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Reversible inactivation of bovine plasma amine oxidase by cysteamine and related analogs

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ABSTRACT

Cysteamine (**1**) was reported many years ago to reversibly inhibit lentil seedling amine oxidase, through the formation of a complex with thioacetaldehyde, the turnover product of **1**. Herein, cysteamine (**1**) and its analogs 2-(methylamino)ethanethiol (**3**) and 3-aminopropanethiol (**6**) were found to be reversible inhibitors of bovine plasma amine oxidase (BPAO), but 2-(methylthio)ethylamine (**7**) was determined to be a weak irreversible inhibitor of BPAO. Based on our results, indicating the necessity of a sulfhydryl-amine for reversible inactivation of BPAO, the failure of inhibited BPAO to recover activity after gel filtration, the first-order kinetics of activity recovery upon dialysis, and 2,4,6-trihydroxyphenylalanine quinine (TPQ) cofactor transformation which indicated from the results of phenylhydrazine titration and substrate protection, we propose a mechanism for the reversible inactivation of BPAO by **1** involving the formation of a cofactor adduct, thiazolidine, between BPAO and **1**.

1. Introduction

The quinone-dependent amine oxidases (EC 1.4.3.6), which contain tightly bound Cu(II), catalyze the oxidative deamination of unbranched primary amines to the corresponding aldehydes. The recent elucidation of the structures of the quinine cofactors has attracted interest in the mechanistic aspects of oxidative deamination and in the development of useful inhibitors. Most of these enzymes utilize a 2,4,6-trihydroxyphenylalanine quinine (TPQ) cofactor derived from an active site tyrosine to catalyze the oxidative deamination [1,2].

The mechanism of the deamination half-reaction [3] begins with condensation of the substrate amine with TPQ to give the "substrate Schiff base" (SSB). A conserved active-site catalytic base (Asp) abstracts the C_α proton, allowing tautomerization to the "product Schiff base" (PSB), which hydrolyzes to yield the aldehyde product and reductively aminated cofactor (an aminoresocinol). The latter subsequently is reoxidized at the expense of reduction of O_2 to H_2O_2, with the hydrolytic release of NH_3 or displacement of NH_3 by another substrate amine (Scheme 1).

The human plasma amine oxidases, which are often known as the soluble semicarbazide-sensitive amine oxidases (SSAOs), deaminate endogeneous and exogeneous amines and have been extensively investigated with respect to their role in various pathophysiological conditions [4–6]. SSAO-mediated production of toxic aldehydes has been proposed to contribute to the pathophysiology of atherosclerosis and diabetes [7–9], and brain SSAOs might play a role in controlling energy balance in adipocytes [10]. In addition, cell surface SSAOs that appear to be involved in regulating glucose uptake, signaling, and cell–cell adhesion have been identified [11]. Overall, potent and selective inhibitors could provide important tools for enzymologic probes of pharmacologic intervention and potential therapeutic agents.

In the 1970s, Abeles and co-workers first reported that amines bearing an unsaturated C–C bond at the β-position could inactivate bovine plasma amine oxidase (BPAO) [12,13]. Recently, inactivation of BPAO by various propargylamine, homopropargylamine, and chloroallylamine analogues was studied extensively by Sayre and co-workers [14–17]. Additionally, cysteamine (**1**) was reported to be a good inhibitor of pig kidney amine oxidase [18] and lentil seedling amine oxidase [19]. In these papers, the researchers proposed that inhibition by cysteamine (**1**) involved the formation of a stable enzyme complex with the product of turnover, thioacetaldehyde.

Since little work has been carried out on the interaction of sulfur-containing amine compounds with BPAO, we investigated the effects of several aminothiol compounds, including cysteamine (**1**), on BPAO and report the results in this paper.

2. Materials and methods

2.1. General procedure

NMR spectra were obtained at a ^1H resonance frequency of 300 MHz (^{13}C NMR at 75 MHz), with chemical shifts referenced to the solvent peak. UV–Vis spectra were obtained using a jacketed

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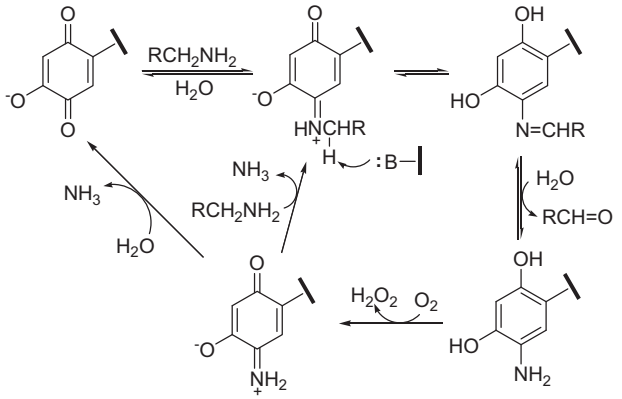

Scheme 1.

(temperature-controlled) cell compartment, from a Perkin-Elmer Lambda 3 spectrophotometer. All column chromatography was carried out with flash-grade silica gel. BPAO (100 U/g of protein) was purchased from Sigma.

2.2. Thiazolidine hydrochloride (2·HCl)

To a solution of 4.0 g (35 mmol) of 2-aminoethanethiol hydrochloride in 25 mL of water was added 3.0 mL of 37% formaldehyde solution. The mixture was stirred overnight at room temperature. The reaction mixture was then concentrated in vacuo to give a white solid, which was recrystallized from EtOH to give **2·HCl** at 80% yield: ^{1}H NMR (DMSO-d_6) δ 3.09 (t, 2H), 3. 39 (t, 2H), 4.21 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 29.2, 47.3, 47.9.

