THE SPECIFICITY OF CYSTEAMINE OXYGENASE

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1. Introduction

Oxidation of cysteamine to hypotaurine is catalyzed by cysteamine oxygenase (EC 1.13.11.19), a non-heme iron enzyme of large distribution [1–3]. A similar oxidation of cysteine to cysteine sulfinic acid has been reported to require a different oxygenase [4,5]. Specificity of cysteamine oxygenase has been studied earlier in this laboratory by assaying a limited number of sulfhydryl-containing compounds. The enzyme was found to be specific for cysteamine under the conditions used in this work [2]. In the present paper we report the results of a more thorough investigation on the oxidation of various sulfhydryl-containing, and one selenhydryl-containing, compounds aimed at establishing the specificity of this oxygenase.

2. Materials and methods

The following compounds have been prepared in the laboratory: 1-amino-4-mercaptobutane hydrochloride [6]; 1-amino-3-mercaptopropane (homocysteamine) hydrochloride by reduction with $NaBH_4$ of the corresponding disulfide [7]; β-mercaptopyruvate sodium salt [8]; β-alanylcysteamine hydrochloride [9]; selenocysteamine by reduction of commercial selenocystamine [10]. *N*-acetylcysteamine was a gift from Professor F. Lynen. All the remaining compounds were obtained commercially.

Oxidation of substrates in the presence of cysteamine oxygenase has been followed by the determination of O_2 uptake in a conventional Warburg apparatus. Since the enzyme is able, under certain conditions, to oxidize a sulfhydryl group to the disulfide level (oxidasic reaction) we have assumed that the oxidation to the sulfinate level had occurred only when the final O_2 uptake was higher than that calculated for the oxidation to the disulfide. Principles and calculation involved in the procedure used have been reported in a previous paper [11].

Cysteamine oxygenase has been prepared as described earlier [12]. The enzyme was purified up to the step 6 described in this paper. Specific activity was in the range of 0.4–0.5 units per mg.

Cysteamine oxygenase oxidizes cysteamine to hypotaurine at relatively high enzyme/substrate ratios (oxygenasic reaction). When the ratio is decreased, either by increasing the substrate concentration or by decreasing the enzyme concentration, the oxidation of cysteamine to cystamine (oxidasic reaction) is prevailing [11,13] Under the latter conditions the addition of certain compounds, named cofactor-like compounds, restore the oxidation to hypotaurine [11,13,14]. We have assayed the oxidation of various substrates in the conditions where the enzyme oxidizes cysteamine to hypotaurine both in the presence and in the absence of the cofactor-like compound. In other words the ratio of enzyme/substrate was such that in any case the oxygenasic reaction was prevailing, as judged by the oxidation of cysteamine to hypotaurine. As a representative of a cofactor-like compound we have used phenazine methosulfate [14].

3. Results and discussion

Substrates oxidized by cysteamine oxygenase may be divided in three groups.

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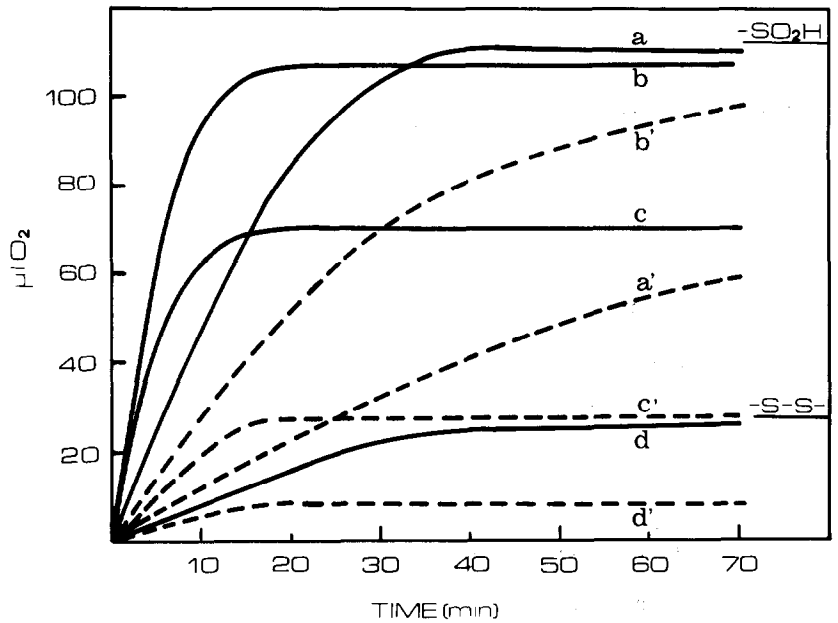

Fig.1. Oxidation of sulfhydryl-containing compounds by cysteamine oxygenase. Each Warburg vessel contained: 10 mg enzyme; 5 μmol neutralized substrate; 0.2 μmol phenazine methosulfate: 300 μmol K-phosphate buffer pH 7.6; water to 3 ml; gas, air; temp. 38°C. a = mercaptoethanol; b = cysteamine; c = cysteine ethyl ester; d = cysteine. a', b', c' and d' like a, b, c, and d with phenazine methosulfate omitted. –S–S– and $-SO_2H$ indicate the theoretical values calculated for the oxidation of 5 μmol of substrate respectively to the disulfide and to the sulfinate level.

