation of the SSAO in an atmosphere of N_2 or O_2 , or in the presence of tyrosinase, did not significantly change the activation pattern with respect to the control. Thus it is unlikely that the formation of this cofactor plays a role in the activation process.

Activation was paralleled by an increase in the tryptophan fluorescence of SSAO, suggesting a conformational change

Figure 4 Effects of varying the concentration of benzylamine on the initial rates of the reaction catalysed by microsomal SSAO

Upper panel: double-reciprocal plots with benzylamine as the varied substrate (25–4000 μM) against fixed concentrations of O_2 : 51 μM (Δ), 207 μM ($\circ$), 510 μM ($\diamond$) and 1020 μM ($\square$). Enzyme activity ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) was determined spectrophotometrically. Lower panel: analysis of the high-substrate inhibition by plotting reciprocal initial velocities against the concentration of benzylamine. Each point represents the mean for three separate determinations.

Figure 5 Effects of varying the concentration of methylamine on the initial rates of the reaction catalysed by microsomal SSAO

Double-reciprocal plots with methylamine as the varied substrate (100–3333 μM) against fixed concentrations of O_2 : 51 μM (Δ), 207 μM ($\circ$) and 1020 μM ($\square$). Enzyme activity ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) was determined spectrophotometrically. Each point represents the mean for two separate determinations.

affecting the environments of these residues (Figure 2). Treatment of SSAO with 6 M guanidinium hydrochloride followed by dialysis against 50 mM potassium phosphate buffer, pH 7.2, indicated that the denaturation that presumably occurred was reversible by removal of the denaturant, with little loss of total activity (results not shown). When the enzyme that had been

Figure 6 Effects of varying the concentration of oxygen on the initial rates of benzylamine and methylamine oxidation by microsomal SSAO measured spectrophotometrically

Upper panel: double-reciprocal plots with O_2 as the varied substrate against fixed concentrations of benzylamine: 25 μM ($\blacktriangle$), 50 μM ($\blacksquare$), 100 μM ($\diamond$), 250 μM ($\square$), 500 μM ($\circ$), 1000 μM (Δ), 2000 μM ($\blacklozenge$) and 4000 μM ($\bullet$). Each point represents the mean for two separate determinations. Lower panel: double-reciprocal plots with O_2 as the varied substrate against fixed concentrations of methylamine: 100 μM ($\blacktriangle$), 250 μM ($\bullet$), 500 μM ($\blacklozenge$), 1000 μM (Δ), 2000 μM ($\circ$) and 3333 μM ($\diamond$). Each point represents the mean for three separate determinations.

activated at 37 $^{\circ}\text{C}$ for 36 h was taken through this procedure, both the activity and tryptophan fluorescence were similar to those of the unactivated enzyme (Figure 2). Incubation of enzyme samples that had been treated in this way at 37 $^{\circ}\text{C}$ resulted in increases in activity and tryptophan fluorescence that were similar to those seen with the untreated enzyme (Figure 2).

Kinetic initial-rate studies showed benzylamine to be a competitive inhibitor of methylamine oxidation (Figure 3, upper panel) and that methylamine competitively inhibited benzylamine oxidation (Figure 3, lower panel). When SSAO was assayed at K_m concentrations of benzylamine and 2-phenylethylamine (40 μM and 312 μM respectively), the respective activities obtained were 1130 ± 32 and 1410 ± 35 d.p.m. $\cdot \text{min}^{-1}$. Determination of the activity towards a mixture of these substrates, each at the same concentration, gave an initial velocity of 1680 ± 90 d.p.m. $\cdot \text{min}^{-1}$. Thus the activity with the mixture was 66.6% of the sum of the activities for the two substrates assayed separately, a value not significantly different from the 66.7% that would be expected for two substrates competing for the same active site (see [24]). Similar experiments with methylamine ($K_m = 260 \mu\text{M}$) showed the activity of a mixture of benzylamine and 2-phenylethylamine to be $66.6 \pm 0.8\%$ and $65.2 \pm 0.6\%$ of the sum of the activities of the substrate pairs assayed separately. Identical results were obtained for both activated

Table 2 Kinetic parameters for benzylamine and methylamine oxidation by SSAO

Values were determined by non-linear regression analysis using the values that are shown in double-reciprocal form in Figures 4 and 5 respectively. Each value is the mean $\pm$ S.D. for three (benzylamine) or two (methylamine) separate experiments. K_s is the dissociation constant for the enzyme–substrate complex.

