Human Aldehyde Dehydrogenase
ACTIVITY WITH ALDEHYDE METABOLITES OF MONOAMINES, DIAMINES, AND POLYAMINES*

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(Received for publication, January 22, 1991)

Two isozymes (E1 and E2) of human aldehyde dehydrogenase (EC 1.2.1.3) were purified to homogeneity 13 years ago and a third isozyme (E3) with a low $K_m$ for $\gamma$-aminobutyraldehyde only recently. Comparison with a variety of substrates demonstrates that substrate specificity of all three isozymes is broad and similar. With straight chain aliphatic aldehydes (C1-C6) the $K_m$ values of the E3 isozyme are identical with those of the E1 isozyme. All isozymes dehydrogenate naturally occurring aldehydes, 5-imidazoleacetalddehyde (histamine metabolite) and acrolein (product of $\beta$-elimination of oxidized polyamines) with similar catalytic efficiency. Differences between the isozymes are in the $K_m$ values for aminoa aldehydes. Although all isozymes can dehydrogenate $\gamma$-aminobutyraldehyde, the $K_m$ value of the E3 isozyme is much lower: the same appears to apply to aldehyde metabolites of cadaverine, agmatine, spermidine, and spermine for which $K_m$ values range between 2–18 $\mu$M and $k_{cat}$ values between 0.8–1.9 amol/min/mg. Thus, the E3 isozyme has properties which make it suitable for the metabolism of aminoa aldehydes. The physiological role of E1 and E2 isozymes could be in dehydrogenation of aldehyde metabolites of monoamines such as 3,4-dihydroxyphenylacetaldehyde or 5-hydroxyindoleacetalddehyde; the catalytic efficiency of these substrates is better with E1 and E2 isozymes than with E3 isozyme. Isoelectric focusing of liver homogenates followed by development with various physiological substrates together with substrate specificity data suggest that aldehyde dehydrogenase (EC 1.2.1.3) is the only enzyme in the human liver capable of catalyzing dehydrogenation of aldehydes rising via monoamine, diamine, and plasma amine oxidases. Although the enzyme is generally considered to function in detoxication, our data suggest an additional function in metabolism of biogenic amines.

NAD-linked dehydrogenation of short chain aliphatic aldehydes such as acetaldehyde and propionaldehyde has been found in virtually every organ of the mammalian body. It has since been determined that the reaction is catalyzed by a large variety of enzymes. Some of these enzymes have a specific physiological substrate which has been identified, others have wide substrate specificity with physiological substrate un

* This work was supported in part by National Institute on Alcohol Abuse and Alcoholism Grant AA00186, Research Scientist Award AA00046, and the Charles and Johanna Busch Memorial Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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suggested by Seiler et al. (9), who used liver homogenates in their experiments. In this paper we present evidence suggesting that aldehyde dehydrogenase from human liver may be specifically involved in the metabolic oxidation of biogenic aldehydes arising from biogenic amines and polyamines.

**EXPERIMENTAL PROCEDURES**

**Materials—**Chemicals were reagent grade. Putrescine-HCl, cadaverine-HCl, spermine-HCl, spermine-HCl, agmatine sulfate, amionoguanidine hemisulfate, hydroxyamine-HCl, formaldehyde (37% (v/v) aqueous solution), benzylamine-HCl, indole-3-acetaldehyde bisulfite, vitamin A1 aldehydes (13 cis and all trans), monoamine oxidase from bovine plasma, and diamine oxidase from pig kidney were from Worthington and NAD (grade 1) by Boehringer Mannheim. 4-Methylpyrazole was from Research Plus Inc. Straight-chain saturated aliphatic aldehydes, γ-aminobutyraldehyde diethyl acetal, and acrolein diethyl acetal were obtained from Aldrich. All buffers used (except where indicated) were exhaustively evaporated at room temperature to remove air and then saturated with nitrogen for El and E2 isozymes or with argon for the E3 isozyme which was more sensitive to atmospheric oxygen (2).

**Enzyme Preparation—**El and E2 isozymes of aldehyde dehydrogenase were purified from human liver following the procedure of Hempel et al. (11) and E3 isozyme as described by Kurys et al. (12). Homogeneity was confirmed by isoelectric focusing and specific activity. El and E2 isozymes were stored at 4 °C in nitrogen-saturated buffer for vitamin A1 aldehyde also contained 0.25% (v/v) Triton X-100 (0.25% (v/v)), ascorbate (1 mM), and serotonin (3 mM), to quinones (16). Control assays were performed in the presence of atmospheric oxygen. With an excess of aldehyde dehydrogenase (determined experimentally by adding aldehyde dehydrogenase until there was no change in the slope of NADH produced), the rate of aldehyde production by diamine oxidase and vitamin A1 aldehyde was measured. Controls contained no substrate or contained substrate and specific inhibitors, 100 μM aminoguanidine for diamine oxidase and 2 μM hydroxyamine for plasma amine oxidase. Control activity assays with propionaldehyde (1 mM) for El and E2 isozymes and γ-aminobutyraldehyde (100 μM) for E3 isozyme were performed in the presence of amines and inhibitors.

