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S-OXIDATION OF THIOUREYLENES CATALYZED BY A MICROSOMAL FLAVOPROTEIN MIXED-FUNCTION OXIDASE

LAWRENCE L. POULSEN,* RICHARD M. HYSLOP and DANIEL M. ZIEGLER†

Clayton Foundation Biochemical Institute and Department of Chemistry,
The University of Texas at Austin, Austin, Texas 78712, U.S.A.

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Abstract—A flavoprotein mixed-function oxidase that catalyzes NADPH-dependent oxidation of thioureylenes by oxygen was isolated from pig liver microsomes and shown to be identical with the flavoprotein amine oxidase isolated earlier. The oxidation products obtained from the thioureylene, methimazole, were identified as *N*-methylimidazole and sulfite. The stoichiometry of the reaction indicated that 2 moles NADPH and 2 moles oxygen were required for each mole of methimazole oxidized. In addition to all of the thioureylenes tested, the flavoprotein also catalyzes the oxidation of dithiothreotol and its cyclic disulfide. At the pH optimum for the flavoprotein mixed-function oxidase, virtually all of the methimazole metabolized by homogenates of pig liver appears to be catalyzed by this membrane-bound oxidase. The methimazole oxidase activity also appears to correlate with the concentration of this oxidase in microsomes isolated from homogenates of rat, rabbit and guinea-pig liver, but species differences in the response of this mixed-function oxidase to activators were observed.

METHIMAZOLE (1-methyl-2-mercaptoimidazole) is a member of a class of compounds known as thioureylenes that have in common a thiocarbamide (—NH—C(=S)—NH—) moiety. Many thioureylenes possess antithyroid activity and are used in the treatment of thyrotoxicosis. Because of their importance as antithyroid drugs, a number of studies have been conducted on the metabolism of thioureylenes *in vivo*. Marchant and Alexander[1] reported that approximately 40 per cent of methimazole, one of the most commonly used antithyroids, was excreted in the urine of rats as a polar metabolite. The polar metabolite was not fully characterized, but its properties were different from those of methimazole, inorganic sulfate or the glucuronic acid conjugate of methimazole. In addition to this metabolite, small amounts of radiolabeled sulfate were excreted in the urine after the administration of ^{35}S-labeled methimazole.

Studies on the tissue distribution of thioureylene metabolites have shown that the thyroids contain the highest concentrations of inorganic sulfate formed from the metabolism *in vivo* of methimazole,[1] propylthiouracil[2] and thiourea.[3] It has been suggested[1] that, in thyroids, the sulfate is formed from the thioureylenes by an oxidative attack on the sulfur atom followed by hydrolytic cleavage to yield inorganic sul-

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fate. While most of the earlier studies on thioureylene metabolism have focused on thyroid tissue, the methimazole–metabolite tissue distribution studies of Marchant and Alexander[1] demonstrated that the concentration of metabolites per g of tissue was higher in the liver than in plasma or in tissues other than thyroid. This study suggested that metabolism of methimazole may occur in liver tissue. However, they were unable to demonstrate oxidation of this compound by unfortified liver homogenates, and they did not test the homogenate for mixed-function oxidases, which might be capable of catalyzing the oxidation of thioureylenes.

The work presented in this report demonstrates that the oxidation of thioureylenes is catalyzed by a mixed-function oxidase concentrated in the microsomal fraction of hepatic homogenates. This mixed-function oxidase has been isolated and is identical with the flavoprotein mixed-function oxidase described earlier[4] that also catalyzes the *N*-oxidation of amines. In addition to the *S*-oxidation of thioureylenes, this oxidase also catalyzes the oxidation of a few sulfhydryl compounds and at least one cyclic disulfide. While the *S*-oxidase activity of this microsomal flavoprotein has not been described previously, naphthylthiourea,[4] dithiothreotol[5] and cysteamine[5] are reported to inhibit the *N*-oxidation of amines catalyzed by this oxidase.