Substrate	K_m (μ M)	K_s (μ M)	V_{max} (nmol $\cdot$ min $^{-1}$ $\cdot$ mg $^{-1}$)
Benzylamine	51 $\pm$ 11	176 $\pm$ 32	6.02 $\pm$ 0.54
Methylamine	403 $\pm$ 25	1045 $\pm$ 86	9.25 $\pm$ 0.76

and non-activated enzyme. These results are not consistent with the existence of two enzymes or two active-sites, one specific for benzylamine and susceptible to activation, and another with activity towards methylamine and 2-phenylethylamine that is not so affected.

Oxidation of benzylamine was subject to inhibition by high concentrations of that substrate (Figure 4, upper panel). This inhibition was uncompetitive with respect to oxygen (Figure 4, lower panel). Neither the nature of the inhibition nor the K_{si} value for high-substrate inhibition was affected by thermal activation (Table 1). No such high-substrate inhibition was evident with methylamine at concentrations up to 10 mM (Figure 5), 2-phenylethylamine (up to 10 mM) or histamine (up to 50 mM). With both benzylamine and methylamine as substrates, the dependence of the initial rate on oxygen concentration did not follow simple Michaelis–Menten kinetics (Figure 6), and the behaviour with benzylamine as substrate was further complicated by the effects of high-substrate inhibition of this amine (Figure 6, upper panel). The values of the kinetic parameters, calculated from the data in Figures 4 and 5 for benzylamine and methylamine, were calculated according to the initial-rate relationship (see [25], pp. 133–137),

$$v = V_{max}/(1 + K_m^A/[A] + K_m^B/[B] + K_s^A K_m^B/[A][B])$$

where [A] and [B] are the amine and oxygen concentrations, and are shown in Table 2.

DISCUSSION

The time-dependent increase in the activity towards benzylamine when ox-lung microsomal SSAO was preincubated at 37 °C was also observed with microsomal preparations from human lung (results not shown) and with purified preparations of the ox lung enzyme. The last observation would tend to argue against the process resulting from the thermal destruction of an inhibitor present in the tissue, although it is noteworthy that the lag period before activation became apparent was increased by purification of the enzyme, perhaps indicating that factors in the crude preparation were capable of modulating the process. Our earlier work indicated that the activation was not likely to involve oxidation of thiol groups [16]. Furthermore, there is no evidence that enzyme aggregation or proteolysis is involved. Indeed the reversibility of the activation, by guanidinium-hydrochloride treatment followed by dialysis, suggests that irreversible modification, such as by proteolysis, is not occurring. The reversible changes in tryptophan fluorescence would be consistent with activation involving a change in the conformation of SSAO.

Activation was substrate specific, affecting the oxidation of benzylamine but not methylamine, 2-phenylethylamine or histamine. However, the mixed-substrate and competition experiments suggested that all these substrates interacted with the

same enzyme active-site. Thus it is improbable that there are two enzymes in the preparation with one of them being specific towards benzylamine and subject to activation; a situation also unlikely in view of our studies on the characterization of the purified enzyme [3]. The changes in limiting velocity (V_{max}) without significant change in the K_m value is not inconsistent with the involvement of a common active site for these substrates and might suggest that the thermally-induced conformational change affects a catalytic step that is limiting for the oxidation of benzylamine but not for the other substrates.

The observation of high-substrate inhibition by benzylamine but not by the other substrates might also suggest a difference in the kinetic mechanisms involved. Uncompetitive high-substrate inhibition would indicate that the substrate is capable of binding to an enzyme–substrate or enzyme–product complex in a sequential kinetic mechanism (see [24]). The kinetic behaviour with both methylamine and benzylamine (Figures 4 and 5) would also be consistent with a sequential reaction mechanism. It is possible that the corresponding intermediate species to which excess benzylamine binds is present in much lower concentrations than the other substrates because of differences in rate-controlling steps affecting intermediate distribution, perhaps reflecting the different electronic structure of benzylamine from that of the other substrates. However, any change in rate-controlling steps resulting from thermal activation clearly does not greatly affect the availability of that species, in view of the fact that high-substrate inhibition by benzylamine was not significantly affected.