**Measurement of Kinetic Constants—**For aldehyde derivatives of polyamines, a two-step procedure was used. In step 1, aldehydes were generated from the polyamines by the action of diamine oxidase and from polyamines by plasma amine oxidase in conditions described for “Enzyme-coupled Reaction System.” The enzymatic reaction was terminated at different time intervals by using a centrifugal ultrafiltration technique (Amicon Centricon 30 Microconcentrators) to separate quickly (usually 3–5 min at 4 °C) newly formed aldehydes from the enzyme solution. The aliquots of protein-free ultrafiltrate were immediately used to initiate the second enzymatic reaction (step 2) with E3 aldehyde dehydrogenase in the presence of 1 mM NAD and 1 mM EDTA in nitrogen-saturated 100 mM sodium phosphate buffer, pH 7.4, and the reaction was allowed to go to completion in anaerobic conditions at 25 °C. The amount of E3 isozyme and the concentration of aldehydes were selected so that the total reaction time was about 10 min. A similar two-step procedure was also employed for 5-hydroxyindoleacetaldehyde. Monoamine oxidase (0.15 unit) and serotonin (3 mM) were incubated in 50 mM phosphate buffer, pH 7.4, containing 1 mM ascorbate, at 30 °C for 2 h (step 1). The reaction was terminated by centrifugation in Amicon Centricon 30 Microconcentrators, and the protein-free ultrafiltrate was used immediately for determination of kinetic constants (step 2) as above.

**Enzyme-Coupled Reaction System—**Aldehydes were generated from monoamines, diamines, and polyamines with their aldehydic or carbinolic derivative Structure

**TABLE I**

| Compound and aldehyde derivative | Structure |
|----------------------------------|-----------|
| Putrescine                        | NH₂(CH₃)₂NH₂ |
| Aldehyde                         | NH₂(CH₃)₂CHO |
| Cadaverine                       | NH₂(CH₃)₂NH₂ |
| Aldehyde                         | NH₂(CH₃)₂CHO |
| Agmatine                         | NH₂(NH₂)₃NH₂ |
| Aldehyde                         | NH₂(NH₂)₃CHO |
| Spermine                         | NH₂(CH₃)₂NH₂(NH₂)₂NH₂ |
| Aldehyde                         | NH₂(CH₃)₂NH₂(NH₂)₂CHO |
| Spermine                         | NH₂(CH₃)₂NH₂(NH₂)₂NH₂ |
| Aldehyde                         | NH₂(CH₃)₂NH₂(NH₂)₂CHO |
| Monoaldehyde                     | NH₂(CH₃)₂NH₂(NH₂)₂CHO |
| Dialdehyde                       | OHC(CH₃)₂NH₂(NH₂)₂CHO |
| Acrolein                         | H₂C=CHCHO |

**Standard Activity Assay—**Aldehyde dehydrogenase activity was determined spectrophotometrically by monitoring NADH production at 340 nm (ε = 6.22 mM⁻¹ cm⁻¹) at 25 °C. The assay system for El and E2 isozymes (in 3 ml of total volume) contained, 100 mM sodium pyrophosphate, pH 9.0, 0.5 mM NAD, 1 mM EDTA, and 1 mM propionaldehyde as substrate. The E3 isozyme was assayed in 100 mM sodium phosphate buffer, pH 7.4, containing 0.5 mM NAD, 1 mM EDTA and 100 μM γ-aminobutyraldehyde as substrate. All buffers were evacuated and nitrogen-saturated. Protein concentrations were determined using the procedure of Lowry et al. (14) with bovine serum albumin as a standard and by 280-nm absorption using previously determined extinction coefficients (2, 15).

