Differential susceptibility of human alcohol dehydrogenase isoenzymes to anions

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Human liver alcohol dehydrogenase (ADH) isoenzymes $\beta_1\beta_1$, $\gamma_1\gamma_1$ from Caucasian individuals with 'typical' ADH and $\beta_2\beta_2$-Bern from Caucasian individuals with 'atypical' phenotype differed in their susceptibility to anions. At pH 7.0 $\beta_1\beta_1$ and $\gamma_1\gamma_1$ were more active in Tris–HCl buffer than in sodium phosphate buffer but less active in Hepes–NaOH and Mops–NaOH. $\beta_2\beta_2$-Bern showed the same activity in all these buffers. At pH 7.0 and at low concentrations (50–100 mM) chloride activated the ethanol oxidation by $\beta_1\beta_1$ and $\gamma_1\gamma_1$, whereas sulfate showed no effect. At anion concentrations above 100 mM all isoenzymes were inhibited. At pH 10.5 $\beta_1\beta_1$ and $\gamma_1\gamma_1$ were not activated. Measuring the acetaldehyde reduction, no comparable activation by chloride was observed; all three isoenzymes were inhibited, at significantly lower anion concentrations. These anion effects can be correlated with the different primary structures of the isoenzymes around the active site and the coenzyme binding site.

Alcohol dehydrogenase *Human liver isoenzyme* *Anion effect*

1. INTRODUCTION

Human alcohol dehydrogenase (ADH, EC 1.1.1.1) occurs in multiple, electrophoretically distinct molecular forms [1,2]. Class I isoenzymes [3] are determined by 3 different gene loci, ADH-1, ADH-2, and ADH-3 which code for the different polypeptide chains α, β_1, and γ_1, which randomly associate to the dimeric isoenzymes [4,5]. Alleles can occur at the ADH-2 and ADH-3 locus, coding for β_2 (atypical ADH) and γ_2 [4–7].

The different class I isoenzymes can be isolated by double ternary complex affinity chromatography on Sepharose–4-[3-(*N*-6-aminocaproyl)aminopropyl]-pyrazole (CapGapp–Sepharose) followed by CM–cellulose ion-exchange chromatography [8–10]. Isoenzymes purified this way not only differ in their electrophoretic mobility and subunit composition, but also exhibit marked differences in substrate specificities and kinetic properties [10–12].

Here we report differential effects of the anions chloride and sulfate on the activity of 3 human liver isoenzymes $\beta_1\beta_1$ and $\gamma_1\gamma_1$, derived from Caucasian human livers with typical phenotype and $\beta_2\beta_2$-Bern, derived from livers with atypical phenotype, also of Caucasian origin. Marked differences in susceptibility of the isoenzymes to these reagents were observed. The results suggest that the different behavior of these isoenzymes reflects differences in the structure of the active sites and the coenzyme binding sites.

2. MATERIALS AND METHODS

2.1. *Chemicals*

NAD^+ and NADH (both grade 1) were obtained from Boehringer (Mannheim). The buffer substances 3-(cyclohexamine)propane-3-sulfonic acid (CAPS) and Hepes were from Serva (Heidelberg), and 3-(*N*-morpholino)propanesulfonic acid (Mops) from Fluka (Buchs, Switzerland). All other

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chemicals were of analytical grade and purchased locally. Acetaldehyde was redistilled before use.

2.2. *Purification of human liver alcohol dehydrogenase isoenzymes*

Caucasian human livers of typical and atypical phenotype were obtained from autopsies, frozen 10–20 h after death and stored at −20°C. Class I isoenzymes were isolated by double ternary complex affinity chromatography [9] at pH 8.0 (typical isoenzymes) or at pH 9.0 (atypical isoenzymes). Homogeneous isoenzymes were isolated by subsequent chromatography on CM–cellulose (Whatman, Maidstone, England) [10].

Purity of the isoenzymes was tested by SDS-gel [13] and starch gel electrophoresis [4]. The isoenzymes and their subunit composition were identified as in [10].