There have been few studies on the kinetic mechanisms of the semicarbazide-sensitive amine oxidases, although Bardsley et al. [26] reported plasma diamine oxidase to follow a double-displacement (Ping Pong) mechanism. The initial-rate behaviour observed for both benzylamine and methylamine oxidation by ox lung SSAO (Figures 4 and 5) was not consistent with a double-displacement reaction mechanism. Furthermore, the behaviour with respect to varying oxygen concentration did not follow simple Michaelis–Menten kinetics for either methylamine or benzylamine oxidation (Figure 6), indicating a complex kinetic mechanism, perhaps involving more than one oxygen-binding site or a random-order of substrate binding to the enzyme under steady-state conditions. The initial-rate equations describing the latter process (see [25], pp. 80–83 and 89–90),

$$v = (\alpha_1[A][B] + \alpha_2[A]^2[B] + \alpha_3[A][B]^2)/(\beta_0 + \beta_1[A] + \beta_2[B] + \beta_3[A]^2 + \beta_4[B]^2 + \beta_5[A][B] + \beta_6[A]^2[B] + \beta_7[A][B]^2)$$

could give rise to the complex kinetics observed with oxygen and the apparently hyperbolic kinetic behaviour, with or without high-substrate inhibition, with the amine substrates, according to the values of the individual rate-constants contained within the α and β parameters. Although Bardsley et al. [26] reported that plasma diamine oxidase followed Michaelis–Menten kinetics with oxygen as the varied substrate, a brief report has indicated that the variation in the rate of oxidation of some amine substrates by rat lung SSAO is a complex function of the oxygen concentration [27].

Although the fact that this activation phenomenon has only been observed with the non-physiological substrate benzylamine might suggest a limited metabolic significance, further work will be necessary to investigate whether it does occur with any naturally occurring amines that are substrates for the enzyme or whether the conformational change involved affects one of the other functions of this protein, such as its vascular-adhesion function.