MATERIALS AND METHODS

Materials. NAD^+, NADPH and dithiothreotol were obtained from P-L Biochemicals, Inc.; glucose 6-phosphate and $NADP^+$, from Sigma Chemical Co.; glucose 6-phosphate dehydrogenase, from Worthington Biochemical Corp.; methimazole, phenylthiourea (1-phenyl-2-thiourea) and naphthylthiourea (1-(1-naphthyl)-2-thiourea), from J. T. Baker Chemical Co.; propyl thiouracil (4-hydroxy-2-mercapto-6-propyl-pyrimidine), from Aldrich Chemical Co.; α-thioglycerol (B-grade), from Calbiochem.; dodecylguanidine, from Alfred Bader; and Sephadex® G-15, from Pharmacia. *N,N*-dimethylaniline, octylamine (Eastman Organic Chemicals) and *N*-methylimidazole (K & K Fine Products) were purified by gas–liquid chromatography. The diethylaminoethanol ester of diphenylpropylacetic acid (SKF-525A) and 2,4-dichloro-6-phenylphenoxyethylamine (DPEA) were gifts from Smith Kline & French and Eli Lilly Inc. respectively. The cyclic disulfide of dithiothreotol was prepared by the procedure described by Cleland.[6]

Methods. Microsomes were separated from pig liver homogenates by differential centrifugation and the flavoprotein mixed-function oxidase was isolated from the microsomes by the method described earlier.[4]

The enzymic *N*-oxidation of *N,N*-dimethylamine was measured as described previously.[7] The thioureylene oxidase activity of liver homogenates, microsomes and the isolated oxidase was measured routinely by following the substrate-dependent increase in oxygen consumption. Oxygen uptake was measured polarographically with a Clark oxygen electrode mounted in a temperature-controlled 1·9-ml reaction vessel. The signal from the electrode was recorded with a Heathkit model EN-2058 strip chart recorder. Unless specified otherwise, the reaction media contained 1 mM NADPH (or an NADPH-generating system consisting of 2·5 mM glucose-6-phosphate, 0·5 mM $NADP^+$ and 1·0 i.u./ml of *L. mesinteroides* glucose-6-phosphate dehydrogenase) in a 16 mM potassium phosphate–32 mM sodium pyrophosphate buffer, pH 8·4, at 37°. With the purified oxidase, the oxidation of sulfur compounds

was also measured by following the substrate-dependent oxidation of NADPH spectrophotometrically at 37° with a Gilford recording spectrophotometer. In these experiments the reaction medium contained buffer, pH 8·4, 0·2 mM NADPH, and 0·05 to 0·1 mg of the purified oxidase. The concentrations of the different substrates used are given in the tables.

The principal organic oxidation product of methimazole was isolated from the reaction medium by the following method. Thirty ml of reaction medium containing buffer, NADPH-generating system (5 mM glucose-6-phosphate), 12 mg of the purified oxidase and 1·67 mM methimazole was incubated at 37° for a few min. The reaction was monitored at regular intervals by diluting 0·01 ml of the reaction medium to 1·0 ml with water and the concentration of methimazole was measured spectrophotometrically at 258 nm against a reference blank that contained all components except methimazole. The oxidation products, unlike methimazole, do not absorb at this wavelength. After more than 90 per cent of the methimazole was oxidized, the reaction mixture was deproteinized by pressure filtration. The pH of the filtrate was adjusted to 3·5 and then freeze-dried. The product was extracted from the dry powder with three successive 2-ml aliquots of dry methanol and the volume of the combined methanol extracts was reduced to approximately 0·5 ml under vacuum. The major product in the extract was identified by analytical mass spectroscopy and by thin-layer chromatography (TLC) with known compounds on EM Laboratory Silica gel plates.

