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SHORT COMMUNICATION

Kinetic Properties of the Human Liver Cytosolic Aldehyde Dehydrogenase for Retinal Isomers

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ABSTRACT. Retinoic acid exerts pleiotropic effects by acting through two families of nuclear receptors, RAR and RXR. All-*trans* and 9-*cis* retinoic acid bind RARs, whereas 9-*cis* retinoic acid binds and activates only the RXRs. To understand the role of human liver cytosolic aldehyde dehydrogenase (ALDH1) in retinoic acid synthesis, we examined the ability of ALDH1 to catalyze the oxidation of the naturally occurring retinal isomers. ALDH1 catalyzed the oxidation of all-*trans*, 9-*cis*, and 13-*cis* retinal with equal efficiency. However, the affinity to all-*trans* retinal (K_m = 2.2 μM) was twofold higher than to 9-*cis* (K_m = 5.5 μM) and 13-*cis* (K_m = 4.6μM) retinal. All-*trans* retinol was a potent inhibitor of ALDH1 activity, and inhibited all-*trans* retinal oxidation uncompetitively. Comparison of the kinetic properties of ALDH1 for retinal isomers with those of previously reported rat kidney retinal dehydrogenase showed distinct differences, suggesting that ALDH1 may play a different role in retinal metabolism in liver. BIOCHEM PHARMACOL **57**;2:195–197, 1999. © 1998 Elsevier Science Inc.

KEY WORDS. aldehyde dehydrogenase; retinal oxidation; retinoic acid

The vitamin A metabolite RA† is important for vertebrate embryogenesis and development [1, 2], and for the maintenance of several epithelial tissues [3]. The regulatory effects of RA are mediated through gene transcription via RA-receptor families [4]. RA is biosynthesized from retinol via two sequential oxidation steps, the first step being the oxidation of retinol to retinal and the second step being the oxidation of retinal to RA [5, 6]. Numerous enzymes involved in the oxidation of retinol to retinal and retinal to RA have been identified, purified, and characterized. These enzymes belong to the members of four distinct families: the alcohol dehydrogenases, the short-chain alcohol dehydrogenases, the aldehyde dehydrogenases, and the cytochrome P450s [7].

ALDHs (aldehyde:NAD oxidoreductase, EC 1.2.1.3) are a superfamily of $NAD(P)^+$-dependent enzymes, which are comprised of at least three classes, based on sequence similarities. The class 1 gene for ALDH is expressed in the cytosolic fractions of mammalian tissues, and is considered to play a major role in the biosynthesis of retinoic acid [8, 9]. We have characterized a cytosolic ALDH from rat kidney that functions as a retinal dehydrogenase, and have cloned the corresponding gene [10, 11]. ALDH1 (which is highly expressed in the human liver) has been shown to be active in all-*trans* retinal oxidation [9]. However, it is not known whether ALDH1 catalyzes the oxidation of retinal isomers. In this report, we show that ALDH1 oxidized all-*trans*, 9-*cis*, and 13-*cis* retinal with equal efficiency.

MATERIALS AND METHODS

Enzyme Preparation

Using a human liver obtained at autopsy, cytosolic ALDH1 was purified to homogeneity by the method described previously [12]. The pure enzyme was provided by Dr. A. Yoshida. The purity of the enzyme was re-checked by polyacrylamide gel electrophoresis and also by western blot.

Enzyme Assay

Enzyme activity was assayed by measuring the retinoic acids produced in the reaction mixture by HPLC as described previously [13]. The standard reaction mixture contained 100 mM phosphate buffer (pH 7.5), 0.02% Tween-80, 161 mM dithiothreitol (DTT), and 603 μM NAD. Reactions were initiated by the addition of the substrates (1.34 to 16 μM) 9-*cis*, all-*trans*, and 13-*cis* retinal in 2.5 μL DMSO. The reaction mixture was incubated at 25° for 60 min and was terminated by cooling in ice-cold water. The retinoids in the reaction mixture were extracted with 400 μL of butanol:acetonitrile (1:1), and an aliquot was injected directly onto the HPLC apparatus.

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† *Abbreviations*: ALDH, aldehyde dehydrogenase; RA, retinoic acid; and RALDH, retinal dehydrogenase.

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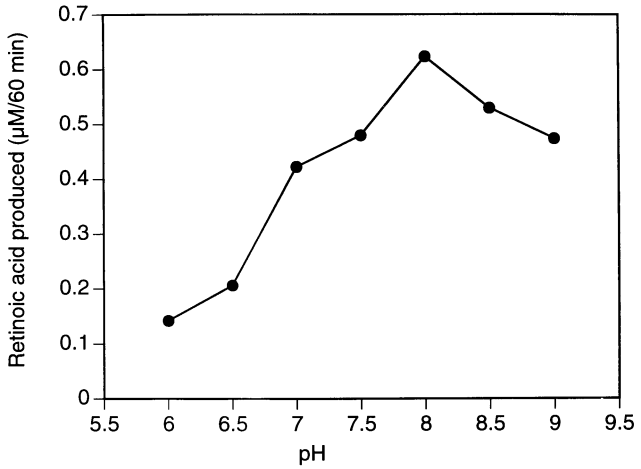

FIG. 1. Effect of pH on ALDH1 activity for all-*trans* retinal oxidation. Each point in the curve represents the average of four replicates (less than 5% variation between each replicate).