2.3. *Enzymatic assay*

ADH activity was measured photometrically at 340 nm by monitoring the production or utilization of NADH at 25°C. Assay conditions in a total volume of 3 ml were 67 mM glycine–NaOH buffer (pH 10.5 or 8.8) and 67 mM sodium phosphate buffer (pH 7.0), containing 1.6 mM NAD^+ and 16.7 mM ethanol when the oxidation reaction was measured or 67 mM sodium phosphate buffer (pH 7.0), containing 0.1 mM NADH and 5 mM acetaldehyde, when the reduction reaction was measured. Sodium chloride and sodium sulfate were added to the assay mixture at various concentrations. The reaction was started by the addition of enzyme.

3. RESULTS

When we tested other buffers (Tris–HCl, Hepes–NaOH, Mops–NaOH, or CAPS–NaOH) than the commonly used sodium phosphate and glycine–NaOH buffers we observed that the 3 human ADH isoenzymes showed significantly different activities in these buffers (table 1). At pH 7.0 all typical isoenzymes were more active in Tris–HCl and distinctly less active in Hepes–NaOH and Mops–NaOH. At pH 10.5 these isoenzymes were less active in CAPS–NaOH than in the normally used glycine–NaOH. The atypical isoenzyme $\beta_2\beta_2$-Bern was not affected by the different buffer ions, or was slightly more active in Mops–NaOH. This led us to investigate the effects of chloride and sulfate on the activity of human liver ADH isoenzymes because all the buffers used had the bulky sulfonate group as the proton donor with the exception of Tris–HCl where chloride was present.

Table 1

Activity of human liver alcohol dehydrogenase isoenzymes in various buffers

Buffer	pH	% relative activity		
		$\beta_1\beta_1$	$\gamma_1\gamma_1$	$\beta_2\beta_2$
Sodium phosphate	7.0	100	100	100
Tris–HCl	7.0	115	142	100
Hepes–NaOH	7.0	73	71	103
Mops–NaOH	7.0	75	74	112
Glycine–NaOH	10.5	100	100	–
CAPS–NaOH	10.5	58	65	–

The effects of chloride and sulfate on the enzymatic activity of ADH isoenzymes at pH 7.0 are shown in fig.1 and table 2. In the oxidative direction the isoenzymes $\beta_1\beta_1$ and $\gamma_1\gamma_1$ were more active at low sodium chloride concentrations in contrast to $\beta_2\beta_2$-Bern that was slightly inhibited. Compared

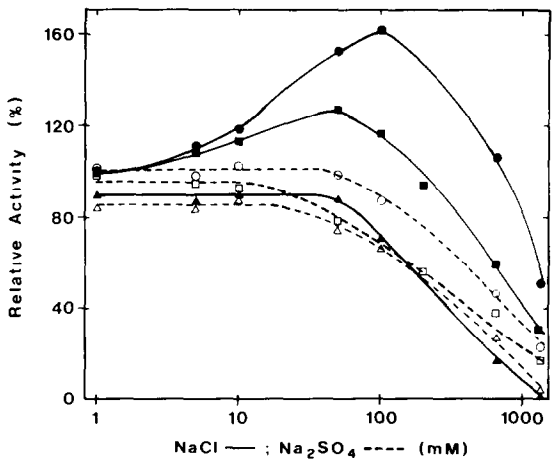

Fig.1. Effect of increasing concentrations of chloride and sulfate on the activity of human liver alcohol dehydrogenase isoenzymes ($\beta_1\beta_1$: ■, □; $\gamma_1\gamma_1$: ●, ○; and $\beta_2\beta_2$-Bern: ▲, △) determined in 67 mM sodium phosphate buffer, pH 7.0. The relative activity (100% = absence of chloride or sulfate) is plotted *vs* the chloride and sulfate concentration.

