The metabolic role of human ADH3 functioning as ethanol dehydrogenase

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Received 19 March 2003; revised 24 April 2003; accepted 25 April 2003

First published online 12 May 2003

Edited by Stuart Ferguson

Abstract **Human class III alcohol dehydrogenase (ADH3), also known as glutathione-dependent formaldehyde dehydrogenase, exhibited non-hyperbolic kinetics with ethanol at a near physiological pH 7.5. The $S_{0.5}$ and k_{cat} were determined to be 3.4 ± 0.3 M and 33 ± 3 min^{-1}, and the Hill coefficient (h) 2.21 ± 0.09, indicating positive cooperativity. Strikingly, the $S_{0.5}$ for ethanol was found to be 5.4×10^6-fold higher than the K_m for *S*-(hydroxymethyl)glutathione, a classic substrate for the enzyme, whereas the k_{cat} for the former was 41% lower than that for the latter. Isotope effects on enzyme activity suggest that hydride transfer may be rate-limiting in the oxidation of ethanol. Kinetic simulations using the experimentally determined Hill constant suggest that gastric ADH3 may highly effectively contribute to the first-pass metabolism at 0.5–3 M ethanol, an attainable range in the gastric lumen during alcohol consumption. The positive cooperativity mainly accounts for this metabolic role of ADH3.**

Key words: Alcohol dehydrogenase, class III; Formaldehyde dehydrogenase; Ethanol dehydrogenase; Kinetic cooperativity; First-pass metabolism

1. Introduction

Alcohol dehydrogenase (ADH) constitutes a complex enzyme family [1,2]. Class III ADH (ADH3) is unique in its ancient origin [3], identification as a glutathione-dependent formaldehyde dehydrogenase [4] and as a constant type with regard to enzyme function and overall structure [5]. Expression of ADH3 appears ubiquitous in mammalian tissues [6]. ADH3 is highly active toward a wide variety of substrates, notably *S*-(hydroxymethyl)glutathione (HMGSH) [4], *S*-nitrosoglutathione [7,8], ω-hydroxyfatty acids [9], and 20-hydroxyleukotriene B4 [10]. Recent studies with the gene knocked out in mice support that ADH3 may contribute to detoxification of formaldehyde [11], protection against nitrosative stress, and the maintenance of *S*-nitrosothiol homeostasis [12] that appears central to nitric oxide signaling and host defense. Despite its extremely poor activity with all-*trans*-retinol, ADH3 may also be involved in the production of retinoic acid, a morphogen and transcriptional regulator, as revealed from the *ADH3*$^{-/-}$ mice studies [13].

Human ADH3 oxidizes ethanol very poorly at physiologically attainable blood alcohol levels (< 50 mM) and the activity appeared directly proportional to the substrate concentration up to 2 M ethanol at alkaline pH [9,14,15]. It has thus been long thought that ADH3 plays only a very minor role in the hepatic metabolism of ethanol [16]. First-pass, or presystemic, metabolism (FPM) of alcohol in stomach, however, presents a unique situation since the gastric ethanol levels can reach virtually molar range during alcohol consumption [17]. A recent study [18] correlating ethanol pharmacokinetics in blood and ADH3 activity in stomach suggested that the gastric enzyme may contribute to the FPM in humans. We previously demonstrated by kinetic simulation that at near physiological pH the high-K_m members of the human ADH family, ADH2 in liver and ADH4 in stomach, may effectively contribute to the FPM [19]. FPM of alcohol influences its bioavailability in the systemic blood and hence the pharmacodynamic effects on the target tissues. We report here for the first time that human ADH3 exhibits positive cooperativity with ethanol and the metabolic significance in FPM by kinetic simulation.

