Oxidation of methanol, ethylene glycol, and isopropanol with human alcohol dehydrogenase family and the inhibition by ethanol and 4-methylpyrazole

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ABSTRACT

Human alcohol dehydrogenase (ADH) family comprises multiple isozymes with wide substrate specificity and ethnic distinct allozymes. ADH catalyzes the rate-limiting step in metabolism of various primary and secondary aliphatic alcohols. The oxidation of common toxic alcohols, that is, methanol, ethylene glycol, and isopropanol in the context of human ADH family remains poorly understood. Kinetic studies were performed in 0.1 M sodium phosphate buffer, at pH 7.5 and 25 ^{o}C, containing 0.5 mM NAD^{+} and varied concentrations of substrate. K_M values for ethanol with recombinant human class I ADH1A, ADH1B1, ADH1B2, ADH1B3, ADH1C1, and ADH1C2, and class II ADH2 and class IV ADH4 were determined to be in the range of 0.12 to 57 mM, for methanol to be 2.0 to 3,500 mM, for ethylene glycol to be 4.3 to 2,600 mM, and for isopropanol to be 0.73 to 3,400 mM. ADH1B3 appeared to be inactive toward ethylene glycol, and ADH2 and ADH4, inactive with methanol. The variations for V_{max} for the toxic alcohols were much less than that of the K_M across ADH family. 4-methylpyrazole (4MP) was competitive inhibitor with respect to ethanol for ADH1A, ADH1B1, ADH1B2, ADH1C1 and ADH1C2, and noncompetitive inhibitor for ADH1B3, ADH2 and ADH4, with the slope inhibition constants (K_{is}) for the whole family being 0.062 to 960 μM and the intercept inhibition constants (K_{ii}), 33 to 3,000 μM. Computer simulation studies using inhibition equations in the presence of

alternate substrate ethanol and of dead-end inhibitor 4MP with the determined corresponding kinetic parameters for ADH family, indicate that the oxidation of the toxic alcohols up to 50 mM are largely inhibited by 20 mM ethanol or by 50 μM 4MP with noticed exceptions. The above findings provide an enzymological basis for clinical treatment of methanol and ethylene glycol poisoning by 4MP or ethanol with pharmacogenetic perspectives.

Keywords:

Human alcohol dehydrogenase family

Methanol

Ethylene glycol

Isopropanol

4-Methypyrazole

Treatment of poisoning

1. Introduction

Human alcohol dehydrogenase (ADH) constitutes a complex enzyme family that is unique with wide variability of kinetic characteristics and allelic variations among racial populations [1–3]. Primarily based on the homology of primary structure and chromosomal organization of the ADH gene cluster, and also on the electrophoretic mobility, the Michaelis constants for ethanol, the sensitivity to pyrazole inhibition and the immunochemical features, human ADH family members have been categorized into five classes [4,5]. The class I ADH contains multiple forms, that is, ADH1A (previously denoted $\alpha\alpha$), ADH1B ($\beta\beta$), and ADH1C ($\gamma\gamma$). The class II to IV ADHs contain a single form each, that is, ADH2 ($\pi\pi$), ADH3 ($\chi\chi$), and ADH4 ($\mu\mu$ or $\sigma\sigma$), respectively. ADH1B and ADH1C exhibit functional polymorphisms [3,6]. Alleles *ADH1B*1* (encoding the β_1 subunit polypeptide) and *ADH1B*2* (encoding β_2 subunit) are predominant among Caucasians and East Asians, respectively; *ADH1B*3* (encoding β_3 subunit) is found exclusively in Africans and some tribes of American Indians. *ADH1C*1* (encoding γ_1 subunit) and *ADH1C*2* (encoding γ_2 subunit) are about equally distributed among Caucasians and American Indians, but the former is highly prevalent among the East Asian and African populations. The ADH family has been involved in the metabolism of a wide variety of physiological and pharmacological substrates, such as retinoids, steroids, biogenic amines,

S-nitrosothiols, lipid peroxidation products, ω-hydroxyfatty acids as well as xenobiotic primary and secondary alcohols and aldehydes [1–3,7]. Currently, class V ADH is the only family member having no available data for catalytic function due to its extremely labile activity [7].

Intoxications with ethylene glycol, methanol, and isopropanol are among the most common ingestions [8]. Toxicity is related to the production of toxic metabolites by ADH and aldehyde dehydrogenase, that is, glycolate and oxalate in ethylene glycol poisoning and formate in methanol poisoning [9,10]. In contrast, metabolite acetone appears less toxic than the parent compound isopropanol [8]. ADH is the rate-limiting step in metabolism of these common industrial toxic alcohols [8–10]. Inhibition by alternate substrate ethanol and dead-end inhibitor 4-methylpyrazole (4MP; fomepizole) of ADH in combination with hemodialysis has been widely used for treatment of severe ethylene glycol and methanol poisonings [8–14]. Hemodialysis is recommended for severe isopropanol intoxication which can remove both isopropanol and acetone effectively [8–10]. Although ethanol and 4MP are accepted antidotes, their enzymological basis for inhibition of ethylene glycol and methanol oxidation with individual human ADH isozymes and allozymes remains unclear. In this report, we determined the corresponding kinetic parameters and simulate effectiveness of the

inhibitions at pharmacologically relevant alcohol levels in the context of ADH family. Isopropanol was also included as a comparison of secondary toxic alcohol.

