*Free Rad. Res.*, Vol. 27(5), pp. 501–504
Reprints available directly from the publisher
Photocopying permitted by license only



# A Peroxidase-catalyzed Sulfoxidation of Promethazine

LAURO GALZIGNA*, MARIA P. SCHIAPPELLI, MARINA SCARPA and ADELIO RIGO

*Department of Biological Chemistry and Institute of Laboratory Medicine, University of Padua, Via Trieste, 75, 35121 Padova, Italy, Department of Physics, University of Trento, Italy*

Accepted by Prof. M. G. Grisham

*(Received 12 February 1997; In revised form 25 July 1997)*

Lactoperoxidase, when incubated with increasing amounts of promethazine (P) and promethazine sulfoxide (PO) catalyzes the formation of promethazine sulfoxide accompanied by oxygen consumption. An intermediate radical of PO can be detected by electron spin resonance (ESR). Catalase or superoxide dismutase do not inhibit the reaction while dopamine does. The lactoperoxidase-catalyzed formation of dopaminochrome in the presence of hydrogen peroxide is inhibited by P. Both P and PO inhibit acetyl- and butyrylcholinesterase. Purified enzymes were used throughout the study and horseradish peroxidase but not myeloperoxidase had an activity similar to that of lactoperoxidase.

*Keywords:* Lactoperoxidase, promethazine sulfoxide, dopamine, acetylcholinesterase, butyrylcholinesterase

*Abbreviations:* Promethazine P, promethazine sulfoxide PO, electron spin resonance ESR

## INTRODUCTION

In a previous paper[1] we have described the sulfoxidation of promethazine (P) catalyzed by horseradish peroxidase. The sulfoxide, hitherto considered as the main catabolite of phenothiazines,[2] was devoid of several actions associated with the parent compound but, rather unexpectedly, maintained the neuroleptic action. We hypothesized that such an action was mostly associated to a radical species which might originate from both the sulfoxide and its parent phenothiazine.

We have investigated the possibility that promethazine sulfoxide (PO) acts as an oxidizing agent, or as a co-oxidant, in a peroxidase-catalyzed reaction.

## MATERIALS AND METHODS

A Beckman DU-640 recording spectrophotometer (Epsom FX 850 recorder), an oxygraph with a Clark's electrode and an ESR Bruker 200-D spectrometer were used.

All reagents and purified enzymes were purchased from Sigma Chem. Co. (St. Louis, MO)

* Corresponding author. Tel.: Fax.: +39 49—8276142.

and were used without further purification. PO was chemically synthesized as described.[1]

An ordinary incubation mixture contained in a final volume of 1 ml, thermostatized at 25°C, 0.1 M acetate buffer pH 5.5, 100 $\mu$M P, 100 $\mu$M PO, 1mM EDTA and 3 U of lactoperoxidase. The blank contained the same except lactoperoxidase. PO formation was measured at 335 nm ($E_m$ of 5,000).

Acetyl- and butyrylcholinesterase activities were measured according to Ellman[3] with acetylthiocholine and butyrylthiocholine as substrates.

## RESULTS AND DISCUSSION

When lactoperoxidase was incubated with P and PO, formation of further PO was observed and quantitated, with concomitant oxygen consumption and disappearance of P. The reaction could also be followed under nitrogen by ESR and a radical intermediate was detected. Figure 1A shows the ESR spectrum of a radical which we suppose is $P^{+\prime}$, together with the kinetics of PO formation and the oxygen consumption (1B).

The reaction has a pH optimum between 4 and 8. At pH 5.5 an apparent $K_m$ of 127 $\mu$M was found for P and an apparent $K_m$ of 41 $\mu$M was found for PO.

The formation of PO was linear with lactoperoxidase concentration from 0 to 5 U.

The sulfoxidation was unaffected by superoxide dismutase (10 $\mu$g) and catalase (10 $\mu$g).

P sulfoxidation was also inhibited by dopamine ($K_i$ of 3 $\mu$M), while dopaminochrome formation catalyzed by lactoperoxidase in the presence of hydrogen peroxide (followed at 475 nm in 0.1M phosphate buffer at pH 7.5) was inhibited by P ($K_i$ of 300 $\mu$M) and slightly activated by PO.

The following mechanism, to be confirmed by further experiments, may be comptible with our observations:

$$2PO + 2P + 4H^+ \rightarrow 4P^{+\prime} + 2H_2O$$
$$4P^{+\prime} + O_2 + 4H^+ \rightarrow 4P^{2+} + 2H_2O$$
$$4P^2 + 4H_2O \rightarrow 4PO + 8H^+$$

Overall: $2P + O_2 \rightarrow 2PO$

The rapid formation of low amounts of a colored intermediate ($P^{2+}$) was also observed, while no significant pH change could be documented in the course of the reaction.

The interaction of PO with lactoperoxidase was checked spectroscopically and the hypochromic effect together with the red shift of the Soret band document the formation of a complex I between PO and the enzyme (Figure 2). This is in agreement with the monoelectronic oxidation of P proposed in the scheme.