2. Materials and methods

Human recombinant ADH3 was expressed in *Escherichia coli* as described previously [20]. The resulting enzyme was initially isolated from the lysate supernatant by DEAE-cellulose chromatography (DE52, Whatman; 150 ml of DEAE/l of culture) in 10 mM Tris, pH 8.0, 0.1% (v/v) 2-mercaptoethanol at 4°C. The unbound proteins were eluted off the 2.6×28-cm DEAE-cellulose column with the equilibrating buffer. The eluate was concentrated and then dialyzed into 50 mM sodium phosphate, pH 7.4, 0.1% 2-mercaptoethanol and loaded onto a 1.6×10-cm 5′-AMP-Sepharose (Amersham Biosciences) column equilibrated in the same buffer. The protein was eluted with a linear gradient from 0 to 1 mM NADH in 50 mM sodium phosphate, pH 7.4, 0.1% 2-mercaptoethanol. The isolated recombinant enzyme exhibited a single protein-staining band with a molecular mass of 40 kDa on sodium dodecyl sulfate–polyacrylamide gel electrophoresis by a PhastSystem according to the manufacturer's protocol (Amersham Biosciences). Total protein concentration was determined by the Lowry method [21]. ADH3 (> 1 mg/ml) appeared stable for at least 5 days in 10 mM sodium phosphate, pH 7.5, when it was kept in an ice bath.

Formaldehyde was prepared by hydrolyzing hexamethylenetetramine with sulfuric acid and standardized after steam distillation by the chromotropic acid method as described previously [22]. During activity measurements, glutathione and formaldehyde were allowed to reach equilibrium with the hemithioacetal adduct HMGSH for 2 min in assay buffer (pH 7.5) at 25°C before addition of the remaining components to initiate the assay. The concentration of HMGSH in

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Abbreviations: ADH, alcohol dehydrogenase; ADH3, class III ADH; HMGSH, *S*-(hydroxymethyl)glutathione; FPM, first-pass metabolism

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00492-7

the assay mixture was calculated using a K_{eq} of 1.77 mM^{-1} [20]. Due to very low Michaelis constant (< 1 μM) for HMGSH, enzyme activity was measured by monitoring fluorescence of the produced NADH (excitation at 340 nm; emission at 460 nm) in the K_m determination experiments. Enzyme activity with ethanol was assayed by monitoring the production of NADH at 340 nm using an A_{340} of 6.22 mM^{-1} cm^{-1}. The reaction was initiated with addition of the enzyme. Stability of ADH3 with ethanol in 100 mM sodium phosphate at pH 7.5 and 25°C was examined for a duration of 2–3 min that was required in the linear initial velocity measurements and the reaction was then initiated with addition of the coenzyme NAD^+. Controls for the examined ethanol concentrations were those in the absence of incubation with the enzyme, i.e. reaction mixture of the substrate and coenzyme was directly initiated with addition of the enzyme, and the linear initial velocity was measured. The ethanol concentration up to 3.4 M was found with reasonable stability (> 90% activity) and thus was employed as the upper limit in experiments of substrate saturation.

Kinetic studies with ethanol and HMGSH were performed in 100 mM sodium phosphate (pH 7.5) at 25°C containing 0.5 mM NAD^+ and varied concentrations of substrate. It has been reported that cytosolic NAD^+ concentration in rat hepatocytes was ca. 0.5 mM [35]. Steady-state kinetic data were analyzed by non-linear regression using the programs of Cleland [23] or the formulating equations that were written according to the statistical package program of SigmaPlot (version 7.0). Initial velocity data were fit with the Michaelis–Menten equation (Eq. 1) or the Hill exponential equation (Eq. 2).

$$v = \frac{V_{max} \times S}{K_m + S} \quad (1)$$

$$v = \frac{V_{max} \times S^n}{S_{0.5}^n + S^n} \quad (2)$$

where V_{max} is the maximum velocity; S is the substrate concentration; K_m is the Michaelis constant; $S_{0.5}$ is the substrate concentration at half-maximal velocity; and n is the Hill coefficient (also denoted h). Maximal velocity is expressed as turnover number (min^{-1}) based on a subunit molecular mass of 40 kDa. To visualize kinetic cooperativity, initial velocity data were also fit with equations for the Hanes plot (Eq. 3) and the Hill plot (Eq. 4).