2. Materials and methods

2.1. Expression and purification of human ADH family

The expression of recombinant enzyme in *Escherichia coli* and purification to apparent homogeneity for human ADH1B1, ADH1B2, ADH1B3, ADH1C1, ADH1C2, ADH2, and ADH4 were as described previously [15,16]. Human ADH1A was expressed and isolated essentially as those procedures for ADH2 [16] with slight modifications in the final purification step of 5'-AMP-Sepharose affinity chromatography, which was equilibrated with 10 mM Hepes, pH 7.0, at 4 ^{o}C instead of the 10 mM sodium phosphate, pH 6.5 [17]. All of the isolated recombinant ADH forms exhibited a single Coomassie blue–staining protein band with a molecular mass of 40,000 Da on sodium dodecyl sulfate–polyacrylamide gel electrophoresis by a PhastSystem according to the manufacturer's protocol (Amersham Biosciences, Bucks, UK). Protein concentration was determined by the method of Lowry et al. [18] using bovine serum albumin as the standard.

2.2. Kinetic analysis

Kinetic studies were performed in 0.1 M sodium phosphate at pH 7.5 and 25 ^{o}C, containing 0.5 mM NAD^{+} and varied concentrations of substrate in the absence or

presence of inhibitor. It has been reported that cytosolic NAD^+ concentration in rat hepatocytes was ca. 0.5 mM [19]. The enzyme activity was determined by monitoring the production of NADH at 340 nm using an absorption coefficient of 6.22 $mM^{-1}cm^{-1}$ or at 460 nm for emission of the fluorescence. Enzyme activity units (U) are expressed as micromoles of NADH formed per minute. Steady-state kinetic data were analyzed by nonlinear least-squares regression using the Cleland programs of HYPER, COMP, NONCOMP, and UNCOMP [20]. Initial velocity data were fit with HYPER program to the Michaelis–Menten equation.

$$v = \frac{V_{max} \times S}{K_M + S} \qquad (1)$$

The data from dead-end inhibition studies were fit with one of the following linear inhibition equations, that is, the COMP program for competitive inhibition, the NONCOMP program for noncompetitive inhibition, or the UNCOMP program for uncompetitive inhibition, respectively.

$$v = \frac{V_{max} \times S}{K_M (1 + I/K_{is}) + S} \qquad (2)$$

$$v = \frac{V_{max} \times S}{K_M (1 + I/K_{is}) + S (1 + I/K_{ii})} \qquad (3)$$

$$v = \frac{V_{max} \times S}{K_M + S (1 + I/K_{ii})} \qquad (4)$$

where V_{max} is the maximum velocity, S is the substrate concentration, K_M is the Michaelis constant, I is the inhibitor concentration, and K_{is} and K_{ii} are the slope inhibition and intercept inhibition constants, respectively. The best fit was determined

by evaluating the standard errors of the kinetic constants and the residual variance [20]. In cases where the intercepts and/or slopes did not vary greatly with inhibitor concentration, Student's *t*-tests were applied to determine if they were significantly different. All of the kinetic measurements were performed in duplicate. Values represent means ± standard error of the mean (SEM). The coefficients of variation of the K_M and V_{max} values were usually less than 15% and those of the inhibition constants were less than 19%.

For evaluation of competitive inhibition of oxidation of toxic alcohols by ethanol, the following equation for enzymes catalyzing two reactions simultaneously was employed [21].

$$v_a = \frac{V_a \times S_a}{K_a\,(1 + S_b/K_b) + S_a} \qquad (5)$$

where V_a is the maximum velocity of toxic alcohol, S_a and S_b are the concentrations of toxic alcohol and ethanol, respectively; and K_a and K_b are the Michaelis constants for toxic alcohol and ethanol, respectively.

Assuming toxic alcohol and ethanol are present in the same concentration, the activity ratio for the toxic alcohol to the ethanol with ADH isozymes/allozymes will be

$$\frac{v_a}{v_b} = \frac{V_a/K_a}{V_b/K_b} \qquad (6)$$

where V_a and V_b are the maximum velocities, and K_a and K_b are the Michaelis constants for toxic alcohol and ethanol, respectively.

3. Results

K_M and V_{max} for ethanol, methanol, ethylene glycol, and isopropanol with human ADH family are shown in Tables 1 and 2, respectively. The K_M values varied tremendously larger than did the V_{max} values for the ADH members. With respect to ethanol and isopropanol, ADH isozymes/allozymes exhibited 2,500 and 4,700-fold variations in K_M while showed 130 and 80-fold variations in V_{max}, respectively. ADH1B3 was virtually inactive toward ethylene glycol, and both ADH2 and ADH4 were inactive with methanol. ADH1A, ADH1B1, ADH1B2, ADH1C1, and ADH1C2 displayed 3,600, 190, 650, 4,800, and 1,100-fold variations, respectively, for K_M for ethanol and the three toxic alcohols. The corresponding catalytic efficiencies, V_{max}/K_M, for ADH family are shown in Table 3. The ratios of the catalytic efficiency for toxic alcohol to that for ethanol represent relative activity of the toxic alcohol to the ethanol with ADH, as the two alternate substrates are present in equimolar concentrations. Methanol, ethylene glycol, and isopropanol are tremendously less efficient than ethanol for the ADH isozymes and allozymes studied (the relative V_{max}/K_M ranging from 0.0063% to 2.3%), with the only exception that ADH1A exhibited 5.9-fold greater catalytic efficiency for isopropanol than that for ethanol.

