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Hydride Transfer Stereospecificity of Rat Liver Aldehyde Dehydrogenases*

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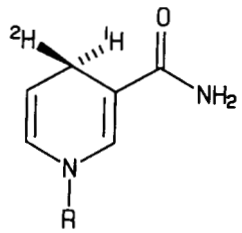
The stereospecificity of hydride transfer to NAD^+ by several forms of rat liver aldehyde dehydrogenase was determined by a nuclear magnetic resonance method. The forms included several mitochondrial and microsomal isozymes from normal liver, as well as isozymes from xenobiotic-treated and tumor cells. The proton added to NAD^+ comes exclusively from the aldehyde substrate and in all cases was A (pro-*R*)-stereospecific.

Hepatic aldehyde dehydrogenases (aldehyde:NAD^+ oxidoreductase (EC 1.2.1.3) and aldehyde:$NAD(P)^+$ oxidoreductase (EC 1.2.1.5)) are pyridine nucleotide-linked enzymes catalyzing the oxidation of aldehydes to carboxylic acids.

$$RCHO + NAD(P)^+ + H_2O \rightarrow RCOOH + NAD(P)H + H^+$$

They have been found in most mammals in multiple molecular forms (1–4). In rat liver, further aldehyde dehydrogenases are induced by xenobiotics (see Ref. 5 and references therein) or during hepatocarcinogenesis (see Refs. 6 and 7 and references cited therein). A rat hepatoma cell line has been developed as an *in vitro* model for studying the regulation of aldehyde dehydrogenase activity in the latter case (7).

Pyridine nucleotide-linked enzymes are well known to transfer hydride stereospecifically to (or from) the pyridine 4-position of the oxidized (reduced) coenzyme (for a review see Ref. 8). A-stereospecific enzymes transfer hydride to or from the A side (pro-*R*, top side of Structure 1), while B-stereospecific (pro-*S*) enzymes transfer at the opposite side. Known pyridine nucleotide-linked enzymes seem to be approximately equally distributed in their stereospecificity. In rat liver the large number of basal and inducible enzymes have been shown to have distinct properties (5, 7, 9). Differences exist in subcellular localization (microsomal, mitochondrial, or cytosolic), subunit composition, stability, and, most importantly, kinetic properties including velocity constants and affinities for different aldehydes and NAD^+ or $NADP^+$ as coenzyme. Therefore, it was possible that differences in stereospecificity also existed. This present communication reports the stereospecificity of the multiple forms of rat liver aldehyde dehydrogenase with respect to hydride transfer.



STRUCTURE 1

There has been an earlier report on the stereospecificity of a liver acetaldehyde dehydrogenase by a non-NMR method (10). However, the earlier work used a crude preparation. In light of the multiple forms now known to exist of aldehyde dehydrogenases, the earlier report is subject to some ambiguity. The authors reported a stoichiometry that was nonintegral (65% of one isomer, 20% of the other, and 15% unexplained), and hypothesized a variety of side reactions that might account for their results. A reinvestigation of the stereospecificity seemed warranted.

EXPERIMENTAL PROCEDURES

From normal rat liver, mitochondrial aldehyde dehydrogenase isozymes I and II (MTI and MTII), and microsomal isozymes I and II (MCI and MCII) were purified as described previously (9). The phenobarbital-induced enzyme (PB) and the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced enzyme (TCDD) were also purified as described previously (5), as was the dehydrogenase from rat hepatoma cells (HTC) (7) and a plasmid-encoded hepatic tumor-associated aldehyde dehydrogenase (P3A1) isolated from *Escherichia coli* strain HB101 (11).

The stereospecificity of $NAD(P)^+$ reduction was determined by a ^1H NMR technique (12, 13) with several *conceptually* important modifications. The procedure of Arnold and co-workers (12, 13) starts with deuterium-labeled NAD^+ at the pyridine 4-position. In the present study, normal protic NAD^+ and $NADP^+$ (Sigma) were used, and the aldehyde substrate was prepared with deuterium in the aldehyde proton position. In this fashion, it was possible to demonstrate that the H atom transferred to the oxidized coenzyme came exclusively from the aldehyde and not from solvent water.