The first group contains compounds oxidized at a very slow rate both in the presence and in the absence of phenazine. The final O_2 uptake is never higher than that calculated for the oxidation to the disulfide level. The oxidation in the presence of phenazine is faster than in its absence, however part of the oxidation in the presence of phenazine is non-enzymatic being catalyzed by phenazine itself even in the absence of the enzyme. For this group of compounds the enzyme acts as an oxidase of very low activity both in the presence and in the absence of phenazine. The oxidation of a typical representative of this group is exemplified in fig.1 by cysteine (curves d and d').

The second group includes compounds which in the absence of phenazine are oxidized by the enzyme at the level equal or lower than that calculated for the oxidation to the disulfide level, while in the presence of phenazine they show a final O_2 uptake higher than this level. As a representative of this group we have reported in fig.1 the oxidation of cysteine ethyl ester (curves c and c').

The third group contains compounds which are oxidized to a level higher than that calculated for the disulfide both in the presence and in the absence of phenazine. All the components of this group show a final O_2 uptake in the presence of phenazine which is in the range of that calculated for the oxidation to the sulfinate level. The oxidation of cysteamine (curves b and b') and mercaptoethanol (curves a and a') is reported in fig.1 as representative of this group. The conversion of cysteamine into the sulfinate derivative has been documented in our earlier papers [1–3]. Evidence that also in the case of mercaptoethanol the oxidation product is a sulfinate derivative has been obtained chromatographically. An aliquot of the final incubate, either in the presence or in the absence of phenazine, has been run in paper chromatography by using butanol–acetic acid–water (50/10/40) as solvent. The presence of a sulfinate derivative has been evidentiated by spraying reagents specific for sulfinates [15].

Table 1 lists all the substrates investigated divided in three groups according to the criteria explained above. Table 2 reports the comparative oxidation rate and the K_M of the compounds of group 3 which appear the real substrates of the enzyme.

Table 1
Sulfhydryl-containing compounds oxidized by cysteamine oxygenase

Group 1.	Compounds oxidized to the level of disulfide or lower both in the presence and in the absence of phenazine methosulfate: Cysteine, homocysteine, selenocysteamine, β-alanylcysteamine, pantetheine, thioglycolic acid, β-mercaptopyruvic acid, glutathione, thiolactic acid, 1-mercaptopropane, 2-mercaptopropane, ethylmercaptane, 2-aminoethylisothiouronium, dithiothreitol.
Group 2.	Compounds oxidized to the level of disulfide or lower in the absence of phenazine methosulfate but oxidized to the level higher than disulfide in the presence of phenazine methosulfate: Cysteine ethyl ester, 1-amino-4-mercaptobutane, 2,3-dithiopropanol.
Group 3.	Compounds oxidized to the level higher than that of disulfide both in the presence and in the absence of phenazine methosulfate: Cysteamine, homocysteamine, *N*-acetylcysteamine, mercaptoethanol, β-mercaptopropionic acid.

Experimental conditions reported in fig.1.

Of particular interest appears the detection of mercaptoethanol and homocysteamine as substrates of cysteamine oxygenase. Mercaptoethanol is generally used as a chemical reagent and very little is known about its metabolic role. The occurrence of an enzymatic oxidation of mercaptoethanol as reported above opens the question of the possible metabolic role of this compound. The enzymatic conversion of β-mercaptopyruvate into mercaptoethanol [16] and the occurrence in tissues of oxidation products, like isethionic acid [17], suggests a careful investigation on the metabolism of mercaptoethanol in animals. The interest of the oxidation of homocysteamine is evident in the fact that the products of its oxidation, homohypotaurine and homotaurine, are used as substrates by other enzymes [18,19].

Table 2
Initial oxidation rate and K_M for compounds listed in table 1 under group 3

	Oxidation rate (μl O_2/min/mg)		K_M	
	Phenazine		Phenazine	
	Added	Omitted	Added	Omitted
Cysteamine	1.16	0.26	6.2×10^{-4}	5.7×10^{-4}
Homocysteamine	0.86	0.20	8.3×10^{-4}	
N-acetylcysteamine	0.74	0.17	7.1×10^{-4}	
Mercaptoethanol	0.50	0.11	1.2×10^{-3}	1.2×10^{-3}
β-mercaptopropionic acid	0.4	0.07	5.0×10^{-3}	

Experimental conditions reported in fig.1.

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