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Oxygenation of mitochondrial membranes by the reticulocyte lipoxygenase. Action on monoamine oxidase activities A and B *

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Incubation of isolated rat liver mitochondria with the pure rabbit reticulocyte lipoxygenase caused a time-dependent inactivation of the monoamine oxidase activities A and B. Furthermore, a conversion of the monoamine oxidase into a diamine oxidase was observed. The inactivation kinetics for both monoamine oxidase activities A and B showed a biphasic behaviour; a reversible short-term inhibition during the first 5 min of incubation was followed by an irreversible inactivation of the enzyme. The kinetic studies suggest that the slow irreversible inactivation of the monoamine oxidase activities is due to secondary reactions subsequent to the initial attack of the lipoxygenase on the mitochondrial outer membrane. During the interaction of the lipoxygenase with the mitochondria, only about 1.5% of the polyenoic fatty acids present in the mitochondrial membranes were oxygenated. The predominant products formed during the interaction of the lipoxygenase with the mitochondrial membranes are (13*S*)-hydro(pero)xy-9*Z*,11*E*-octadecadienoic acid and (15*S*)-hydro(pero)xy-5,8,11,13(*Z*,*Z*,*Z*,*E*)-eicosatetraenoic acid.

Introduction

Lipoxygenases oxygenate polyunsaturated fatty acids containing a (1*Z*,4*Z*)-pentadiene system to their corresponding hydroperoxy derivatives [1]. Most plant and animal lipoxygenases preferentially attack free polyenoic fatty acids [2]. The intracellular action of most animal lipoxygenases is proposed to be controlled by the liberation of the polyenoic fatty acids from membrane-bound phospholipids, in particular by the action of a phospholipase A_2 [3]. In contrast, the lipoxygenase of rabbit reticulocytes effectively attacks phospholipids and biological membranes without preceding action of a phospholipase [4,5]. The oxygenation of mitochondrial membranes in the intact reticulocytes was proposed to be an essential step in the breakdown of these organelles during the maturation of red blood cells [6]. During the reaction with rat liver mitochondria the lipoxygenase acts on both the inner and outer mitochondrial membrane as indicated by an inactivation of respiratory enzymes localized in the mitochondrial inner membrane [7,8] and by a destruction of the iron-sulfur clusters of the mitochondrial outer membrane [9]. Moreover, drastic changes in the passive electric membrane properties were observed [10].

The monoamine oxidase which is localized in the mitochondrial outer membrane requires an intact phospholipid environment for its catalytical function [11,12]. It has been shown before that non-enzymatic lipid peroxidation causes a strong inhibition of the monoamine oxidase. Mechanistic studies suggest that the oxidative modification of the phospholipid environment of the enzyme was responsible for the inactivation [13].

Here we report that the oxygenation of rat liver mitochondria by the pure rabbit reticulocyte lipoxygenase is accompanied by an inhibition of the monoamine oxidase activities A and B and by a conversion to a diamine oxidase activity. The oxygenation products formed during the interaction of the lipoxygenase with rat liver mitochondria were identified.

Materials and Methods

Chemicals. The chemicals used were from the following sources. Hydroxytryptamine creatine sulfate, pargy-

* Dedicated to Prof. H. Funder on the occasion of his 70th birthday.

Abbreviations: 13-HOD, 13-hydroxy-9,11-(*Z*,*E*)-octadecadienoic acid; 15-HETE-15-hydroxy-5,8,11,13-(*Z*,*Z*,*Z*,*E*)-eicosatetraenoic acid; RP-HPLC, reversed-phase high-pressure liquid chromatography; SP-HPLC, straight-phase high-pressure liquid chromatography; 1 nkat, 1 nmol/s.

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line, sodium borohydride, and β-phenylethylamine from FERAK (F.R.G.), benzylamine was from Merck (F.R.G.), putrescine, soybean lipoxygenase (grade IV), α-D,L-tocopherol and linoleic acid were from Serva (F.R.G.), salicylhydroxamic acid was from VEB Pharmazeutisches Werk Oranienburg, sodium cholate was from Reanal (Hungary), KCN was from FERAK (F.R.G.). All solvents used were of analytical grade and distilled prior to use.