**Enzyme-Coupled Reaction System—**Aldehydes were generated from polyamines putrescine, cadaverine, and agmatine via the action of diamine oxidase (3, 12) and spermidine and spermine via the action of plasma amine oxidase (8) and oxidized by an excess of aldehyde dehydrogenase. The assay system containing a 3-ml total volume of 100 mM sodium phosphate buffer, pH 7.4 (not evacuated and not nitrogenated), monoamine oxidase (15 μg) or plasma amine oxidase (0.5 mg), amine (3-5 mM), NAD (1 mM), and aldehyde dehydrogenase. Reaction was initiated by the addition of amine, at 25 °C in the presence of atmospheric oxygen. With an excess of aldehyde dehydrogenase (determined experimentally by adding aldehyde dehydrogenase until there was no change in the slope of NADH produced), the rate of aldehyde production by diamine oxidase and vitamin A1 aldehyde was measured. Controls contained no substrate or contained substrate and specific inhibitors, 100 μM aminoguanidine for diamine oxidase and 2 μM hydroxyamine for plasma amine oxidase. Control activity assays with propionaldehyde (1 mM) for El and E2 isozymes and γ-aminobutyraldehyde (100 μM) for E3 isozyme were performed in the presence of amines and inhibitors.
activity at the beginning of the experiment.

Identification of Aldehyde Derivatives of Diamines and Polyamines—The formation of carbonyl compounds in the incubation mixture (step 1) was also evaluated qualitatively using 2,4-dinitrophenylhydrazine. Small aliquots of protein-free ultrafiltrates were treated with saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl and then mixed with 10% NaOH solution. Carbonyl compounds gave a yellow color which turned red-brown when treated with alkali; both were seen as dark spots in the ultraviolet. Aliquots of protein-free ultrafiltrates were also chromatographed on thin layer chromatography silica plates (40 X 80 mm) in butanol/acetic acid/H$_2$O (12:3:5) solvent system. The carbonyl compounds were visualized by spraying with 2,4-dinitrophenylhydrazine while amines and carbonyl amine derivatives were detected with 0.1% ninhydrin in butanol. Due to instability of aldehydes formed by oxidation of spermidine and spermine preliminary identification was made after reduction of aldehydes to corresponding alcohols. The protein-free ultrafiltrates from different incubation times (15, 40, 75, 90, 120 min) were treated with an excess of sodium borohydride, and the corresponding alcohols after extraction with butanol were subjected to thin layer chromatography on cellulose in butanol/acetic acid/pyridine/H$_2$O (4:1:1:2) system as described by Kimes et al. (19). Reduced derivatives of oxidized spermidine were also detected chromatographically on Whatman No. 1 paper in propanol/H$_2$O/HCl (6:2:4) solvent as described by Tabor et al. (8). Amines, putrescine, spermidine, and spermine were used as controls. Chromatograms were sprayed with 0.3% ninhydrin in 90% propionaldehyde containing 4% pyridine and heated at 110 °C for 5-10 min. Samples of human liver (~5 g) were homogenized in 30 mM sodium phosphate buffer, pH 7.0 (1:2 w/v), containing 1 mM EDTA and 0.1% (v/v) 2-mercaptoethanol and then centrifuged at 100,000 X g for 30 min at 4 °C. Isoelectric focusing was carried out on agarose plates (114 X 225 mm) composed of 1% (w/v) agarose, 12% (w/v) sorbitol, 6% (v/v) Pharmalyte, pH 3-10 (Pharmacia Fine Chemicals), at 8 °C and at 1500 V for 0.5 h at 5 mA followed by 0.5 h at 10 mA and then 1 h at 15 mA. The enzyme activity was visualized after incubation in the dark and under nitrogen in 0.1 M sodium phosphate buffer, pH 7.4 (evacuated and nitrogenated), containing NAD (10 mg/30 ml), nitroblue tetrazolium (2 mg/30 ml), 3-acetaldehyde (2 mg/30 ml), 4-methylpyrazole (1 mM), and different substrates (100 µM). Control gels were obtained by omitting the substrate from the staining solution. Other controls used high concentrations of propionaldehyde (13 mM) or y-aminobutyraldehyde (2 mM) with or without 4-methylpyrazole (1 mM). Homogeneous E1, E2, and E3 isozymes were used as standards. Due to observed reaction of 3,4-dihydroxyphenylacetaldehyde and indole-3-acetaldheyde with the staining reagents, two to three changes of the developing buffer were made in the course of activity staining. Isoelectric points were determined using standards provided in a Isoelectric Focusing Calibration Kit (broad range pH 3-10) from Pharmacia Fine Chemicals. The standards were applied according to instructions of the manufacturer.

