Biochemical Pharmacology, Vol. 27, pp. 1296–1297.

0006-2952/78/0415-1296$02.00/0

Alcohol dehydrogenase from human and horse liver—Substrate specificity with diols*

(*Received* 5 *May* 1977; *accepted* 7 *September* 1977)

1,2-Ethanediol, a common solvent of commercial antifreezes, constitutes a hazard when ingested. 1,2-Propanediol is less toxic and is employed as an antifreeze in breweries and also as a solvent for medicaments. Metabolism of glycols that are important industrial chemicals has been studied in rabbits [1] and other animals [2].

1,3-Butanediol is nontoxic and has been studied in the rat as a potential synthetic food substance [3]. It has also been demonstrated that it may have therapeutic value in alleviating withdrawal after alcohol [4]. Metabolism of 1,3-butanediol [3] was inhibited *in vivo* by both ethanol and pyrazole, demonstrating the involvement of alcohol dehydrogenase (ADH). In this paper, we report on the Michaelis and catalytic rate constants with diols of 2- to 6-carbon chain length for horse and human liver ADH.

ADH from horse liver was obtained from Boehringer und Soehne, Mannheim, Germany. The enzyme was dialyzed in three changes of 0.05 M phosphate buffer, pH 7.5, before use.

Human ADH was prepared from a single liver obtained from a 70-year-old male approximately 10 hr after death from a heart attack. The liver was extracted and fractionated as described previously [5]. The enzyme was stored at 4° in 0.05 M phosphate buffer containing 10% saturated ammonium sulphate; before use, the enzyme was thoroughly dialyzed against 0.05 M phosphate buffer, pH 7.5. The normality of the horse and human ADH was determined by fluorometric titration with standard NADH in the presence of 0.1 M isobutyramide in 0.1 M phosphate buffer, pH 7.0, employing an Aminco–Bowman recording spectrofluorometer at an excitation wavelength of 330 nm and emission wavelength of 410 nm [6].

Kinetic measurements were made with a Beckman DB-GT recording spectrophotometer at 340 nm in a 3-ml volume in cuvettes of 1 cm light path, at 25°. All measurements were made in 0.1 M phosphate buffer, pH 7.0, and 500 μM NAD. The Michaelis and catalytic rate constants were calculated from the linear regression [7] of substrate concentration on velocity (e/v vs $1/S$), using at least four points obtained from duplicate determinations at concentrations well below those resulting in substrate inhibition. Regression coefficients and their standard errors (in all cases less than 5 per cent of the coefficients) and the respective values of K_m and maximal velocity were calculated by the method of least squares.

1,2-Ethanediol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 1,5-pentanediol, 1,6-hexanediol and 1-hexanol were obtained from MCB Manufacturing Chemists, East Rutherford, NJ; 1,4-butanediol was from Eastman Organic Chemicals, Rochester, NY. The purity of each of the diols was investigated by gas–liquid chromatography, using a Gow–Mac 750C chromatograph with a flame ionization detector. A 1-μl sample of a 1% solution in water of each of the substrates was injected into a Tenex-GC column, first at 80° and then at 150°–200°; the first injection revealed contaminants with boiling points of less than 125°, while the second injection showed the major peak as well as any contaminants with boiling points above 125°. Had any volatile substrates for ADH (other than diols themselves) been present, it is estimated that concentrations of about 2 parts/100,000 of the diol would have been detectable. No materials other than the diols were detected in our samples of 1,2-ethanediol, 1,2-propanediol, 1,3-butanediol, 1,4-butanediol, 1,5-pentanediol and 1,6-hexanediol. Chromatography of 1,3-propanediol showed a peak of an unidentified compound that was less volatile than ethanol but more volatile than *n*-propanol and whose concentration was about 2–4 parts/10,000 of the diol. Less volatile materials than the diol itself were also detected: such materials appeared after the diol peak and were estimated to comprise less than 2 per cent of the diol. Since 1,3-propanediol is known to undergo decomposition at the temperature used, it is not unlikely that the peaks appearing after the diol are higher molecular weight ethers arising from dehydration of the diol.

The kinetic parameters for horse and human liver ADH and 2- to 6-carbon diols are presented in Table 1; Michaelis

* Financial support of the National Institute on Alcohol Abuse and Alcoholism Grants AA-00186, AA-00216 and AA-01849 is gratefully acknowledged.

Table 1. Kinetic parameters for various diols with horse and human liver ADH

	Horse liver ADH			Human liver ADH		
Substrate	K_m (mM)	K_{cat}*	Substrate range used (mM)	K_m (mM)	K_{cat}	Substrate range used (mM)
1,2-Ethanediol	50.5	29	7–120	30.2	4.1	7–120
1,2-Propanediol	36.2	90	5–90	31.8	3.5	5–90
1,3-Propanediol	12.9	107	6–46	19.9	6.5	6–93
1,3-Butanediol	6.9	94	4–37	6.8	6.4	2–75
1,4-Butanediol	4.2	171	1–37	1.8	9.4	0.5–18
1,5-Pentanediol	0.7	168	0.25–8	0.3	12.0	0.1–8
1,6-Hexanediol	0.2	180	0.09–2.8	0.07	10.8	0.02–0.7
Ethanol†	0.8	180		0.4	10.7	
1-Hexanol†	0.1	180		0.06	9.7	

* K_{cat} = turnover number at maximal velocity in moles/mole of active sites/min.
† See Ref. 5.

and catalytic rate constants for ethanol and hexanol are included for comparison. As with saturated and unsaturated monohydric alcohols [5], the K_m values of the diols decrease with an increase of their carbon chain length. While 1,2-ethanediol is a relatively poor substrate, 1,5-pentanediol is as good as ethanol.

