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IDENTIFICATION OF 4-(*N,N*-DIPROPYLAMINO)BENZALDEHYDE AS A POTENT, REVERSIBLE INHIBITOR OF MOUSE AND HUMAN CLASS I ALDEHYDE DEHYDROGENASE

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Abstract—As the physiologic roles for the different classes of aldehyde dehydrogenase (ALDH) enzymes are elucidated, the identification of specific, reversible inhibitors becomes of great pharmacologic interest. Previous structure–function studies identified dialkylamino substituted benzaldehyde compounds as a novel class of reversible inhibitors of class I ALDH. To examine further structural requirements for inhibition, we tested a series of 4-(*N,N*-dialkylamino)benzaldehyde analogs as inhibitors of propanal oxidation by mouse liver and human erythrocyte class I ALDH. 4-(*N,N*-dipropylamino)benzaldehyde (DPAB) was identified as the most potent, reversible inhibitor of propanal oxidation by class I ALDH in spectrophotometric enzyme assays. In kinetic studies, DPAB showed mixed-type inhibition with respect to the aldehyde substrates propanal, phenylacetaldehyde, benzaldehyde, and aldophosphamide. DPAB exhibited uncompetitive inhibition with respect to the cofactor NAD. Inhibition constants (K_i) for DPAB, estimated from Dixon plots, were 10 nM (propanal) and 77 nM (phenylacetaldehyde) for mouse ALDH and 3 nM (propanal) and 70 nM (phenylacetaldehyde) for human ALDH. These K_i values are 100-fold lower than those reported for class I specific inhibitors. At low ($< 1\ \mu M$) DPAB concentrations, inhibition of propanal and aldophosphamide oxidation was $> 75\%$, whereas inhibition of benzaldehyde (32%) and phenylacetaldehyde (19%) oxidation was reduced markedly. These results indicate that DPAB exhibits potent, reversible inhibition of mouse and human class I ALDH. The degree of inhibition was highly dependent on the structure of the aldehyde substrate.

Key words: aldehyde dehydrogenase; mouse; human; 4-(*N,N*-dipropylamino)benzaldehyde; inhibition

NAD-dependent aldehyde dehydrogenases (aldehyde:NAD oxidoreductase, ALDH†) catalyze the oxidation of a variety of aliphatic and aromatic aldehyde substrates to their corresponding carboxylic acids. Key conversions include the oxidation of acetaldehyde to acetic acid as a detoxification step in ethanol metabolism, oxidation of lipid peroxidation products such as 4-hydroxynonenal [1], oxidation of retinal to retinoic acid as a step in the regulation of the retinoid hormone signalling pathway [2, 3], and oxidation of aldophosphamide, an activated aldehyde-containing metabolite of the antitumor drug cyclophosphamide (Cytoxan), to the inactive compound carboxyphosphamide [4]. The purification and characterization of multiple ALDH enzymes from mouse, rat, and human tissues, including those important in acetaldehyde and aldophosphamide oxidation in mouse and human liver, have been described [5–7].

The pharmacologic inhibition of the oxidation of aldehydes by ALDH has been pursued for over 40 years. Disulfiram (tetraethylthiuram disulfide; Antabuse) was first used clinically in an attempt to deter people from ethanol consumption [8]. Disulfiram inhibits the ALDH-catalyzed oxidation of acetaldehyde to acetate, resulting in the accumulation of the more toxic aldehyde metabolite. Unfortunately, disulfiram not only inhibits ALDH enzymes in the liver and erythrocytes, but it also interacts with metalloenzymes and proteins having reactive cysteine thiol side chains, including dopamine β-hydroxylase and drug-metabolizing monooxygenase enzymes [9]. Although identification of the active metabolite responsible for enzyme inhibition *in vivo* has proven difficult, bioactivation of disulfiram appears to be essential. Hart and Faiman [10] have presented strong evidence that *S*-methyl *N,N*-diethylthiolcarbamate sulfoxide is the disulfiram metabolite that acts as the *in vivo* inhibitor of rat liver mitochondrial ALDH.

