Table 2. Net changes in glycerol and FFA

	Glycerol (μmol/hr/g)	FFA (μmol/hr/g)
Adipose tissue		
Control	0.31 ± 0.2	0.21 ± 0.3
POCA-treated	0.99 ± 0.43*	0.51 ± 0.63
	nmol/hr/10^5 cells	
Adipocytes		
No addition	8.0 ± 5.3△	
POCA 0.05 mM	13.0 ± 5.3	

Measurements were made in the medium, adipose tissue and adipocytes at the beginning and end (1 hr for fat pads, 4 hr for cells) of the incubations. The glycerol and FFA production calculated as net change in medium + tissue or medium + cells. Values are given as means ± SD for 6 animals for adipose tissue and for 4 different cell batches. * $P < 0.01$; △ significant by paired *t*-test.

not increase the plasma free fatty acid concentration which was 0.54 ± 0.24 mM in the drug-treated animals compared to 0.49 ± 0.14 mM in controls (N = 12). This indicates that either there is no increase in the rate of release of FFA from adipose tissue *in vivo* or that the rate of FFA utilization is increased proportionately. Increased lipolysis would fit with the finding of lower total body fat content of rats fed 0.2% POCA in their diet for 12 weeks [2].

After POCA treatment there was no significant change in the activity of glycerol phosphate acyltransferase in the microsomal fraction of liver (control, 3.9 ± 2.6; POCA-treated, 7.9 ± 2.3; nmol product/min/mg protein, N = 8). The addition of POCA-CoA to the assay of microsomal glycerol phosphate acyltransferase did not alter the enzyme's activity (control, 7.2 ± 2.8; with 0.05 mM POCA-CoA, 6.5 ± 3.1; with 0.1 mM POCA-CoA, 6.1 ± 2.0; nmol product/min/mg protein, N = 4).

The work presented here shows that, compared with controls, feeding 0.2% POCA for 3 nights increased LPL activity in heart and post heparin plasma with no change in adipose tissue enzyme activity, suggesting increased triacylglycerol removal in drug-treated animals. POCA-feeding and incubation of isolated adipocytes with 0.05 mM POCA increased triacylglycerol lipolysis as evidenced by increased glycerol production. Glycerol phosphate acyltransferase activity was not inhibited on incubation with 0.05 mM POCA-CoA showing that the drug does not inhibit all acyl CoA reactions.

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Organosulfur oxygenation and suicide inactivation of lactoperoxidase*

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Our recent report demonstrated the involvement of peroxidases in the oxidative metabolism of organosulfur compounds [1]. Lactoperoxidase (LPX) catalyzed the oxygenation of organosulfide and thioamide functional groups to the respective sulfoxide. The enzymatic reactivity correlated with the peak potential for electrochemical oxidation of the substrates.

These observations provided a clue to the mechanism of thiocarbamide goitrogen-induced inactivation of LPX and the closely related thyroid peroxidase [2]. The results of our subsequent study supported a suicide inactivation mechanism for the action of thiocarbamides on LPX [3].

This communication presents data that extend the range of observations on the S-oxygenation reactions catalyzed by LPX and give additional support to a causal relationship between thiocarbamide oxygenation and suicide inactivation.

Methods

2-Mercaptobenzothiazole (I), obtained from the Pfaltz & Bauer Co., was recrystallized from methanol. 6-Propyl-2-thiouracil (II), obtained from the Sigma Chemical Co., was recrystallized from aqueous ethanol. Phenyldisulfide [4], 2,2′-bis-dithiobenzothiazole (MBTS) [4], phenacyl-phenyl sulfide (PPS) [5], and the corresponding sulfoxide (PPSO) [6] were synthesized according to published procedures. Bovine LPX was obtained from the Sigma Chemi-

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cal Co., the concentration was determined spectrophotometrically [3], and the activity was 3×10^4 moles iodide ion oxidized per mole LPX per min.

Polyacrylamide gel electrophoresis was performed under nondenaturing conditions (11.25% polyacrylamide, pH 4.3), and bands were visualized by either Coomassie blue or peroxidase activity (30 mM guaiacol plus 0.2 mM hydrogen peroxide). The LPX preparation was estimated to be greater than 95% homogenous, and the peroxidase activity was associated with only the major protein band.

Cyclic voltammetry was used to measure the anodic peak potentials ($E_{p,a}$) of I, II, thioanisole (III), thiobenzamide (IV), 5-nitro-2-mercaptobenzimidazole (V), 2-mercaptobenzimidazole (VI) and 1-methyl-2-mercaptoimidazole (VII), employing an $Ag/AgNO_3$ reference electrode [1]. These voltages are neither standard oxidation or reduction potentials but operationally-defined peak potentials derived from the cyclic voltammograms.