RESULTS

Identification of Aldehyde Derivatives of Diamines and Polyamines Generated by Diamine and Plasma Amine Oxidases—Aldehyde derivatives of diamines and polyamines are not commercially available, therefore an enzymatic procedure employing diamine oxidase and plasma amine oxidase was used to generate aldehydes. The oxidative products of putrescine, cadaverine, agmatine, and spermine were detected with 0.1% ninhydrin in butanol. Due to instability of aldehydes, formation of a new carbonyl compound in the incubation mixture from amine was shown qualitatively when protein-free ultrafiltrates were chromatographed on thin layer chromatography silica plates in butanol/acetic acid/H$_2$O (12:3:5) solvent, and chromatograms were sprayed with 1% ninhydrin or 2,4-dinitrophenylhydrazine. Two main spots were observed when putrescine, cadaverine, agmatine, spermidine, or spermine were used. The faster migrating carbonyl compound was sensitive to 2,4-dinitrophenylhydrazine and to ninhydrin and increased in concentration with time of incubation while the slower migrating amine was sensitive only to ninhydrin.

When the products resulting from the incubation of spermidine or spermine with plasma amine oxidase were reduced with sodium borohydride (which reduces aldehydes to alcohols) and after extraction with butanol chromatographed on thin layers of cellulose in butanol/acetic acid/pyridine/H$_2$O (4:1:1:2) solvent, they no longer reacted with 2,4-dinitrophenylhydrazine. In extracts of the incubation mixture with spermine, after reduction to alcohols and extraction with butanol, only one main component was visualized with an R$_f$ value ~0.4. Two main spots were observed on chromatograms with extracts from incubation mixture with spermine after reduction to alcohols: the faster migrating component with an R$_f$ value ~0.7, and the slower migrating one with an R$_f$ value ~0.4. Chromatographic localization and distribution of these components when compared with standard amines putrescine, spermidine, and spermine was similar to that described by Kimes and Morris (19) and their R$_f$ values corresponded to dialcohol [N,N'-bis(3-hydroxypropyl)-1,4-diaminobutane] and monoalcohol [N-3-hydroxypropyl-1,4-di-aaminobutane], respectively. When borohydride-reduced derivatives of spermine were chromatographed on Whatman No 1 paper in propanol/H$_2$O/HCl (6:2:2) solvent, two components were also observed with R$_f$ values ~0.73 and 0.62. These values are in good agreement with R$_f$ values 0.77 and 0.60, reported by Tabor et al. (8), respectively, for reduced di and mono derivatives of oxidized spermine. Both reduced components were observed in all extracts from incubation mixtures with spermine (set up for 15, 40, 75, 90, and 120 min) but more monoaldehyde derivative was seen in the samples after 15 and 40 min of incubation and more dialdehyde derivative after 1 h of incubation. Alcohol derived from monoaldehyde was light blue in color and that of dialdehyde white-pink after spraying with ninhydrin.

Enzyme-coupled Reaction System with E3 Aldehyde Dehydrogenase and Diamine Oxidase or Plasma Amine Oxidase—The coupled reaction system was at first verified employing benzylamine as substrate with both diamine oxidase and plasma amine oxidase. Following this, other amines such as putrescine, cadaverine, agmatine, spermidine, and spermine were used. The activity of all three isozymes in the system was observed with all amines tested. The E3 isozyme, which was most active, was used for standardization of the system and for assay of diamine and plasma amine oxidases. The reaction was initiated by the addition of amine and after a lag period and non-linear time course, linear steady-state was approached. All controls were carefully checked. No activity was observed in the coupled reaction system in the absence of amine or in the presence of specific inhibitors: 100 µM aminoguanidine for diamine oxidase and 2 µM hydroxylamine for plasma amine oxidase. Instantaneous inhibition of plasma amine oxidase upon addition of hydroxylamine (2 µM) and diamine oxidase upon addition of aminoguanidine (100 µM) to the coupled reaction system with amine, NAD, and E3 isozyme (also E1 and E2 isozymes) was observed. Instantaneous inhibition of plasma amine oxidase by hydroxylamine (2 µM) was also confirmed by spectrophotometric assay of plasma amine oxidase activity with benzylamine at 250 nm, at 25 °C. The amount of hydroxylamine employed had no effect on levels of aldehyde substrate. The presence of amines at a concentration up to 5 mM, aminoguanidine at 100 µM, and hydroxylamine at 2 µM concentration was without effect on aldehyde dehydrogenase activity, when the isozymes were assayed with 100 µM y-aminobutyraldehyde (E3 isozyme) or 1 mM propionaldehyde (E1 and E2 isozymes) and 1 mM NAD in 100 mM sodium phosphate buffer, pH 7.4.
Due to the fact that plasma amine oxidase can generate monoaldehyde from spermine and dialdehyde from monoaldehyde (8, 19), two experiments were set up. In the first one, a coupled enzyme reaction system with plasma amine oxidase, spermine (0.05 mM) and an excess of E3 isozyme (ratio of activity ca. 3:1) was used to oxidize in situ arising aldehyde(s). A 1:1 ratio of NADH formed to total spermine oxidized was observed after reaction was completed. No further activity was detected, after addition of more plasma amine oxidase or E3 isozyme. Remaining activity of E3 isozyme in the assay system, after completion of reaction, was in the range 90–95% of the control. The second experiment contained plasma amine oxidase and spermine (0.5 mM) at the same conditions as first experiment, but E3 isozyme and NAD were omitted from the system. Aliquots were removed at various time intervals and amounts of aldehydes were determined with E3 isozyme and NAD (1 mM). The amount of NADH formed increased with time up to the point where the ratio of NADH/spermine was 1.3–1.8:1. This ratio could not be increased by longer incubation.