Several agents that can block the oxidation of broad classes of aldehydic substrates or specific aldehydes, including chloral hydrate, cyanamide, and metronidazole, have been identified. More recently, a series of structurally diverse compounds have been identified as inhibitors of the rat and human class II (mitochondrial) ALDH. These compounds include nitroxyl analogs [11], cinnamic acid analogs [12], and the natural products citral [13] and daidzin [14]. Kinetic studies of the different

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† Abbreviations: ALDH, aldehyde dehydrogenase; DEAB, 4-(*N,N*-diethylamino)benzaldehyde; DMAB, 4-(*N,N*-dimethylamino)benzaldehyde; DPAB, 4-(*N,N*-dipropylamino)benzaldehyde; DBAB, 4-(*N,N*-dibutylamino)benzaldehyde; MPAB, 4-(*N*-methyl,*N*-propylamino)benzaldehyde; and IPB, 4-(isopropyl)benzaldehyde.

ALDH enzymes have determined several mechanisms of inhibition, including competitive inhibition of aldehyde or NAD binding and noncompetitive inhibition involving disulfide bridges at the enzyme active site or those residues important for quaternary structure.

Previously, we screened a series of non-thiol, aromatic compounds with dialkylamino and carbonyl substituents for ALDH inhibition. DEAB strongly inhibits propanal and aldophosphamide oxidation by class I ALDH from mouse liver and a mouse leukemic cell line (L1210/CPA) that is resistant to cyclophosphamide by virtue of its overexpression of class I ALDH [15]. Treatment of L1210/CPA cells *in vitro* with 50 μM DEAB abolished the resistance of the tumor cells to an activated form of cyclophosphamide. The potency of class I ALDH inhibition increased as the alkyl chain length increased from methyl in DMAB to ethyl in DEAB. Likewise, the inhibition potency increased as the alkyl chain length increased from methyl in tetramethylthiuram disulfide (Thiuram) to ethyl in disulfiram.* The 4-(*N,N*-dialkylamino)benzaldehyde compounds are structurally similar to disulfiram or the putative active metabolite by virtue of the common dialkylamino group. However, evidence for inhibition by disulfiram has suggested interaction between thiol groups of inhibitor and enzyme. Since the dialkylamino benzaldehyde compounds contain no thiol groups, they likely inhibit ALDH via a different mechanism.

DEAB has also been an effective class I ALDH inhibitor *in vivo*. Studies in mice showed that in the presence of DEAB, cyclophosphamide becomes profoundly toxic to the intestinal crypt cells [16], whereas no toxicity was observed in mice receiving up to 20 mg/kg DEAB alone via intraperitoneal injection. Recently, Mahmoud *et al.* [17] reported that 50 mg/kg doses of DEAB inhibit ethanol metabolism in mice, as measured by an increased blood acetaldehyde concentration.

To obtain a better understanding of the structural requirements for ALDH inhibition by DEAB, we investigated the steric and electronic properties of the *para* substituent. We synthesized analogs of DEAB, in which we varied the dialkyl chain lengths, mixed the chain length in the dialkyl substitution, or eliminated the amino group. In this paper, we report the identification and kinetic characterization of DPAB as the most potent of these as a class I ALDH inhibitor.

MATERIALS AND METHODS

Materials. NAD, aprotinin, DMSO, leupeptin, pyrazole, Cibacron Reactive Blue agarose, and CM-50 Sephadex were obtained from the Sigma Chemical Co., St. Louis, MO. Benzaldehyde, phenylacetaldehyde, DEAB, IPB, and propanal were purchased from the Aldrich Chemical Co., Milwaukee, WI. Sephadex G-25, Polybuffer 96, and Polybuffer Exchanger 94 were from Pharmacia LKB Biotechnology, Piscataway, NJ. Centriflo Ultrafiltration membrane cones and Centricon-30 microconcentrators were obtained from Amicon, Danvers, MA. 4-Hydroperoxycyclophosphamide was provided by Dr. J. Hilton, Johns Hopkins Oncology Center, Baltimore, MD.

Synthesis of 4-N,N-*dialkylanilines.* DPAB was prepared as follows: 1-bromopropane (55 mL, 0.60 mol) and aniline (140 mL, 1.5 mol) were heated at reflux for 10 hr. The mixture was cooled, treated with NaOH, and combined with a mixture of 92 g $ZnCl_2$ in 92 g H_2O. The resulting paste was stirred for 12 hr, filtered, and extracted with petroleum ether. The extracts were combined, washed with H_2O and dilute NH_3, and dried over $MgSO_4$. The petroleum ether was evaporated, and the mixture was distilled. The *N*-propylaniline, collected at 218–220°, (59 mL, 0.41 umol) was combined with 1-bromopropane (62 mL, 0.68 mol) and refluxed for 30 hr. The liquid was cooled and treated with excess NaOH, and the organic layer was collected and dried. Unreacted 1-bromopropane was removed *in vacuo*, leaving a golden, oily liquid, *N,N*-dipropylaniline in 92% yield.

