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
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 retinol 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.