The enzyme kinetics of substrate oxygenation or suicide inactivation were determined as previously described [1, 3]. The kinetics of I oxygenation catalyzed by LPX were measured spectrophotometrically at 315 nm and 22°. The product MBTS was determined from dichloromethane extracts of reaction mixtures containing LPX and I (100 ml of pH 7.0 phosphate buffer containing 1 mM I, 25 nM LPX and 1 mM hydrogen peroxide added in five equal aliquots at 6-min intervals). The product was identified by u.v. spectrophotometry and thin-layer chromatography (silica gel, dichloromethane elution) in comparison with authentic synthetic MBTS and by high resolution mass spectrometry (Varian MAT 311; molecular ion: 332 *m/e*; molecular formula as determined by exhaustive computer matching: C:14, H:8, N:2, S:4 as required for MBTS).

The products of an incubation of 0.5 mg/ml LPX with 1.0 mM PPS and 0.2 mM hydrogen peroxide for 2 hr at 0° were quantitated by HPLC (5 micron Novapak 8 mm ×·10 cm cartridge in a Z-module, Waters Associates; elution with 40% acetonitrile/water for PPSO and 80% acetonitrile/water for phenyldisulfide at a flow rate of 1.5 ml/min and detection at 246 nm).

Results and discussion

The sole product from the oxidation of I by LPX in the presence of hydrogen peroxide was determined to be MBTS, and Scheme 1 shows the proposed S-oxygenation mechanism. The action of LPX on the therapeutic antithyroid agent II produced suicide inactivation in agreement with our previous results from other thiocarbamide goitrogens [3].

Substrate oxygenation and suicide inactivation reactions of LPX are linked by a common linear dependence of the apparent binding constants derived from steady-state enzyme kinetics (K_m for substrates or K_i for inhibitors) with the peak potentials for electrochemical oxidation of the respective organosulfur compound. This dependence holds over a range of four orders of magnitude of the apparent binding constants in Fig. 1.

The linear correlation between the free energy-related parameter ($E_{p,a}$) and the apparent binding constants (K_m or K_i) constitutes an empirically derived linear free energy relation [7]. This relationship between organosulfur $E_{p,a}$ and the apparent enzyme binding constant is consistent

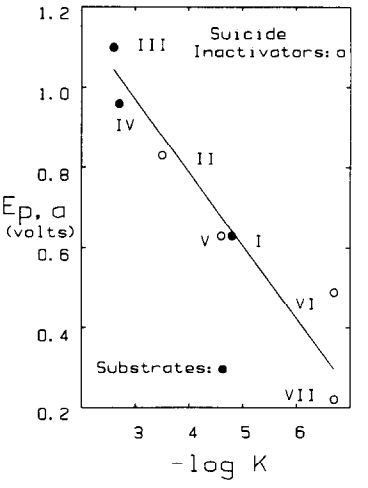
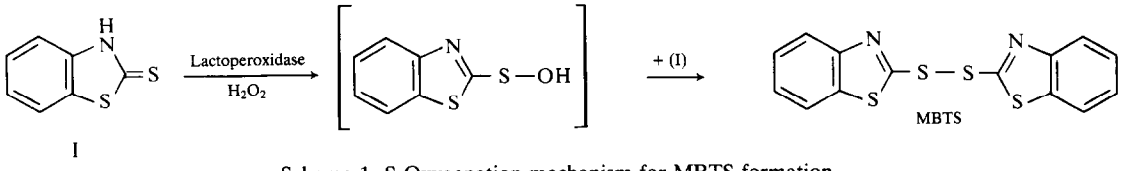


Fig. 1. Linear free energy relationship between the electrochemical oxidation potentials of organosulfur compounds and the apparent LPX binding constants.

with an interaction of the sulfur-containing compound with an electron-deficient oxo-ferryl heme intermediate formed by the action of hydrogen peroxide on LPX as previously proposed [1, 3]. The correlation strongly suggests a common mechanism of LPX-catalyzed S-oxygenation for both substrates and suicide inactivators and supports the previous hypothesis that reactive products derived from thiocarbamides are responsible for active site heme modification and LPX inactivation [3].

