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On the role of microsomal aldehyde dehydrogenase in metabolism of aldehydic products of lipid peroxidation

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To elucidate a possible role of membrane-bound aldehyde dehydrogenase in the detoxication of aldehydic products of lipd peroxidation, the substrate specificity of the highly purified microsomal enzyme was investigated. The aldehyde dehydrogenase was active with different aliphatic aldehydes including 4-hydroxyalkenals, but did not react with malonic dialdehyde. When Fe/ADP-ascorbate-induced lipid peroxidation of arachidonic acid was carried out in an in vitro system, the formation of products which react with microsomal aldehyde dehydrogenase was observed parallel with malonic dialdehyde accumulation.

Aldehyde dehydrogenase; 4-Hydroxyalk-2-enal; Detoxication; Lipid peroxidation

1. INTRODUCTION

Lipid peroxidation in cellular membranes is accompanied by accumulation of various aldehydic products which include, apart from the well-known malonic dialdehyde, a number of aliphatic aldehydes with chain lengths from 3 to 10 carbon atoms [1]. Among them the most toxic contain a double bond and an OH group such as 4-hydroxyhexenal and 4-hydroxynonenal. There is increasing evidence indicating the important role of 4-hydroxyalkenals in the deteriorative action of lipid peroxidation on cell structure and function [2,3]. Recently, it has been demonstrated that certain isozymes of glutathione *S*-transferase catalyze the reaction of 4-hydroxyalkenals with reduced glutathione [4,5]. Moreover, the toxic aldehydes can be readily reduced to the corresponding alcohols by liver alcohol dehydrogenase [6]. It is suggested that these reactions are responsible for intracellular detoxication of 4-hydroxyalkenals and some other aldehydes. Nevertheless, it should be emphasized that both processes are catalyzed by soluble cytosolic enzymes. At the same time, molecules of the majority of aliphatic aldehydes derived from lipid peroxidation possess hydrophobic properties and may accumulate in the lipid bilayers of membranes [2,3]. We have suggested previously that membrane-bound microsomal and peroxisomal aldehyde dehydrogenases may be implicated in the metabolism of 4-hydroxyalkenals and other lipid-soluble aldehydes [7,8]. Here, we report on the substrate specificity of highly purified aldehyde dehydrogenase from rat liver microsomes using some aldehydes previously identified as products of lipid peroxidation [1].

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2. MATERIALS AND METHODS

Aliphatic and aromatic aldehydes were obtained from Aldrich or Sigma. The 4-hydroxyalkenals were generously provided by Dr H. Esterbauer (Institut für Biochemie, Universität Graz, Graz,

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Austria). Acetaldehyde and propionaldehyde were redistilled before use. The sources of all other reagents were as given in [7,8].

Adult male Wistar rats (200–250 g) were injected i.p. with clofibrate at a dose of 400 mg/kg daily for 10 days. Animals were anesthetized and killed by decapitation after 16–18 h starvation. Livers were perfused with saline to wash out erythrocytes, homogenized and the microsomal fraction isolated as in [7]. The microsomal suspension was resedimented in 50 mM Tris-HCl (pH 7.5) containing 0.15 M KCl. Aldehyde dehydrogenase was purified from microsomes as described by Lindahl and Evces [9]. Electrophoresis in the presence of SDS was performed according to Weber and Osborn [10] using 10% polyacrylamide gels. Aldehyde dehydrogenase activity was determined by following NAD^+ reduction, spectrophotometrically (37°C) or fluorimetrically (25°C) [7]. Some aldehydes were prepared as stock solutions (final added volume 5 μl) in methanol which was without effect on the microsomal aldehyde dehydrogenase. Kinetic constants (K_m and V_{max}) were measured in 50 mM pyrophosphate buffer (pH 8.6), containing 0.5 mM NAD^+ and 0.2% Triton X-100. The reaction was initiated by the addition of aldehydes. Data were analyzed on Lineweaver-Burk plots. Peroxidation of arachidonic acid (Sigma) was performed in 50 mM Tris-HCl (pH 7.5) containing 0.15 M KCl. The suspension (final arachidonic acid concentration 13 mM) was treated by using an ultrasonic disintegrator. Immediately after sonication the suspension was incubated for 30 min at 37°C in the presence of lipid peroxidation initiators (35 μM $FeSO_4$, 1.5 mM ADP, 0.1 mM ascorbic acid). In some cases the reaction was stopped by addition of 0.01% butylated hydroxytoluene. Malondialdehyde formation was measured by means of the thiobarbituric acid assay at

