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Silicon-Mediated Inactivation of Semicarbazide-Sensitive Amine Oxidase

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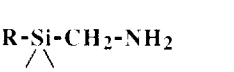
Abstract: Several organosilicon amines were synthesized as potential mechanism-based inhibitors of semicarbazide-sensitive amine oxidase (SSAO) prepared from rat aorta. 2-[Dimethyl(2-phenylethyl)silyl]-methanamine, hydrochloride produced potent time-dependent inhibition of SSAO. A potential mechanism is proposed to explain SSAO inactivation by organosilicon amines.

It was recently reported that (aminoalkyl)trimethylsilanes and derivatives produce mechanism-based inactivation of the flavin-containing enzymes monoamine oxidases[1-6] and of the copper-containing amine oxidases[7] for which recent studies have shown that the active-site cofactor is 2,4,5-trihydroxyphenylalanine[8-10] (TOPA). Here, we show that silicon-mediated enzyme inactivation can also be applied to a third class of amine oxidases referred to as benzylamine oxidase[11] or, simply, as the semicarbazide-sensitive amine oxidases[12] (SSAO's) since these enzymes are inhibited by semi-carbazide. SSAO is insensitive to copper-chelating agents[13] and its cofactor (presumably containing a carbonyl group) is unknown[10,14,15]. The physiological function of SSAO is unclear but its prevalence in vascular tissues suggests that the enzyme may play a role in the maintenance of cardiovascular function[14]. A specific inhibitor of SSAO could be a very important tool to further study physiological significance of the enzyme (s).

Based on the putative mechanism of SSAO[15]

$$E_{ox} + H_3\overset{+}{N} - CH_2 - R \rightleftharpoons E_{red} - \overset{+}{N}H = CH - R$$
$$E_{red} - \overset{+}{N}H = CH - R + H_2O \rightarrow E_{red} - NH_2 + O = CH - R$$
$$E_{red} - NH_2 + O_2 \rightarrow E_{ox} + H_2O_2 + \overset{+}{N}H_4$$

we envisioned that processing of (aminoalkyl)trimethylsilanes or derivatives by SSAO might result either in transaminative desilylation and possibly attachment of $R(Me_2)Si^+$ to a nucleophilic residue in the active site (Scheme I, path a) or in simple transamination (Scheme I, path b) which would produce $R(Me_2)Si\text{-}CH = \overset{+}{N}H\text{-}E$, a potential alkylating agent[1,3]. We found (aminomethyl)trimethylsilane derivatives[16] **1**, **3** and **4** to produce pseudo-first-order time-dependent inactivation of SSAO partially purified from rat aorta[17]. Kitz and Wilson[18]



replots of the data indicated that saturation was attained. K_I and k_{inact} values are given in Table 1. Values of k_{inact} for **2-4,** the phenyl and the phenylalkyl analogues of **1** are increasing when the distance between the aromatic ring and the silicon atom increases, suggesting that steric hindrance at the silicon atom may be an important limiting factor in the inactivation process[19].

Scheme I. Potential Pathways for Inactivation of SSAO by (Aminomethyl)trimethylsilane derivatives

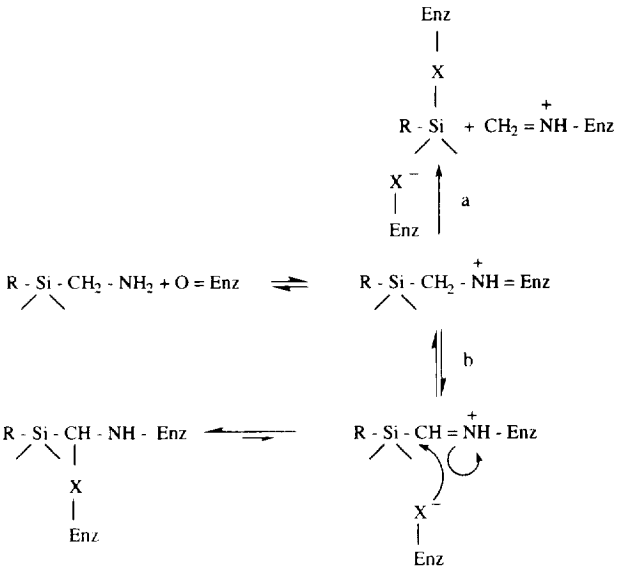


Table I. SSAO Inactivation by R-Si(/\)-CH_2-NH_2

R	Cpd	Inhibition constants K_I (M)	k_{inact} (min^{-1})	$t_{1/2}$ at 2 mM (min)
CH_3	**1**	ND*	ND	17
Ph	**2**	ND	ND	>100
Ph-CH_2	**3**	$5x10^{-4}$	$4.2x10^{-2}$	21
Ph-$(CH_2)_2$	**4**	$1.7x10^{-5}$	$3.6x10^{-1}$	2

