Biochemical Pharmacology, Vol. 27, pp. 805–806. Pergamon Press, 1978. Printed in Great Britain.

Effect of mitochondrial lipid peroxidation on monoamine oxidase

(*Received* 14 *March* 1977; *accepted* 10 *June* 1977)

Studies using partially purified monoamine oxidase (MAO) have shown that oxidized oleic acid inhibits the enzyme [1]. It was found that after incubation of MAO with the oxidized fatty acid the quantity of thiol groups decreased and a peroxide–enzyme thiol group interaction was suggested as the mode of inhibition [2]. In addition, it was shown that MAO bound to mitochondria was also inhibited by oxidized oleic acid. However, high concentrations of the oxidized fatty acid as high as 0.01 M were needed to obtain inhibition possibly due to the presence of other mitochondrial proteins which have been shown to interact with peroxides [3]. Since mitochondria contain substantial quantities of unsaturated fatty acids that can be oxidized to form peroxides [4], the present work was performed to investigate ferrous ion-induced mitochondrial lipid peroxidation and its effect on MAO activity.

Rat liver mitochondria were isolated as described by Hunter *et al.* [5]. The lipid peroxide content was determined as described by Hunter *et al.* [4]. In plotting lipid peroxide formation, the absorbance of the 2-thiobarbituric acid (TBA) color formed was graphed directly rather than converting to malonaldehyde equivalents. Peroxidation experiments were performed essentially as described by McKnight *et al.* [6] except that an incubation volume of 600 ml was used containing 0.2 mg protein/ml. Incubations were carried out at 4° and, in some experiments, 0.2 mg/ml of BSA or 0.01 M mercaptoethanol was present. After 60 min EDTA was added to give a final concentration of 50 μM and the suspensions were centrifuged at 10,000 *g* for 10 min. The pellet was suspended in 50 mM sodium phosphate buffer, pH 7.4, and assayed for protein [7], phospholipid [8] and thiol content [9].

MAO activity was determined by incubating 50 μl of enzyme preparation (0.1 to 2.5 mg protein/ml), 100 μl of 0.1 M phosphate buffer, pH 7.4, containing 2 mM EDTA and 100 μl of either tyramine, serotonin or benzylamine. The final substrate concentration was 1 mM containing 25 nCi [^{14}C]tyramine (55 mCi/m-mole, New England Nuclear), 50 nCi [^{14}C]serotonin (55 mCi/m-mole, New England Nuclear) or 50 nCi [^{14}C]benzylamine (5.6 mCi/m-mole, Mallinckrodt Chemical Works). Incubations were performed at 37° in a shaking water bath for 20 min. At the end of the incubation, 0.3 ml of 2 N HCl was added and the reaction products were extracted and counted as described by Wurtman and Axelrod [10].

In Fig. 1, the accumulation of malonaldehyde, a measure of the lipid peroxide content [11], and the decrease in thiol groups are compared as a function of time during treatment of mitochondria with ferrous ions. Up to 45 min of incubation, there was an increase in lipid peroxide formed and a 40 per cent decrease in the relative thiol content, after which plateaus were reached. The loss in reactive thiol groups was expected, as it has been known for some time that lipid peroxides react with thiol groups [12]. However, it was surprising that similar results, as shown in Fig. 1, were obtained when incubations were performed at room temperature, as it has been shown that the reaction of peroxides with thiol groups decreases with decreasing temperature [12]. Increasing the ferrous ammonium sulfate concentration to 50 μM had no effect on the total amount of peroxide formed, suggesting that at 20 μM this agent has induced the production of the maximum amount of peroxides.

At the end of the incubations, the suspensions were centrifuged at 10,000 *g* for 10 min, and Table 1 shows the effect of lipid peroxidation on the protein, phospholipid and thiol content of the pellets. As compared to controls, the pellets after lipid peroxidation lost 67 per cent of the protein and 47 per cent of the phospholipids. This finding is in agreement with the results obtained by McKnight *et al.* [6]. In addition, the ferrous ion-treated pellet lost 46 per cent of the reactive thiol groups/mg of protein. The presence of mercaptoethanol, which had no effect on the production of lipid peroxides, did not protect the thiol groups from reaction with peroxides, although recovery of phospholipid increased to 61 per cent. When peroxidation experiments were carried out in the presence of BSA, the rate and extent of peroxide formation were unaffected. However, the recovery of protein, phospholipid and thiol groups increased to 40, 63 and 63 per cent respectively.