The common aldehyde substrate benzaldehyde-1-*d* was used in all assays. Although it is not an optimum substrate for all enzyme forms, it is an acceptable one that undergoes reaction at reasonable rates. The labeled compound was synthesized by a published procedure (14) and contained 94% deuterium at the aldehyde position.

Assays were performed at room temperature in 60 mM potassium phosphate buffer, pH 8.5. Concentrations of benzaldehyde-1-*d* and NAD^+ or $NADP^+$ were typically 1 mM each, although in some experiments the concentrations were raised as high as 4 mM. Enzyme (3.6 up to 534 mIU) was added as a concentrated aliquot either in the same assay buffer or in buffer containing in addition 1 mM EDTA, 1 mM mercaptoethanol, and 0.2% (v/v) Triton X-100. Reactions were followed either spectrophotometrically at 340 nm or by NMR spectroscopy, as will be discussed. After the reaction had progressed to between 30 to 99+% completion, the assay mixture was frozen, lyophilized, and redissolved in the NMR solvent deuterium oxide (99.8% deuterium, Aldrich Chemical Co.) containing 0.5 mM 3-(trimethyl)-tetradeutero-sodium propionate as internal reference. Very slight differences (0.02 ppm) for chemical shifts for NADH and its specifically deuterated form were observed in the present study compared to the literature (12, 13) because of different temperatures and concentrations employed. It was not necessary to purify products because, in the critical region of 2–3 ppm, only the reduced pyridine nucleotides contributed an NMR detectable signal. Spectra were obtained on a Nicolet NTC200 spectrometer at 200.06 MHz.

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RESULTS AND DISCUSSION

The HTC aldehyde dehydrogenase was active in 99+% deuterium oxide buffered with 60 mM phosphate to pH 8.5. At 1 mM in both substrates, it was 57% as active compared to assays conducted in normal protic water. Therefore, it was possible to observe the appearance of NADH produced from benzaldehyde-1-*d* and NAD^+ in NMR spectra as a function of time. Typical results are shown in Fig. 1. The resonance that grows in at a chemical shift of 2.65 ppm is the 4B methylene proton of NADH arising from the 4 aromatic proton of NAD^+. (The chemical shift of the latter is at 8.95 ppm.) The deuterium transferring from benzaldehyde-1-*d* is itself spectrally invisible, although its presence is certain because the scalar coupling pattern and chemical shift for the observed proton (originally on the NAD^+) is definitive for a methylene pair ^1H, ^2H. The observed spectrum matched that previously reported for Structure 1, which is [4A-^{2}H, 4B-^{1}H] NADH (12, 13). The observed shift is clearly distinct from a shift of 2.77 ppm which would correspond to the other isomer. Hence, the enzyme has A-stereospecificity.

Additional conclusions were possible in light of the following observations. When the reaction was conducted in ^1H_2O, the same final spectrum was obtained. By integration of appropriate NMR peaks in other regions of the spectrum, it was observed that 0.97 ± 0.05 eq of [4A-^{2}H, 4B-^{1}H]NADH were formed per equivalent of NAD^+ consumed or per equivalent of benzaldehyde-1-*d* consumed. This confirms the stoichiometry of the reaction and that the hydrogen transferred comes from the substrate aldehyde and not from solvent. When $NADP^+$ was substituted for NAD^+, A-stereospecificity was again observed.