Inhibitions of oxidation of a wide range of methanol, ethylene glycol, and isopropanol by the fixed alternate substrate ethanol with human ADH family are shown in Fig. 1. Between 10 to 50 mM methanol, the inhibitions by 20 mM ethanol for ADH1B1, ADH1C1, and ADH1C2 were calculated to be 99.4 to 97.1%, for ADH1A to be 80.9–80.7%, for ADH1B2 to be 95.1–94.5%, and for ADH1B3 to be 25.9–25.7%. Between 10 to 50 mM ethylene glycol, the inhibitions by 20 mM ethanol for ADH1B1, ADH1C1, and ADH1C2 were calculated to be 99.6 to 98.4%, for ADH1A to be 80.6–79.3%, for ADH1B2 to be 95.2–94.9%, for ADH2 to be 58.3–56.1%, and for ADH4 to be 30.2–29.9%. At 10, 20, and 50 mM isopropanol, the inhibitions by 20 mM ethanol for ADH1B1, ADH1C1, and ADH1C2 were calculated to be 98.9 to 93.7%, for ADH1A to be 22.5, 13.0, and 5.8%, respectively, for ADH1B2 to be 95.0–93.8%, for ADH1B3 to be 25.7–24.8%, for ADH2 to be 58.3–56.1%, and for ADH4 to be 30.2–30.0%.

Steady-state kinetic studies showed that 4-methylpyrazole (4MP) was a competitive inhibitor versus ethanol for ADH1A, ADH1B1, ADH1B2, ADH1C1, and ADH1C2, and a mixed-type noncompetitive inhibitor against ethanol for ADH1B3, ADH2, and ADH4. The determined corresponding inhibition constants are shown in Table 4. The slope inhibition constants (K_{is}) varied 15,000-fold across ADH family. Figure 2

illustrates inhibitions of oxidation of a wide range of ethanol and toxic alcohols by the fixed dead-end inhibitor 4MP. Between 20 to 50 mM ethanol, the inhibitions by 50 μM 4MP for ADH1A, ADH1B3, and ADH1C1 were calculated to be 88.8 to 65.9%, for ADH1B2 and ADH1C2 to be 72.6–50.5%, for ADH1B1 to be 9.6–4.1%, for ADH2 to be 3.6–3.2%, and for ADH4 to be 7.9–6.1%. Between 20 to 50 mM methanol, the inhibitions by 50 μM 4MP for ADH1A, ADH1B2, ADH1C1, and ADH1C2 were calculated to be 99.9 to 97.6%, and for ADH1B1 and ADH1B3 to be 89.4–78.1%. Between 20 to 50 mM ethylene glycol, the inhibitions by 50 μM 4MP for ADH1A, ADH1B1, ADH1B2, ADH1C1, and ADH1C2 were calculated to be 99.8 to 88.0%, for ADH2 to be 4.9–4.7%, and for ADH4 to be 10.4–10.3%. Between 20 to 50 mM isopropanol, the inhibitions by 50 μM 4MP for ADH1B2, ADH1C1, and ADH1C2 were calculated to be 99.4 to 97.5%, for ADH1B1 and ADH1B3 to be 89.2–66.6%, for ADH1A to be 59.5–37.5%, for ADH2 to be 4.9–4.7%, and for ADH4 to be 10.4–10.3%.

4. Discussion

To our knowledge, in the context of human ADH family this is the first report on kinetic properties of methanol, ethylene glycol, and isopropanol which are some of the most common ingested toxic alcohols in comparison with that of ethanol, and on kinetic parameters of 4MP inhibition that is the only current drug approved by the

Food and Drug Administration of the US as antidote for the toxic alcohol poisoning, at a near physiological pH and cytosolic NAD^+ concentration. We found a considerable large variation for K_M and V_{max} for the toxic alcohols and also for inhibition pattern and constants for 4MP within class I ADHs and an even larger variation across class I, II, and IV ADHs. Most interesting is the significant variation in K_M for the toxic alcohols for class I ADH1B allozymes, suggesting that there may be ethnic distinctions for metabolism of the toxic alcohols. It has been firmly documented that the *ADH1B*2* allele can protect against development of alcoholism across ethnic groups [3,6].

Class I, II, and III ADHs are predominantly expressed in human liver, the major organ for metabolism of ingested alcohols [3,22]. Class IV ADH is uniquely expressed in stomach and upper digestive tract that it contributes to gastric first-pass metabolism of ethanol [22,23] and toxic alcohols. Class III ADH was not included in the present study because of its negligible role in metabolism of ethanol and the toxic alcohols. It has been reported that human ADH3 was unsaturable with ethanol [24], inactive toward methanol and ethylene glycol at the pH-optimum 10.0 [25], and insensitive to 4MP inhibition [25].

