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MOUSE HEPATIC MICROSOMAL OXIDATION OF ALIPHATIC ALDEHYDES (C_6 to C_{11}) TO CARBOXYLIC ACIDS

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SUMMARY: Addition of saturated and α, β-unsaturated aliphatic aldehydes (C_6 to C_{11}) significantly increased NADPH oxidation with mouse hepatic microsomes, and the aldehydes themselves were oxidized to the corresponding carboxylic acids. When these aldehyde substrates were incubated similarly under oxygen-18 gas and the carboxylic acids formed were analyzed by GC-MS after methylation, it was indicated that oxygen-18 was significantly incorporated into the carboxylic acids formed from α, β-unsaturated aldehydes, but not significantly into the carboxylic acids formed from saturated aldehydes. These results indicate that enzyme and/or mechanism responsible for the oxidation of these two types of aldehydes is different from each other.

Saturated and α, β-unsaturated aliphatic aldehydes have been regarded as the lipid peroxidation products of microsomal membranes (1-3). Some of these aldehydes have the adverse effects on the biological system (4-6), and exert mutagenic activity (7,8). It is well known that these toxic aldehydes are metabolized to nontoxic alcohols or carboxylic acids by alcohol dehydrogenase or aldehyde dehydrogenase (3,9), which is mainly localized in the cytosolic or the mitochondrial fraction, respectively.

Our previous studies demonstrated that hepatic microsomal aldehyde oxygenase (MALDO), which is a particular form of cytochrome P450, catalyzes the oxidation of various aldehydes to the corresponding carboxylic acids (10-12).

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Hepatic microsomal fractions also contain membrane bound aldehyde dehydrogenases, which are reported to catalyze the oxidation of aldehydic products of lipid peroxidation (13).

The present communication describes hepatic microsomal oxidation of saturated and α, β-unsaturated aliphatic aldehydes having medium chain length of eight to eleven carbons.

MATERIALS AND METHODS

1-Octanal, 1-nonanal, 1-decanal and 1-undecanal were purchased from Wako Pure Chem. Ind., Ltd.; 2-octenal and 2-nonenal were from Aldrich Chem. Co. Inc.; 2-decenal and 2-undecenal were from Tokyo Kasei Kogyo Co., Ltd.; NADP and glucose-6-phosphate were from Boehringer Mannheim GmbH.; glucose-6-phosphate dehydrogenase (type V) was from Sigma Chem. Co. and oxygen-18 gas (97 atom%) was from Amersham International plc. Other chemicals used were of analytical reagent grade. Hepatic microsomes from male ddN mice (30 to 35 g) were prepared by the different centrifugation force described previously (14). Microsomal NADPH oxidation was assayed by the previous method (15). A typical incubation mixture for the microsomal oxidation of aldehyde substrates consisted of washed microsomes (0.4 g liver equivalent), 0.5 mM NADP, 10 mM glucose-6-phosphate, 10 mM $MgCl_2$, 1.2 units glucose-6-phosphate dehydrogenase, 1 mg aldehyde substrate and 100 mM sodium-potassium phosphate buffer (pH 7.4) to make a final volume of 1 ml. The mixture was incubated at 37 ℃ for 30 min under oxygen-18 gas, and the carboxylic acid metabolites formed were analyzed by GC-MS as described previously (11). GC-MS was performed with a JEOL GCG-06 gas chromatograph coupled with a JEOL JMS-DX-300 mass spectrometer and a JEOL JMA-DA 5000 mass data system. The conditions were as follows: column, 2% OV-17 on Chromosorb W (60-80 mesh, 3 mm x 2 m); column temperature 120 ℃ for 1-octanal, 2-octenal, 1-nonanal and 2-nonenal as substrates or 130 ℃ for 1-decanal, 2-decenal, 1-undecanal and 2-undecenal as substrates; carrier gas, He 40 ml/min; ionization energy, 70 eV; ionizing current, 300 μA. Under these conditions, the retention times of methyl ester of the carboxylic acids were 1.9 min (1-octanal), 2.9 min (2-octenal), 3.2 min (1-nonanal), 3.4 min (1-decanal), 4.7 min (2-nonenal), 5.1 min (2-decenal), 5.8 min (1-undecanal) and 8.7 min (2-undecenal).