$$\frac{S}{v} = \frac{S}{V_{max}} + \frac{K_m}{V_{max}} \quad (3)$$

$$\log\frac{v}{V_{max} - v} = n \times \log S - n \times \log S_{0.5} \quad (4)$$

FPM of ethanol by ADH3 in the stomach and liver was simulated using a combination of two Hill exponential equations for two substrate concentrations at different compartments.

$$\text{FPM} = \frac{V_{max} \times S_1^n}{S_{0.5}^n + S_1^n} - \frac{V_{max} \times S_2^n}{S_{0.5}^n + S_2^n} \quad (5)$$

where S_1 is the ethanol concentration in the gastric lumen or the portal vein and S_2 is the ethanol concentration in the systemic blood. All of the kinetic measurements were run in duplicate for HMGSH and in triplicate for ethanol. The values represent the means ± S.E.M.

3. Results

Substrate saturation profiles of human ADH3 displayed Michaelian kinetics with the classic substrate HMGSH but non-hyperbolic kinetics with ethanol at pH 7.5 (Fig. 1). The Hanes plots show a straight line with positive slope for HMGSH (Fig. 1B) and a concave-up curvature for ethanol (Fig. 1A) that is typical of positive cooperativity [24]. Both substrates fit well to the linear Hill plot over the entire concentration range (regression coefficient $R^2 > 0.99$) (Fig. 2). The kinetic parameters for ethanol and HMGSH of ADH3 are compared in Table 1. Standard errors for all determined

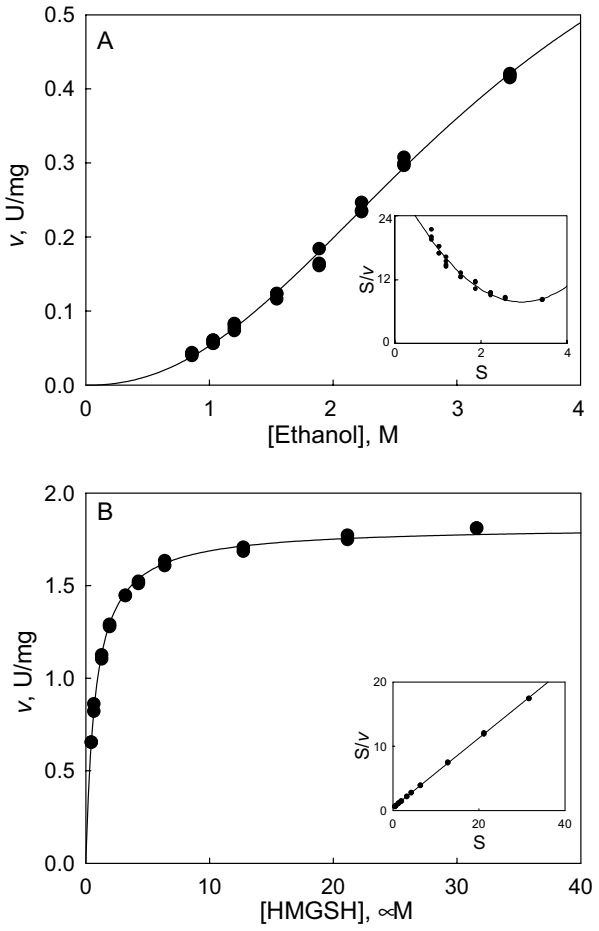

Fig. 1. Saturation profiles of ADH3 toward oxidation of ethanol (A) and HMGSH (B). Enzyme activity was determined in 0.1 M sodium phosphate, pH 7.5, at 25°C, containing 0.5 mM NAD^+. Specific activity is measured as μmol/min/mg (U/mg). The lines are the fit of data to Eqs. 2 and 1 in A and B, respectively. Inset is the data fit to Eq. 3. The Hill coefficient, h, for ethanol was calculated to be 2.21 ± 0.09.