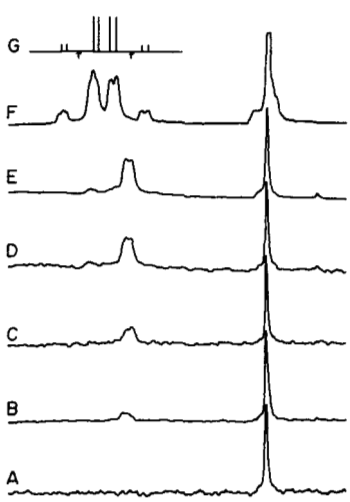

FIG. 1. **Production of [4A-^{2}H, 4B-^{1}H] NADH by HTC aldehyde dehydrogenase as a function of time.** The assay mixture contained, in a final total volume of 0.5 ml, 1 mM benzaldehyde-1-*d*, 1 mM NAD^+, 60 mM potassium phosphate, 0.5 mM 3-(trimethyl)-tetradeutero-sodium propionate, and 0.28 mg of enzyme all in 99.8% ^2H_2O. The spectral region between 2 and 3 ppm is displayed, and the single peak on the *right* is from residual acetone (2.04 ppm) that is a contaminant in the stock NAD^+ preparation. *Spectrum A* was taken within 10 min of the addition of enzyme to the assay and is a nominal time 0 trace. *Spectra B–E* were taken at 3-h intervals. *Spectrum F* is pure NADH with both methylene protons ^1H, taken at pH 8.5. *Inset G* is a computer-generated simulation of the expected line positions for the 4A and 4B methylene protons of NADH, whereby the chemical shift of the 4A proton is 2.77 ppm and is coupled to the pyridine proton 5 (J(4A,5) = 3.1 Hz), as well as to 4B (J(4A,4B) = 18 Hz), and the chemical shift of the 4B proton is 2.65 ppm and is coupled to proton 5 (J(4B,5) = 3.8 Hz) as well as 4A. The tick marks indicate the chemical shifts of 4A and 4B. Because of the strong scalar coupling, there is no "peak" at these chemical shifts in the fully protic NADH, while in [4A-^{2}H, 4B-^{1}H]NADH the peaks form a doublet (because of the still present 4B-5 coupling) symmetrical about the chemical shift.

TABLE I

Summary of kinetic parameters for rat liver aldehyde dehydrogenase

Benzaldehyde K_m values are millimolar while coenzyme values are micromolar. V_{max} values are relative to NAD^+. Isozyme designations are: MTI and MTII, mitochondrial aldehyde dehydrogenase isozymes I and II; MCI and MCII, microsomal isozymes I and II; PB, phenobarbital-induced enzyme; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced enzyme; P3A1, plasma-encoded hepatic tumor-associated aldehyde dehydrogenase; HTC, dehydrozenase from rat hepatoma cells.

Isozyme	Benzaldehyde		NAD^+		$NADP^+$		Stereospecificity
	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	
MTI	0.35	1.6	1.00	36	0.49	910	A
MTII	0.80	50	1.00	22	0.55	440	A
MCI	1.40	5.9	1.00	97	0.60	440	A
MCII	0.34	3.0	1.00	95	0.27	270	A
PB	0.02	1.3	1.00	2.3	0.01	100	A
TCDD	4.54	0.5	1.00	73	4.44	210	A
P3A1	5.66	0.8	1.00	74	5.85	420	A
HTC	5.66	0.8	1.00	74	5.85	420	A

Table I summarizes some kinetic parameters and the determined stereospecificity for the other enzyme forms. A-stereospecificity was observed in all cases tested. The conformity is not as exciting as it would have been to observe a clear difference between, for example, the normal and tumor-related forms. However, firm data now experimentally establish the stereospecificity and eliminate speculation or assumption.

The exact ancestral relationships between aldehyde dehydrogenase and other dehydrogenases have yet to be defined. Two other dehydrogenases of interest for comparison are alcohol and glyceraldehyde-3-phosphate dehydrogenase (15–17). In many pathways alcohol dehydrogenase provides the substrate for aldehyde dehydrogenase, especially in ethanol metabolism. In this context it is interesting to note that alcohol dehydrogenase is a dimer, requires a bound zinc atom for activity, and has full site reactivity (15). Both alcohol and aldehyde dehydrogenases are A (pro-*R*)-specific. Aldehyde dehydrogenase is more closely analogous to glyceraldehyde-3-phosphate dehydrogenase structurally. Both function as tetramers of approximately equal size, both show half-site reactivity with aldehyde substrates, and following substrate binding, the complex is oxidized to an intermediate which is then hydrolyzed to an acid (16, 18). Interestingly, glyceraldehyde-3-phosphate dehydrogenase is a B (pro-*S*)-specific enzyme.

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