Preparations. The reticulocyte lipoxygenase was purified to homogeneity as described in Ref. 14. Peak fractions of the isoelectric focussing were used. Rat liver mitochondrial membranes were prepared according to Ref. 15. The lipoxygenase products formed during the incubation of rat liver mitochondrial membranes with the reticulocyte lipoxygenase were prepared as follows: after incubation of the enzyme with the mitochondria in 0.1 M phosphate buffer, (pH 7.4) for 1 or 20 min, the hydroperoxy fatty acids formed were reduced by addition of sodium borohydride. The membrane lipids were extracted [16], hydrolyzed under alkaline conditions and the resulting free fatty acid derivatives were analyzed by RP- and SP-HPLC. Authentic standards of (15*S*)-HETE and (13*S*)-HOD were prepared with the soybean lipoxygenase [17]. Racemic standards of 15 (*R/S*)-HETE and 13(*R/S*)-HOD were obtained by vitamin E-supported autooxidation of methyl arachidonate and methyl linoleate, respectively, and subsequent HPLC purification.

Assays. The monoamine oxidase activity of the rat liver mitochondrial membranes was assayed oxygraphically with a Clark-type oxygen electrode: the suspension of rat liver mitochondria was diluted with phosphate buffer (pH 7.4) to adjust a protein concentration of 2.5 mg/ml. Endogenous respiration was inhibited by addition of 2 mM KCN. The monoamine oxidase reaction was started by addition of 1 mM 5-hydroxytryptamine (MAO-A) and 1 mM benzylamine or 1 mM β-phenylethylamine (MAO-B). After preincubation of the mitochondria with monoamine oxidase inhibitors, no increase in the oxygen uptake was observed after addition of monoamine oxidase substrates. These data indicate that the oxygen uptake measured was due entirely to the action of the monoamine oxidase, but not to non-enzymatic reactions. To assay the diamine oxidase activity, 10 mM putrescine was used as substrate.

Lipoxygenase activity was assayed oxygraphically using sodium linoleate as substrate [18]. Salicylhydroxamic acid (0.1 mM) was used as lipoxygenase inhibitor.

Analytics. HPLC was performed on a Shimadzu instrument coupled with a Hewlett-Packard diode array detector 1040 A. RP-HPLC was carried out on a Nucleosil C-18 column (Macherey-Nagel, KS-system, 250 × 4 mm, 5 μm particle size) with a pre-column (30 × 4 mm, 5 μm particle size). A solvent system of methanol/water/acetic acid (85/15/0.1, v/v) and a flow rate of 1 ml/min was used. For detection of the conjugated dienoic fatty acids, the absorbance at 235 nm was recorded. SP-HPLC was performed on a Zorbax-SIL column (250 × 4.6 mm, 5 μm particle size) with a solvent system of *n*-hexane/isopropanol/acetic acid (100/2/0.1, v/v) and a flow rate of 1 ml/min. The absorbances at 235 nm (detection of lipoxygenase products) and 205 nm (detection of polyenoic fatty acids) were recorded. The chromatogrammes were quantified by peak area. Calibration curves (five point measurements) for 13-HOD, arachidonic acid and linoleic acid were established. Compounds were generally identified by coinjection with authentic standards.

To quantify the oxidative modification of the membranes, the hydroxy fatty acid/polyenoic fatty acid ratio was determined. This ratio was calculated from the content of linoleic acid, arachidonic acid, 13-HOD and 15-HETE.

Chiral phase-HPLC [19] was performed on a Baker-bond (*R*)-dinitrobenzoylphenylglycine column (250 × 4.6 mm; 5 μm particle size; covalently linked chiral phase) with the solvent system *n*-hexane/isopropanol (100/0.5, v/v). The absolute configuration of the chiral centres was determined by coinjections with racemic and chiral standards.