The K_M values for ethanol for the studied human ADH family are in following decreasing order: ADH4 > ADH2 > ADH1A > ADH1B2 > ADH1C2 ≈ ADH1C1 > ADH1B1, with the exception of ADH1B3 being the highest K_M. X–ray crystallographic studies indicate that class I and class IV ADH isozymes/allozymes possess grossly similar but clearly discernible topology at the bottom of the hydrophobic substrate binding site adjacent to the catalytic zinc ion [26–29], that can largely explain the substrate affinity to the enzyme. Class II and IV ADHs in general exhibit higher K_M for ethylene glycol and isopropanol than do the class I enzymes with some exceptions. ADH1A uniquely exhibits the largest K_M for methanol, except that of ADH1B3, among class I ADHs but displays the smallest K_M for isopropanol among all of the ADH family studied. This can largely be attributed to a single amino acid substitution of Ala in ADH1A for Phe-93 in all of the remaining ADH family members except Tyr-93 in ADH2. Smaller alanyl residue at this position allows effective binding to more bulky secondary alcohol isopropanol and hence a much less effective binding to the smallest substrate methanol [29,30]. Indeed, ADH1A exhibits the highest catalytic efficiency for isopropanol compared with that for ethanol, methanol, and ethylene glycol. Furthermore, the catalytic efficiency for isopropanol for ADH1A is greatest among those for the class I, II, and IV ADHs studied. It has been well accepted that dissociation of NADH is the rate-limiting step in catalysis of

ethanol oxidation in human ADH family [3]. For those ADH forms exhibiting considerably lower V_{max} for some of the toxic alcohols compared with that of ethanol, it suggests a shift of rate-limiting step in catalysis, possibly to the hydride transfer.

Human class I ADH isozymes/allozymes exhibit a 95-fold variation in slope inhibition constants (0.062–5.9 μM) for 4MP with respect to ethanol. Previous studies using a mixture of human class I isozymes isolated from the autopsy liver, reported K_{is} for 4MP, 0.21 μM [31] and 0.09 μM [32]. Class II and class IV ADHs exhibit much higher K_{is} for 4MP than do the class I enzymes. X–ray crystallographic and site-directed mutagenesis studies provide evidence that Met-141 directly influences the binding of 4MP in ADH4 [28,33]. It is interesting to note that ADH1B3, ADH2, and ADH4 revealed a mixed-type noncompetitive inhibition of 4MP versus ethanol. This result confirms the previous observation with ADH2 [34]. The noncompetitive inhibition pattern suggests that 4MP may reversibly bind to both the E–NAD^+ and E–NADH binary complexes in catalytic cycle, as demonstrated by inhibition studies with substrate analogs of malic enzyme [35]. The formation of E–NADH–inhibitor complex prevents release of NADH, that is, the rate-limiting step, and hence giving rise to the intercept inhibition effect. For competitive and noncompetitive inhibitions of 4MP with respect to ethanol, the K_{is} represents dissociation constant for E–NAD^+

and inhibitor in an Ordered Bi Bi mechanism. Recently formamide derivatives, potent uncompetitive inhibitor against ethanol, has been developed to inhibit ethanol metabolism in mice [36] and yet no available data for clinical trials.

The accepted target plasma ethanol concentration for treatment of ethylene glycol and methanol poisoning is approximately 100 mg/dl (21.7 mM) [10]. The reported plasma levels of ethylene glycol [11], methanol [13], and isopropanol [10] in intoxicated patients can reach a high 50 mM. Our simulation results indicate that inhibitions of oxidation of 50 mM toxic alcohols by 20 mM ethanol for human ADH family are quite effective (inhibition ≥ 80%), except for methanol for ADH1B3 (25.7%) and for ethylene glycol for ADH2 (56.1%) and ADH4 (29.9%). At therapeutically attainable plasma levels of 4MP [9], inhibition of oxidation of 50 mM toxic alcohols by 50 μM 4MP is highly effective (inhibition ≥ 88.0%), except for methanol for ADH1B1 (78.1%) and for ethylene glycol for ADH2 (4.7%) and ADH4 (10.3%). Of human ADH family, ADH1B and ADH2 appeared to be with the highest and the second highest protein contents in liver, respectively [17], and ADH4 is a high-activity isozyme expressed in the stomach [23]. Therefore, the efficacy for treatment of the toxic alcohol poisoning by ethanol or 4MP may potentially vary for patients carrying the polymorphic *ADH1B* gene alleles or with different expression levels of hepatic

ADH2.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

[1] S.J. Yin, C.L. Han, A.I. Lee, C.W. Wu, Human alcohol dehydrogenase family: functional classification, ethanol/retinol metabolism, and medical implications, Adv. Exp. Med. Biol. 463 (1999) 265–274.

[2] H. Jornvall, J.O. Hoog, B. Persson, X. Pares, Pharmacogenetics of the alcohol dehydrogenase system, Pharmacology 61 (2000) 184–191.

[3] H.J. Edenberg, W.F. Bosron, Alcohol dehydrogenases, in: C.A. McQueen (Ed.), Comprehensive Toxicology, vol. 4, Academic Press, Oxford, 2010, pp. 111–130.

[4] G. Duester, J. Farres, M.R. Felder, R.S. Holmes, J.O. Hoog, X. Pares, B.V. Plapp, S.J. Yin, H. Jornvall, Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family, Biochem. Pharmacol. 58 (1999) 389–395.