RESULTS AND DISCUSSION

All aldehyde substrates except for 1-undecanal significantly stimulated NADPH oxidation with hepatic microsomes (Table I). The enhancement of the NADPH oxidation by these aldehydes may be partially due to their effects on the NADPH-dependent electron transport system involving cytochrome P450 by which these aldehydes could be metabolized. Hepatic microsomal fractions contain other NADPH-dependent enzyme responsible for metabolism of carbonyl compounds, carbonyl reductase (16), which may also contribute to the stimulation of the NADPH oxidation by the aldehyde substrates.

Table I. Effects of Aliphatic Aldehydes on NADPH Oxidation in Mouse Hepatic Microsomes

Saturated aldehydes			α, β-Unsaturated aldehydes		
Additions	NADPH oxidation (nmol/min/mg)	% of control	Additions	NADPH oxidation (nmol/min/mg)	% of control
Control	9.7 ± 0.3	100			
1-Octanal	14.7 ± 0.7 [b]	152	2-Octenal	14.0 ± 0.1 [b]	144
1-Nonanal	12.5 ± 0.2 [b]	129	2-Nonenal	14.3 ± 0.1 [b]	147
1-Decanal	11.5 ± 0.1 [b]	119	2-Decenal	12.7 ± 0.2 [b]	131
1-Undecanal	10.3 ± 0.3	106	2-Undecenal	11.8 ± 0.4 [a]	122

Values represent the mean ± S.E. of three experiments.
Aldehydes added to the incubation mixture were 4 mM.
[a] Significantly different from control ($p < 0.05$).
[b] Significantly different from control ($p < 0.01$).

Our previous studies demonstrated that MALDO catalyzed the oxidation of aromatic aldehydes (9-anthraldehyde, cuminaldehyde and veratrum aldehyde), α, β-unsaturated alicyclic aldehydes (myrtenal and perillaldehyde) and an aliphatic aldehyde having aromatic ring (3-phenylpropionaldehyde)(11). Gans and Werringloer reported NADPH-dependent metabolism of acetaldehyde, propionaldehyde and butyraldehyde by rat hepatic microsomes (17). Recently, Terelius et al. reported that rat cytochrome P450 IIE1 metabolized acetaldehyde (18). These findings suggest that aliphatic aldehydes may be common substrates for MALDO.

MALDO which catalyzes oxidation of aldehydes to carboxylic acids could be confirmed by measuring the incorporation of molecular oxygen into the carboxylic acids. Figure 1 shows the mass chromatograms and mass spectra of methyl esters of carboxylic acid metabolites formed from 1-octanal and 2-octenal by mouse hepatic microsomes under oxygen-18 gas. Only a single molecular ion at m/z 158 was apparently observed on the mass spectrum of methyl ester of the carboxylic acid formed from 1-octanal, whereas double molecular ions at m/z 156 and m/z 158 were shown for that from 2-octenal. The molecular ion at m/z 158 indicated that molecular oxygen was incorporated into 2-octenoic acid formed from 2-octenal. The relative abundance of the molecular ions at m/z 158 and m/z 160 was 100 : 13 for the metabolite from 1-octanal, and that at m/z 156 and