Miscellaneous. The fatty acid derivatives were methylated by treating with diazomethane at room temperature for 15 min. For quantification of the chromatogrammes a molar absorption coefficient of 23,000 $M \cdot cm^{-1}$ for the conjugated dienes was used [20]. Protein concentration was determined according to Ref. 21.

Results

Characterization of the monoamine oxidase activities A and B in rat liver mitochondria

Monoamine oxidase activities of rat liver mitochondria were assayed oxygraphically using a Clark-type oxygen electrode. Endogenous respiration of the mitochondria suspension was excluded by addition of 0.1 M KCN and the monoamine oxidase reaction was started by the addition of 1 mM hydroxytryptamine and 1 mM benzylamine as substrate for monoamine oxidase activities A and B, respectively (Fig. 1). From the oxygraphic traces specific activities of 86.5 pkat/mg mitochondrial protein and 72.2 pkat/mg mitochondrial protein for the monoamine oxidase activities A and B, respectively, were calculated. These values are two orders of magnitude higher than those reported for bovine heart mitochondria [13].

Both activities A and B of the monoamine oxidase show a linear dependence on the amount of the mitochondrial protein added to the assay mixture in range between 0.5 and 6 mg/ml. Similar behaviour has been reported for the monoamine oxidase B activity of rat

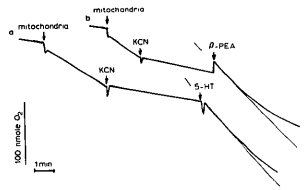

Fig. 1. Measurement of the monoamine oxidase activities of rat liver mitochondria with a Clark-type oxygen electrode. 6.6 mg mitochondrial protein were preincubated in phosphate buffer for 4 min. After the inhibition of the endogenous respiration of the mitochondria with 2 mM KCN, the reaction of the monoamine oxidase was started by addition of the substrate. For assay of the monoamine oxidase A, 1 mM hydroxytryptamine was used (trace a), for the assay of the monoamine oxidase B (trace b) 1 mM benzylamine was used. Measurements were carried out at 37 °C; the assay volume was 2 ml.

liver mitochondria when assayed spectrophotometrically [22].

Preincubation of the mitochondria with the suicidal monoamine oxidase inhibitor pargyline [23] strongly inhibited the monoamine oxidase of rat liver mitochondria (Table I). A 10 min preincubation proved to be necessary to obtain maximal and irreversible inactivation of the enzyme [24].

Action of the pure reticulocyte lipoxygenase on the monoamine oxidase activities of rat liver mitochondria

In order to investigate whether the oxygenation of rat liver mitochondria by the reticulocyte lipoxygenase causes an inhibition of the monoamine oxidase, the mitochondria were preincubated with the pure lipoxygenase. After a preincubation period of 10 min, the lipoxygenase reaction was stopped by addition of the lipoxygenase inhibitor salicylhydroxamic acid. Afterwards, the monoamine oxidase reaction was started by adding hydroxytryptamine or benzylamine. Salicyl-

TABLE I

Inhibition of the monoamine oxidase activities A and B by pargyline

Rat liver mitochondria (2 mg mitochondrial protein/ml) were preincubated with 0.1 mM pargyline for 10 min. The monoamine oxidase activities were assayed as described in the legend to Fig. 1. The control incubations were performed without addition of pargyline.

	Monoamine oxidase activity (pkat/mg protein)		Inhibition (%)
	without pargyline	with pargyline	
Monoamine oxidase A	61.2	2.2	97
Monoamine oxidase B	78.0	0	100

TABLE II

Inhibition by pargyline of the residual monoamine oxidase activity B after treatment of the mitochondria with small amounts of the reticulocyte lipoxygenase

4 mg mitochondrial protein were incubated with 6.0 nkat lipoxygenase (LOX) at 37 °C. The lipoxygenase reaction was stopped with 1 mM salicylhydroxamic acid (SHAM). Afterwards the sample was incubated for 10 min with or without pargyline. To measure the residual monoamine oxidase activity 0.1 mM benzylamine was added. Similar values were obtained for the monoamine oxidase activity A.

