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LIPID ALDEHYDE OXIDATION AS A PHYSIOLOGICAL ROLE FOR CLASS 3 ALDEHYDE DEHYDROGENASES

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Abstract—A large number of different unsaturated, saturated and hydroxylated aliphatic aldehydes can be generated during the peroxidation of cellular lipids. This study examined the kinetic properties of purified Class 3 rat aldehyde dehydrogenase (ALDH) with respect to the oxidation of various lipid aldehyde substrates. It also compared the substrate preference of the prototypic Class 3 ALDH with that of the constitutive rat microsomal aldehyde dehydrogenase. The results suggest that (1) microsomal ALDH is a member of the Class 3 aldehyde dehydrogenase family, and (2) the physiological role of the Class 3 ALDHs, including the microsomal form, is the oxidation of medium (6 to 9 carbon) chain length saturated and unsaturated aldehydes generated by the peroxidation of cellular lipids. Short chain aliphatic aldehydes, such as a malondialdehyde and 4-hydroxyalkenals, are not substrates for the Class 3 aldehyde dehydrogenases.

Our laboratories are interested in defining the physiological roles of the various mammalian aldehyde dehydrogenase (ALDH, EC 1.2.1.3) enzymes. It is generally agreed that, based on primary structural data, at least three major classes of ALDHs can be identified in mammalian tissues [1]. Class 1 ALDHs are commonly found in the cytosolic fraction, Class 2 ALDHs are found in the mitochondrial fraction, and Class 3 ALDHs are also cytosolic. Class 1 and 2 forms are generally constitutive, preferring small aliphatic aldehydes as substrates and NAD^+ as cofactor. Both constitutive and xenobiotic-inducible forms of Class 3 ALDH are known, depending on the tissue. Class 3 forms generally show a much broader substrate-oxidizing ability, including aromatic as well as aliphatic aldehydes. *In vitro*, $NADP^+$ is often as effective a cofactor as NAD^+ for Class 3 ALDH.

Lindahl and colleagues have demonstrated that rat Class 3 aldehyde dehydrogenase from a variety of sources, including hepatocellular carcinomas [2], 3-methylcholanthrene- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated livers [2, 3] and rat cornea [4], preferentially oxidizes benzaldehyde-like aromatic and medium chain length (C4 to C9) aliphatic aldehydes. On the basis of these studies, we have hypothesized that Class 3 aldehyde dehydrogenase may play a role in the oxidation of lipid aldehydes, especially those generated by lipid peroxidation. However, none of these studies examined the kinetic parameters for all of the major aldehydic products of lipid peroxidation using highly purified preparations of the Class 3 enzyme.

Mitchell and Petersen [5] have shown that many of the major medium chain length saturated and unsaturated aliphatic aldehydes generated from lipid peroxidation are oxidized by highly purified preparations of a constitutive rat liver microsomal aldehyde dehydrogenase. The kinetic constants indicate that these aldehydes are likely physiological substrates for this particular aldehyde dehydrogenase. It has also been demonstrated that Class 1 and Class 2 rat aldehyde dehydrogenases may oxidize certain lipid aldehydes under physiological conditions [6–8].

The purpose of the present study was to determine which of the major aldehyde products of lipid peroxidation are likely physiological substrates for the Class 3 aldehyde dehydrogenase. The results indicate that Class 3 ALDH may play an important role *in vivo* in the oxidation of saturated and unsaturated 6 to 9 carbon aliphatic aldehydes generated from lipid peroxidation. We also propose, based on the functional and structural similarities between the prototypic Class 3 and microsomal aldehyde dehydrogenases, that the microsomal enzyme is a member of the Class 3 family of ALDHs and that the major physiological role for this entire class of aldehyde dehydrogenase is the oxidation of medium chain length aldehydes generated from lipid metabolism.

MATERIALS AND METHODS

Materials. All commercially available aldehydes used in this study except malondialdehyde were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Malondialdehyde was prepared by hydrolysis of malonaldehyde tetramethyl acetal (Sigma Chemical Co., St. Louis, MO) as described [6]. 4-Hydroxynonenal was synthesized as described [9]. The purity of 4-hydroxynonenal was determined by GC/MS analysis and ^{1}H-NMR spectroscopy prior to use. NAD^+, $NADP^+$, CM-Sepharose 6B and 5′-AMP Sepharose were obtained from Sigma. All other reagents were of the highest purity available.

