Inhibition of Bovine Plasma Semicarbazide-Sensitive Amine Oxidase by Caffeine

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ABSTRACT: Semicarbazide-sensitive amine oxidase (SSAO) is a copper-containing enzyme that catalyzes the oxidative deamination of endogenous and exogenous primary amines. SSAO exists in mammals both as a plasma-soluble and as a membrane-bound form, and its active site is able to come into contact with numerous xenobiotic, amine-containing compounds. The kinetic studies performed in this work showed that caffeine inhibition of bovine serum amine oxidase was noncompetitive when benzylamine was used as substrate and mixed when the substrate used was methylamine. Since caffeine contains an imidazole ring, it cannot be excluded that it might bind to an inhibitory imidazoline-binding site on SSAO. © 2010 Wiley Periodicals, Inc. J Biochem Mol Toxicol 25:26–27, 2011; View this article online at wileyonlinelibrary.com. DOI 10:1002/jbt.20356

KEYWORDS: **Semicarbazide-Sensitive Amine Oxidase (SSAO); Caffeine; Enzyme Inhibition; Imidazoline Binding Sites**

BRIEF COMMUNICATION

Semicarbazide-sensitive amine oxidase (SSAO) is a common name for primary-amine oxidase (EC 1.4.3.21), previously classified as EC 1.4.3.6 [1]. SSAO, a copper-containing enzyme that catalyzes the oxidative deamination of endogenous and exogenous primary amines, is found as a membrane-bound protein in mammalian species [2], although a plasma soluble form of the enzyme (pSSAO) also exists, resulting from the proteolytic cleavage of membrane-bound SSAO [3]. In some tissues, it functions as a vascular adhesion protein, VAP-1, that mediates the slow rolling and adhesion of lymphocytes to endothelial cells, during inflammation [4]. The active site of the tissue-bound SSAO is located in the extracellular domain [5], making the enzyme, together with its plasma-soluble form, a scavenger of potentially toxic amines in the blood. Some authors reported the presence of different imidazoline-binding sites on SSAO [6,7]. Caffeine, an alkaloid containing an imidazole ring and therefore somewhat related to imidazolines, is probably the most widely consumed drug, and its effect on the activity of SSAO was investigated in this study.

Bovine plasma SSAO was obtained by the supplier BioVar Ltd (Yerevan, Armenia). Caffeine and other chemicals used in this study were obtained from Sigma-Aldrich (Arklow, Ireland).

The activity of SSAO was determined following the production of hydrogen peroxide at 498 nm, by the method of Holt and Palcic [8], in the presence of benzylamine or methylamine at the appropriate concentration and SSAO (10 μg/mL of protein). The chromogenic solution for the detection of H_2O_2 was prepared as previously reported [8]. Control assays of the coupling system, in the presence of 10 μM H_2O_2, 1 mU/mL HRP but in the absence of SSAO, showed that caffeine did not affect the chromogenic detection system.

The IC_{50} value (± SD) was determined by plotting the initial rates of reaction, obtained in the presence of 5-mM benzylamine, against the $\log_{10}$ [caffeine] and fitting the resulting plot to a sigmoidal curve (not shown). The curve fit and the value of $IC_{50} = 0.8 \pm 0.3$ mM were obtained with the aid of the computer software GraphPad Prism, version 5.00. To determine the dissociation constant K_i, samples were assayed in the presence of increasing concentrations of inhibitor and at different concentrations of substrate. The initial rates of hydrogen peroxide formation were determined at 37°C and pH 7.2, in the presence of HEPES physiological buffer, prepared as previously reported [8]. Data were then fitted to the Michaelis–Menten equation by nonlinear regression (obtained with the aid of GraphPad Prism 5.00), and the value of K_i was determined from the dependence of the kinetic parameters on the inhibitor concentration.

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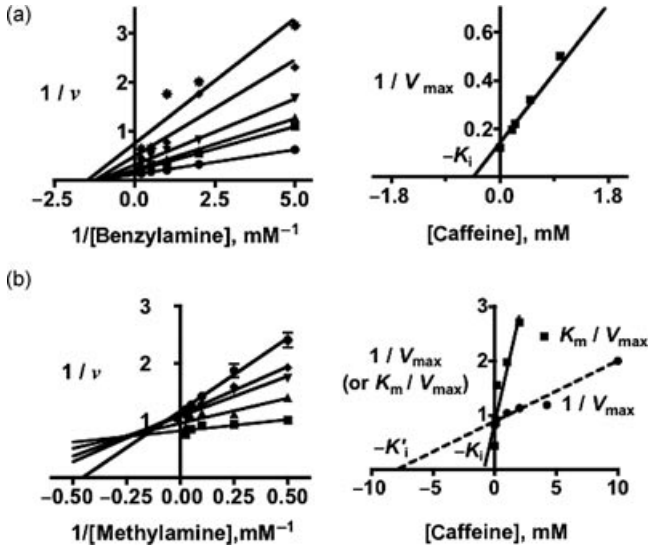

FIGURE 1. Substrate-dependent patterns of inhibition of SSAO by caffeine. (a) Noncompetitive type pattern. All samples contained increasing concentrations of caffeine (from 0 to 2 mM) and benzylamine (from 0.2 to 5 mM). A K_i of approximately 1.0 mM could be obtained by plotting the values of $K_m$app/V_{max} app against the concentration of inhibitor used, as shown on the right. Similarly, a K_i' of approximately 8 mM could be obtained by plotting $1/V_{max}$app against the concentration of inhibitor used. Double reciprocal plots are used for illustrative purpose only. Data shown are the mean values ± SEM of four different determinations; error bars not evident were less than the representation of the points. (b) Mixed type pattern. All samples contained increasing concentrations of caffeine (from 0 to 2 mM) and methylamine (from 2 to 40 mM). The initial rates ($v = \text{abs}_{498\ nm} \times 10^{-3}$ min^{-1}) of hydrogen peroxide formation were determined at 37°C and pH 7.2. Data were fitted to the Michaelis–Menten equation with the aid of computer software GraphPad Prism, 5.0. The values of $1/V_{max}$ app were plotted against the concentration values of inhibitor used to obtain a K_i value ($-K_i$ = intercept on the X axis of the plot on the right) of approximately 1 mM.

Caffeine, in the concentration range of 0.1–10 mM, was found to inhibit SSAO activity (IC_{50} = 0.8 ± 0.3 mM). The kinetic studies performed on caffeine showed that its inhibition of SSAO was noncompetitive ($K_i \approx K_i' \approx 1.0$ mM) when benzylamine was used as the substrate (Figure 1a) and mixed ($K_i \approx 1.0$ mM; $K_i' \approx 8.0$ mM) when the substrate used was methylamine (Figure 1b). Since caffeine contains an imidazole ring, it cannot be excluded that it might bind to an inhibitory imidazoline binding site on SSAO [6,7]. Although Mu et al. [6] reported that the imidazoline-binding site ligand amiloride competitively inhibited SSAO, Holt et al. [7] found that clonidine, guanabenz, and 2-(2-benzofuranyl)-2-imidazoline showed different affinities for each of different binding sites on SSAO, and that these differences were apparently substrate dependent. An alternative explanation might involve caffeine binding to both the oxidized and reduced forms of the enzyme.

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