The catalytic rate constants increase with an increase of chain length until a virtual plateau is reached at 1,4-butanediol, where the values for horse and our preparation of the human ADH were *ca.* 180/active site/min and 11/active site/min, respectively; these values are determined by the rate of NADH dissociation from both enzymes [8, 9]. Diols of short chain length are interconverted at high concentrations with low catalytic rate constants in comparison with monohydric alcohols of the same chain length. There is a great similarity between horse and human ADH in catalysis of dehydrogenation of diols.

The distance in carbon atoms by which the hydroxyl groups are separated is another feature which appears to determine the effectiveness of diols as substrates. Thus, 1,3-propanediol seems to be a better substrate than 1,2-propanediol: its K_m is less than that of 1,2-propanediol and its turnover number is greater; an analogous comparison holds for 1,4-butanediol and 1,3-butanediol. This comparison, however, is not exact because in the first case both hydroxyls are primary alcohol groups, while in the second case one of the hydroxyl groups is a secondary alcohol.

The current preparation of human liver ADH is the same as that used by us previously for determining substrate specificity with saturated and 2-enoic alcohols [5]. The results obtained here with the diols, therefore, are directly comparable with those obtained previously.

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Biochemical Pharmacology, Vol. 27, pp. 1298–1299.

0006-2952/78/0415-1298$02.00/0

Effects of allopurinol on purine metabolism in cultured heart cells

(*Received* 2 *July* 1977; *accepted* 8 *September* 1977)

The release of myocardial adenosine appears to be involved with the adjustments of coronary blood flow to the metabolic requirements of the heart [1]. The loss of purines from the releasing cells must be replenished by either *de novo* synthesis or reutilization (salvage synthesis), or both. The salvage pathway is quite active in the heart [1], and in the presence of allopurinol, a xanthine oxidase inhibitor, it has been postulated that the nucleotide polyphosphate pool is increased during or after periods of oxygen depletion [2, 3]. The present study is concerned with the effects of allopurinol on the uptake and release of adenine and hypoxanthine and their metabolites, and on the status of the intracellular purine nucleotide pool in cultured fibroblasts (F-cells) and beating myocardial cells (M-cells) segregated from the same neonatal heart preparation.

Methods. Hearts from 1- to 2-day-old Sprague–Dawley rats were collected in the sterile nutrient medium employed by Mark and Strasser [4]. The same nutrient medium was used subsequently for the incubation of the isolated heart cells. The apical half of each ventricle was minced and transferred to a flask to which was added 2.5 ml per heart equivalent of 0.125% trypsin in Ca^{2+}- and Mg^{2+}-free Hank's basic salt solution. Gentle stirring was continued for 20 min at 37°, after which the supernatant was discarded. Cells were collected by low-speed centrifugation (1000 rev/min, 4 min) at room temperature after three subsequent periods of digestion of 15 min each in the presence of the same volume of trypsinizing solution. A nearly total maceration of the tissue resulted after this treatment. The sedimented cells were resuspended in the nutrient medium, combined, and 2-ml aliquots layered in a sterile centrifuge tube on 10 ml of nutrient medium containing 2% ficoll (mol. wt 400,000). A low-speed centrifugation (1000 rev/min, 4 min) at room temperature resulted in the sedimentation of intact cells in the loose pellet. The supernatant, containing cellular and other debris, was discarded. The pelleted cells were then washed once by gentle suspension in fresh medium and centrifugation. This preparation represented a purified mixture of viable F- and M-cells obtained from the trypsinized ventricles. The passage through the ficoll–nutrient medium mixture did not affect the viability of the cells and the ability of the myocardial cells to continue beating for at least 4 weeks, and it had the added advantage of reducing initial bacterial contamination.

Cultures enriched with either F-cells or beating M-cells were obtained from the same heart tissue preparation by a differential rate of attachment [5]. Primed culture plates were inoculated with the above mixed cell population containing approximately 2×10^6 cells, and the cultures were incubated in a water-saturated 5% CO_2–95% air incubator at 37°. The F-cells were found to attach to the surface of the culture plate more rapidly than the M-cells. Optimally, the unattached M-cells could be aspirated after 60 min of incubation. Fresh nutrient medium was added to the attached F-cells, and the M-cells in the aspirated medium were concentrated by centrifugation and replated in fresh medium. The nutrient medium was replaced after 24 hr, and the cultures were assessed for morphology and rate of contraction. After 3 days the F-cell cultures were confluent and were essentially pure (99 per cent or better). M-cell cultures represented an enrichment from about 60