The acid-labile reducing compound formed during the enzymic oxidation of methimazole was detected by the following method. Aliquots of the reaction mixture were deproteinized either by pressure filtration through an XM-100 filter in a Aminco Diaflo® cell, or by mixing with chloroform and centrifuging. Between 0·1 and 0·2 ml of the protein-free aliquot was added to 0·9 ml of a solution containing 0·1 M phosphate buffer, pH 7·4, and 0·8 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB). The concentration of reduced DTNB was measured spectrophotometrically at 410 nm against a reagent blank. Under these conditions neither methimazole, NADPH, nor components of the NADPH-generating system reduce DTNB.

RESULTS

Methimazole oxidase activity of the purified flavoprotein. Like *N,N*-dimethylaniline and all other known substrates whose oxidation is catalyzed by the purified oxidase, the addition of methimazole to the complete reaction medium produces an increase in the rate of oxygen consumption (Table 1). The increased rate of oxygen uptake observed with the thioureylene, like the tertiary amine, is dependent upon NADPH or NADH and, at pH 8·4, NADH is only 80 per cent as effective a reductant as NADPH. The addition of primary alkylamines or alkyl guanidines, compounds known to increase the catalytic activity of this oxidase,[8,9] also stimulate methimazole-dependent oxygen uptake. The optimum pH for the oxidation of methimazole, 8·4, is also identical with that of other substrates for this oxidase and the curve obtained by plotting the rate of methimazole oxidation as a function of pH is identical with that reported for tertiary amine substrates.[10]

The information given in Table 1 is good presumptive evidence that the isolated flavoprotein can catalyze the NADPH-dependent oxidation of methimazole by

TABLE 1. METHIMAZOLE- AND *N,N*-DIMETHYLANILINE-DEPENDENT O_2 UPTAKE CATALYZED BY THE PURIFIED FLAVOPROTEIN OXIDASE

Changes in standard reaction medium*		Oxygen uptake† (nmoles/min/mg enzyme)	
Deletions	Additions	Methimazole	*N,N*-dimethylaniline
SM		886	895
NADPH	$NADP^+$	0	0
NADPH	NADH	705	740
Enzyme	Heat-denatured enzyme	0	0
SM	3·0 mM octylamine	1600	1880
SM	0·2 mM dodecylguanidine	1613	1880
SM	0·25 mM SKF-525A	825	940
SM	0·5 mM DPEA	1755	1969

* Standard media contained 16 mM phosphate–32 mM pyrophosphate buffer, pH 8·4; 1 mM nucleotide(s) added as indicated and 30–50 μg enzyme, SM = no change in the standard medium.

† Measured polarographically at 38° as described under Methods. Rates were calculated from the increase in oxygen uptake observed after the addition of 1·0 mM methimazole or 1·0 mM dimethylaniline.

oxygen. More direct evidence that the flavoprotein catalyzes the oxidation of methimazole was obtained by isolating the principal oxidation product as described under Methods. The major organic product was first identified as *N*-methylimidazole by analytical mass spectroscopy and then shown to be identical with this compound by co-chromatography on thin-layer plates in four solvent systems. The R_f values of the oxidation product, *N*-methylimidazole, and of the parent thioureylene, methimazole, were respectively 0·13 and 0·84 in tetrahydrofuran–formic acid (10:1), 0·35 and 0·64 in tetrahydrofuran–concentrated NH_4OH (10:0·5); 0·70 and 0·75 in methanol–concentrated NH_4OH (10:0·5); and 0·67 and 0·74 in methanol. *N*-methylimidazole was formed from methimazole only upon incubation of the complete reaction mixture. The product could not be detected in reaction mixtures incubated without NADPH or enzyme.

In addition to *N*-methylimidazole, a reducing compound is also formed during the flavoprotein-catalyzed oxidation of methimazole. This reducing compound, estimated as described in Methods, appears to be identical with sulfite. It escapes from acid solution into the vapor phase and, like sulfite, its oxidation by oxygen at pH 7·0 to 8·5, measured polarographically, is catalyzed by traces of ferric or cupric ions.