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Preparation of cell extracts. The source of Class 3 aldehyde dehydrogenase in this study was *Escherichia coli* strain DH5α carrying pTALDH, a plasmid containing a full-length cDNA to rat liver Class 3 aldehyde dehydrogenase. Previous work has shown that *E. coli* strains carrying pTALDH synthesize functional Class 3 ALDH indistinguishable from authentic rat liver class 3 ALDH [10, 11]. Five hundred milliliter cultures of *E. coli* DH5α containing pTALDH were grown overnight with aeration and harvested as described [10] except that the final washed pellets were resuspended in 25 mL of the phosphate buffer system lacking Triton X-100. The resuspended pellets were frozen at −80° until needed.

Purification of Class 3 aldehyde dehydrogenase. Class 3 aldehyde dehydrogenase was purified from *E. coli* extracts in a modification of the procedure described by Harper *et al.* [11]. Triton X-100 was omitted from the buffer system. Additionally, the sonicate supernatant was passed through a CM-Sepharose 6B column prior to the 5′-AMP Sepharose step. Briefly, frozen *E. coli* were thawed on ice, sonicated and centrifuged. The supernatant was dialyzed against several changes of 10 mM sodium acetate buffer, pH 5.5, containing 2 mM EDTA and 0.1% 2-mercaptoethanol (2-ME). The dialyzed supernatant was applied to the 2.5 × 30 cm CM-Sepharose 6B column equilibrated with the same buffer. After washing with starting buffer, aldehyde dehydrogenase activity was eluted by washing the column with a linear NaCl gradient to 1 M in starting buffer. Fractions containing aldehyde dehydrogenase were pooled and dialyzed against 25 mM sodium phosphate buffer, pH 7.5, containing 1 mM EDTA and 0.1% 2-ME. The dialyzed eluant was applied to a 2 × 8 cm 5′-AMP Sepharose column equilibrated with dialysis buffer. After washing with starting buffer, aldehyde dehydrogenase activity was eluted with the 25 mM phosphate buffer system containing 0.5 mg/mL NAD^+. Active fractions were pooled and either used immediately or frozen overnight at −80°.

Determination of aldehyde dehydrogenase activity. Aldehyde dehydrogenase activity was assayed at 25° by monitoring the change in A_{340} caused by NADH or NADPH production during the oxidation of aldehyde substrate. The 3-mL assay contained 50 mM sodium phosphate buffer, pH 8.5, containing 1 mM EDTA and 0.1% 2-ME, 1.0 mM NAD^+ or $NADP^+$, approximately 1.5–2.0 μg of protein and various concentrations of substrate. The kinetic parameters, K_m, V_{max} and V_{max}/K_m were determined from Lineweaver and Burk plots. Protein concentrations were determined by the method of Lowry *et al.* [12] with bovine serum albumin as the standard.

RESULTS

The purification procedure employed in the present study is a slight modification of the procedure developed previously for the purification of Class 3 aldehyde dehydrogenase from cultured rat hepatoma cells [13], rat cornea [4] and from *E. coli* carrying pTALDH [11]. Inclusion of the CM-Sepharose step, while not improving the ultimate purity or yield, removes the vast majority of the proteins in the original extract, greatly improving the performance of the 5′-AMP Sepharose affinity column. The changes in pH occurring during the purification and the sensitivity of Class 3 ALDH to pH [2] make analysis of the efficacy of the CM-Sepharose column impossible to quantify, but the purity of Class 3 ALDH produced was comparable to the single-step procedure used previously (Table 1 and Fig. 1).

We have reported that both Class 3 [2, 4] and microsomal ALDH [5] can use either NAD^+ or $NADP^+$ as cofactor in the oxidation of aldehyde substrates. For Class 3 ALDH, both the K_m (6 vs 112 μM) and V_{max}/K_m (1.2×10^6 vs 5.2×10^5) values indicate NAD^+ is the preferred coenzyme [4]. The V_{max}/K_m values (5.7×10^4 vs 2.9×10^4) also indicate that NAD^+ is the preferred coenzyme for the microsomal enzyme, although the K_m is lower for $NADP^+$ (22 vs 67 μM) [5]. In subsequent kinetic studies, all substrates were tested with both cofactors. Only the data for NAD^+ is presented because, with the exception noted below, NAD^+ was the better coenzyme for all substrates.