Sample	Monoamine oxidase activity B (pkat/mg protein)	Inhibition
Mitochondria	56.0	0
Mitochondria + pargyline	0	100
Mitochondria + SHAM	55.7	0.6
Mitochondria + LOX	40.0	28.6
Mitochondria + LOX + pargyline	5.5	90.2

hydroxamic acid was chosen as lipoxygenase inhibitor because it does not influence the monoamine oxidase activities of the mitochondria (Table II). Incubation of rat liver mitochondria with the pure reticulocyte lipoxygenase led to inhibition of both monoamine oxidase activities A and B (Table II). Preincubation of the lipoxygenase with the lipoxygenase inhibitor salicylhydroxamic acid protected the monoamine oxidase activities from inactivation (not shown). The residual oxygen consumption remaining after treatment of the mitochondria with small amounts of lipoxygenase was inhibited by pargyline indicating that it was due to the monoamine oxidase rather than to oxygen-consuming secondary reactions initiated by the lipoxygenase (Table II).

The inhibition of the monoamine oxidase by the lipoxygenase-catalyzed oxygenation of the mitochondrial membranes showed biphasic kinetics (Fig. 2). Short-term incubation (1–5 min) of the mitochondria with the lipoxygenase led to a strong but reversible inhibition of the monoamine oxidase activities A and B. Long-term incubation (10 min or longer) led to an irreversible inactivation of both monoamine oxidase activities. In additional experiments, we preincubated the rat liver mitochondria with the lipoxygenase for 1 min. Then the lipoxygenase activity was stopped by addition of salicylhydroxamic acid, and, after different time periods, aliquots were taken off and the activities of the monoamine oxidases A and B were assayed. In these experiments, biphasic inactivation kinetics of the monoamine oxidase activities were also observed. Further addition of lipoxygenase after 20 min did not lead to an increase in the inhibition of the monoamine oxidase activities despite the fact that further oxygenation of membrane-bound lipids had taken place, as indicated by the analysis of the lipoxygenase products (not shown). These data indicate that for inactivation of

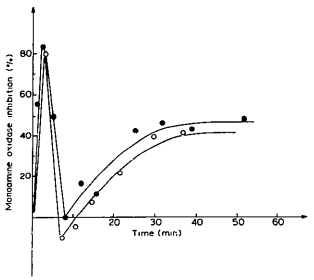

Fig. 2. Inhibition of the monoamine oxidase activities A and B by the action of the reticulocyte lipoxygenase. Rat liver mitochondria (2 mg mitochondrial protein/ml) were incubated with the pure reticulocyte lipoxygenase (0.94 nkat/ml mitochondrial protein) at 37 °C in the assay chamber. After the times indicated, the lipoxygenase reaction was stopped by addition of 1 mM salicylhydroxamic acid. Simultaneously, 2 mM KCN was added to inhibit respiration of the mitochondria. This took about 15 s. Subsequently, the monoamine oxidase reaction was started by addition hydroxytryptamine (1 mM) or benzylamine (1 mM). From the oxygen uptake during the first 20 s of the reaction, the initial rate was determined.

the monoamine oxidase, an active lipoxygenase is necessary only at the very beginning of the reaction. During this time, the inactivation of the monoamine oxidase is rather small. The irreversible inactivation per se seems not to require an active lipoxygenase. Therefore the lipoxygenase reaction might be regarded as initiator for the inactivation of the monoamine oxidase activities. The typical inactivation kinetics, however, are proposed to be due to lipoxygenase-initiated secondary reactions most probably to decomposition processes of the hydroperoxy lipids formed during the lipoxygenase reaction.

The extent of inhibition of the monoamine oxidase by the reticulocyte lipoxygenase depends on the amount of the lipoxygenase added. As shown in Fig. 3A, the reversible short-term inhibition and the irreversible long-term inactivation (Fig. 3B) show a similar curve except for a higher slope of the curves for the short-term inhibition. For the short-term inhibition, a lipoxygenase concentration necessary for a half-maximal inhibition of 0.55 nkat/ml mitochondrial protein for monoamine oxidase activity A and 1.0 nkat/ml for the monoamine oxidase activity B were determined. These data indicate that monoamine oxidase activity A is somewhat more sensitive to inhibition by the reticulocyte lipoxygenase than the monoamine oxidase activity B. For the long-term inactivation, a half-inhibition concentration of 3 nkat/mg mitochondrial protein for both activities was determined.