2.3. 2-(Methylamino)ethanethiol hydrochlodide (*3·HCl*)

In a portion-wise manner, 0.85 g of $LiAlH_4$ was added to a solution of 2.0 g (16 mmol) of thiazolidine (**2**) in anhydrous THF (80 mL) under Ar. The mixture was heated at reflux for 2 h under Ar. To decompose unreacted hydride, 10 mL of water was carefully added, and the mixture was filtered using a Celite pad. The filtrate was dried over Na_2SO_4 and concentrated in vacuo. The residue was recrystallized from EtOH and Et_2O to give **3·HCl** at 63% yield: ^{1}H NMR (D_2O) δ 2.75 (s, 3H), 3.01 (t, 2H), 3.31 (t, 2H).

2.4. 3-Aminopropanethiol hydrochlodide (*6·HCl*)

To a solution of 40 mL of CS_2, 2.0 g (11.5 mmol) of 3-bromopropylamine hydrochloride was added. The mixture was stirred for 2 h, and the solid that formed was filtered to give perhydro-1,3-thiazine-2-thione (**5**). Compound **5** (1.2 g, 9.0 mmol) was heated at reflux for 1 h in a 2*N* aqueous NaOH solution (20 mL). The reaction mixture was concentrated in vacuo, and the residue was partitioned between CH_2Cl_2 and brine. The organic layer was separated, dried over Na_2SO_4, and concentrated. To the residue, 10 mL of MeOH and 2 mL of concentrated HCl were added. The mixture was stirred for 10 min and then concentrated. The residue was recrystallized from MeOH and Et_2O to give **6·HCl** at 75% yield: ^{1}H NMR (D_2O) δ 2.06 (m, 2H), 2.78 (t, 2H), 3,08 (t, 2H). ^{13}C NMR (D_2O) δ 28.6, 36.4, 40.7.

2.5. 2-(Methylthio)ethylamine hydrochlodide (*7·HCl*)

Under Ar, 1.0 g of sodium was added to a solution of 1.2 g (15.6 mmol) of 2-aminoethanethiol (**1**) in absolute EtOH (25 mL). After complete dissolution of sodium by stirring for 30 min at room temperature under Ar, 2.8 g (20 mmol) of methyl iodide was added dropwise, and the reaction mixture was stirred for 24 h. Sodium iodide that had formed was filtered off, the filtrate was concentrated, and the residue was dissolved in aqueous NaOH. The resulting solution was extracted with chloroform, and the organic layer was separated, dried over Na_2SO_4, and concentrated. To the residue were added 10 mL of MeOH and 2 mL of concentrated HCl. The mixture was stirred for 10 min and then concentrated. The residue was recrystallized from MeOH and Et_2O to give **7·HCl** at 40% yield: ^{1}H NMR (D_2O) δ 2.15 (s, 3H), 2.85 (t, 2H), 3.26 (t, 2H). ^{13}C NMR (D_2O) δ 13.8, 30.4, 37.7.

2.6. Time-dependent inactivation of BPAO by candidate inhibitors

A 0.9 mL aliquot of a stock solution of candidate inhibitor in 100 mM potassium phosphate buffer, pH 7.2, was mixed with BPAO (0.1 mL) suspension (Sigma, 100 U/g of protein, final concentration ~2 μM) and aerobically incubated at 30 °C. Aliquots (0.1 mL) were periodically withdrawn using disposable calibrated Drummond micropipets and diluted with 1.0 mL of benzylamine (10 mM in 50 mM sodium phosphate buffer, pH 7.2) in a 1 cm cuvette (1.5 mL). The rate of oxidation of benzylamine to benzaldehyde was measured by recording the increase in absorbance at 250 nm for 1 min, and compared to the rate of benzylamine oxidation for a companion control solution of enzyme without the inhibitor. The concentration of active BPAO was estimated from the rate of benzylamine oxidation (1 U oxidizes 1.0 μmol of benzylamine to benzaldehyde per minute at 25 °C), using an activity of 0.48 U/mg protein for the pure enzyme of molecular weight 85,000 Da and $\Delta\varepsilon_{250}$ = 12,800 M^{-1} cm^{-1} for benzaldehyde (corresponding to a A_{250} of 12.8/min/U of activity for 1 mL volume).

2.7. Irreversibility of BPAO inhibition

(*a*) *By gel filtration.* The irreversibility of the inhibition for inactivators was checked by applying enzyme preparations (0.5 mL) that were 50–90% inhibited to a PDX G. F. 25 column (1 × 7.8 cm) equilibrated with 100 mM sodium phosphate buffer (pH 7.2) to separate non-covalently bound small molecules. Percent activity following gel filtration was determined by comparing the activity between two gel-filtered incubations of enzyme in the absence and presence of inhibitor. The same initial protocol had to be worked out each time the column was changed.

(*b*) *By dialysis.* The irreversibility of the inhibition for inactivators was also checked by adding 0.5 mL enzyme preparations that were 50–90% inhibited to dialysis tubing (6.4 mm Spectrum Spectro/Por membrane, MW cutoff 12,000–14,000), followed by dialysis against 100 mM sodium phosphate buffer, pH 7.2, at room temperature for up to 24 h. Percent activity was determined by comparing enzyme activity between two dialysis experiments following incubation of enzyme in the absence and presence of inhibitor.

2.8. Phenylhydrazine titration of active site after inactivation of BPAO with inactivators

Incubation of the enzyme with the test inactivator in 100 mM phosphate buffer, pH 7.2, was allowed to proceed at 30 °C until there was more than 80% loss of activity after gel filtration or dialysis. A control reaction was run for the same time. The gel filtered or dialyzed enzyme fractions were titrated with 4 μL of phenylhydrazine hydrochloride (2 mM) in H_2O, and the absorbance at 440 nm was measured. The percent decrease in the absorbance at 440 nm was determined compared to the control experiment.