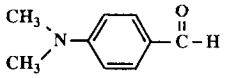
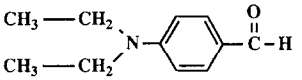
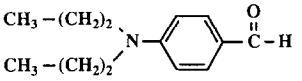
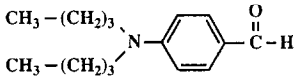
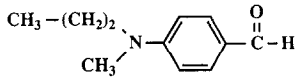
Synthesis of 4-(N,N-*dialkylamino)benzaldehydes.* Glacial acetic acid (20 g, 0.33 mol) and hexamine (20 g, 0.14 mol) were mixed and heated on a boiling water bath. A mixture of *N,N*-dipropylaniline (20 mL, 0.11 mol) and glacial acetic acid (44 mL, 0.78 mol) was added dropwise to this solution over a 1-hr period. The reaction mixture was stirred at 100° for 2 hr. The reaction mixture was poured into 310 mL of 2.5 M HCl. The organic layer was extracted into CH_2Cl_2, and made alkaline with NaOH. The CH_2Cl_2 was removed on a water bath, and the product was distilled *in vacuo*. DPAB was obtained in 40% yield as a golden oil, boiling at 160–162° at 1.5 mm Hg. DBAB and MPAB were prepared using analogous procedures, beginning with 1-bromobutane and bromomethane, respectively. DBAB was obtained in 30% yield as a yellow oil, boiling at 153–156° at 2 mm Hg. MPAB was obtained in 28% yield, boiling at 133–137° at 1.5 mm Hg.

Product analyses were performed using a Mattson Polaris FT-IR, a Perkin–Elmer model R-24 60 MHz NMR, and a Hewlett–Packard 5890 gas chromatograph with an HP 1 column coupled to a model 5971A mass spectrometer (GC-MS). DPAB was obtained at 99.5% purity via GC-MS. Spectral analysis for DPAB was as follows: ^1H NMR (CCl_4): 0.7 (t, CH_3), 1.3 (m, CH_2CH_2), 3.2 (t, NCH_2), 6.6 (d, ArH), 7.5 (d, ArH), 9.6 (s, CHO). IR (neat): 3018 (C=C—H), 2806 (C—C), 1666 (CHO), 1600 (Ar C=C). GC-MS: 206, (10%), 205 (61.7%), 178 (3.3%), 177 (36.7%), 176 (100.0%). The purity for DBAB and MPAB was comparable.

Enzyme kinetic assays. ALDH activity was measured via spectrophotometric assay on a Shimadzu UV1201 spectrophotometer at 36.5° (±0.5°) by following the appearance of NADH at 340 nm. Standard assay conditions were as follows: 1 mL final reaction volume; assay buffer (0.1 M sodium phosphate, pH 7.4, 1.0 mM EDTA, 5.0 mM 2-mercaptoethanol); 1.0 mM NAD; 10 mM pyrazole. The order of addition in the assay was as follows: buffer, pyrazole, NAD, ALDH (10 mU mouse liver ALDH or 5 mU human erythrocyte ALDH), inhibitor (if any), and, lastly, the aldehyde substrate.

* Russo J, unpublished result.

Table 1. Inhibition constants (K_i) and percent inhibition of mouse class I ALDH for 4(*N*,*N*-dialkylamino)benzaldehyde analogs

Inhibitor		K_i (μM)	% inhibition
	DMAB	> 10	33
	DEAB	0.12	80
	DPAB	0.010	84
	DBAB	> 10	26
	MPAB	NT	66

ALDH assays were performed as described in Materials and Methods with 1 mM NAD. Percent inhibition assays were performed with 500 μM propanal and 5 μM inhibitor. NT = not tested.