kinetic parameters were less than 10% of the values, suggesting a reasonable precision. Strikingly, the $S_{0.5}$ for ethanol was 5.4×10^6-fold higher than the K_m for HMGSH, whereas the k_{cat} for the former was 41% lower than that for the latter. The Hill coefficient, h, for ethanol oxidation was determined to be 2.21 ± 0.09, clearly indicating a positive cooperativity. Kinetic measurements for ethanol were also performed at a higher NAD^+ (5 mM) in the same buffer, and k_{cat} and $S_{0.5}$ were determined to be 28 ± 2 min^{-1} and 3.0 ± 0.2 M, and h 2.26 ± 0.11. The kinetic parameters for ethanol were similar at 0.5 and 5 mM NAD^+. It was attempted to study kinetic isotope effects for ethanol at 0.5 mM NAD^+. The k_{cat} and $S_{0.5}$ for deuterio ethanol failed to show acceptable statistical precision as fitting the data to Eq. 2, though it still exhibited a reliable positive cooperativity of $h = 1.93 \pm 0.05$. This may be in part due to much lower enzyme activity with deuterio ethanol compared with protio ethanol in the paired assays. The activity ratio profile of protio ethanol to deuterio ethanol showed that $v^{H/D}$ ranged over 5.1–3.3 for 0.86–3.4 M substrate, demonstrating a significant isotope effect on the enzyme activity (Fig. 3).

FMP of ethanol through the ADH3 pathway in human stomach and liver was quantitatively assessed using Eq. 5

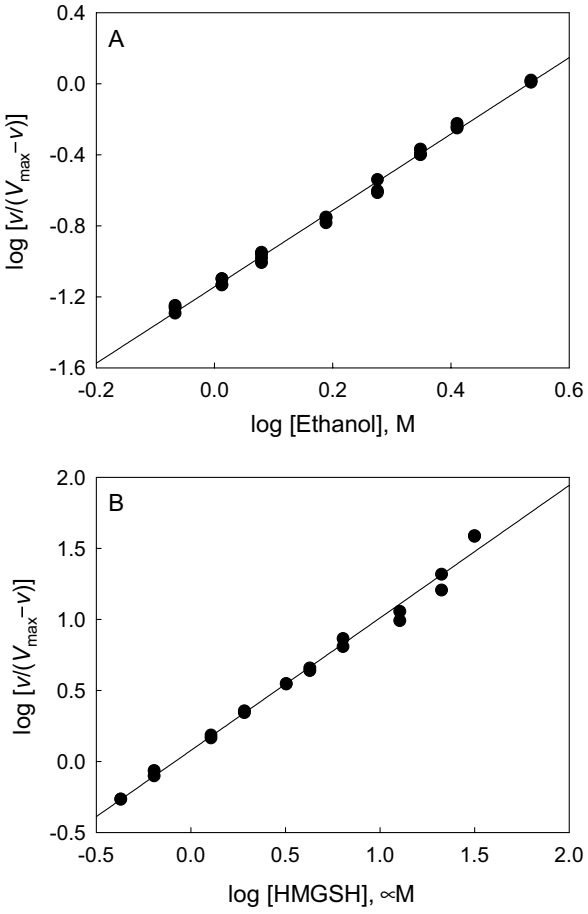

Fig. 2. Hill plots for ethanol (A) and HMGSH (B). The line is the linear regression of data to Eq. 4. The regression coefficients, R^2, are 0.997 and 0.992 for ethanol and HMGSH, respectively. The Hill coefficient, h, for HMGSH was calculated to be 1.01 ± 0.03.

on the basis of a reasonable estimation of the enzyme contents in the tissues as well as blood ethanol levels in different compartments. It was reported following purification and the correction for the yield that human liver contained ~20 mg ADH3 per 100 g tissue [15]. Hence the content of ADH3 in a liver (~1500 g) was estimated as 300 mg. Based on previous reports for the specific activity of ADH3 in human stomach mucosa (~6 nmol/min/mg mucosal protein) [18], the protein concentration of the human stomach mucosa (~60 mg/g tissue) [25], and the specific activity of the purified ADH3 which was measured according to the assay condition in [18] (i.e. 1.6 μmol/min/mg; unpublished data), it was estimated that human stomach mucosa contained ~0.2 mg ADH3 per g tissue.