Conversion of the monoamine oxidase activity to a diamine oxidase activity during the lipoxygenation of the mitochondrial membranes

The monoamine oxidase of rat liver mitochondria is known to exhibit a high substrate specificity for monoamine substrates. However, after exposure to non-enzymatic lipid peroxidation the enzyme also oxygenates diamines such as histamine or putrescine [25]. We confirmed these findings using an enzymatic lipid peroxidating system. After incubation of rat liver mitochondria with large amounts of lipoxygenase (20 nkat/mg protein) a diamine oxidase activity of 12–30 nkat/mg protein with 10 mM putrescine [26] as substrate was measured. Control incubations without lipoxygenase did not reveal any measurable diamine oxidase activity (data not shown).

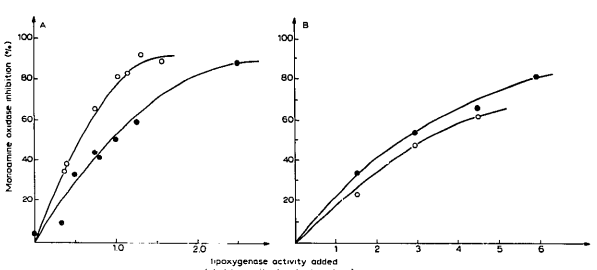

Fig. 3. Dependence of the inhibition of the monoamine oxidase on the amount of the lipoxygenase added. Different amounts of the lipoxygenase were incubated with 4.5 mg mitochondrial protein for 2 min (A) and 30 min (B) at 37 °C. The monoamine oxidase activities A and B were assayed as described in the legend to Fig. 1.

Identification of the lipoxygenase product

The oxygenation products formed by the lipoxygenase reaction were isolated as described in Material and Methods and analyzed by RP-HPLC recording the ultraviolet absorbance at 235 nm (detection of the lipoxygenase products) and at 205 nm (detection of the polyenoic fatty acids). For quantification, the hydroxy polyenoic fatty acid/linoleic acid ratio was calculated. The results shown in Table III indicate that during the first minute of the reaction, about 703 ng lipoxygenase products per incubation sample were formed. A hydroxy fatty acid/polyenoic fatty acid ratio of 0.016 was calculated indicating that only a small percentage of the polyenoic fatty acids were oxygenated under the conditions of the short-term inhibition of the monoamine oxidase activities. In control incubations (without addition of lipoxygenase) only traces of hydroxy polyenoic fatty acids were detected, which most probably originate from non-enzymatic lipid peroxidation during the preparation of the mitochondria or the work-up procedure of the lipids. If the lipoxygenase reaction with the mitochondrial membranes was stopped after 1 min by the addition of salicylhydroxamic acid, but the incubation was continued over a period of 20 min, the long-term inactivation of the monoamine oxidase activities were observed (Fig. 2). Under these conditions, only 415 ng lipoxygenase product per incubation sample was detected. A hydroxy fatty acid/polyenoic fatty acid ratio of 0.011 was calculated. These data indicate a decrease in the content of hydroxy fatty acids during the incubation after having stopped the lipoxygenase reaction. It is proposed that processes decomposing hydroperoxy lipids, most probably iron-catalyzed hydroperoxidase reactions, are responsible for these changes. It is well known that the mitochondrial membranes contain iron as iron-sulfur clusters [27]. The iron of the outer membrane was shown to be released during the interaction of the reticulocyte lipoxygenase with rat liver mitochondria [9] and may act as a catalyst for hydroperoxidase-like reactions.