Background activity was determined in the first 2 min of the assay prior to the addition of any inhibitor or propanal. Inhibitors were dissolved in DMSO and diluted in water. DMSO concentration did not exceed 0.1% (v/v) in the assay, and this concentration had no effect on enzyme activity. Substrate and/or inhibitor was added between min 2 and 3, and the initial rate for analysis was recorded from min 3 to 5. The initial rate of phenylacetaldehyde oxidation was recorded from 0.5 to 2 min. Propanal concentration was varied from 500 to 10 μM (20 to 0.4 times K_m), and phenylacetaldehyde concentration was varied from 40 to 5 μM (5 to 0.6 times K_m), both with constant (1 mM) NAD concentration. Likewise, NAD concentration was varied from 500 to 30 μM with constant (5 mM) propanal concentration. DPAB concentrations were varied from 20 to 0.05 μM. One unit of activity is defined as 1 μmol NADH formed/min. Protein concentrations were determined by using the Bio-Rad Protein Dye Reagent and assay (Bio-Rad Laboratories, Richmond, CA). Dixon plots and inhibition constants were obtained using the *Enzyme Kinetics* v 1.11 program from Trinity Software. The Dixon plots contain the averages of duplicate trials.

Purification of class I ALDH from mouse liver. Female BDF-1 mice were obtained from Charles River Laboratories, Wilmington, MA. Livers were excised from 8 to 12-week-old mice (20–25 g) following cervical dislocation. The livers were rinsed in PBS (pH 7.4) and frozen at −70° until used. For purification, 3–5 g of tissue was thawed, and aprotinin (200 U/mL) and leupeptin (50 μg/mL) were added as protease inhibitors. The tissue was homogenized in 12–20 mL of homogenization buffer (0.25 M sucrose in 10 mM sodium phosphate, 5 mM 2-mercaptoethanol, 1 mM EDTA, pH 7.2) using a Biospec homogenizer. The homogenate was then centrifuged at 100,000 *g* for 60 min, in a Beckman L7-65 ultracentrifuge. The lipid layer was removed by aspiration, and the cytosolic fraction was collected. The cytosolic fraction was loaded onto a Sephadex G-25 column equilibrated at pH 7.4 with Buffer A (0.1 M sodium phosphate, 5 mM 2-mercaptoethanol, and 1 mM EDTA). The protein-containing fractions were pooled, diluted to 40 mL in Buffer A, and loaded onto a 1.6 × 20 cm Cibacron Reactive Blue agarose affinity column, equilibrated with buffer A, at a flow rate of 0.2 mL/min. The column was washed with several column volumes of buffer A, and enzyme was eluted with 100 mL of a 0–1 mM NAD gradient. The active fractions were pooled and concentrated, and the buffer was changed to Pharmacia Polybuffer 96 (1:10 dilution, pH 7.0) using Amicon concentration cones. Protein was loaded onto a 1.0 × 35 cm column of Pharmacia Polybuffer Exchanger 94, equilibrated at pH 9.4 with 25 mM Tris–Cl, 5 mM 2-mercaptoethanol, and 1 mM EDTA. The column was eluted with Polybuffer 96, pH 7.0. The mouse class I ALDH eluted from pH 8.8 to 9.0. The fractions collected were concentrated in Centricon-30 microconcentrator units, and the buffer was changed to 20 mM sodium phosphate, pH 7.4, with 5 mM 2-mercaptoethanol, and 1 mM EDTA. The enzyme was stored in 25% glycerol at −20°. Enzyme purity was confirmed by

Table 2. Substrate specificity of ALDH inhibition by DPAB

Substrate	Percent inhibition [DPAB] 5.0 μM	0.5 μM
Propanal	> 99	> 99
Aldophosphamide	NT	79 ± 3.0
Phenylacetaldehyde	90 ± 2.3	19 ± 3.1
Benzaldehyde	87 ± 1.8	32 ± 3.3
4-(Isopropyl)benzaldehyde	NT	17 ± 2.9

ALDH assays were performed as described in Materials and Methods with 1 mM NAD and 40 μM aldehyde substrate. Each percent inhibition value is the mean ± SEM for at least three trials. NT = not tested.

detection of a single band at M_r 51,000 by silver stain detection following SDS–PAGE.