PPS was chosen as an organosulfide substrate containing acidic alpha-protons to test for the presence of a sulfur cation-radical intermediate during LPX-catalyzed S-oxygenation. Oae and coworkers [8] showed that proton loss from intermediates of this type is facile and leads to S-dealkylation products in cytochrome P-450-catalyzed reactions. When PPS was incubated with LPX and hydrogen peroxide, the products of the reaction were PPSO (the S-oxygenation product) and phenyldisulfide (the stable S-dealkylation product derived from thiophenol under aerobic conditions) in a ratio of 1.1 to 1 equivalents respectively (see Scheme 2). This result provides support for the previously proposed electron transfer mechanism of LPX-catalyzed S-oxygenation and is consistent with the linear free energy relationship shown in Fig. 1. However, incubation of III with LPX and hydrogen peroxide gave only thioanisole sulfoxide, and no phenyldisulfide was detected. It is therefore likely that, in the absence of acidic alpha-protons, S-oxygenation is the preferred pathway for LPX catalysis.

Our understanding of the S-oxygenation and suicide inactivation reactions of LPX was extended in this study. A linear free energy relationship was observed between the apparent enzyme binding constant and the electrochemical oxidation potential of LPX substrates and inactivators. The



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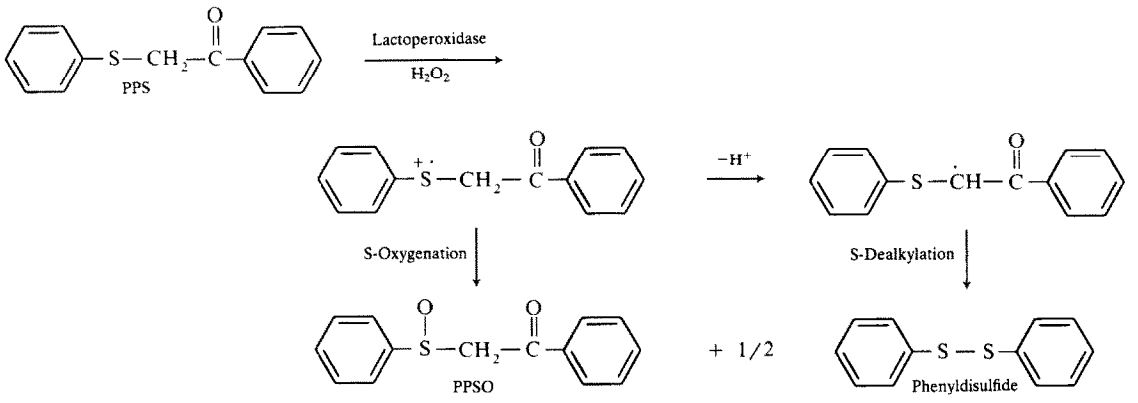
Scheme 1. S-Oxygenation mechanism for MBTS formation.



Scheme 2. S-Oxygenation and S-dealkylation reactions of PPS.

presence of a one-electron S-oxidized intermediate was inferred from the use of an organosulfide substrate containing acidic alpha-protons. These results provide support for a common S-oxygenation mechanism for LPX substrates and inactivators and are consistent with the formation of reactive S-oxygenated products by the action of LPX on thiocarbamide goitrogens [3].

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Effect of histamine on $^{45}Ca^{2+}$ uptake in rat brain synaptosomes

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Ca^{2+} is regarded as an intracellular messenger involved in coupling stimulus to response in a wide variety of biological reactions [1]. Among other functions, it is well established that calcium ions play a central role in the stimulus–neurosecretion coupling [2, 3]. It has been shown that Ca^{2+} is accumulated by nerve terminals during depolarization and this Ca^{2+} entry subsequently results in neurotransmitter (NT) release [4, 5]. Although the regulatory mechanisms involved in the neurosecretory process are still largely unknown, evidence has been provided that at least certain NT or neuromodulators exert their control on this process by regulating Ca^{2+} fluxes into nerve terminals, thereby increasing or restricting the availability of Ca^{2+} necessary for the release process [5–8].

Accumulating evidence strongly suggests that histamine (HA) acts as a NT in the CNS [9]. HA has been shown to either inhibit its own release [10] or to stimulate that of catecholamines [11, 12] and serotonin [13] in the mammalian brain. The mechanism by which HA regulates NT release has yet to be elucidated. Indirect evidence indicates, however, that it is a Ca^{2+}-dependent process [10, 12]. With this insight, we studied, as a preliminary approach, the effect of HA on the uptake of $^{45}Ca^{2+}$ into rat brain synaptosomes.

Materials and methods

Materials. $^{45}CaCl_2$ (10–40 mCi/mg calcium) was purchased from Amersham International. Histamine, mepyr-