The kinetic properties of Class 3 aldehyde dehydrogenase with various substrates are shown in Table 2. The data of Mitchell and Petersen [5] for rat liver microsomal aldehyde dehydrogenase are presented for comparison. Both Class 3 and microsomal aldehyde dehydrogenases oxidized saturated and unsaturated aldehydes from 3 to 9 carbons in length. Affinity for these aldehydes, as measured by V_{max}/K_m, increased with increasing chain length, especially between 3 and 6 and 6 and 8 carbons (Table 2). The saturated aldehyde was the preferred substrate relative to its corresponding unsaturated form. For Class 3 ALDH, this difference was due to significantly higher K_m values for the unsaturated aldehyde. For the microsomal enzyme the differences were due to changes in V_{max}. Longer chain length aldehydes (10–12 carbons) were very poor substrates, with maximal velocities less than that for propionaldehyde (data not shown). 4-Hydroxynonenal was a very poor substrate as indicated by its very high K_m with NAD^+ as cofactor. Interestingly, with the Class 3 ALDH 4-hydroxynonenal consistently showed a much lower K_m with $NADP^+$ as cofactor (1.30 vs 1290 μM), although the V_{max}/K_m continued to reflect that 4-hydroxynonenal was a poor substrate relative to other lipid aldehydes (V_{max}/K_m) of 3.4×10^4). Neither malondialdehyde nor acrolein was a substrate for Class 3 or microsomal aldehyde dehydrogenases.

All aldehydes were tested at final concentrations from 5 to 1000 μM. For all aldehydes but propionaldehyde and 4-hydroxynonenal, substrate inhibition was observed at concentrations above approximately 250 μM with either NAD^+ or $NADP^+$ as cofactor.

DISCUSSION

Our results indicate that both Class 3 and microsomal aldehyde dehydrogenases have very high affinities for medium chain length aliphatic aldehydes which are normally produced during cellular lipid metabolism. The kinetic constants suggest that

Table 1. Purification of Class 3 aldehyde dehydrogenase

Step	Total activity* (mIU)	Specific activity (mIU/mg)	Yield (%)	Purification (fold)
Untransformed *E. coli* DH5α	1,062	6.5		
E. coli DH5α carrying pTALDH	623,675	3,386		
CM-Sepharose 6B	ND†	ND	ND	ND
5'-AMP Sepharose	224,150	128,085	36	37.8

* Assayed with benzaldehyde and $NADP^+$; 1 mIU = 1 nmol NADPH produced/min.
† ND = not determined.

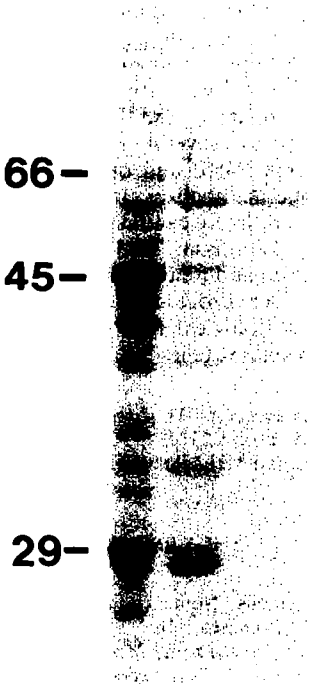

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of Class 3 aldehyde dehydrogenase purified from *E. coli* DH5α. From left to right: lane 1, crude *E. coli* extract; lane 2, NaCl gradient eluate from the CM-Sepharose column; lane 3, NAD^+ eluate from the 5'-AMP Sepharose column. Approximately 50 μg protein was loaded in lane 1, and 20 μg in lanes 2 and 3. Gel was stained with Coomassie Blue. Numbers represent molecular weight standards in kDa.

octanal and nonanal and their unsaturated forms are especially good substrates. The six carbon aldehydes are also very good substrates, particularly when compared to the short chain aliphatics. *n*-Hexanal is a major metabolite of the peroxidation of linoleic and arachidonic acids and longer chain aliphatics are also produced in significant amounts during the oxidation of these and other polyunsaturated fatty acids [14, 15]. The efficiency with which the Class 3 and microsomal aldehyde dehydrogenases oxidize C6 and C9 aliphatic aldehydes may account for these aldehydes being considered minor products of lipid peroxidation as they would be metabolized at the site of formation.

Interestingly, 4-hydroxynonenal and malondialdehyde, the so-called major products of lipid peroxidation, were not substrates for either the Class 3 or microsomal aldehyde dehydrogenases. It is especially surprising that 4-hydroxynonenal was not oxidized by these enzymes since nonanal and nonenal were among the best substrates. This observation suggests that aldehydic products of lipid peroxidation such as 4-hydroxynonenal undergo detoxification by other forms of aldehyde dehydrogenase. In this context, 4-hydroxynonenal has been demonstrated to be a very good substrate for rat Class 1 and Class 2 aldehyde dehydrogenases [8]. Likewise, it has been established that Class 1 and Class 2 ALDHs are capable of oxidizing malondialdehyde [6]. The possibility that these aldehydic products may also undergo reductive metabolism has been documented by the observation that 4-hydroxynonenal can be reduced by alcohol dehydrogenase [9]. Collectively, the results of these studies suggest the presence of multiple enzyme systems within the cell which are capable of metabolizing potentially toxic aldehydic products of lipid peroxidation.