Unfortunately, the formation of neither of these oxidation products can be used to follow the course of the enzyme-catalyzed reaction. A sensitive method for measuring the concentration of *N*-methylimidazole could not be developed and sulfite added to the reaction medium cannot be recovered quantitatively. At the pH optimum of the purified oxidase, the oxidation of sulfite is quite rapid in the presence of trace amounts of ferric ions. By complexing with metal ions, methimazole retards but does not block this reaction. In addition to the metal-catalyzed oxidation of sulfite, other components in the reaction mixture also interfere with the recovery of added sulfite. While these were not identified, both $NADP^+$ and flavoproteins are known to form addition compounds with sulfite, and all attempts to quantitatively recover nanomolar quantities of sulfite from the reaction medium failed.

Although the products formed could not be measured quantitatively, their identification suggests that the flavoprotein catalyzes the oxidation of methimazole to the corresponding sulfinate, which hydrolyzes to sulfite and *N*-methylimidazole. Sul-

finates are known to be extremely unstable and readily hydrolyze to sulfite and the parent organic compound.[11]

The stoichiometry of the enzyme-catalyzed reaction is also consistent with the formation of the sulfinate from methimazole. In the presence of limiting amounts of methimazole, 2 moles oxygen are consumed and 2 moles NADPH are oxidized for every mole of methimazole added (Fig. 1). With the large amounts of enzyme used in these experiments, the oxidation of methimazole was essentially complete in less than 2 min. Under these conditions the uptake of oxygen by sulfite was negligible, and the change in the oxygen uptake (Fig. 1A) upon the addition of methimazole can be attributed to the oxidation of methimazole. The data in Fig. 1 show that the methimazole–O_2–NADPH stoichiometry is 1:2:2, which is consistent with the formation of methimazole sulfinate.

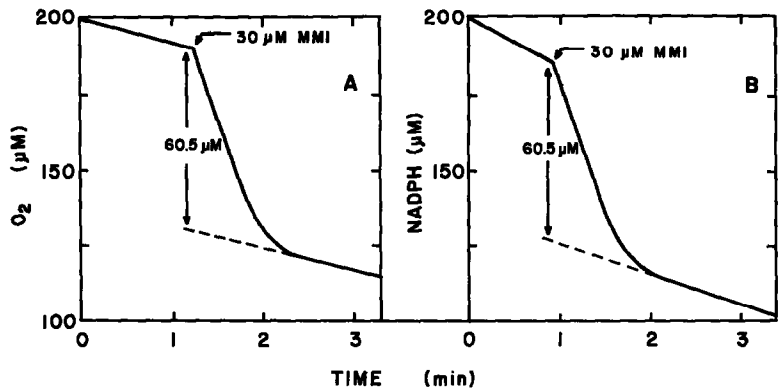

FIG. 1. Amount of oxygen reduced measured polarographically (A) and NADPH oxidized (B) by 30 μM methimazole. The reaction media contained phosphate–pyrophosphate buffer, pH 8·4, NADPH (1·0 mM in A and 0·2 mM in B) and 0·1 mg/ml purified flavoprotein oxidase. After temperature equilibration, enzyme was added first to establish the endogenous rate and then methimazole (MMI) in 5 μl was added as indicated. The reaction was followed until the rate returned to the endogenous rate. The methimazole-dependent oxygen reduced and NADPH oxidized were calculated from the increment indicated by the arrows.