* ND: Not Determined

Further experiments were performed on SSAO inactivation produced by **4**. The substrate benzylamine protected the enzyme from inactivation in a concentration-dependent manner, thus confirming that the inactivation takes place in the enzyme active-site. Furthermore, addition of 2-mercaptoethanol (2 mM) to the preincubation medium did not modify significantly inactivation rates. These results, as well as the absence of a lag-time before

the onset of inhibition, rule out the possibility that the species responsible for inactivation was released from the enzyme active site[20]. Incubation of SSAO with 3.3 μM **4** for 30 min at 37°C resulted in 90% inactivation of the enzyme activity. Prolonged dialysis at pH 7.8 (100 mM sodium phosphate buffer) of this inactivated SSAO preparation for 24 h at 4°C did not produce partial regeneration of SSAO activity, suggesting a stable covalent linkage of the inhibitor to the enzyme active-site. Such a stable linkage and the fact that steric hindrance at the silicon atom slows down the inactivation process suggest that the transaminative desilylation pathway, accompanied by silylation of a nucleophilic residue of the enzyme (Scheme I, path a), may be responsible for the enzyme inactivation by **4**.

Additional work will be required to clarify the nature of interaction of (aminoalkyl)trimethylsilane derivatives with SSAO, including mainly purified enzyme preparations and radiolabelled inhibitors. Nevertheless, our work demonstrates that the principle of a silicon-mediated suicide inhibition, initially applied to cytochrome P-450[20] inactivation and to two classes of amine oxidases[1-7], can be generalized to SSAO, even if the cofactor of this third important class of amine oxidases is still unknown[10,14,15]. A one-electron transfer to amine radical cation was involved for MAO inactivation[3]; a transamination mechanism was proposed for plasma amine oxidase[7]; a carbonium mechanism was suggested for cytochrome P450 inactivation[21]. However, radical mechanism can not be ruled out for the two latter enzymes[21,22] and remains a possible clue for the silicon-mediated enzyme inactivation. Confirmation of different molecular mechanisms involved in inactivation of these various oxidative enzymes should lead to a broader use of silicon-containing compounds as enzyme inactivators.

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(4) When administered to animals some (aminoalkyl)trimethylsilane derivatives have been shown to produce selective and long-duration inhibition of MAO-B relative to MAO-A in brain and, therefore, represent a new class of potential anti-Parkinsonian agents (see ref. 5 and 6).

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(16) All compounds were hydrochloride salts. a) Compound **1** is commercially available (Fluka Chemie AG). b) Compound **3** was synthesized as described[5]. c) Compound **2** was prepared by conversion of chloromethyldimethylphenylsilane (Aldrich Chemical Co, Inc.) to N-[(dimethylphenylsilyl)-methyl]phthalimide with potassium phthalimide (dimethylformamide, 70°C, 5 h.) followed by hydrazinolysis. **2** was purified as its hydrochloride salt: mp 196-197°C (lit[23] mp 198-200°C). Anal. Calcd. for $C_9H_{15}NSi$, HCl: C, 53.57; H, 7.99; N, 6.94. Found: C, 53.58; H, 8.10; N, 6.97; ^1H NMR (D_2O) δ, 0.47 (s, 6H), 2.67 (s, 2H), 7.45-7.75 (m, 5H). e) Compound **4** was prepared as described for **2**, from chloromethyldimethyl-2-(phenyl)ethylsilane[23]: mp 105-106°C; Anal. Calcd. for $C_{11}H_{19}NSi$, HCl: C, 57.49; H, 8.77; N, 6.09. Found: C, 57.45; H, 8.92; N, 5.96. ^1H NMR ($CDCl_3$) δ 0.20 (s, 6H), 1.05-1.15 (m, 2H), 2.15-2.25 (m, 2H), 2.28 (s, 2H), 7.15-7.35 (m, 5H); 8.22 (s, 3H).

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