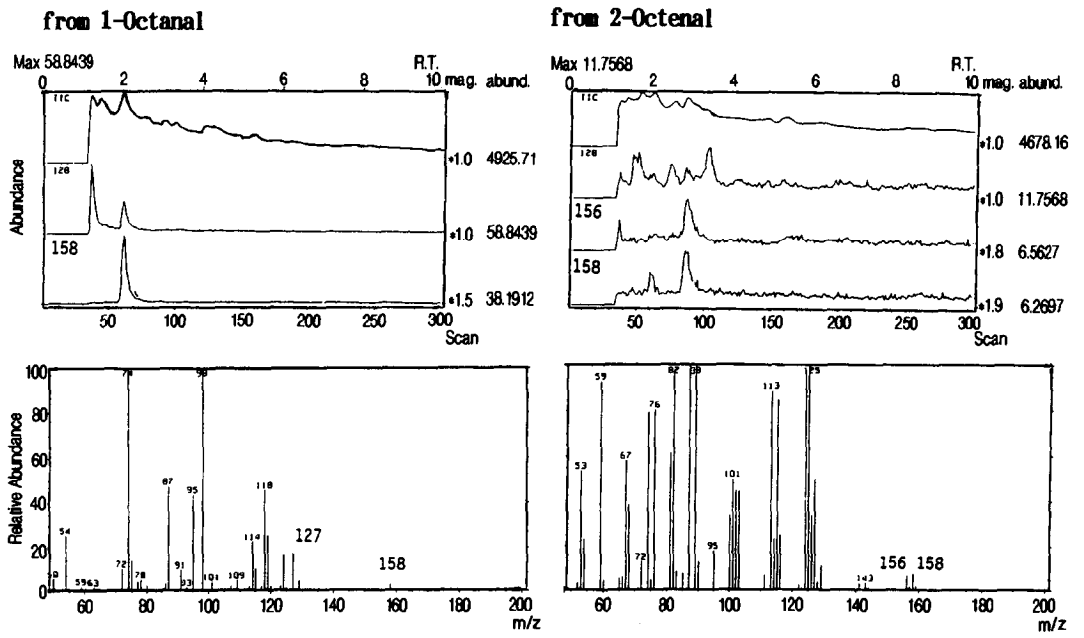

Fig. 1. Mass Chromatograms and Mass Spectra of Methylated Carboxylic Acid Metabolites Derived from 1-Octanal and 2-Octenal by Mouse Hepatic Microsomes under Oxygen-18 Gas.

m/z 158 was 88 : 100 for the metabolite from 2-octenal. The result indicates that 2-octenal is biotransformed to the carboxylic acid by MALDO to some extent, whereas other enzymes may contribute to the biotransformation of 1-octanal in the hepatic microsomes. Other aldehyde substrates were also examined in a similar manner and found to be metabolized to the corresponding carboxylic acid metabolites.

The relative abundance of molecular ions of carboxylates formed under oxygen-18 gas is summarized in Table II. The results indicate that molecular oxygen was significantly incorporated into the carboxylic acid metabolites derived from the α, β-unsaturated aldehydes, but not significantly into the acids derived from the saturated aldehydes.

The present study suggests that the α, β-unsaturated aldehydes could be biotransformed to the corresponding carboxylic acids by MALDO, whereas other enzymes may contribute to the microsomal oxidation of the saturated aldehydes, although the possibility that the reaction mechanism may different from each other could not entirely be ruled out at present. MALDO catalyzing oxidation

Table II. Isotope Ratios in Molecular Ions of Carboxylic Acid Metabolites Formed by Mouse Hepatic Microsomes under Oxygen-18 Gas

Saturated aldehydes		α, β-Unsaturated aldehydes	
Substrates	Relative abundance of M^+ Ions	Substrates	Relative abundance of M^+ Ions
1-Octanal (m/z 158:160)	100 : 13	2-Octenal (m/z 156:158)	88 : 100
1-Nonanal (m/z 172:174)	100 : 37	2-Nonenal (m/z 170:172)	77 : 100
1-Decanal (m/z 186:188)	100 : 5	2-Decenal (m/z 184:186)	63 : 100
1-Undecanal (m/z 200:202)	100 : 4	2-Undecenal (m/z 198:200)	98 : 100

of aliphatic aldehydes is important for understanding the toxicological significance of lipid peroxidation in the microsomal membrane.

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