[5] S.P. Lee, C.P. Chiang, S.L. Lee, Y.J. Hsia, T.L. Chuang, J.C. Lin, S.C. Liang, S. Nieh, S.J. Yin, Immunochemical features in the classification of human alcohol dehydrogenase family, Alcohol 39 (2006) 13–20.

[6] Y.C. Chen, G.S. Peng, M.F. Wang, T.P. Tsao, S.J. Yin, Polymorphism of ethanol-metabolism genes and alcoholism: correlation of allelic variations with the pharmacokinetic and pharmacodynamic consequences, Chem. Biol. Interact. 178 (2009) 2–7.

[7] J.O. Hoog, P. Stromberg, J.J. Hedberg, W.J. Griffiths, The mammalian alcohol dehydrogenases interact in several metabolic pathways, Chem. Biol. Interact. 143–144 (2003) 175–181.

[8] S. Abramson, A.K. Singh, Treatment of the alcohol intoxications: ethylene glycol, methanol and isopropanol, Curr. Opin. Nephrol. Hypertens. 9 (2000) 695–701.

[9] B. Megarbane, S.W. Borron, F.J. Baud, Current recommendations for treatment of severe toxic alcohol poisonings, Intensive Care Med. 31 (2005) 189–195.

[10] J.A. Kraut, I. Kurtz, Toxic alcohol ingestions: clinical features, diagnosis, and management, Clin. J. Am. Soc. Nephrol. 3 (2008) 208–225.

[11] J. Brent, K. McMartin, S. Phillips, K.K. Burkhart, J.W. Donovan, M. Wells, K. Kulig, Fomepizole for the treatment of ethylene glycol poisoning, N. Engl. J. Med. 340 (1999) 832–838.

[12] S.W. Borron, B. Megarbane, F.J. Baud, Fomepizole in treatment of uncomplicated ethylene glycol poisoning, Lancet 354 (1999) 831.

[13] J. Brent, K. McMartin, S. Phillips, C. Aaron, K. Kulig, Fomepizole for the treatment of methanol poisoning, N. Engl. J. Med. 344 (2001) 424–429.

[14] D.G. Barceloux, G.R. Bond, E.P. Krenzelok, H. Cooper, J.A. Vale, American Academy of Clinical Toxicology practice guidelines on the treatment of methanol poisoning, J. Toxicol. Clin. Toxicol. 40 (2002) 415–446.

[15] C.F. Chou, C.L. Lai, Y.C. Chang, G. Duester, S.J. Yin, Kinetic mechanism of human class IV alcohol dehydrogenase functioning as retinol dehydrogenase, J. Biol. Chem. 277 (2002) 25209–25216.

[16] S.L. Lee, J.O. Hoog, S.J. Yin, Functionality of allelic variations in human alcohol dehydrogenase gene family: assessment of a functional window for protection against alcoholism, Pharmacogenetics 14 (2004) 725–732.

[17] S.L. Lee, G.Y. Chau, C.T. Yao, C.W. Wu, S.J. Yin, Functional assessment of human alcohol dehydrogenase family in ethanol metabolism: significance of first-pass metabolism, Alcohol. Clin. Exp. Res. 30 (2006) 1132–1142.

[18] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[19] T. Bucher, B. Brauser, A. Conze, F. Klein, O. Langguth, H. Sies, State of oxidation-reduction and state of binding in the cytosolic NADH-system as

disclosed by equilibration with extracellular lactate-pyruvate in hemoglobin-free perfused rat liver, Eur. J. Biochem. 27 (1972) 301–317.

[20] W.W. Cleland, Statistical analysis of enzyme kinetic data, Methods Enzymol. 63 (1979) 103–138.

[21] M. Dixon, E.C. Webb, Enzymes, 3rd ed., Academic Press, New York, 1979, pp. 72–75.

[22] S.J. Yin, Alcohol dehydrogenase: enzymology and metabolism, Alcohol Alcohol. 29(Suppl. 2) (1994) 113–119.

[23] S.J. Yin, C.F. Chou, C.L. Lai, S.L. Lee, C.L. Han, Human class IV alcohol dehydrogenase: kinetic mechanism, functional roles and medical relevance, Chem. Biol. Interact. 143–144(2003) 219–227.

[24] S.L. Lee, M.F. Wang, A.I. Lee, S.J. Yin, The metabolic role of human ADH3 functioning as ethanol dehydrogenase, FEBS Lett. 544 (2003) 143–147.

[25] F.W. Wagner, X. Pares, B. Holmquist, B.L. Vallee, Physical and enzymatic properties of a class III isozyme of human liver alcohol dehydrogenase: chi-ADH, Biochemistry 23 (1984) 2193–2199.

[26] T.D. Hurley, W.F. Bosron, C.L. Stone, L.M. Amzel, Structures of three human beta alcohol dehydrogenase variants: correlations with their functional differences, J. Mol. Biol. 239 (1994) 415–429.

[27] G.J. Davis, W.F. Bosron, C.L. Stone, K. Owusu-Dekyi, T.D. Hurley, X-ray structure of human beta3 beta3 alcohol dehydrogenase: the contribution of ionic interactions to coenzyme binding, J. Biol. Chem. 271 (1996) 17057–17061.