Table 1
Kinetic parameters of ADH3 at pH 7.5

Substrate	Kinetic parameter	
Ethanol	$S_{0.5}$ (M)	3.4 ± 0.3
	k_{cat} (min^{-1})	33 ± 3
	h	2.21 ± 0.09
HMGSH	K_m (μM)	0.63 ± 0.03
	k_{cat} (min^{-1})	56 ± 1
	k_{cat}/K_m (min^{-1} μM^{-1})	89 ± 3
	h	1.01 ± 0.03

For experimental conditions and the fitting equations, see Figs. 1 and 2. h, Hill coefficient. Values represent means ± S.E.M.

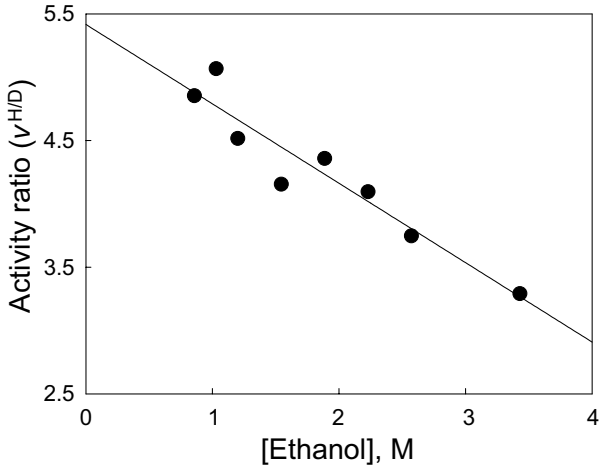

Fig. 3. Deuterium isotope effects for the ethanol-oxidizing activity. $v^{H/D}$ represents ratios of the mean activity of protio ethanol to that of deuterio ethanol (ethanol-d_5). Activity measurements were run in triplicate for each concentration point. The linear equation of best fit for the data was estimated to be $y = -0.627x + 5.416$; $R^2 = 0.905$.

Hence the ADH3 content in the mucosa of the stomach (assuming one third of the stomach is mucosa, i.e. 50 g) can be estimated as 10 mg. The capacity of FPM by ADH3 in stomach and liver was then simulated using Eq. 5 (Table 2). Tremendous rises of the enzyme activity (7600–180 000-fold) in comparison with the corresponding substrate concentration ratios (75–250) demonstrated that gastric ADH3 may effectively contribute to FPM of ethanol on account of the positive cooperativity.

4. Discussion

Human ADH3 exhibits hyperbolic saturation kinetics toward a wide range (0.7–50 K_m) of HMGSH (Fig. 1B). This finding is in agreement with the report by Sanghani et al. [20] but in conflict with that by Uotila and Mannervik [27] who observed a non-hyperbolic behavior. Steady-state kinetic and equilibrium binding studies indicate that the oxidation of HMGSH with human ADH3 is consistent with a rapid equilibrium random sequential mechanism [20]. The structural explanation for the mechanism has been ascribed to a semi-open structure of the substrate/coenzyme domain [28]. In contrast, human ADH3 displays a non-hyperbolic saturation kinetics toward the far smaller substrate ethanol at pH 7.5 (Fig. 1A) with a positive cooperativity of $h = 2.2$ (Table 1). Previous studies [9,14,15] based on the observations of a linear saturation profile up to 2 M ethanol and the corresponding linear double reciprocal plot suggested that human ADH3 may follow the Michaelian kinetics with ethanol at alkaline pH 10. The k_{cat} and K_m for ethanol could not be determined in these studies simply because of a presumed extremely high K_m [9,15]. In the present study at pH 7.5, the k_{cat} and $S_{0.5}$ for ethanol of ADH3 were determined to be 33 min^{-1} and 3.4 M, respectively (Table 1). The $S_{0.5}$ is strikingly 100–150 000-fold greater than the K_ms for human ADH1A, ADH1B1, ADH1B2, ADH1C1, ADH2 and ADH4 [19]. The k_{cat} is 1.3–8.7-fold higher than those of the ADH1A, ADH1B1, ADH1C1 and ADH2, but 7.3- and 45-fold lower than those of the ADH1B2 and ADH4, respectively [19]. ADH1B2 and ADH4 belong to the high-k_{cat} members of the family [29].