Purification of class I ALDH from human erythrocytes. Purification of human erythrocyte ALDH was modified from the method of Rawles *et al.* [18]. Blood (100 mL) was collected by venipuncture from two adult males and diluted in PBS containing 5 mM EDTA at pH 6.0. The pooled samples were centrifuged at 2000 *g* for 15 min at 5°. The supernatant was decanted, and the pellet was resuspended and washed twice in PBS, pH 7.4. The packed cells were then lysed with 3 vol. of ice-cold distilled water, and the pH was adjusted to 6.0 with 1 M HCl. The sample was centrifuged at 2000 *g* for 15 min. The supernatant was brought to 5 mM 2-mercaptoethanol and 1 mM EDTA. The lysate was then loaded onto CM-50 Sephadex (350 mL, equilibrated with 30 mM sodium phosphate, pH 6.0) in a 500-mL Nalgene filtration unit. The ALDH activity was eluted in the same buffer. Active fractions were pooled, concentrated, transferred into Buffer A, and loaded onto a Cibacron Reactive Blue agarose affinity column (1.5 × 10 cm), equilibrated with buffer A, at a flow rate of 0.2 mL/min. The column was washed with several column volumes of buffer A, and enzyme was eluted with 50 mL of a 0–1 mM NAD gradient. The active fractions were pooled and concentrated in Centriflo ultrafiltration membrane cones, and the buffer was changed to 20 mM sodium phosphate, pH 7.4, with 5 mM 2-mercaptoethanol and 1 mM EDTA. The enzyme was stored in 25% glycerol at −20°.

RESULTS

The alkyl chain length of the 4-(*N,N*-dialkylamino)benzaldehyde compounds plays a profound role in the reversible inhibition of mouse class I ALDH. Inhibition potency increased (K_i decreased 10-fold) as the alkyl chain length increased from ethyl to propyl in DEAB to DPAB (Table 1). However, introduction of butyl groups in DBAB resulted in a significant drop in inhibition potency, as seen by the large increase in the K_i value and drop in percent inhibition from 84 to 26%, when compared with DPAB. The presence of a single propyl group in MPAB retained inhibitory properties for this analog, although the potency was less than that observed in DEAB or DPAB. We also investigated the necessity of the nitrogen atom at the 4-position on the aromatic ring for inhibition with the compound IPB. IPB is a substrate, rather than an inhibitor, for class I ALDH. This suggests that the dialkyl groups are necessary, but not sufficient to confer inhibitory properties to these analogs. Although DPAB contains the benzaldehyde moiety, DPAB does not undergo oxidation to the corresponding acid. When presented as the sole aldehydic compound *in vitro* to ALDH, reduction of NAD to NADH is undetectable. However, benzaldehyde is readily oxidized to benzoic acid by class I ALDH with NAD as cofactor. Therefore, we examined DPAB inhibition with respect to several aliphatic and aromatic ring-containing aldehydes. DPAB selectively inhibited the oxidation of the aliphatic aldehydes propanal and aldophosphamide, when compared with the aromatic (benzaldehyde) and aromatic ring-containing (phenylacetaldehyde) aldehydes. At a high DPAB concentration (5 μM), potent inhibition (> 85%) of propanal, benzaldehyde, and phenylacetaldehyde oxidation was observed (Table 2). However, at 0.5 μM DPAB, propanal oxidation remained completely inhibited (> 99%), and aldophosphamide oxidation was inhibited 79%, whereas inhibition of benzaldehyde (32%) and phenylacetaldehyde (19%) was reduced markedly. In addition, very weak inhibition (17%) was seen with IPB as substrate.

Since DPAB exhibited the lowest K_i value of the 4-(*N,N*-dialkylamino)benzaldehyde compounds tested, we investigated further the kinetics of inhibition by DPAB of class I ALDH from mouse liver with respect to aldehyde substrate and NAD cofactor. Lineweaver–Burk and Dixon plots were used to determine inhibition type and to estimate K_i values. Linear mixed-type inhibition was observed for DPAB with respect to propanal (Fig. 1, A and B) and phenylacetaldeyde (Fig. 1C) oxidation by mouse class I ALDH. Uncompetitive inhibition was observed for DPAB inhibition with respect to the NAD cofactor (Fig. 1D).

The high affinity of DPAB for mouse class I ALDH was evident by the remarkably low K_i/K_m ratio (0.0004) with propanal as substrate (Table 3). DPAB inhibited human (erythrocyte) class I ALDH with a slightly greater potency than mouse ALDH. However, inhibition of phenylacetaldehyde oxidation was reduced markedly, as shown by the higher K_i values (0.077 and 0.070 μM for mouse and human class I ALDH, respectively). In addition, the K_i/K_m ratio of phenylacetaldehyde oxidation increased 35-fold for mouse ALDH and 60-fold for human ALDH, when compared with the K_i/K_m ratio of propanal oxidation obtained with DPAB.