TABLE III

Hydroxy polyenoic fatty acid content in rat liver mitochondrial membranes oxygenated by the reticulocyte lipoxygenase

Rat liver mitochondria were incubated with the reticulocyte lipoxygenase at 37 °C for 1 min. The hydroperoxy lipids were reduced with borohydride and the lipids were extracted according to Ref. 16 and hydrolyzed under alkaline conditions. The hydrolyzed lipid extracts were analyzed by RP-HPLC with a solvent system of methanol/water/acetic acid (85/15/0.1, v/v) and a flow rate of 1 ml/min. The absorbances at 205 and 235 nm were recorded simultaneously in order to detect the polyenoic fatty acids and the hydroxy polyenoic fatty acids, respectively. The compounds were quantified by the peak areas. Calibration curves for linoleic acid, arachidonic acid and (13*S*)-hydroxy-9*Z*,11*E*-octadecadienoic acid were established.

Sample	Hydroxy fatty acids (ng)	Hydroxy fatty acid/polyenoic fatty acid ratio
Mitochondria	34	0.08
Mitochondria + 15 nkat lipoxygenase	703	1.60
Mitochondria + 15 nkat lipoxygenase [a]	415	1.10

[a] After a 1 min incubation of the mitochondria with the lipoxygenase, the oxygenation reaction was stopped by addition of 1 mM salicylhydroxamic acid, reduction of the hydroperoxy lipids and lipid extraction were performed 20 min later.

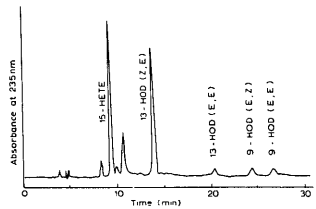

Fig. 4. SP-HPLC of the products formed during the reaction of the reticulocyte lipoxygenase with rat liver mitochondria. Rat liver mitochondria were incubated with the pure reticulocyte lipoxygenase (6 nkat/mg mitochondrial protein). After 10 min, the reaction was stopped by addition of sodium borohydride, the lipids were extracted, hydrolyzed under alkaline conditions and analyzed by RP-HPLC on a C-18 Macherey and Nagel column with a solvent system of methanol/water/acetic acid (85/15/0.1, v/v). Since, under the conditions described, most of the hydroxy fatty acids were not separated, the material absorbing at 235 nm was prepared and further analyzed by SP-HPLC on a Zorbax-SIL column (250 × 4.6 mm, 5 μm particle size). Solvent system: *n*-hexane/isopropanol/acetic acid (100/2/0.1, v/v); flow rate, 1 ml/min. The absorbance at 235 nm was recorded.

In order to obtain more detailed information on the lipoxygenase products formed under the conditions of inactivation of the monoamine oxidase, SP-HPLC of the hydroxy fatty acids isolated by RP-HPLC were performed. As shown in Fig. 4, 15-HETE and 13-HOD are the main products formed. Chiral phase HPLC indicated the predominant formation of the L_S-isomer (optical purity higher than 90%). Small amounts of other lipoxygenase products (9-HOD, all-E isomers of 13- and 9-HOD) were identified by chiral phase HPLC as racemic mixtures. Beside these main products we detected three isomers of keto polyenoic fatty acids which amount to about one-fifth of the hydroxy compounds (not shown). The chemical structure of these products has not been studied in detail, but the ultraviolet spectra with its characteristic maximum of strong absorbance at 270 nm and the co-migration in SP-HPLC with authentic standards of 9- and 13-ketooctadecadienoic acid suggest ketooctadecadienoic acids. Such products have been described before as decomposition products of hydroperoxy fatty acids formed via enzymatic and non-enzymatic hydroperoxidase reac-

tions. The occurrence of these products indicates secondary reactions decomposing hydroperoxy lipids.

Discussion

The pure reticulocyte lipoxygenase effectively oxygenates mitochondrial membranes leading to irreversible inactivation of both NADH- and succinate oxidase activities [7,10] and to a reversible inhibition of the cytochrome *c* oxidase [8]. The cytochrome *c* oxidase is known to require an intact phospholipid environment for its enzymatic activity [27]. It was suggested that the oxygenation of the lipid component of the enzyme by the lipoxygenase was responsible for the inactivation. These data prompted us to check whether the monoamine oxidase as an enzyme of the mitochondrial outer membrane, which also requires a native phospholipid environment for its activity, is also inactivated by the reticulocyte lipoxygenase. The data presented here indicate that the lipoxygenase-catalyzed oxygenation of rat liver mitochondria leads to an inactivation of both activities of the monoamine oxidase.