**Comparison of E3 Isozyme and E1 and E2 Isozymes Activity in the Coupled Reaction System**—When aldehydes were generated from putrescine, cadaverine, agmatine, spermidine, and spermine in the coupled reaction system in the presence of E1 or E2 or E3 isozymes, the formation of NADH at 340 nm was observed with all amines tested (Table II). The observed activity in comparative studies (the same concentration of amine, the same level of diamine oxidase, or plasma amine oxidase and aldehyde dehydrogenase) was much higher with E3 isozyme than that for E1 and E2 isozymes (Table II). Even when a large excess of E1 and E2 isozymes was used, activity was still considerably higher with the E3 isozyme. When a specific inhibitor of diamine oxidase (100 µM amidoguanidine) or plasma amine oxidase (2 µM hydroxylamine) was added at different time periods after initiation of the reaction, the reaction proceeded to completion very fast with E3 isozyme and very slowly with E1 and E2 isozymes. Remaining aldehyde concentration which reflected a pool of steady-state level of biogenic aldehydes, determined from NADH formed after instantaneous inhibition of diamine oxidase or plasma amine oxidase, was considerably higher with E1 and E2 isozymes (tens of µM) than with E3 isozyme (few µM).

**Kinetic Constants for E3 Isozyme with Biogenic Aldehydes Arising from Diamines and Polyamines**—The $K_n$ and $k_{cat}$ values for E3 isozyme and aldehydes arising from putrescine, agmatine, cadaverine, and spermidine were obtained by employing aliquots of protein-free ultrafiltrate, following generation of aldehydes from parent amines. The $K_n$ values with all aldehydes (Table III) were micromolar (2–18.5 µM); the $k_{cat}$ values ranged from 0.8 to 1.9 µmol/min/mg. Aldehyde from spermidine (N-3-propanal-1,4-diaminobutane) was the best substrate; other aldehydes however were also good substrates as judged by comparison of $k_{cat}/K_n$ ratios. The kinetic data for oxidized spermine were more complicated. Results of kinetic measurements of aliquots from the incubation mixture with spermine after 15-, 40-, 75-, 90-, and 120-min periods were compared (1/v versus 1/[S] plots). When determination of kinetic constants was attempted following a short incubation time (15 min), the plots were linear giving a single $K_n$ value (~16 µM). Based on the results of chromatographic experiments (described above) this $K_n$ value was assigned to monoaldehyde. At longer times of incubation (40 min), the plots became biphasic giving two $K_n$ values: one similar to that described and another new $K_n$ of about 2 µM. At this point of incubation time, chromatography showed presence of approximately equal amounts of monoaldehyde and dialdehyde allowing assignment of this new $K_n$ to dialdehyde. When incubation was continued for 90 min and longer, no monoaldehyde $K_n$ could be detected but dialdehyde $K_n$ could still be approximately calculated. Also another change in a slope pattern occurred from which a $K_n$ value ~120–150 µM could be calculated. This suggested that further dehydrogenation of the dialdehyde to acid might have occurred. When the incubation reaction was nearing completion, there were additional unidentified spots on the chromatograms; there was also a loss of enzyme activity during the reaction making further interpretation of the slope pattern changes impossible.