DISCUSSION

Our results indicate that the three-carbon propyl group present in DPAB provides the optimal alkyl chain length for inhibition of propanal oxidation by class I ALDH in the series of dialkylamino-substituted benzaldehyde compounds studied.

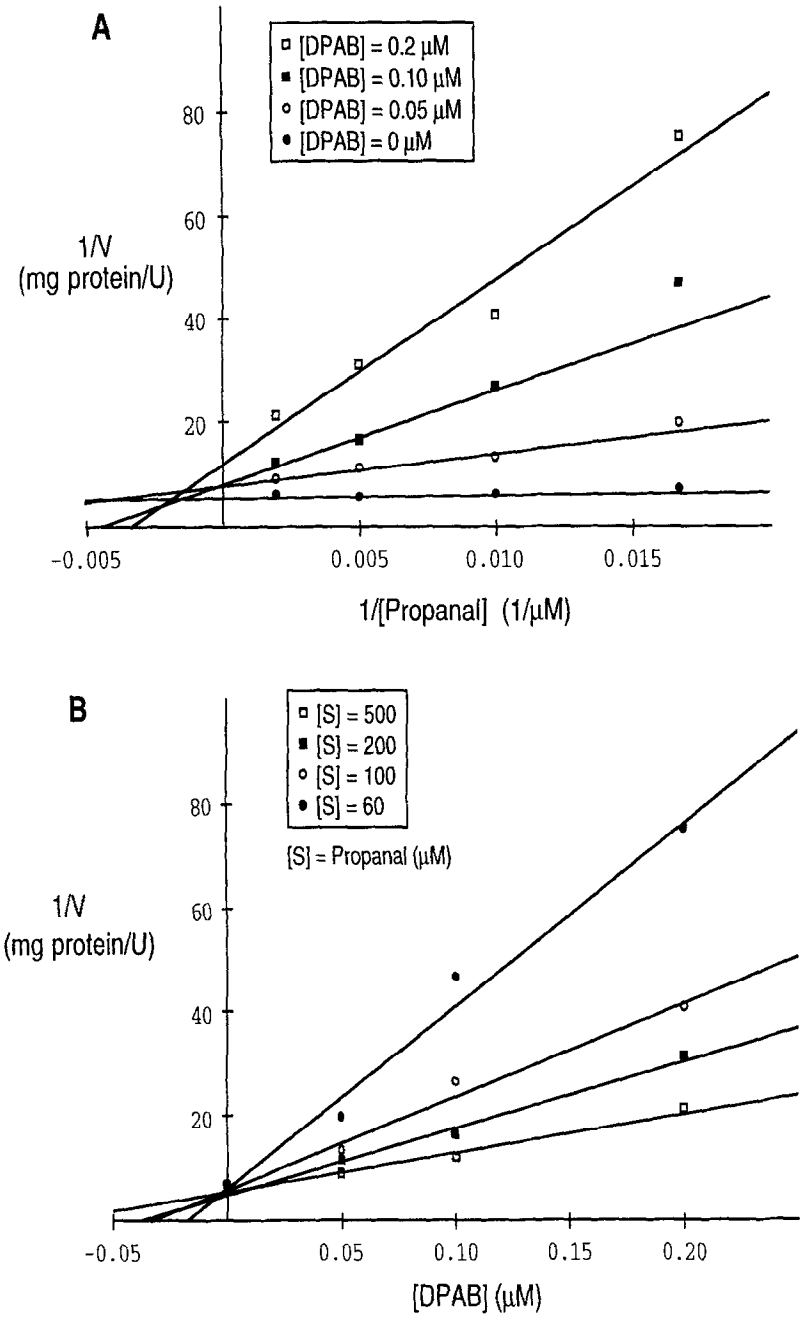


Fig. 1. Kinetics of inhibition of mouse class I ALDH by DPAB. Assays were performed as described in Materials and Methods. Lineweaver–Burk plot for inhibition of propanal oxidation (panel A); Dixon plots for inhibition of propanal oxidation (panel B).