Table 1 shows the effect of lipid peroxidation on the recovery of MAO activity. After centrifugation, no MAO activity was detected in the supernatant. In the pellet, lipid peroxidation reduced the MAO activity by 28, 23 and 28 per cent when tyramine, serotonin and benzylamine were used as substrates respectively. When mercaptoethanol was present during lipid peroxidation, no significant difference was observed in the recovery of enzyme activity. In contrast, Rapava *et al.* [1] found that MAO in the presence

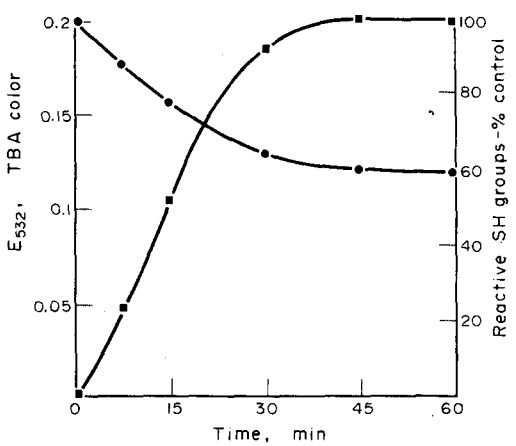

Fig. 1. Production of lipid peroxides and decrease in protein thiol groups during ferrous ion-induced lipid peroxidation. Mitochondrial suspensions (0.2 mg protein/ml) were incubated at 4° with 20 μM ferrous ammonium sulfate (see text). At the times indicated, samples were removed and assayed for lipid peroxides, measured as 2-thiobarbituric acid color produced (■) and thiol content (●). Each point on the graph is the mean of three determinations. Individual assays for peroxide and thiol content varied by less than 3 and 6 per cent respectively.

Table 1. Mitochondrial recovery of protein, phospholipid, thiol content and MAO activity after lipid peroxidation*

Mitochondrial content and MAO activity	Per cent recovery after additions during Fe^{2+}-induced lipid peroxidation		
	Fe^{2+}	Mercaptoethanol (0.01 M) $+Fe^{2+}$	BSA (0.2 mg/ml) $+Fe^{2+}$
Protein (% total mg)	33 ± 5 ($P < 0.01$)	33 ± 6 ($P < 0.01$)	40 ± 6 ($P < 0.01$)
Phospholipid (P) (% P/mg protein)	53 ± 6 ($P < 0.01$)	61 ± 5 ($P < 0.01$)	63 ± 4 ($P < 0.01$)
Thiol content (SH) (% SH/mg protein)	54 ± 7 ($P < 0.01$)	48 ± 7 ($P < 0.01$)	63 ± 3 ($P < 0.01$)
MAO substrates			
Tyramine	72 ± 3 ($P < 0.01$)	72 ± 7 ($P < 0.05$)	100 ± 11 (NS)
Serotonin	77 ± 11 ($P < 0.01$)	77 ± 10 ($P < 0.01$)	95 ± 6 (NS)
Benzylamine	72 ± 7 ($P < 0.01$)	69 ± 11 ($P < 0.01$)	85 ± 8 ($P < 0.01$)

* Mitochondrial suspensions were incubated at 4° with 20 μM ferrous ammonium sulfate for 60 min and then centrifuged at 10,000 *g* for 10 min (see text). The pellets were resuspended in phosphate buffer, pH 7.4, and assayed for protein, phospholipid, thiol content and MAO activity. Results shown are per cent of control ± standard deviation values obtained from three separate experiments, each carried out in duplicate. Control values have been set at 100 per cent. NS indicates not significant.

of glutathione was partially protected from inhibition by oxidized oleic acid. When lipid peroxidation was performed in the presence of BSA, no significant tyramine or serotonin MAO activity was lost, whereas MAO activity toward benzylamine was reduced by 15 per cent.