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REFERENCES

- Precious, E. and Lyles, G. A. (1988) Deamination of methylamine by semicarbazide-sensitive amine oxidase in human umbilical artery and rat aorta. *J. Pharm. Pharmacol.* **40**, 627–633
- Raimondi, L., Pirisino, R., Ignesti, G., Capecci, S., Banchelli, G. and Buffoni, F. (1991) Semicarbazide-sensitive amine oxidase activity (SSAO) of rat epididymal white adipose tissue. *Biochem. Pharmacol.* **4**, 456–470
- Lizcano, J. M., Tipton, K. F. and Unzeta, M. (1998) Purification and characterization of membrane-bound semicarbazide-sensitive amine oxidase (SSAO) from bovine lung. *Biochem. J.* **331**, 69–78
- Lyles, G. A. (1996) Mammalian plasma and tissue-bound semicarbazide-sensitive amine oxidases: biochemical pharmacological and toxicological aspects. *Int. J. Biochem. Cell Biol.* **28**, 259–274
- Elliot, J., Callingham, B. A. and Sharman, D. F. (1989) Semicarbazide-sensitive amine oxidase (SSAO) of the rat aorta. Interactions with some naturally occurring amines and their structural analogues. *Biochem. Pharmacol.* **38**, 1507–1515
- Lizcano, J. M., Fernández de Arriba, A., Lyles, G. A. and Unzeta, M. (1994) Several aspects on the amine oxidation by semicarbazide-sensitive amine oxidase (SSAO) from in bovine lung. *J. Neural Trans. (Suppl.)* **41**, 415–420
- Enrique-Tarancón, G., Martí, L., Morin, N., Lizcano, J. M., Unzeta, M., Sevilla, L., Camps, M., Palacín, M., Testar, X., Carpené, C. and Zorzano, A. (1998) Role of semicarbazide-sensitive amine oxidase in glucose transport and GLUT4 recruitment to the cell surface in adipose cells. *J. Biol. Chem.* **273**, 8025–8032
- Schreck, R., Rieber, P. and Baeuerle, P. A. (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J.* **10**, 2247–2258
- Meyer, M., Schreck, R. and Baeuerle, P. A. (1993) H₂O₂ and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J.* **12**, 2005–2015
- Feng, L., Xia, Y., García, G. E., Hwang, D. and Wilson, C. B. (1995) Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. *J. Clin. Invest.* **95**, 1669–1675
- Smith, D. J., Salmi, M., Bono, P., Hellman, J., Leu, T. and Jalkanen, S. (1998) Vascular adhesion protein-1 reveals a novel multifunctional adhesion molecule. *J. Exp. Med.* **188**, 17–27
- Bono, P., Salmi, M., Smith, D. J. and Jalkanen, S. (1998) Cloning and characterization of mouse vascular adhesion protein-1 (mVAP-1) reveals a novel molecule with enzymatic activity. *J. Immunol.* **160**, 5563–5571
- Salmi, M. and Jalkanen, S. (1992) A 90-kilodalton endothelial cell molecule mediating lymphocyte binding in humans. *Science (Washington, D.C.)* **257**, 1407–1409
- Jaakola, K., Kaunismäki, Tohka, S., Yegutkin, G., Vääntinen E., Havia, T., Pelliniemi, L. J., Virolainen, M., Jalkanen, S. and Salmi, M. (1999) Human vascular adhesion protein-1 in smooth muscle cells. *Am. J. Pathol.* **155**, 1953–1965
- Buffoni, F. and Blascko, H. (1964) Benzylamine oxidase and histaminase: purification of an enzyme from pig plasma. *Proc. R. Soc. Biochem.* **161**, 153–167
- Lizcano, J. M., Fernández de Arriba, A., Tipton, K. F. and Unzeta, M. (1998) Studies on the time-dependent activation of microsomal semicarbazide-sensitive amine oxidase. *J. Neural Trans. (Suppl.)* **52**, 251–257
- Tipton, K. F. (1985) Determination of monoamine oxidase. *Methods Find. Exp. Clin. Pharmacol.* **7**, 361–367
- Precious, E., Gunn, C. E. and Lyles, G. A. (1988) Deamination of methylamine by semicarbazide-sensitive amine oxidase in human umbilical artery and rat aorta. *Biochem. Pharmacol.* **37**, 707–713
- Lizcano, J. M., Unzeta, M. and Tipton, K. F. (2000) A spectroscopic method for determining the oxidative deamination of methylamine by the amine oxidases. *Anal. Biochem.*, in the press
- Tabor, C. W., Tabor, H. and Rosenthal, S. M. (1954) Purification of amine oxidase from beef plasma. *J. Biol. Chem.* **208**, 644–661
- Crosbie, A. and Callingham, B. A. (1994) Semicarbazide sensitive amine oxidase in sheep plasma: interaction with some substrates and inhibitors. *J. Neural Transm. (Suppl.)* **41**, 427–432
- Markwell, M. A. K., Hass, S. M., Bieber, L. L. and Tolbert, N. E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **14**, 206–210
- Mu, D., Janes, S. M., Smith, A. J., Brown, D. E., Dooley, D. M. and Klinman, J. P. (1992) Tyrosine codon corresponds to topa quinone at the active site of copper amine oxidase. *J. Biol. Chem.* **267**, 7979–7982
- Tipton, K. F. (1996) Patterns of enzyme inhibition. In *Enzymology LabFax* (Engel, P.C., ed.), pp. 115–174, Bios Scientific Publishers, Oxford and Academic Press, San Diego
- Dixon, M. and Webb, E. C. (1979) *Enzymes*, 3rd edn. Longmans, London
- Bardsley, W. G., Crabbe, M. J. C. and Shindler, J. S. (1973) Kinetics of the diamine oxidase reaction. *Biochem. J.* **131**, 459–469
- Ueda, T. and Kinemuchi, H. (1984) Determination of some biogenic monoamines in rat lung by monoamine oxidase and benzylamine oxidase. *Biogenic Amines* **1**, 179–192

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