Hydride Transfer Stereoespecificity 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:NADP* oxidoreductase (EC 1.2.1.5)) are pyridine nucleotide-linked enzymes catalyzing the oxidation of aldehydes to carboxylic acids.

\[ {\text{RCRO}} + \text{NAD}^* + \text{H}_2\text{O} \rightarrow \text{RCOOH} + \text{NAD}^*(\text{H}) + \text{H}^* \]

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.

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 'H 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 to 504 μU) 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)-tetra-deutero-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 290.06 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 H3, H3. The observed spectrum matched that previously reported for Structure 1, which is [4A-2H, 4B-2H] 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 2H2O, the same final spectrum was obtained. By integration of the resonance, the complex is oxidized to an intermediate which is then contained, in a final total volume of 0.5 ml, 1 mM 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 H3, H3. The observed spectrum matched that previously reported for Structure 1, which is [4A-2H, 4B-2H] 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.

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Stereospecificity of Aldehyde Dehydrogenase