Table 1

Kinetic constants of microsomal aldehyde dehydrogenase for various aldehydes

Aldehydes	K_m (μM)	V_{max} (μmol·min^{-1}·mg protein^{-1})	K_{cat}/K_m ($s^{-1}\cdot\mu M^{-1}$)
Aliphatic aldehydes			
Acetaldehyde (C_2)	1700	2.4	0.004
Propionaldehyde (C_3)	1380	3.5	0.008
Butyraldehyde (C_4)	600	5.4	0.028
Hexanal (C_6)	24	9.7	1.26
Heptanal (C_7)	6.0	9.3	4.83
Octanal (C_8)	6.5	9.3	4.45
Nonanal (C_9)	4.4	9.8	6.94
Decanal (C_{10})	2.6	9.9	11.82
Undecanal (C_{11})	2.5	9.7	12.10
Alkenals			
4-Hydroxynonenal	70	2.3	0.104
4-Hydroxyhexenal	2520	2.4	0.003
Aromatic and other aldehydes			
Phenylacetaldehyde	500	3.9	0.024
Benzaldehyde	730	2.0	0.008
Glutaraldehyde	1130	3.1	0.009

Michaelis constants (K_m) and maximal velocities (V_{max}) were determined from Lineweaver-Burk plots. The K_{cat} values were calculated on the basis of $M_r = 190000$

535–570 nm [11]. Protein was determined by the method of Lowry et al. [12].

3. RESULTS

Microsomal aldehyde dehydrogenase was purified from the livers of rats treated with the hypolipidaemic drug clofibrate. Previously we have shown a 1.5–2.0-fold increase in the enzyme's specific activity in liver microsomes under the action of this drug [7]. After the affinity chromatography step the specific activity of aldehyde dehydrogenase increased 50-fold compared to that in the microsomes. SDS electrophoresis of the final enzyme preparation revealed one major band which constituted about 95% of the total protein content in the polyacrylamide gel. Attempts at additional purification of aldehyde dehydrogenase by gel filtration on Sephadex G-200 led to significant enzyme inactivation and served only for molecular mass estimates. According to our present data microsomal aldehyde dehydrogenase has a molecular mass of 190 kDa.

Table 1 lists data on the substrate specificity of microsomal aldehyde dehydrogenase. It is noteworthy that with aliphatic aldehydes as substrates the increase in size of their molecules is accompanied by a decrease in the K_m values and a rise in V_{max}. The differences were far more discernible when the catalytic efficiency (K_{cat}/K_m) was calculated. This parameter gradually increases 3000-fold in the row from acetaldehyde (C_2) to undecanal (C_{11}). The introduction of an α,β-unsaturated bond and a 4-OH group into the molecules of aliphatic aldehydes remarkably lowers the values of the catalytic efficiency obtained with microsomal aldehyde dehydrogenase. The K_{cat}/K_m value for 4-hydroxynon-2-enal ($0.1\ s^{-1}\cdot\mu M^{-1}$) is about 70-fold lower than that obtained for nonanal. These data can be explained by a decrease in the 4-hydroxyalkenals' hydrophobicity compared to the corresponding alkenals. It should be emphasized that