**Substrate Specificity of E3 Isozyme with Other Naturally Occurring Aldehydes**—Kinetic constants for E3 isozyme with other physiological substrates are shown in Table IV. Comparison of $k_{cat}/K_n$ values demonstrates that y-aminobutyraldehyde is the best substrate in this group. The values for the E3 isozyme obtained during this investigation are compared with kinetic constants for E1 and E2 isozymes, some of which were previously published (12, 16, 20). It can be seen from this comparison that the $K_n$ value for 5-imidazolacetaldehyde for the three isozymes is identical, as is the $K_n$ value for 3,4-dihydroxyphenylacetaldehyde. Activity of the E3 isozyme with 5-hydroxindoleacetaldehyde, when compared with that of the E1 and E2 isozymes, was extremely low (~5% of the activity of the standard assay with y-aminobutyraldehyde). Thus, 5-hydroxindoleacetaldehyde is a much better substrate for E1 and E2 isozymes than for E3 isozyme. Because of low activity, kinetic constants with 5-hydroxindoleacetaldehyde not determined using the method of Yun and Suelter (17). Values are means ± S.D. for n experiments. With dialdehyde $k_{cat}$ could not be obtained with sufficient accuracy to be quoted in this table.

### Table II

**Comparison of activity of E3 with E1 and E2 isozymes with metabolites of diamines and polyamines in the enzyme-coupled reaction system**

| Coupled reaction system | Parent amine | Observed dehydrogenase activity with isozymes | Observed dehydrogenase activity with isozymes |
|-------------------------|--------------|---------------------------------------------|---------------------------------------------|
| E1                      | E2           | E3                                         | E1                                         |
| DA + ALDH               | Putrescine   | +                                          | +                                          |
| DA + ALDH               | Cadaverine   | +                                          | +                                          |
| DA + ALDH               | Agmatine     | +                                          | +                                          |
| PAO + ALDH              | Spermidine   | +                                          | +                                          |
| PAO + ALDH              | Spermine     | +                                          | +                                          |

### Table III

**Substrate specificity of E3 isozyme with aldehydes arising from diamines and polyamines**

Aldehydes were generated from parent amines by the action of diamine oxidase or plasma amine oxidase (step 1), and protein free aliquots were used in aldehyde dehydrogenase assay (step 2), in 100 mM sodium phosphate buffer, pH 7.4 (evacuated and nitrogenated) containing 1 mM EDTA and 1 mM NAD. Kinetic constants were determined using the method of Yun and Suelter (17). Values are means ± S.D. for n experiments. With dialdehyde $k_{cat}$ could not be obtained with sufficient accuracy to be quoted in this table.
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TABLE IV

Substrate specificity of E3 isozyme with some naturally occurring aldehydes and comparison with E1 and E2 isozymes

| Aldehyde                         | n   | E3 isozyme | E1 isozyme | E2 isozyme |
|----------------------------------|-----|------------|------------|------------|
|                                  |     | IrM       | K,        | kcat/K,    | IrM       | K,        | kcat/K,    | IrM       | K,        | kcat/K,    |
|                                  |     | µM        | µmol/min/mg|           | µM        | µmol/min/mg|           | µM        | µmol/min/mg|           |
| γ-Aminobutyraldehyde             | 3   | 4.6 ± 0.8 | 1.53 ± 0.06| 0.33       | 760       | 0.13       | 0.001      | 512       | 0.15      | 0.001      |
| 3,4-Dihydroxyphenylacetaldehyde  | 5   | 2.6 ± 1.9 | 0.19 ± 0.09| 0.08       | 0.4       | 0.20       | 0.5        | 1.0       | 0.30      | 0.3        |
| 5-Hydroxyimidazoleacetaldehyde   |     | Active    |            |            | 2.4      | 0.22       | 0.09       | 0.8       | 0.21      | 0.26       |
| 5-Imidazolacetaldehyde           | 3   | 59.0 ± 8.5| 0.92 ± 0.11| 0.02       | 39       | 0.18       | 0.005      | 30        | 0.28      | 0.009      |
| Acrolein                         | 6   | 4.9 ± 2.5 | 0.38 ± 0.06| 0.08       | Active    | (low K,)|           | 1.5       | 0.23      | 0.15       |
| Vitamin A1 aldehyde              |     | All trans | Active     |            |           |            |            |           |           |            |
| 13 cis                           |     | Active     |            |            |           |            |            |           |           |            |

* E3 and E2 isozymes, 100 mM sodium phosphate buffer, pH 7.0; 1 mM EDTA; 0.5 mM NAD (12); all values determined at 25 °C.
* E1 and E2 isozymes, 30 mM phosphate buffer, pH 7.1; 1 mM EDTA; 0.5 mM NAD (20).
* K, values could not be determined.
* Enzyme inactivation occurred during K, determination, K, values not as accurate as other K, values (measured at 0–10 µM acrolein).