Table 2
Simulations of gastric and hepatic contribution to FPM of ethanol by ADH3

Organ	Assumed ethanol concentration				Activity increase (fold)	FPM capacity (μmol/min/organ)
	Gastric lumen (mM)	Portal vein (mM)	Systemic blood (mM)	Concentration ratio		
Stomach	500		4	125	40 000	0.12
	1000		4	250	180 000	0.52
	3000		40	75	7 600	3.6
Liver		50	4	12.5	260	0.023
		200	40	5	34	0.47

Enzyme activity was simulated using Eq. 2 and the capacity for FPM was simulated using Eq. 5. For values of the kinetic parameters, see Table 1. Total contents of ADH3 were estimated as 10 mg in the stomach and 300 mg in the liver (see Section 3). It was reported that in humans the blood ethanol levels may reach ~4 mM during 30–90 min after postprandial ingestion of alcohol (0.3 g/kg) [18] and may reach ~40 mM during 2–8 h after ingestion (1.75 g/kg) [26]. Ethanol levels in the human gastric lumen may reach ~1.5 M after ingestion (0.8 g/kg) [17]. 3 M ethanol is equivalent to 17.5% (v/v). The cytosolic NAD^+ concentration in rat liver was reported to be ~0.5 mM [35].

Therefore, the kinetic features for ethanol oxidation for ADH3 in the human ADH family are a tremendously large $S_{0.5}$, and the highest k_{cat} among the member group with lower catalytic constants.

Human ADH1C1 and ADH1C2 allozymes exhibit a negative cooperativity toward ethanol oxidation [19,30]. This has been explained by an ordered sequential mechanism with alternative pathways, including abortive enzyme–NADH–ethanol and binary enzyme–NADH complexes, which differ in the rate of dissociation of NADH [31]. The NADH release appears to be rate-limiting in ethanol oxidation for most mammalian ADHs such as the prototype horse ADH1E [32]. In contrast, human ADH3 exhibits positive cooperativity with ethanol. It seems unlikely through the same mechanism of alternative pathways for coenzyme dissociation which determines the catalytic rate. Deuterium isotope effect results (Fig. 3) suggest that hydride transfer rather than coenzyme release may be rate-limiting for ethanol oxidation. This inference is supported by the finding that k_{cat} for ethanol appears to be even lower than that for HMGSH (Table 1) and that the conversion of central ternary complexes is rate-limiting in HMGSH oxidation for human ADH3, consistent with a rapid equilibrium random mechanism [20]. X-ray studies [28,33] provide no evidence of a subunit interaction in human ADH3 resulting from binding with the substrate ligand which would account for a site–site cooperativity. Kinetic studies of homodimer and the heterodimer of human ADH1B1 and ADH1B2 allozymes indicate that the constituent subunits act independently [34]. To examine a possible steady-state random mechanism [24] that might cause the observed kinetic cooperativity at 0.5 mM NAD^+ (Fig. 1A), ethanol saturation kinetics was also performed in the presence of 5 mM NAD^+ (i.e. 455-fold greater than the K_d [20] for the enzyme–NAD^+ complex). This potential kinetic cause seems to be ruled out by the finding of the very similar kinetic parameters (i.e. $S_{0.5}$, k_{cat} and h) for ethanol that were determined at 0.5 and 5 mM NAD^+, since under such a huge excess of coenzyme (5 mM) the path of binding NAD^+ prior to ethanol should be predominant, i.e. the reaction would become ordered for a random mechanism [24]. The exact kinetic mechanism of ADH3 for ethanol remains unclear. It was difficult to approach using initial velocity study owing to the fairly low activity with such a high $S_{0.5}$ enzyme in the experiments. We speculate that one possible explanation would be binding of multiple ethanol molecules in the relatively spacious active site as alternative pathways when increasing ethanol concentration that could facilitate to yield a binding mode better for hydride transfer in the enzyme–NAD^+–ethanol complex. It obviously requires further studies to test this hypothetical mechanism for the observed kinetic cooperativity.