The kinetics of the inactivation of the monoamine oxidase revealed an initial reversible phase followed by an irreversible inactivation of the enzyme. The reasons for these biphasic kinetics are not well understood. It might be speculated that the initial reversible inhibition of the monoamine oxidase activities is due to a change of the phospholipid environment owing to an oxygenation of polyunsaturated phospholipids. Substitution of oxidized phospholipids in the lipid environment of the monoamine oxidase for non-oxygenated species via lateral diffusion and/or leaflet flip-flop might be responsible for the reactivation of the enzyme which occur between 5 and 10 min of incubation. During this time, no further formation of hydroperoxy lipids via the lipoxygenase reaction was observed. The irreversible inactivation of the monoamine oxidase might be due to an oxidative modification of the protein itself, perhaps caused by radical intermediates originating from secondary decomposition of the hydroperoxy phospholipids formed during the lipoxygenase reaction. Several lines of experimental evidence support this assumption.

(i) The lipoxygenase reaction itself does not lead to an irreversible inactivation of the monoamine oxidase; rather, it acts as initiator for the inactivation.

(ii) Once the lipoxygenase reaction has taken place the presence of an enzymatically active lipoxygenase is no longer necessary, as judged from the addition of lipoxygenase inhibitors.

(iii) After having stopped the lipoxygenase reaction, a non-enzymatic decomposition process of the hydroperoxy lipids takes place, as evidenced by the decrease in the hydroxy polyenoic fatty acid content and by the appearance of decomposition products, such as ketodienoic fatty acids.

The decomposition of lipohydroperoxides via hydroperoxidase reactions involves the formation of radical intermediates [29]. These radicals may cause oxidative modification of amino acid residues [30,31] of the enzyme and of the loss of the enzymatic activities.

It has been shown before that the changes in the substrate specificity of the pure monoamine oxidase exposed to lipid peroxidation were due to an oxidation of cysteine residues of the enzyme [25]. The change in the substrate specificity reported here might be due to the same. However, since our experiments were carried out with intact mitochondria, the changes in the substrate specificity might also be caused by oxidative modifications of the phospholipid environment of the enzyme.

It has been suggested before that the inactivation of the respiratory enzymes, in particular of the NADH- and succinate oxidase activities is not caused by the oxygenation of the membrane-bound lipids but rather by processes connected with a secondary decomposition of the hydroperoxy compounds [10], possibly via a hydroperoxidase reaction catalyzed by the lipoxygenase. A similar mechanism is proposed for the long-term inactivation of the monoamine oxidase. Under anaerobic conditions, lipoxygenases exhibit a hydroperoxidase activity converting hydroperoxy lipids to secondary products, such as ketodienes, epoxy hydroxy compounds, fatty acid dimers and pentane [1,2]. For the reticulocyte enzyme, absolute anaerobiosis is not a necessary precondition for the hydroperoxidase activity. In model studies it has been shown [32] that this activity can already be detected if the oxygen tension drops below 25 μM. In some of the experiments described here, this critical oxygen concentration was reached. The formation of ketodienoic fatty acids indicate that hydroperoxidase reactions took place during the incubation of the lipoxygenase with the mitochondrial membranes. These data suggest that the lipoxygenase-catalyzed hydroperoxidase reactions might be involved in the inactivation of the monoamine oxidase.

In the present and foregoing studies, the reticulocyte lipoxygenase proved to be a valuable tool for studying the role of lipid peroxidation in the alteration of membrane-bound enzyme systems. In this respect, this lipoxygenase is a useful alternative to non-enzymatic methods to induce lipid peroxidation. An important advantage of the use of the reticulocyte lipoxygenase is the possibility of differentiation between primary and secondary lipid peroxidation products by means of chiral phase-HPLC analysis of the enantiomeric composition of the hydro(pero)xypolyenoic fatty acid residues found.

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