could not be determined for the E3 isozyme. Dehydrogenation of vitamin A1 aldehydes (both cis and trans) could be followed at 400 nm utilizing vitamin A1 absorbance. In these conditions, the reaction was observed which could be abruptly stopped by the addition of propionaldehyde (1 mM), indicating that all three enzymes catalyzed the reaction. However, at substrate concentrations higher than ~10 mM, strong substrate inhibition occurred. Thus, although it could be demonstrated that vitamin A1 aldehydes were substrates for all three isozymes, kinetic constants could not be determined. E1, E2, and E3 isozymes catalyzed dehydrogenation of acrolein at low micromolar concentration (0–10 µM). Estimated values of K, for all isozymes were below 5 µM, and the highest kcat/K, was calculated for E2 isozyme. However, even at low concentrations of acrolein, ~5–10 µM for E2 and E3 isozymes and ~5 µM for E1 isozyme, inactivation of isozymes was observed. The possibility that acrylic acid can inhibit dehydrogenase activity of E3 isozyme or that the reverse reaction may occur was considered. However, no inhibition of enzyme activity by acrylic acid (up to 2 mM) and no reverse reaction with acrylic acid (2 mM) and NADH (0.1 mM) was observed.

Substrate Specificity of E3 Isozyme with Saturated Aliphatic Aldehydes and Comparison with E2 and E2 Isozymes—Kinetic constants for straight chain-saturated aliphatic aldehydes of one to six carbon atoms and E3 isozyme are listed in Table V. The values are averages of several determinations. It can be seen that K, values decrease upon increase of aldehyde chain length from 400 µM at formaldehyde to 0.6 µM at hexanaldehyde; the kcat/K, values increase slightly between formaldehyde and hexanaldehyde. The kcat/K, ratios, however, also increase demonstrating that the longer the chain length of the aldehyde the better the substrate. The K, kcat, and kcat/K, ratios obtained for E3 isozyme are also compared with those of E1 and E2 isozymes in Table V. The values for E1 and E2 isozymes, with the exception of those for acetaldehyde and propionaldehyde, are single determinations obtained using the Lineweaver-Burk (18) procedure. When Michaelis constants are low these values are only approximate. This comparison demonstrates that E3 isozyme resembles both E1 and E2 isozymes in kinetic properties. All three isozymes have low micromolar K, values for aldehydes of three to six carbon atoms and catalyze aldehyde dehydrogenation at similar velocity. The E3 isozyme resembles the E1 isozyme more than E2 isozyme; its K, values for aldehydes of one to six carbon atoms are almost identical with those of E1 isozyme. Comparison of kcat/K, ratios shows that efficiency of aldehyde substrates for cytoplasmic E1 and E3 isozymes increases with the increase of chain length much faster than for mitochondrial E2 isozyme. The E3 isozyme differs from E2 isozyme in K, value for acetaldehyde which is much lower for the E2 isozyme.

Isoelectric Focusing of Liver Homogenates—When liver extracts were subjected to isoelectric focusing in agarose gels and stained for aldehyde dehydrogenase activity with low concentration (100 µM) of γ-aminobutyraldehyde, propionaldehyde, 3,4-dihydroxyphenylacetaldehyde, indole-3-acetaldehyde, and phenylacetaldehyde, only aldehyde dehydrogenases in the pI range 4.9 to 5.5 were visualized (Fig. 1). A multiple banded pattern corresponded to E1, E2, and E3 isozymes. However, when high concentration of propionaldehyde (13 mM) or γ-aminobutyraldehyde (2 mM) were used as substrates in the control gel staining mixture, other aldehyde dehydrogenases were seen at higher pI values. A band corresponding to glutamic-γ-semialdehyde dehydrogenase was identified at pI ~7 and another band, not previously identified, at pI of ~9.5. The presence of 4-methyl pyrazole in the staining mixture had no effect on aldehyde dehydrogenase visualization but eliminated alcohol dehydrogenase bands visualized at high pI.

DISCUSSION

Aldehydes arising from polyamines were reported to be unstable, giving rise to β-elimination products and the formation of oxidized spermidine and acrolein from dioxidized spermine, spermidine, and acrolein from monooxidized spermine, putrescine and acrolein from oxidized spermidine (8, 19). Due to reported instability of aminoaldehydes, it was decided to use an enzyme-coupled reaction system. Aldehydes
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TABLE V
Substrate specificity of E3 isozyme with straight chain-saturated aliphatic aldehydes and comparison with E1 and E2 isozymes

Assay system was 50 mM sodium phosphate buffer, pH 7.0, containing 0.5 mM NAD and 1 mM EDTA, at 25 °C. All values were determined using Lineweaver-Burk plots, except for E3 isozyme with butyraldehyde, pentaldehyde, and hexanaldehyde as substrates, where single reaction curve method of Yun and Suelter (17) was used. Values are means ± S.D. for n determinations with E3 isozyme.