The sulfoxidation of P under these conditions was catalyzed also by horseradish peroxidase, while myeloperoxidase appeared to be uneffective. P inhibits both purified acetyl- and butyrylcholinesterase ($K_i$ of 55 $\mu$M and 3.5) and PO has an even stronger inhibitory action ($K_i$ of 25 $\mu$M and 2.4 $\mu$M respectively).

The anticholinesterase effects shown for different phenothiazines[4] may therefore increase after sulfoxidation. The clinical importance of the peroxidase-catalyzed sulfoxidation is related to the pharmacological effect of P which, as any other phenothiazine, has strong antioxidant and photosensitizing properties. On the other hand, PO appears to act as an oxidizing agent, or as a co-oxidant, replacing hydrogen peroxide when a phenothiazine is the acceptor, in the presence of peroxidase.

The effect of P and PO on the lactoperoxidase-catalyzed oxidation of dopamine is an interesting property, in view of the implication of dopamine in neurotoxicity[5,6] and the dopaminergic cell death involved in Parkinson's disease or other central nervous system alterations. In particular, Parkinson's disease affects the pigmented cells of *substantia nigra* in conditions of low defence against oxidative stress.[7] Since dopaminochrome has neurotoxic properties, the

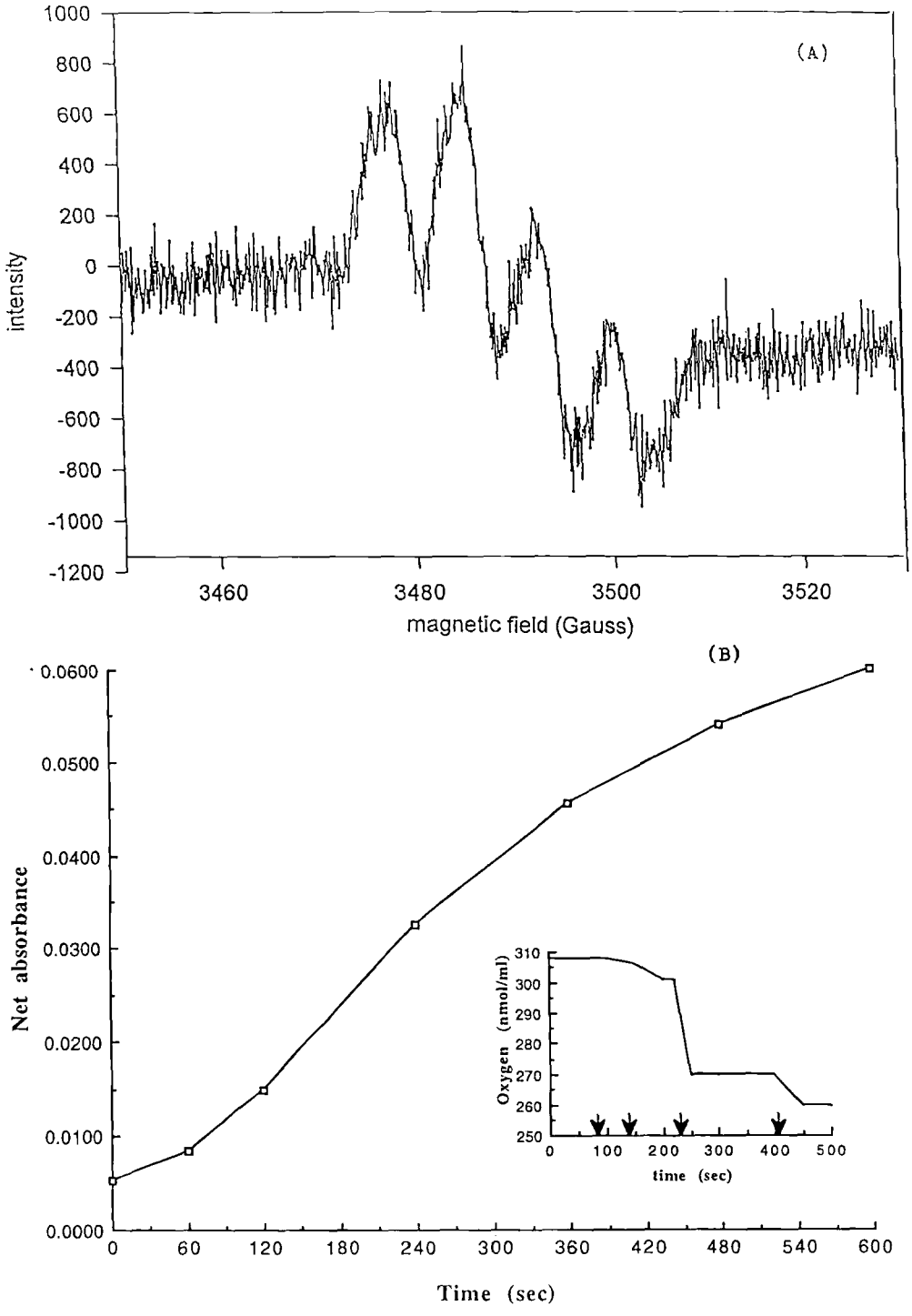


FIGURE 1 (**A**) Typical ESR spectrum obtained by incubating in 0.1 M acetate buffer pH 4.5 lactoperoxidase (3U) with 0.8 mM promethazine, 0.8 mM promethazine sulfoxide, 1 mM EDTA. Central field 3487 G; sweep time 1000 s; modulation field 20 mGpp; microwave power 20 mW; microwave frequency 9.82 GHz. The PO formation documented spectrophotometrically under the same conditions was 4–15 nmoles/min/ml and oxygen consumption was 2–8 nmoles/min/ml. (**B**) Kinetics of PO formation followed spectrophotometrically at 335 nm. In the inset, oxygen consumption measured with the oxygraph. The vertical arrows indicate the additions of P, lactoperoxidase, and PO in that order. A second addition of PO corresponds to a lower oxygen consumption. Conditions are those described in the Materials and Methods section.

