Mammalian Alcohol Dehydrogenase – Functional and Structural Implications

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Abstract
Mammalian alcohol dehydrogenase (ADH) constitutes a complex system with different forms and extensive multiplicity (ADH1–ADH6) that catalyze the oxidation and reduction of a wide variety of alcohols and aldehydes. The ADH1 enzymes, the classical liver forms, are involved in several metabolic pathways beside the oxidation of ethanol, e.g. norepinephrine, dopamine, serotonin and bile acid metabolism. This class is also able to further oxidize aldehydes into the corresponding carboxylic acids, i.e. dismutation. ADH2 can be divided into two subgroups, one group consisting of the human enzyme together with a rabbit form and another consisting of the rodent forms. The rodent enzymes almost lack ethanol-oxidizing capacity in contrast to the human form, indicating that rodents are poor model systems for human ethanol metabolism. ADH3 (identical to glutathione-dependent formaldehyde dehydrogenase) is clearly the ancestral ADH form and S-hydroxymethylglutathione is the main physiological substrate, but the enzyme can still oxidize ethanol at high concentrations. ADH4 is solely extrahepatically expressed and is probably involved in first pass metabolism of ethanol beside its role in retinol metabolism. The higher classes, ADH5 and ADH6, have been poorly investigated and their substrate repertoire is unknown. The entire ADH system can be seen as a general detoxifying system for alcohols and aldehydes without generating toxic radicals in contrast to the cytochrome P450 system.

The mammalian alcohol dehydrogenase (ADH) system is divided into six classes, ADH1–ADH6, whereof five have been identified in man [4, 9]. These dimeric enzymes belong to the protein superfamily of medium-chain dehydrogenases/reductases [13] and are further divided into subgroups (ADH2), isoenzymes (ADH1 and ADH2) and allelic forms (ADH1). All ADH classes catalyze the reversible oxidation of alcohols to aldehydes/ketones using NAD+/NADH as electron acceptor and donor, respectively, and have a broad but only partially overlapping substrate repertoire [5]. Apart from the formaldehyde scavenging of ADH3, functional roles of the other ADHs are not fully established. However, based on their catalytic activities they could play roles in the metabolism of steroids, retinoids, biogenic amines, lipid peroxidation products, ω-hydroxy fatty acids as well as xenobiotic alcohols and aldehydes.

The structure determinations for ADH1–ADH4 have provided a structural basis for the understanding of their different properties. All ADH subunits consist of one catalytic and one coenzyme-binding domain, and both
coenzyme and substrate bind in a cleft between the two domains [6, 15]. Low positional identities between the classes are especially observed in three segments, constituting parts of the substrate-binding pocket and subunit interaction areas [15]. This results in large differences in substrate pocket topology although the overall positional identity is high (~65%) and acceptance of residue exchanges at the substrate-binding site is likely to reflect the functional divergence of the classes.

ADH3, identical to glutathione-dependent formaldehyde dehydrogenase, is clearly the ancestral form of all mammalian ADHs and has been traced in all living species investigated [9, 10]. Further, this is the only ADH that has been ascribed a physiological substrate, S-hydroxymethylglutathione (HMGSH) and is identified as a functional formaldehyde scavenger [7]. ADH1, the major enzyme in the metabolism of ingested ethanol is the only human class where more than one isoenzymic form exists (α-, β- and γ-subunits, ADH1A, ADH1B and ADH1C in the new nomenclature system [4]). Mainly, rodents have been used to study the contributions of various ADHs in the metabolism of ethanol. The rodents do not show a setup of ADH1 isoenzymes, but the set of different classes is present (ADH1–ADH4 and ADH6). The ADH1 enzymes are involved in several metabolic pathways besides the oxidation of ethanol, e.g. norepinephrine, dopamine, serotonin and as recently shown bile acid metabolism [5, 12, 17]. This class is able to further oxidize aldehydes into the corresponding carboxylic acids, i.e. dismutation. The γγ-isoenzyme (ADH1C) shows the highest capacity for dismutation among the human ADHs and it seems to be a common theme that this form is involved in several specific pathways besides its high capacity for ethanol oxidation [12, 16].

Human ADH2 was isolated as a liver enzyme with a high K_m for ethanol [11] that was reinterpreted for the recombinantly isolated enzyme [18]. Reductions of the intermediate aldehydes in serotonin and norepinephrine catabolism are efficiently catalyzed [17] and it has further been shown that the human ADH2 is fairly efficient in retinoid metabolism [3]. For all these reactions, ADH1 and the extrahepatically expressed ADH4 have overlapping activities, and the ADH4 enzyme has been suggested to be the main bioactivator of retinoids [3]. ADH2 can be divided into two subgroups, one group consisting of the human enzyme together with a rabbit form and another consisting of the rodent forms. The ADH2 forms are found almost exclusively in the liver where the rodent forms almost lack ethanol dehydrogenase activity [18].

The higher classes, ADH5 and ADH6, have been poorly investigated, and nothing is known about their substrate repertoire. They are however liver expressed, and at least the human form, ADH5, shows an alternative splicing pattern.

In a further attempt to understand the interactions between different ADHs (classes and isoenzymes) as well as between different substrates, we here focus on the complex interplay between the participants in oxidoreductive cell defense.

**Materials and Methods**

**Enzyme Preparations**

Enzymes were recombinantly expressed in *Escherichia coli*, mainly using pET expression vectors for subcloning of mammalian ADH cDNAs. The recombinant proteins were purified to homogeneity essentially in a three-step procedure including ion exchange, affinity and gel permeation chromatography as described earlier [16, 18]. Protein concentrations were determined with the Bio-Rad protein assay with bovine serum albumin as standard and enzymatic activity was determined spectrophotometrically at pH 10 in glycine-NaOH buffer [16].

**Kinetic Analysis**

Ethanol and all-trans retinol oxidation were determined at pH 7.5 with a Hitachi U-3000 spectrophotometer, by monitoring the formation of NADH at 340 nm for ethanol oxidation and by the formation of retinal at 400 nm for retinol oxidation. Oxidation of HMGSH, spontaneously formed by formaldehyde and glutathione, was determined at pH 8.0 by monitoring the NADH formation. Reactions with serotonin metabolites were quenched by addition of perchloric acid, and metabolites were separated by HPLC and detected electrochemically [17].

**Cloning and Expression**

Human ADH5 and rat ADH6 were cloned with conventional techniques from liver cDNA libraries and the PCR technique with oligonucleotides designed after a published cDNA sequence [19]. Genomic DNA was prepared from blood samples and the ADH5 3'-end region was amplified with the PCR technique for sequence analysis. For *in vitro* translation the cDNA coding for the ADH6 was subcloned into transcription vector pTRIkan. This cDNA was further cloned into pEGFP adjacent to the coding sequence for green fluorescence protein for transfection into COS cells. The harvested cells were fixed and analyzed for protein expression.

**Data Analysis**

To fit lines to kinetic data points and to calculate kinetic parameters, a weighted non-linear-regression analysis program was used (Fig.P for Windows). DNA and deduced protein sequences were analyzed using the University of Wisconsin Genetics Computer Group Program and compared with EMBL data banks. For phylogenetic calculations, the programs Clustal W and Tree View were used.