FPM, i.e. the difference between the quantity of ethanol that reaches the systemic circulation by the intravenous route and the quantity that entered by the oral dose, may mainly occur in the liver and stomach. The contribution of gastric and hepatic ADH3 to the FPM can be simulated using Eq. 5 (Table 2). The simulation results demonstrate that the very high $S_{0.5}$ (3.4 M) in combination with a quite high h (2.2) contributes to a surprisingly significant FPM at the range of 0.5–3 M ethanol that is attainable in the gastric lumen during alcohol consumption. This is evidenced by a striking contrast of the activity increase folds with the corresponding concentration ratios of ethanol. For instance, ADH3 exhibits a huge increase of activity (180 000-fold) at ethanol levels of 1000 mM in the gastric lumen versus 4 mM in the systemic blood (cf. 250, the corresponding ethanol concentration ratio). Thus a 720-fold ($180\,000 \div 250 = 720$) activity increase is mainly attributable to the positive cooperativity for ethanol oxidation. The capacity of hepatic FPM through ADH3 appears much lower than that in the stomach (Table 2). This is because of the much lower ethanol levels in portal vein than in gastric lumen even though the hepatic ADH3 content is estimated to be 30-fold greater than the gastric enzyme. It is worth noting, however, that the amount of FPM contributed by both gastric and hepatic ADH3 (e.g. 1-h average FPM capacity for an ethanol dose of 0.3 g/kg or 4-h average FPM capacity for 1.75 g/kg in a 70-kg person) may be very minor (calculated estimate, $< 0.1\%$) relative to the total amount of ethanol consumed. It thus seems unlikely that the gender difference in postprandial blood ethanol levels is due mainly to the difference in gastric ADH3 activity as proposed by Baraona et al. [18].

In conclusion, human ADH3 exhibits non-hyperbolic saturation kinetics with ethanol at near physiological pH. The high $S_{0.5}$ and in particular the relatively high h account for the effective contribution to FPM by gastric ADH3 at physiologically attainable ethanol levels.

Acknowledgements: We thank Dr. Thomas D. Hurley and Dr. William F. Bosron (Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, USA) for the generous gift of the expression vector for human ADH3. This work was supported by grants from the National Science Council 89-2320-B016-002 and the National Health Research Institutes EX91-8939BP.