The present results and those of Mitchell and Petersen [5] indicate that both the Class 3 and microsomal aldehyde dehydrogenases have as their preferred substrates medium chain length saturated and unsaturated lipid aldehydes. These two aldehyde dehydrogenases are also unqiue among the mammalian ALDHs in that they are the only two forms that can utilize $NADP^+$ as cofactor. The complete primary sequence of the class 3 aldehyde dehydrogenase has been determined [10, 16], as has approximately 50% of the microsomal enzyme

Table 2. Kinetic properties of Class 3 aldehyde dehydrogenases

Substrate	This study			Mitchell and Petersen [5]		
	K_m* (μM)	V_{max}†	V_{max}/K_m‡	K_m* (μM)	V_{max}†	V_{max}/K_m‡
Propionaldehyde	2700	7.1	0.3	808	6.5	8
Hexanal	69	26.7	39	37	14.4	39
Hexenal	180	10.5	6	38	5.4	1
Octanal	3	12.2	410	3	19.2	640
Octenal	23	39.2	170	3	9.4	313
Nonanal	1	12.5	1250	3	23.0	766
Nonenal	27	14.2	53	2	7.7	385
4-Hydroxynonenal	1290	22.2	2		0	0
Malondialdehyde	5000	0.6	0.0		0	0
Acrolein		NT§			0	0

* K_m was determined with 1 mM NAD^+.
† Expressed in μmol NADH produced/min/mg protein.
‡ Aldehyde oxidizing capacity expressed as $V_{max}/K_m \times 10^4$.
§ NT = not tested.

(Hempel J and Lindahl R, unpublished observations). Amino acid sequence data indicate that the Class 3 and microsomal aldehyde dehydrogenases share approximately 80% position identity in overlapping regions (Hempel J and Lindahl R, unpublished observations). Therefore, based on both functional and structural criteria, the microsomal aldehyde dehydrogenase is a Class 3 ALDH. It is possible that the minor differences in substrate and coenzyme kinetic properties of the two enzymes are due to slight differences in the structure of the functional enzyme due to the amino acid differences between them. For example, the prototypic Class 3 enzyme is soluble and functions as a dimer of two identical M_r 51,000 subunits. The microsomal enzyme is membrane-associated and believed to function as a tetramer of identical M_r 54,000 subunits [2]. In this context, it is interesting to note that the two enzymes are immunologically distinct in spite of their high degree of sequence conservation in common regions [17].

Both the subcellular and tissue distributions of the Class 3 ALDHs, including the microsomal enzyme, are consistent with a major physiological function of this class of aldehyde dehydrogenases being the oxidation of certain aldehydes generated by lipid metabolism. The microsomal enzyme is constitutive, being present in the microsomal fraction of all tissues in which microsomes have been assayed. For example, in mammalian liver, the microsomal enzyme may account for up to 50% of the total aldehyde dehydrogenase activity, even when assayed with acetaldehyde or propionaldehyde as substrate [18, 19]. Thus, the microsomal Class 3 ALDH may serve an important role in oxidizing and detoxifying medium chain aldehydes produced during endogenous microsomal lipid peroxidation.

In other tissues, including lung, heart, stomach, colon, urinary bladder and particularly cornea, the cytosolic Class 3 aldehyde dehydrogenase is the major constitutive ALDH [4, 20–23]. These tissues are subject to considerable oxidative stress *in vivo*, including lipid peroxidation. The cytosolic Class 3 ALDH is also inducible in several tissues, including liver, following xenobiotic exposure and during tumorigenesis, two pathophysiological conditions known to involve oxidative damage to cells [23, 24].

Class 3 aldehyde dehydrogenases would appear to afford cells considerable protection from the toxic effects of lipid aldehydes generated both endogenously and by xenobiotic exposures. In this context, Nebert and Gonzalez [25] have proposed that aldehyde dehydrogenase be considered part of the *Ah* receptor-mediated pathway of xenobiotic metabolism. This pathway involves xenobiotic-receptor binding and ligand-receptor mediated activation of several genes, including various cytochrome P450s and other enzymes involved in xenobiotic metabolism. It appears that the Class 3 ALDHs are likely candidates for inclusion in such a scheme. The tissue and subcellular distribution, inducibility, and functional properties of the Class 3 aldehyde dehydrogenases are all consistent with the major physiological role of this class of aldehyde dehydrogenases being lipid aldehyde oxidation.

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