Although a single propyl chain can confer significant inhibition to MPAB, the dipropyl group provides the highest inhibition potency. The lack of inhibition observed with IPB indicates that the nitrogen contributes to the binding of these inhibitors, probably via an ionic interaction as the protonated, quaternary amine.

The mixed-type inhibition observed with respect to aldehyde substrates and the uncompetitive inhibition with respect to NAD suggest that DPAB does not interfere with the binding of NAD, but the dialkylamino group interferes with hydride ion transfer from aldehyde to NAD. DPAB may bind subsequently to NAD, similar to ordered binding observed for aldehyde substrates, or binding of DPAB may occur at a unique site. The specific binding interactions of DPAB with the enzyme active site are unknown, but may be revealed with

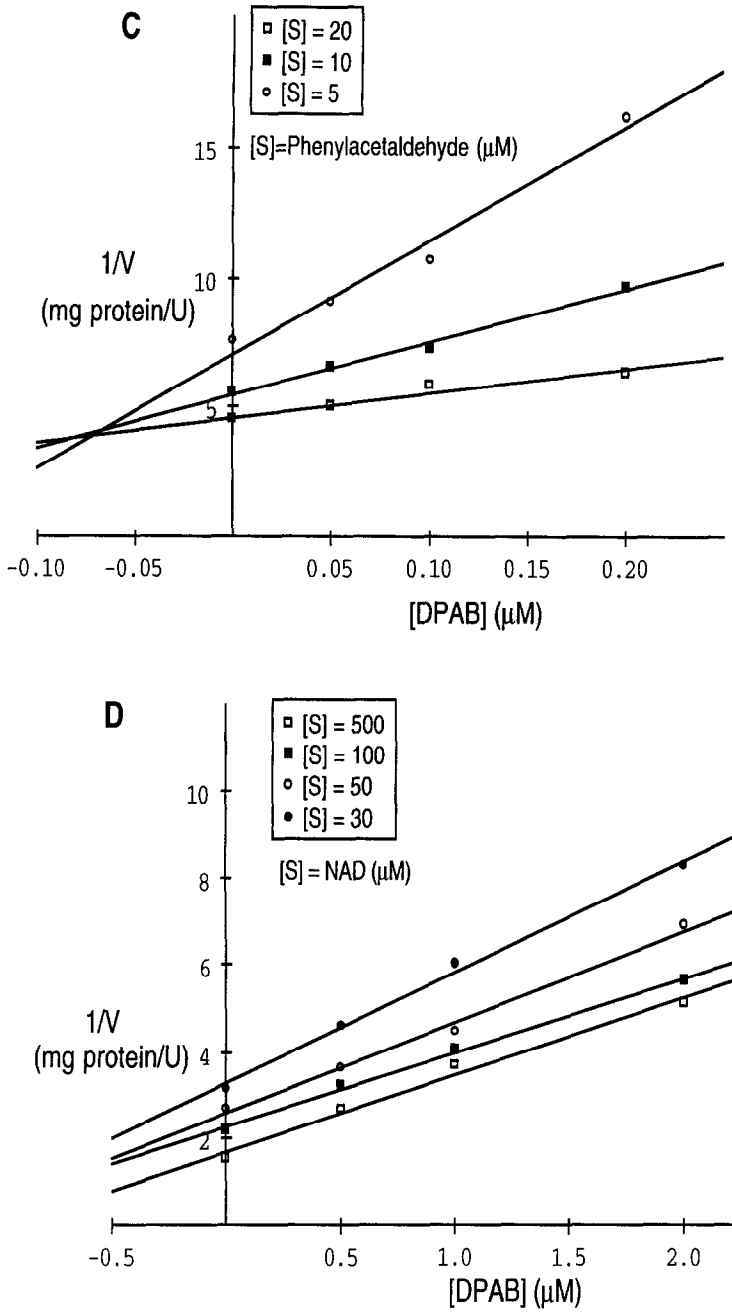


Fig. 1—*contd.* Phenylacetaldehyde oxidation (panel C), and NAD reduction (panel D). NAD concentrations were held constant at 1 mM in panels A–C and propanal concentration was held constant at 500 μM in panel D.

high-resolution crystallographic studies or the development of a DPAB-related affinity label.