References

[1] Duester, G., Farrés, J., Felder, M.R., Holmes, R.S., Höög, J.-O., Parés, X., Plapp, B.V., Yin, S.-J. and Jörnvall, H. (1999) Biochem. Pharmacol. 58, 389–395.
[2] Jörnvall, H., Höög, J.-O., Persson, B. and Parés, X. (2000) Pharmacology 61, 184–191.
[3] Danielsson, O. and Jörnvall, H. (1992) Proc. Natl. Acad. Sci. USA 89, 9247–9251.
[4] Koivusalo, M., Baumann, M. and Uotila, L. (1989) FEBS Lett. 257, 105–109.
[5] Danielsson, O., Atrian, S., Luque, T., Hjelmqvist, L., Gonzalez-Duarte, R. and Jörnvall, H. (1994) Proc. Natl. Acad. Sci. USA 91, 4980–4984.
[6] Edenberg, H.J. (2000) Prog. Nucleic Acids Res. Mol. Biol. 64, 295–341.
[7] Jensen, D.E., Belka, G.K. and Du Bois, G.C. (1998) Biochem. J. 331, 659–668.
[8] Hedberg, J.J., Griffiths, W.J., Nilsson, S.J.F. and Höög, J.-O. (2003) Eur. J. Biochem. 270, 1249–1256.
[9] Wagner, F.W., Parés, X., Holmquist, B. and Vallee, B.L. (1984) Biochemistry 23, 2193–2199.
[10] Gotoh, Y., Sumimoto, H. and Minakami, S. (1990) Biochim. Biophys. Acta 1043, 52–56.
[11] Deltour, L., Foglio, M.H. and Duester, G. (1999) J. Biol. Chem. 274, 16796–16801.
[12] Liu, L., Hausladen, A., Zeng, M., Que, L., Heitman, J. and Stamler, J.S. (2001) Nature 410, 490–494.
[13] Molotkov, A., Fan, X., Deltour, L., Foglio, M.H., Martras, S., Farrés, J., Parés, X. and Duester, G. (2002) Proc. Natl. Acad. Sci. USA 99, 5337–5342.
[14] Parés, X. and Vallee, B.L. (1981) Biochem. Biophys. Res. Commun. 98, 122–130.
[15] Moulis, J.M., Holmquist, B. and Vallee, B.L. (1991) Biochemistry 30, 5743–5749.
[16] Yin, S.-J. and Agarwal, D.P. (2001) in: Alcohol in Health and Disease (Agarwal, D.P. and Seitz, H.K., Eds.), pp. 1–26, Marcel Dekker, New York.
[17] Halsted, C.H., Robles, E.A. and Mezey, E. (1973) Am. J. Clin. Nutr. 26, 831–834.
[18] Baraona, E., Abittan, C.S., Dohmen, K., Moretti, M., Pozzato, G., Chayes, Z.W., Schaefer, C. and Lieber, C.S. (2001) Alcohol. Clin. Exp. Res. 25, 502–507.
[19] Han, C.-L., Liao, C.-S., Wu, C.-W., Hwong, C.-L., Lee, A.-R. and Yin, S.-J. (1998) Eur. J. Biochem. 254, 25–31.
[20] Sanghani, P.C., Stone, C.L., Ray, B.D., Pindel, E.V., Hurley, T.D. and Bosron, W.F. (2000) Biochemistry 39, 10720–10729.
[21] Lowry, O.H., Rosebrough, N.J., Farr, A.I. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[22] Uotila, L. and Koivusalo, M. (1974) J. Biol. Chem. 249, 7653–7663.
[23] Cleland, W.W. (1979) Methods Enzymol. 63, 103–138.
[24] Neet, K.E. (1995) Methods Enzymol. 249, 519–567.
[25] Lai, C.-L., Chao, Y.-C., Chen, Y.-C., Liao, C.-S., Chen, M.-C., Liu, Y.-C. and Yin, S.-J. (2000) Alcohol. Clin. Exp. Res. 24, 1625–1632.
[26] Ylikahri, R.H., Leino, T., Huttunen, M.O., Pösö, A.R., Eriksson, C.J.P. and Nikkilä, E.A. (1976) Eur. J. Clin. Invest. 6, 93–102.
[27] Uotila, L. and Mannervik, B. (1979) Biochem. J. 177, 869–878.
[28] Sanghani, P.C., Robinson, H., Bosron, W.F. and Hurley, T.D. (2002) Biochemistry 41, 10778–10786.
[29] Yin, S.-J., Han, C.-L., Lee, A.-I. and Wu, C.-W. (1999) Adv. Exp. Med. Biol. 463, 265–274.
[30] Bosron, W.F., Magnes, L.J. and Li, T.-K. (1983) Biochemistry 22, 1852–1857.
[31] Charlier Jr., H.A. and Plapp, B.V. (2000) J. Biol. Chem. 275, 11569–11575.
[32] Dalziel, K. and Dickinson, F.M. (1966) Biochem. J. 100, 34–46.
[33] Yang, Z.N., Bosron, W.F. and Hurley, T.D. (1997) J. Mol. Biol. 265, 330–343.
[34] Yin, S.-J., Bosron, W.F., Magnes, L.J. and Li, T.-K. (1984) Biochemistry 23, 5847–5853.
[35] Veech, R.L., Guynn, R. and Veloso, D. (1972) Biochem. J. 127, 387–397.