[28] P. Xie, S.H. Parsons, D.C. Speckhard, W.F. Bosron, T.D. Hurley, X-ray structure of human class IV sigma sigma alcohol dehydrogenase: structural basis for substrate specificity, J. Biol. Chem. 272 (1997) 18558–18563.

[29] M.S. Niederhut, B.J. Gibbons, S. Perez-Miller, T.D. Hurley, Three-dimensional structures of the three human class I alcohol dehydrogenases, Protein Sci. 10 (2001) 697–706.

[30] C.L. Stone, T.K. Li, W.F. Bosron, Stereospecific oxidation of secondary alcohols by human alcohol dehydrogenases, J. Biol. Chem. 264 (1989) 11112–11116.

[31] T.K. Li, H. Theorell, Human liver alcohol dehydrogenase: inhibition by pyrazole and pyrazole analogs, Acta Chem. Scand. 23 (1969) 892–902.

[32] R. Pietruszko, Human liver alcohol dehydrogenase: inhibition of methanol activity by pyrazole, 4-methylpyrazole, 4-hydroxymethylpyrazole and 4-carboxypyrazole, Biochem. Pharmacol. 24 (1975) 1603–1607.

[33] P.T. Xie, T.D. Hurley, Methionine-141 directly influences the binding of 4-methylpyrazole in human sigma sigma alcohol dehydrogenase, Protein Sci. 8 (1999) 2639–2644.

[34] W.F. Bosron, T.K. Li, W.P. Dafeldecker, B.L. Vallee, Human liver pi-alcohol dehydrogenase: kinetic and molecular properties, Biochemistry 18 (1979) 1101–1105.

[35] M.I. Schimerlik, W.W. Cleland, Inhibition and alternate-substrate studies on the mechanism of malic enzyme, Biochemistry 16 (1977) 565–570.

[36] T.H. Venkataramaiah, B.V. Plapp, Formamides mimic aldehydes and inhibit liver alcohol dehydrogenases and ethanol metabolism, J. Biol. Chem. 278 (2003) 36699–36706.

Figure Legends

Fig. 1. Inhibition of oxidation of (a) methanol, (b) ethylene glycol, and (c) isopropanol by alternate substrate ethanol with human ADH family. Enzyme activity ratio was simulated at 0.5 mM NAD^+ and varied concentrations of toxic alcohol in the presence (v_i) to that in the absence (v_o) of 20 mM ethanol using Eq. 5. For the corresponding kinetic constants of ADH family for the simulation, see Tables 1–3.

Fig. 2. Inhibition of oxidation of (a) ethanol, (b) methanol, (c) ethylene glycol, and (d) isopropanol by dead-end inhibitor 4-methylpyrazole with human ADH family. Enzyme activity ratio was simulated at 0.5 mM NAD^+ and varied concentrations of alcohol in the presence (v_i) to that in the absence (v_o) of 0.05 mM 4MP using Eq. 2, except using Eq. 3 for ADH1B3, ADH2 and ADH4. For the corresponding kinetic constants of ADH family for the simulation, see Tables 1–4.

Table 1

K_M values (mM) for ethanol and toxic alcohols of human ADH family

Substrate	Class I						Class II	Class IV
	ADH1A	ADH1B1	ADH1B2	ADH1B3	ADH1C1	ADH1C2	ADH2	ADH4
Ethanol	4.7 ± 0.2	0.023 ± 0.001	1.0 ± 0.1	57 ± 2	0.12 ± 0.01	0.16 ± 0.01	14 ± 1	46 ± 2
Methanol	2,600 ± 500	2.0 ± 0.2	290 ± 20	3,500 ± 300	570 ± 90	180 ± 20	–[b]	–[b]
Ethylene glycol	440 ± 40	4.3 ± 0.4	650 ± 40	–[a]	53 ± 7	49 ± 4	420 ± 20	2,600 ± 300
Isopropanol	0.73 ± 0.05	1.1 ± 0.1	160 ± 10	760 ± 70	5.3 ± 0.7	6.7 ± 0.5	420 ± 60	3,400 ± 300

Enzyme activity was determined in 0.1 M sodium phosphate at pH 7.5 and 25 ^{o}C, containing 0.5 mM NAD^+ and varied concentrations of substrate. Values represent means ± SEM.

[a] Not determined due to the activity too low to be precisely measured up to 2.7 M ethylene glycol.

[b] Not determined due to the activity too low to be precisely measured up to 3.7 M methanol.

Table 2

V_{max} values (U/mg) for ethanol and toxic alcohols of human ADH family

Substrate	Class I						Class II	Class IV
	ADH1A	ADH1B1	ADH1B2	ADH1B3	ADH1C1	ADH1C2	ADH2	ADH4
Ethanol	0.37 ± 0.01	0.11 ± 0.01	4.3 ± 0.2	2.7 ± 0.1	0.65 ± 0.02	0.38 ± 0.01	0.21 ± 0.01	14 ± 1
Methanol	0.025 ± 0.001	0.033 ± 0.002	0.41 ± 0.01	0.029 ± 0.002	0.19 ± 0.02	0.080 ± 0.002	–[b]	–[b]
Ethylene glycol	0.17 ± 0.01	0.10 ± 0.01	1.9 ± 0.1	–[a]	0.27 ± 0.02	0.23 ± 0.01	0.074 ± 0.002	3.1 ± 0.2
Isopropanol	0.34 ± 0.01	0.11 ± 0.01	1.6 ± 0.1	0.35 ± 0.02	0.10 ± 0.01	0.090 ± 0.002	0.045 ± 0.002	3.7 ± 0.3

For assay conditions, see Table 1.