Methimazole oxidase activity of the membrane-bound flavoprotein. The oxidation of methimazole catalyzed by the membrane-bound flavoprotein in hepatic homogenates is readily measured polarographically. At the pH optimum (8·4) of the purified oxidase, the addition of the thioureylene results in an increase in oxygen uptake with both the whole homogenate and isolated microsomes (Fig. 2). As with the purified flavoprotein oxidase, octylamine also stimulates the methimazole-dependent increase in oxygen uptake catalyzed by microsomes or the whole homogenate. However, unlike the purified flavoprotein-catalyzed reaction, octylamine usually stimulates the homogenate- or microsomal-catalyzed oxidation of methimazole more than 2-fold, and 5-fold stimulation of methimazole-dependent oxygen uptake by 3 mM octylamine has been observed with a few preparations of pig liver microsomes. To determine whether this difference between the microsomes and the isolated flavoprotein could be due to the presence of other hepatic enzyme systems that also catalyze the oxidation of methimazole, this activity (± octylamine) was measured in the

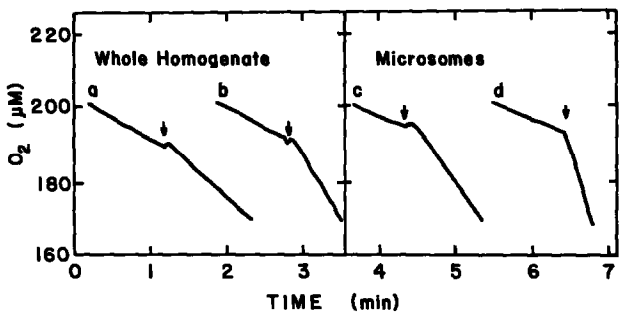

FIG. 2. Increased oxygen consumption measured polarographically in whole homogenates and isolated microsomes from pig liver after addition of 1 mM methimazole minus (a and c) and plus (b and d) 3·0 mM octylamine. After a preliminary temperature equilibration, whole homogenate (20 mg protein) or microsomes (0·4 mg protein) were added and, after recording the endogenous rate, methimazole was added as indicated by the arrows. The basic reaction media contained phosphate–pyrophosphate buffer, pH 8·4, 0·5 mM NADPH, 2·5 mM glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The octylamine was added in tracings b and d before the whole homogenate or microsomes, and the primary amine as shown, has little effect on the endogenous rate.

whole homogenate, in subfractions obtained by differential centrifugation and in intermediate fractions in the isolation of the flavoprotein from microsomes. The enzymic *N*-oxidation of *N*,*N*-dimethylaniline was also measured on all of these fractions.

TABLE 2. METHIMAZOLE OXIDASE (Meth-Ox) AND DIMETHYLANILINE *N*-OXIDASE (DMA-Ox) ACTIVITIES OF WHOLE HOMOGENATE AND SUBFRACTIONS OF PIG LIVER

Preparation	Specific activity* Meth-Ox	Meth-Ox + Octam	DMA-Ox	DMA-Ox + Octam	Ratio Meth-Ox/DMA-Ox	Meth-Ox/DMA-Ox + Octam
Whole homogenate†	2·1	5·9	1·7	5·5	1·2	1·1
Nuclei + debris	3·0	6·4	1·1	5·3	2·7	1·2
Mitochondria	2·5	5·0	2·2	4·2	1·1	1·2
Microsomes	9·8	23·3	8·7	18·6	1·1	1·2
Soluble	0	0	0	0		
Microsomal subfractions‡						
Microsomes	17	50	16	41	1·1	1·2
Triton extract	58	133	50	155	1·2	0·9
Ammonium sulfate fractionation	144	320	194	384	0·7	0·8
Chromatography on Sephadex	886.	1613	895	1880	1·0	0·9

* The oxidation of methimazole measured polarographically at 38°, and activity expressed as substrate-dependent oxygen uptake/min/mg protein. The *N*-oxidation of *N*,*N*-dimethylaniline measured as described earlier[7] and activity expressed as nmoles *N*-oxide formed/min/mg protein. The reaction media contained 16 mM phosphate–32 mM pyrophosphate buffer, pH 8·4; 2·5 mM glucose-6-phosphate; 0·5 mM $NADP^+$: glucose-6-phosphate dehydrogenase; 1·0 mM substrate; and a sufficient amount of each fraction to give reliable rates. Activities of all fractions were measured ± 3·0 mM octylamine (Octam).