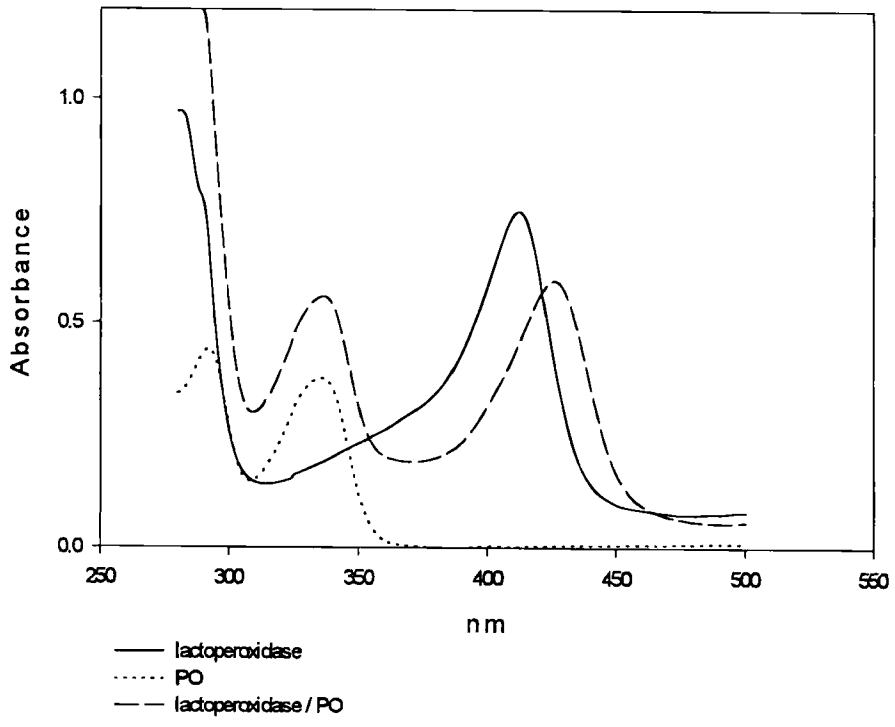


FIGURE 2 0.1 mM lactoperoxidase in 0.1 M acetate buffer pH 5.5 was reacted with a stoichiometric amount of PO. The spectra of lactoperoxidase, PO and the lactoperoxidase/PO complex are shown.

inhibition of its formation by P may be relevant to the therapeutic action of the latter at a central level.

## *References*

[1] Galzigna, L., Rizzoli, V., Schiappelli, M. P., Rigobello, M. P., Scarpa, M. and Rigo, A. (1996). Horseradish peroxidase-catalyzed sulfoxidation of promethazine and properties of promethazine sulfoxide. *Free Radical Biology and Medicine*, **20**, 807–811.

[2] Kelder, P. P., de Mol, N. J., Fischer, M. J. and Jenssen, L. H. M. (1994). Kinetic evaluation of the oxidation of phenothiazine derivatives by methemoglobin and horseradish peroxidase in the presence of hydrogen peroxide. Implications for the reaction mechanism. *Biochimica et Biophysica Acta*, **1205**, 230–238.

[3] Ellman, G. L. (1958). A colorimetric method for determining low concentrations of mercaptans. *Archives of Biochemistry and Biophysics*, **74**, 443–450.

[4] Spinedi, A., Pacini, L., Limatola, C., Lully, P. and Farias, H. N. Phenothiazine inhibits acetylcholinesterase by concentration dependent type kinetics. A study with trifluoperazine and perphenazine. *Biochemical Pharmacology*, 192; **44**, 1511–1514.

[5] Hastings, T. G. (1995). Enzymatic oxidation of dopamine. The role of prostaglandin H synthase. *Journal of Neurochemistry*, **64**, 919–924.

[6] Mattammal, M. B., Strong, R., Lakshmi, V. M., Chung, H. D. and Stephenson, A. H. Prostaglandin H synthetase-mediated metabolism of dopamine. Implication for Parkinson's disease. *Journal of Neurochemistry*, 19; **64**, 164–1654.

[7] Smythies, J. (1996). On the function of neuromelanin. *Proceedings of the Royal Society of London Series B.*, **263**, 487–489.