[a] Not determined due to the activity too low to be precisely measured up to 2.7 M ethylene glycol.

[b] Not determined due to the activity too low to be precisely measured up to 3.7 M methanol.

Table 3

V_{max}/K_M values (mU/(mg mM)) for ethanol and toxic alcohols of human ADH family

Substrate	Class I						Class II	Class IV
	ADH1A	ADH1B1	ADH1B2	ADH1B3	ADH1C1	ADH1C2	ADH2	ADH4
Ethanol	79 ± 2	4,700 ± 200	4,200 ± 200	48 ± 1	5,500 ± 500	2,300 ± 100	15 ± 1	310 ± 10
Methanol	0.0098 ± 0.0008 (0.012%)	17 ± 1 (0.35%)	1.4 ± 0.1 (0.034%)	0.0085 ± 0.0003 (0.018%)	0.35 ± 0.03 (0.0063%)	0.43 ± 0.03 (0.018%)	–[b]	–[b]
Ethylene glycol	0.38 ± 0.03 (0.47%)	24 ± 2 (0.51%)	3.0 ± 0.1 (0.072%)	–[a]	5.0 ± 0.3 (0.090%)	4.8 ± 0.3 (0.20%)	0.18 ± 0.01 (1.2%)	1.2 ± 0.1 (0.39%)
Isopropanol	460 ± 20 (590%)	110 ± 10 (2.3%)	10 ± 1 (0.25%)	0.48 ± 0.03 (0.99%)	20 ± 2 (0.36%)	14 ± 1 (0.58%)	0.11 ± 0.01 (0.72%)	1.1 ± 0.1 (0.36%)

For assay conditions, see Table 1. The ratios of V_{max}/K_M for toxic alcohol to that for ethanol are shown in parentheses. Values of the ratios represent relative activity of toxic alcohol to ethanol assuming the two alternate substrates are present in equimolar concentrations (refer to Eq. 6). Note that milliunits (mU) are used to express the enzyme activity.

[a] Not determined due to the activity too low to be precisely measured up to 2.7 M ethylene glycol.

[b] Not determined due to the activity too low to be precisely measured up to 3.7 M methanol.

Table 4
Inhibition constants of 4-methylpyrazole with respect to ethanol in human ADH family

Class	Isozyme/allozyme	Inhibition pattern	Inhibition constant	
			K_{is} (μM)	K_{ii} (μM)
I	ADH1A	Comp	1.2 ± 0.1	
	ADH1B1	Comp	0.54 ± 0.06	
	ADH1B2	Comp	0.96 ± 0.08	
	ADH1B3	Noncomp	5.9 ± 0.5	33 ± 6
	ADH1C1	Comp	0.062 ± 0.005	
	ADH1C2	Comp	0.15 ± 0.01	
II	ADH2	Noncomp	960 ± 140	1,800 ± 200
IV	ADH4	Noncomp	430 ± 30	3,000 ± 600

Enzyme activity was determined in 0.1 M sodium phosphate at pH 7.5 and 25 ^{o}C, containing 0.5 mM NAD^{+} and varied concentrations of both substrate and inhibitor. Values represent means ± SEM.