† Whole homogenate prepared from 20 g liver in 80 ml of 0·25 M sucrose with a glass–Teflon homogenizer. The fractions were separated by differential centrifugation in the usual manner, but the fractions were not washed.

‡ For a complete description of these fractions see ref. 4. This microsomal preparation was from a liver different from the one used to collect the data in the first part of this table.

This activity serves as a marker for the flavoprotein mixed-function oxidase, since the *N*-oxidation of this tertiary amine is catalyzed exclusively by this oxidase in pig liver homogenates.[4]

As shown in Table 2, in the presence of 3 mM octylamine the ratio of the two activities is constant in the fractions separated from the whole homogenate by differential centrifugation. Like the *N*-oxidase, the methimazole oxidase concentrates in the microsomal fraction and the increase of both activities in this fraction over that in the whole homogenate is virtually identical. The activities present in the nuclei + debris fraction and in the mitochondrial fraction were not studied in detail, but they may be due to unbroken cells in the former fraction and to microsomal contamination in the latter fraction. Neither activity could be detected in the soluble fraction, which contains between 32 and 38 per cent of the protein in whole homogenates of pig liver. In the absence of octylamine, the ratio of the two activities among the different fractions was somewhat more variable and reflects the difficulties encountered in accurately measuring the *N*-oxidase activity in some of the particulate fractions.

The two activities also concentrate together in fractions obtained during the isolation of the flavoprotein oxidase from microsomes (Table 2). There is, however, a 10–20 per cent change in the ratio of the two activities from the microsomes to the isolated flavoprotein. The rate of methimazole-dependent oxygen uptake divided by the rate of dimethylaniline *N*-oxidation decreases by 10–20 per cent after the particles are treated with detergent. This could indicate that microsomes contain more than one oxidase which catalyzes the oxidation of methimazole by oxygen and one of the oxidases is destroyed by the addition of detergents. However, it is also possible that more of the methimazole-dependent oxygen uptake observed with the particulate fractions is due to the nonenzymic oxidation of sulfite by oxygen. Sulfite is oxidized much faster by oxygen, presumably to sulfate, in the presence of microsomes than in buffer or in buffer containing the detergent-extracted fractions. This is not too surprising, since microsomes contain a variety of auto-oxidizable components (e.g. disulfides, hemoproteins, flavins) that are readily reduced by sulfite. If all of the sulfite produced by hydrolysis of the sulfinate is oxidized by oxygen, the rate of methimazole oxidized is $\frac{1}{3}$ the rate of oxygen consumption, whereas with the purified oxidase the rate of methimazole oxidized is $\frac{1}{2}$ the rate of oxygen consumption (Fig. 1A). In any event, the data summarized in Table 2 demonstrate that most, if not all, of the methimazole-dependent oxygen uptake measured at pH 8·4 in the different fractions can be attributed to the activity of the flavoprotein mixed-function oxidase.

Methimazole oxidation catalyzed by hepatic microsomes of different species. The methimazole oxidase activity, measured polarographically, is quite close to the dimethylaniline *N*-oxidase activity in microsomes from guinea-pig and pig liver. However, the methimazole oxidase is consistently 30–40 per cent higher than the *N*-oxidase in rat and rabbit liver microsomes (Table 3). While both activities were measured on only three different preparations of rabbit liver microsomes, these activities were measured in over 20 different rat liver microsomal preparations. The rats varied in age from weanling to over a year old and included both males and females. Regardless of age or sex, the rate of methimazole-dependent oxygen uptake in rat liver microsomes was consistently 1·3 to 1·4 times the rate of dimethylaniline *N*-oxidation. The higher methimazole oxidase activity of rat liver indicates that either the

TABLE 3. METHIMAZOLE OXIDASE AND *N*,*N*-DIMETHYLANILINE *N*-OXIDASE ACTIVITIES OF HEPATIC MICROSOMES FROM DIFFERENT SPECIES*

Species	Methimazole oxidase		Dimethylaniline *N*-oxidase	
	(nmoles O_2/ min/mg prot.)	(% activation by 3 mM oxtylamine)†	(nmoles *N*-oxide/ min/mg prot.)	(% activation by 3 mM oxtylamine)†
Pig	14	146	13	146
Guinea-pig	10	67	9	78
Rabbit	13	0	10	30
Rat	10	0–10	7	0–10

* Oxidase activities were measured as described in the legend to Table 2. The microsomal preparations were obtained from livers of adult male animals except the pig. The pig liver was from an adult female.