Table 2

Effect of chloride and sulfate on the ethanol oxidizing activity of human liver alcohol dehydrogenase isoenzymes

Isoenzyme	% relative activity in the presence of	
	NaCl (50 mM)	Na_2SO_4 (50 mM)
$\beta_1\beta_1$	127	79
$\gamma_1\gamma_1$	152	99
$\beta_2\beta_2$	88	74

The enzymatic activity was determined in 67 mM sodium phosphate buffer (pH 7.0) in the presence or absence of 50 mM sodium chloride or 50 mM sodium sulfate. Values are presented as % relative activity (absence of chloride or sulfate = 100%)

to chloride no activation was obtained with sodium sulfate at the same concentrations. At higher anion concentrations all 3 isoenzymes were inhibited by chloride and by sulfate.

When measuring the reverse reaction (acetaldehyde reduction) none of the isoenzymes were activated at pH 7.0, either with chloride or with sulfate. However, all 3 isoenzymes were inhibited, at concentrations up to 9-times lower than were necessary in the opposite direction.

A distinct pH effect was observed. When the ethanol oxidation activities of the isoenzymes were measured at their respective pH optima (pH 10.5 for $\beta_1\beta_1$ and $\gamma_1\gamma_1$; pH 8.8 for $\beta_2\beta_2$-Bern) no activation was obtained, either with chloride or with sulfate. However, all isoenzymes were inhibited with chloride and sulfate at concentrations comparable to the inhibitory effect at pH 7.0 (table 3). As was the case at pH 7.0, sodium sulfate was more effective an inhibitor than sodium chloride, i.e., lower concentrations were necessary to reach 50% inhibition.

Table 3

Effect of chloride and sulfate on the ethanol oxidizing activity of human liver alcohol dehydrogenase isoenzymes at their respective pH optima

Isoenzyme	50% inhibition at	
	NaCl (mM)	Na_2SO_4 (mM)
$\beta_1\beta_1$	400	260
$\gamma_1\gamma_1$	700	400
$\beta_2\beta_2$	600	500

$\beta_1\beta_1$, $\gamma_1\gamma_1$ were measured in 67 mM glycine-NaOH buffer, pH 10.5; $\beta_2\beta_2$-Bern was measured in the same buffer but at pH 8.8. The values represent % relative activity (absence of chloride or sulfate = 100%)

4. DISCUSSION

Experiments with horse liver ADH showed that anions such as sulfate bind to the anion binding site around arginine 47, the same site that binds the pyrophosphate group of the cofactor [14]. In analogy, the inhibition of human liver ADH isoenzymes at the higher anion concentrations could be explained by the competition of the anions with the coenzyme. Chloride increases the dissociation constants of the horse ADH–NADH complex in a pH-dependent manner [15]. This could explain the activation of $\beta_1\beta_1$ and $\gamma_1\gamma_1$ by chloride. We found maximal activation of both isoenzymes between 50 and 100 mM NaCl, concentrations that are comparable to the 150 mM found with horse liver ADH also at pH 7.0 [15].

The difference in susceptibility to ions found between $\beta_1\beta_1$, $\gamma_1\gamma_1$, and $\beta_2\beta_2$-Bern most likely reflect differences in their primary structures or in the 3-dimensional configuration of the active sites or of the coenzyme binding sites. Differences in primary structures have been reported [16–19]. Thus, Arg-47 in β_1 is replaced by His-47 in β_2 [16]. Both, β_1 as well as γ_1 have Arg-47 [18,19]. In the horse enzyme Ser-48 together with His-51 and the zinc bound water forms a proton release system essential for the catalytic activity [20]. Like the horse ADH subunit E, γ_1 has Ser-48, whereas β_1 has Thr-48 that probably distorts this system [17]. This results in different catalytic activities of $\beta_1\beta_1$ and $\gamma_1\gamma_1$ [10–12]. Therefore, a His-47 (β_2) would result in complete insensitivity to chloride activation, Thr-48 (β_1) would result in a decreased activation and only Arg-47 together with Ser-48 (γ_1), a configuration occurring in the horse isoenzyme E, would yield maximal effects. Of course it cannot be excluded that other structural differences also reported [16–19] participate.

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