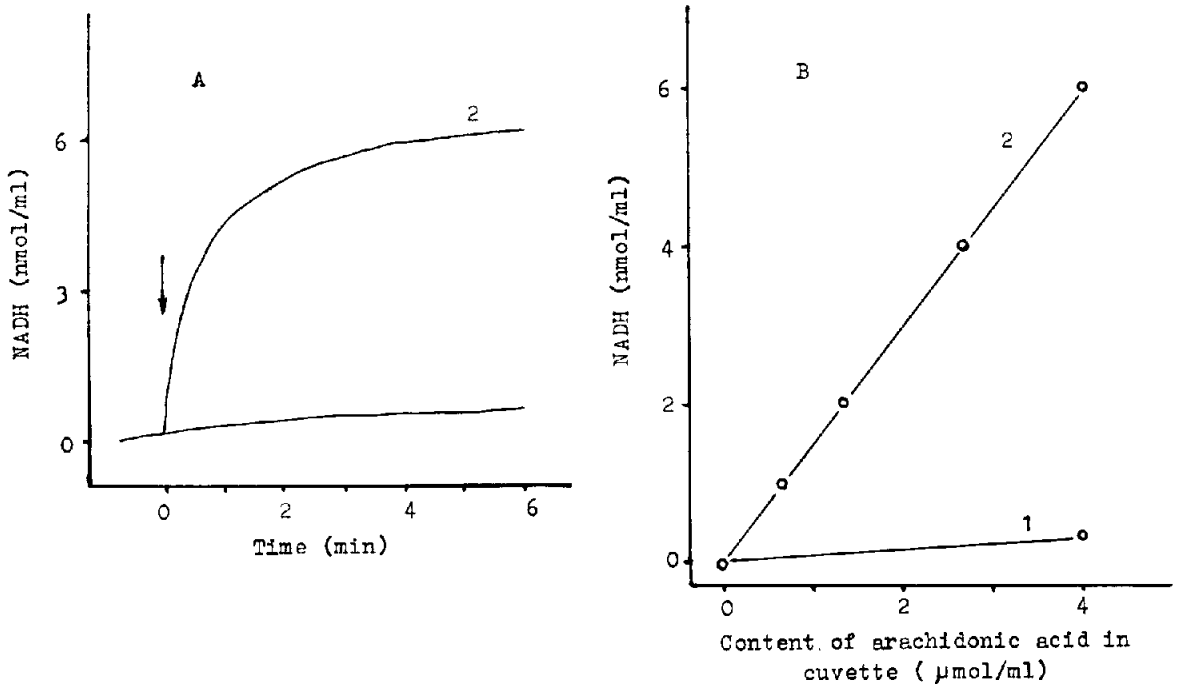

Fig.1. Enzymatic reduction of NAD^+ in the presence of peroxidized arachidonic acid. (A) Assay mixture (1 ml) contained 50 mM pyrophosphate buffer (pH 8.6), 0.5 mM NAD^+, 0.2% Triton X-100 and 4 mM arachidonic acid. The reaction was started by the addition of 5 μg purified microsomal aldehyde dehydrogenase (indicated by the arrow). (B) Relationship between the level of NAD^+ reduction and concentration of peroxidized arachidonic acid. (1) Arachidonic acid before peroxidation, (2) peroxidized arachidonic acid.

4-hydroxynonenal and 4-hydroxyhexenal in the concentration ranges below 1.0 and 4.5 mM, respectively, did not exert inhibitory effects on the purified microsomal enzyme. Apart from the medium-chain aliphatic aldehydes the enzyme also reacts with aromatic aldehydes and glutaric dialdehyde, but the K_{cat}/K_m values for these substrates are very low (table 1). Microsomal aldehyde dehydrogenase was inactive with formaldehyde, glyceraldehyde and malonic dialdehyde.

In in vitro studies we have attempted to elucidate a possible role for aldehyde dehydrogenase in the metabolism of aldehydic products generated in the process of arachidonic acid ($C_{20:4}$) peroxidation (fig.1). The concentration of malonic dialdehyde in the suspension of arachidonic acid rose from 4.7 to 18.9 μM after 30 min incubation with lipid peroxidation initiators (see section 2). At the same time when accumulation of NADH originating from the aldehyde dehydrogenase reaction was traced to assess the content of aldehydic products, the levels registered were 1.3 and 18.5 μM, respectively. Denaturation of the enzyme by heat treatment (100°C, 5 min) prevented reduction of NAD^+. Based on the present results the supposition can be put forward that in vitro microsomal aldehyde dehydrogenase is capable of effective oxidation of aldehydes which, together with malonic dialdehyde, are formed during peroxidation of arachidonic acid.

4. DISCUSSION

The present results suggest that membrane-bound aldehyde dehydrogenases are involved in the metabolism of aldehydic products derived from lipid peroxidation. It is remarkable that aldehydes with apparent hydrophobic properties are the best substrates for the rat liver microsomal enzyme. At the same time, water-soluble aldehydic products of lipid peroxidation, such as propionaldehyde and 4-hydroxyhexenal, are oxidized by microsomal aldehyde dehydrogenase with relatively low catalytic efficiency. The enzyme is also inactive towards malonic dialdehyde. Recently, it has been reported that these aldehydes may be primarily metabolized by the soluble cytosolic and mitochondrial enzymes [13–15]. On the other hand, the known tendency of the lipid-soluble aldehydes to accumulate in the membrane lipid bilayer [2] may render them unavailable for enzymes located in the cytosol. Thus, a certain degree of specialization for the soluble and membrane-bound enzymes in dealing with the metabolism of aliphatic aldehydes can be supposed.

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