† These values were calculated by subtracting the control rate from the rate obtained in the presence of octylamine, then dividing by the control rate and multiplying by 100.

specificity of the flavoprotein is different in this species or there may be a second oxidase in rat liver microsomes that can also catalyze the oxidation of methimazole.

Marked species differences in the activation of both activities by octylamine were also observed (Table 3). Octylamine stimulates both activities in pig and guinea-pig liver microsomes, but octylamine has little or only marginal effects on these activities in rat and rabbit liver microsomes. In a few microsomal preparations from older rats, both activities were stimulated as much as 25 per cent by 3 mM octylamine but, in general, significant activation of this oxidase by octylamine, so consistently observed in pig liver microsomes, is virtually absent in rat liver microsomes.

Other sulfur-containing substrates for the flavoprotein oxidase. In addition to methimazole, the isolated flavoprotein also catalyzes the oxidation of other thioureylenes (Table 4). The concentration of the different thioureylenes required for half-maximal velocity ($[S]_{1/2}$) is in the micromolar range and at infinite substrate concentration all of the thioureylenes are oxidized at approximately the same rate. The oxidation products of the different thioureylenes were not characterized, but the stoichiometry of the reaction with phenylthiourea or naphthylthiourea as substrate was identical

TABLE 4. KINETIC CONSTANTS FOR SULFUR-CONTAINING SUBSTRATES OF THE ISOLATED FLAVOPROTEIN OXIDASE*

Substrate	$[S]_{1/2}$ ($M \times 10^6$)	V_m
Thioureylenes		
Methimazole	13	890
1-Naphthyl(2-thiourea)	4	747
Phenylthiourea	3	811
Sulfides		
Thioglycerol	4900	721
Dithiothreotol	465	685
Disulfide		
trans-4,5-Dihydroxy-*o*-dithiane	270	739

* Constants were calculated from double reciprocal plots of substrate concentration vs velocity. Velocities are expressed as nmoles substrate-dependent NADPH oxidized/min/mg enzyme at 38°, pH 8·4.

with that obtained for methimazole. In addition to the thioureylenes listed in Table 4, pentothal, propylthiourea, thiourea and propylthiouracil are also substrates for the flavoprotein oxidase, but the kinetic constants ($[S]_{1/2}$, V_m) were not determined for these four compounds.

As also shown in Table 4, the flavoprotein appears to catalyze the oxidation of dithiothreotol and its cyclic disulfide. The $[S]_{1/2}$ for these substrates is, however, in the millimolar range and their V_m is somewhat less than values obtained with the thioureylenes. The oxidation products formed during the enzymic oxidation of the thiols or the disulfide were not determined. The high $[S]_{1/2}$ of these substrates also precludes determining the stoichiometry of these reactions by the methods used with methimazole.

DISCUSSION

The data in this report demonstrate that a flavoprotein mixed-function oxidase isolated from pig liver microsomes can catalyze the NADPH-dependent oxidation of thioureylenes by oxygen. The oxidation products obtained with methimazole suggest that the thioureylene is oxidized to the sulfinate and then hydrolyzed to sulfite and *N*-methylimidazole. The formation of the sulfinate requires incorporation of two atoms of oxygen and the addition of the second oxygen could occur by disproportionation or enzymically, as shown by the reactions in Fig. 3 (sequence A or B respectively). From the data available, there is no definitive way of distinguishing between