RESULTS AND DISCUSSION

The pH optimum of ALDH1 for all-*trans* retinal oxidation was 8.0 (Fig. 1). The saturation kinetics of ALDH1 with retinal isomers are shown in Fig. 2; 9-*cis* retinal exhibited the highest activity. However, the K_m for 9-*cis* retinal was two-fold higher than that of all-*trans* retinal (Table 1). Furthermore, ALDH1 also catalyzed 13-*cis* retinal oxidation. ALDH1 catalyzed the oxidations of all-*trans*, 9-*cis*, and 13-*cis* retinal with equal efficiency (Table 1).

We have shown previously that all-*trans* retinol competitively inhibits rat kidney RALDH activity [10]. Therefore, we examined the nature of inhibition by all-*trans* retinol on ALDH1 activity. All-*trans* retinol was a potent inhibitor of ALDH1 activity and inhibited all-*trans* retinal oxidation uncompetitively (Fig. 3). This observation suggests that all-*trans* retinol binds to the enzyme (ALDH1)-substrate complex, but does not bind to the active site region.

TABLE 1. Specificity of ALDH1 for retinal isomers

Retinal isomer	K_m (μM)	V_{max} (μM/60 min)	V_{max}/K_m
9-*cis*	5.52	1.48	0.27
all-*trans*	2.24	0.44	0.20
13-*cis*	4.60	1.0	0.22

Data from the saturation curves were treated with the computer program Enzfitter. Values are the average of two independent determinations, where each point in the curve in each determination represents the average of four replicates.

ALDH1 is an acidic protein that is highly expressed in the human liver [14]. RALDH is a basic protein, and is present predominantly in the rat kidney [11]. RALDH shares 87% amino acid sequence identity with ALDH1 [11]. Comparison of kinetic behavior of ALDH1 toward retinal isomers with that of RALDH showed several differences. RALDH exhibited high specificity for 9-*cis* retinal substrate and did not catalyze 13-*cis* retinal oxidation, whereas ALDH1 had high affinity for all-*trans* retinal substrate and oxidized 13-*cis* retinal with equal efficiency. In addition, all-*trans* retinol competitively and uncompetitively inhibited RALDH and ALDH1 activities, respectively. These distinct differences between ALDH1 and RALDH in the kinetic properties for retinal isomers suggest that the functions of these two ALDHs *in vivo* in retinal metabolism may be different. Because the liver is the major site of retinol (all-*trans* and 13-*cis*) storage and metabolism [15], it is possible that ALDH1 may participate in catabolizing the excess retinol present in the liver via retinoic acid. Further studies on the regulation of expression of ALDH1 and RALDH may provide information about their specific roles in vitamin A metabolism.

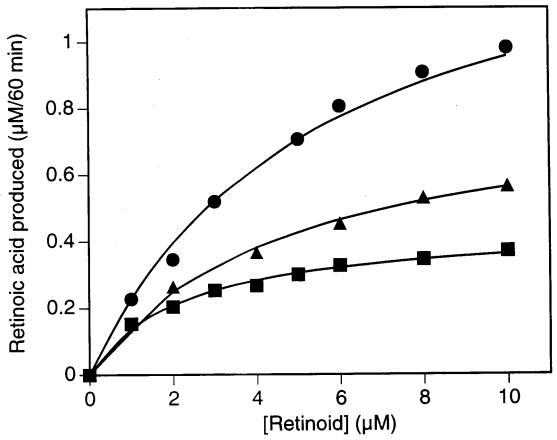

FIG. 2. Saturation curves of ALDH with retinal isomers. Incubation conditions are described in Materials and Methods. Key: 9-*cis* (●), all-*trans* (■), and 13-*cis* (▲). Each point in the curve represents the average of four replicates (less than 5% variation between each replicate).

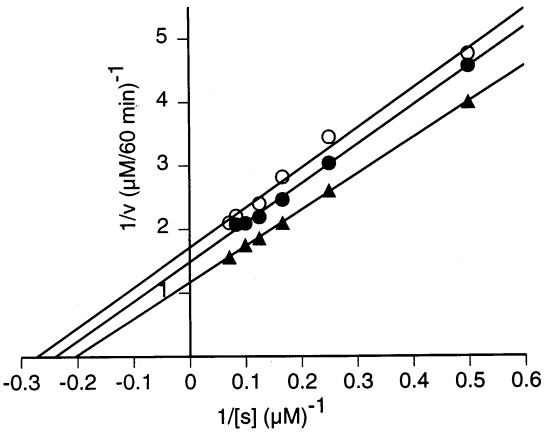

FIG. 3. Double-reciprocal plot of inhibition of ALDH1 activity by all-trans retinol. The substrate used was all-*trans* retinol. All-*trans* retinol concentrations: none (▲), 2 μM (●), and 3 μM (○). Each point in the curve represents the average of four replicates (less than 10% variation between each replicate).

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