It has been shown that MAO contains thiol groups [13], and although BSA largely protects MAO activity from the inactivation observed by lipid peroxidation and reduced the loss of thiol groups by 9 per cent, the present results do not conclusively show that thiol–peroxide interactions are responsible for the loss of MAO activity. Mercaptoethanol, a thiol-protecting agent, was not helpful in elucidating this mode of inhibition, as peroxidation in the presence of mercaptoethanol did not prevent the loss of either thiol groups or MAO activity. Furthermore, Gomes *et al.* [14] found that beef liver MAO retained 70–80 per cent of its activity after stepwise addition of *p*-chloromercuribenzoate in amounts sufficient enough to react with all thiol groups. If the same holds true for the rat liver enzyme, then a peroxide–thiol interaction may not result in the loss of MAO activity. It is known that peroxides react with other protein constituents besides thiol groups [15, 16] and these reactions could account for the loss of MAO activity observed.

When peroxidation was performed in the presence of BSA, it seems likely that some of the peroxides reacted with the BSA rather than mitochondrial protein thus increasing the recovery of protein and phospholipid in the pellets by 7 and 10 per cent, respectively, and largely protecting MAO from inactivation (Table 1). Although peroxidation may have released a phospholipid or protein factor that may be required for MAO activity, addition of supernatant after peroxidation and centrifugation to the enzyme assay did not result in any increase in enzyme activity (results not shown). The finding that BSA greatly protects MAO activity from inactivation by lipid peroxidation is in agreement with the results of Rapava *et al.* [1].

The present results do show that MAO, a marker for the outer mitochondrial membrane [17], is relatively resistant to inactivation by ferrous ion-induced peroxidation. In comparison, peroxidation has been shown to inactivate succinate dehydrogenase, an inner mitochondrial membrane marker, to a similar extent as MAO in the present work, whereas glutamate dehydrogenase, a mitochondrial matrix marker, retained all of its original activity [3]. In the same report, isocitrate dehydrogenase was completely inactivated and 3-hydroxybutyrate dehydrogenase lost 80 per cent of its activity during peroxidation.

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REFERENCES

1. E. A. Rapava, L. B. Klyashtorin and V. Z. Gorkin, *Biokhimiya* **31**, 1047 (1966).
2. I. V. Veryovkina, M. M. Abdel Samed and V. Z. Gorkin, *Biochim. biophys. Acta* **258**, 56 (1972).
3. R. C. McKnight and F. E. Hunter, Jr., *J. biol. Chem.* **241**, 2757 (1966).
4. F. E. Hunter, Jr., J. M. Jebicki, P. E. Hoffsten J. Weinstein and A. Schneider, *J. biol. Chem.* **238**, 828 (1963).
5. F. E. Hunter, R. Malison, W. F. Bridges, B. Schutz and A. Atchison, *J. biol. Chem.* **234**, 693 (1959).
6. R. C. McKnight, F. E. Hunter, Jr and W. H. Oehlert, *J. biol. Chem.* **240**, 3429 (1965).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. R. K. Raheja, C. Kaur, A. Singh and I. S. Bhatia, *J. Lipid Res.* **14**, 695 (1973).
9. A. F. S. A. Habeeb, *Meth. Enzym.* **25**, 457 (1972).
10. R. J. Wurtman and J. Axelrod, *Biochem. Pharmac.* **12**, 1439 (1963).
11. K. M. Wilbur, F. Bernheim and O. W. Shapiro, *Archs Biochem.* **24**, 305 (1949).
12. S. E. Lewis and E. D. Wills, *Biochem. Pharmac.* **11**, 901 (1962).
13. L. B. Klyashtorin and L. I. Gridneva, *Biokhimiya* **31**, 716 (1966).
14. B. Gomes, G. Naguwa, H. G. Kloepfer and K. T. Yasunobu, *Archs Biochem. Biophys.* **132**, 28 (1969).
15. I. D. Desai and A. L. Toppel, *J. Lipid Res.* **4**, 204 (1963).
16. A. L. Toppel, *Fedn Proc.* **24**, 73 (1965).
17. C. Schnaitman, V. G. Erwin and J. W. Greenawalt, *J. Cell Biol.* **32**, 719 (1967).