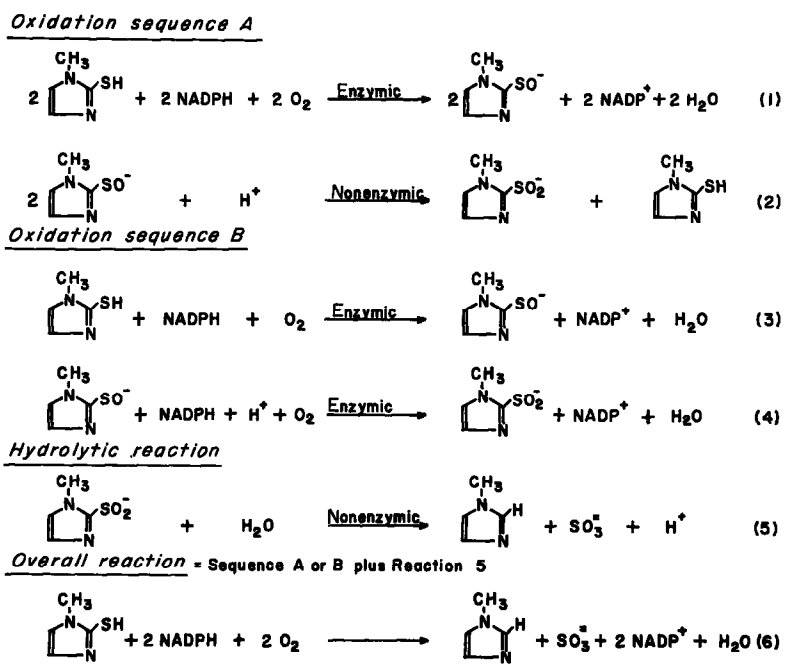

FIG. 3. Proposed alternate reactions for the oxidation of methimazole by oxygen catalyzed by the flavoprotein oxidase. In sequence A, there is an enzymic (1) and a nonenzymic (2) reaction in the formation of the sulfinate; whereas in sequence B, both steps (3,4) from methimazole to the sulfinate are enzymic. The sulfinate formed by either route is hydrolyzed (4) to the same products and the stoichiometry of the overall reaction (6) is the same for either sequence A or B.

these alternate routes for the incorporation of the second oxygen. However, if the sulfenate is enzymically oxidized to the sulfinate, it must be as good a substrate for the oxidase or better than methimazole. since there is no detectable break in the oxygen uptake curve with limiting methimazole (Fig. 1A). Such a low $[S]_{1/2}$ for the sulfenate would be unexpected, since amines with a polar group within a two-carbon radius of the atom oxidized are very poor substrates for the flavoprotein oxidase,[4] and it is unlikely that the flavoprotein can catalyze the oxidation of a substrate as polar as the sulfenate. On the other hand, the curves in Fig. 1 indicate that if the second oxygen is added by disproportionation of 2 moles of the sulfenate the rate of this reaction must be faster than the rate of sulfenate formation catalyzed by the oxidase. Data on the rate of disproportionation of methimazole sulfenate are not available, but the rapid disproportionation of other sulfenates has been described.[12]

The relatively rapid rates of thioureylene oxidation catalyzed by the isolated or membrane-bound flavoprotein (Table 2) suggest that this oxidase may contribute to the metabolism of these compounds *in vivo*. If this is the case, metabolism of thioureylenes in the liver may be much greater than previously suspected, since studies[13,14] on the tissue distribution of the flavoprotein oxidase have shown that it is concentrated in liver tissue, with smaller but detectable amounts present in lung and intestinal mucosa. Despite repeated attempts in this laboratory, the presence of this oxidase could not be demonstrated in homogenates or microsomes isolated from porcine thyroids. If this oxidase is present in thyroids, its concentration is below the limits of the current methods used to detect this enzyme. It is, of course, quite possible that the thyroid thioureylene oxidase is different from that of liver and additional studies will be necessary to clarify this point.

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