Fig. 1

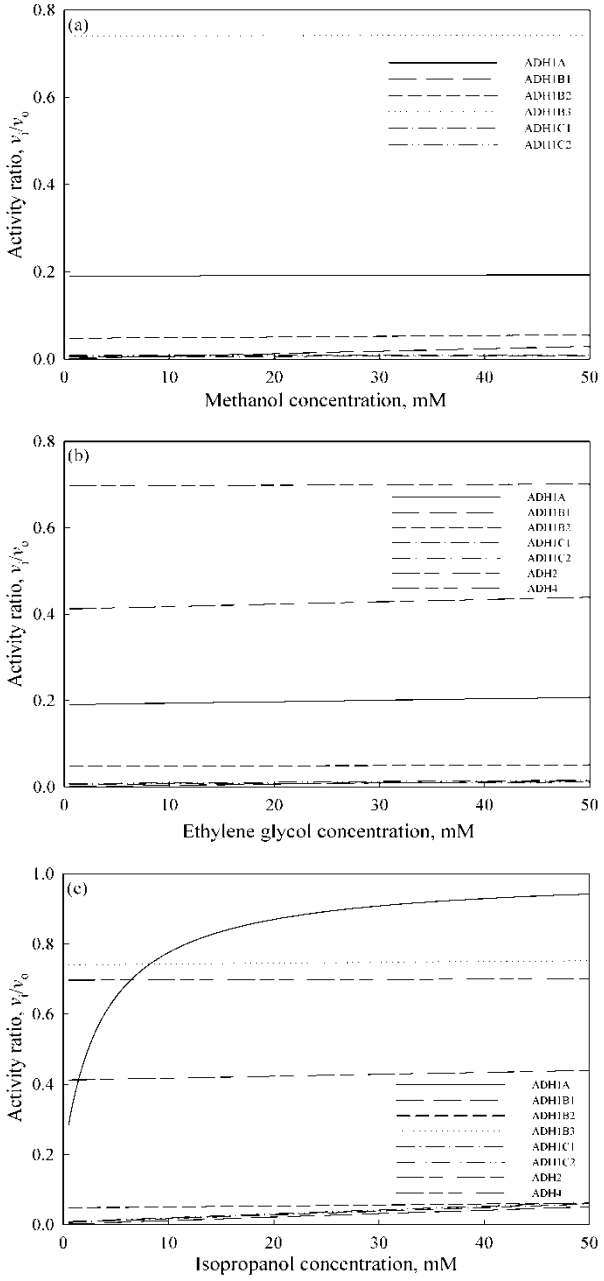
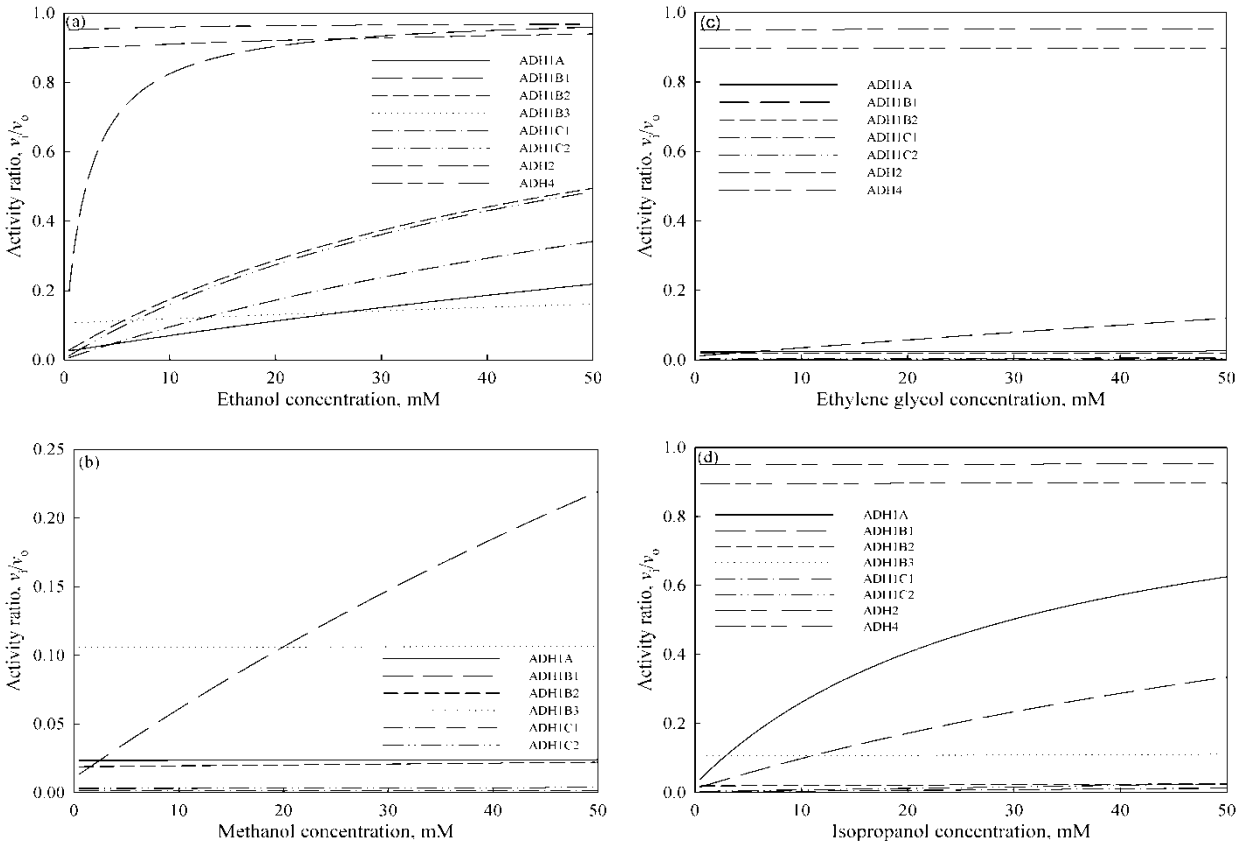

(a)
ADH1A
ADH1B1
ADH1B2
ADH1B3
ADH1C1
ADH1C2
Activity ratio, v_i/v_o
Methanol concentration, mM
(b)
ADH1A
ADH1B1
ADH1B2
ADH1C1
ADH1C2
ADH2
ADH4
Activity ratio, v_i/v_o
Ethylene glycol concentration, mM
(c)
ADH1A
ADH1B1
ADH1B2
ADH1B3
ADH1C1
ADH1C2
ADH2
ADH4
Activity ratio, v_i/v_o
Isopropanol concentration, mM

Fig. 2


(a)
Activity ratio, v_i/v_o
Ethanol concentration, mM
ADH1A
ADH1B1
ADH1B2
ADH1B3
ADH1C1
ADH1C2
ADH2
ADH4
(c)
Ethylene glycol concentration, mM
(b)
Methanol concentration, mM
(d)
Isopropanol concentration, mM