The K_i of DPAB for class I ALDH is more than 100-fold lower than values reported for the inhibition of class I ALDH by the reversible, competitive inhibitor chloral hydrate [7, 19] and 10-fold lower than the K_i reported for the inhibition of class II ALDH by the terpene citral [13]. Interestingly, both DPAB and citral exhibit linear-mixed type inhibition with respect to aldehyde but do not interfere with binding of NAD to enzyme.

DPAB exhibits at least two desirable features for a clinically useful pharmacologic agent: high affinity for the target enzyme (K_i 10 nM) and reversible inhibition. Previous *in vivo* studies with DEAB in mice demonstrated a rapid onset for the inhibition of the oxidation of aldophosphamide [16] and acetaldehyde, but an undesirable rapid clearance of

Table 3. Inhibition constants (K_i) for DPAB inhibition of mouse and human class I ALDH

Substrate	K_i (μM)	K_m (μM)	K_i/K_m
Mouse ALDH			
Propanal	0.010	25	0.0004
Phenylacetaldehyde	0.077	5.4	0.014
Human ALDH			
Propanal	0.003	16	0.0002
Phenylacetaldehyde	0.070	5.7	0.012

ALDH assays were performed as described in Materials and Methods with 1 mM NAD. DPAB concentrations were varied from 0.05 to 0.5 μM. K_i and K_m values are the means for at least four trials.

DEAB from the blood [17]. Daidzin, a natural product isoflavone recently identified as a competitive inhibitor of ALDH [14], also has a high affinity (K_i 40 nM) and is a reversible inhibitor. In addition, daidzin is highly selective *in vitro* for the inhibition of acetaldehyde oxidation by human mitochondrial (class II) ALDH compared with cytosolic (class I) enzyme.* Also, daidzin has been shown to suppress ethanol intake in Syrian Golden hamsters [20]. Thus, daidzin appears to be an effective agent for blocking acetaldehyde metabolism via the specific inhibition of class II ALDH. Although DEAB was shown previously [15] to inhibit ALDH activity in cytosolic (92%), but not mitochondrial (25%) mouse liver extracts, neither DEAB nor DPAB has been tested for inhibition of purified class II ALDH. However, the report from Mahmoud *et al.* [17] on the inhibition of ethanol metabolism by DEAB suggests that DEAB may inhibit both class I and class II ALDH *in vivo*, since class II ALDH is known to play a major role in the oxidation of acetaldehyde to acetate [21, 22]. It is possible that doses of daidzin and DPAB used in combination could provide an effective alcohol aversion drug therapy without inhibiting other key physiologic aldehyde oxidations.

Cellular levels of ALDH expression can play a critical role in the toxicity observed following exposure to a particular aldehyde-containing compound. The oxidation of aldophosphamide by ALDH has been identified as a key determinant in the survival of murine [23, 24] and human [25] hematopoietic progenitor cells following exposure to the antitumor alkylating agents 4-hydroperoxy cyclophosphamide and mafosfamide. However, there are conflicting results on the identification of the specific ALDH enzyme(s) responsible for the survival of these pluripotent blood cells [26, 27]. If class I ALDH provides the major activity for aldophosphamide oxidation in the bone marrow stem cells (or even gastrointestinal stem cells), then DPAB may be of minimal use. Concentrations of DPAB necessary to sensitize tumor cells that are resistant to cyclophosphamide due to expression of high levels of class I ALDH levels would possibly sensitize the bone marrow stem cells, and significantly reduce or eliminate any therapeutic benefit of this alkylating agent. If the bone marrow stem cells were oxidizing aldophosphamide via a different ALDH enzyme, and the tumor cells were resistant due to expression of class I ALDH, then DPAB may be of great therapeutic use, especially in autologous bone marrow purging procedures. Additionally, it will be crucial to determine the effect of DPAB on other known physiologic substrates for class I ALDH, especially retinaldehyde. Since retinaldehyde to retinoic acid conversions play a regulatory role in the hormonal signalling of the retinoids in cell proliferation and differentiation pathways, it is plausible that class I ALDH catalyzed oxidation of retinaldehyde is an important step in the differentiation of bone marrow progenitor cells.

The therapeutic utility of DPAB will depend on its selectivity for binding to the different ALDH enzymes and the potency with which it blocks the oxidation of important xenobiotic and endogenous aldehyde substrates for a given ALDH enzyme. Thus, it will be necessary to examine the inhibitory effect of DPAB on class II and III ALDH enzymes.

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* The designation class II corresponds to ALDH I described in Ref. 14.

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