| Aldehyde     | n | E3 isozyme | E1 isozyme | E2 isozyme |
|--------------|---|------------|------------|------------|
|              |   | K<sub>n</sub> | h<sub>cat</sub> | h<sub>cat</sub>/K<sub>n</sub> | K<sub>n</sub> | h<sub>n</sub> | h<sub>cat</sub>/K<sub>n</sub> | K<sub>n</sub> | h<sub>n</sub> | h<sub>cat</sub>/K<sub>n</sub> |
| Formaldehyde | 4 | 410 ± 58 | 0.19 ± 0.04 | 0.001 | 330 | 0.46 | 0.001 | 1 | 0.31 | 0.31 |
| Acetaldehyde | 4 | 57 ± 11 | 0.29 ± 0.03 | 0.005 | 50 | 0.25 | 0.005 | 0.8 | 0.30 | 0.37 |
| Propionaldehyde | 4 | 9.5 ± 2.5 | 0.30 ± 0.03 | 0.03 | 5 | 0.28 | 0.06 | 4 | 0.34 | 0.08 |
| Butyraldehyde | 5 | 2.8 ± 0.1 | 0.32 ± 0.04 | 0.11 | 4 | 0.34 | 0.08 | <0.5 | 0.39 | <0.5 |
| Pentaldehyde | 4 | 1.4 ± 0.5 | 0.37 ± 0.10 | 0.26 | <0.5 | 0.39 | <0.5 | <0.5 | 0.50 |
| Hexanaldehyde | 3 | 0.6 ± 0.1 | 0.39 ± 0.02 | 0.65 | <0.5 | 0.39 | <0.5 |

FIG. 1. Isoelectric focusing gel of human liver homogenates, developed for activity with low concentration of physiological substrates. The arrow (next to pl) shows the direction of increase of pl. Positions of E1, E2 and E3 isozymes during isoelectric focusing are shown diagrammatically; please note that while E1 and E2 isozyme each consists of one band with pl of 5.3 and 5.0, respectively, the E3 isozyme consists of two bands of pl 5.3 and 5.45. Lane 1, homogeneous; E3 isozyme control developed with 100 μM γ-aminobutyraldehyde, pH 7.4; lane 2, control mixture of homogeneous E1 and E2 isozymes developed with 13 mM propionaldehyde, pH 7.4; lanes 3-6, liver homogenates; lane 3, developed at pH 7.4 with propionaldehyde (100 μM); lane 4, developed with γ-aminobutyraldehyde (100 μM); lane 5, developed with 3,4-dihydroxyphenylacetaldehyde (100 μM); lane 6, developed with indole-3-acetaldehyde (100 μM).

were generated from parent amines by the action of diamine oxidase or plasma amine oxidase and used as substrates by aldehyde dehydrogenase. When aldehyde dehydrogenase isozymes (E1 or E2 or E3) were used in the coupled system, formation of NADH could be observed. Validation of the enzyme-coupled reaction system was also done by using specific inhibitors for diamine oxidase (aminoguanidine) and plasma amine oxidase (hydroxylamine at stoichiometric concentrations), see “Results”) which completely inactivated the whole system. Formation of aldehydes whose concentration increased with the time of incubation was also observed on thin layer chromatography. However, in the case of spermine results were more complicated. Two main spots, corresponding to monoaldehyde and dialdehyde derivatives of spermine were seen when the products resulting from the incubation of spermine with plasma amine oxidase were reduced with sodium borohydride. Formation of a dialdehyde from spermine and an intermediate, presumably the monoaldehyde at earlier period of reaction was shown by Tabor et al. (8) and Kimes et al. (19). During this work, to obtain more information about the sequence of reaction steps, the first product from spermine via plasma amine oxidase was instantaneously oxidized by an excess of aldehyde dehydrogenase (E3 isozyme). Only one form, the monoaldehyde, was generated as judged from 1:1 production of NADH from spermine. When the reaction system was separated into two steps (step 1, generation of aldehydes from spermine by plasma amine oxidase; step 2, oxidation of aldehydes by aldehyde dehydrogenase), formation of the monoaldehyde and then dialdehyde in the incubation mixture (step 1) was seen on thin layer chromatography of reduced aldehydes, after different time periods. In these conditions the ratio of NADH formed to spermine increased from 1:1 to 1.3–1.81. Thus, the results of our investigation confirm those of Tabor et al. (8) and Kimes et al. (19).

In the enzyme-coupled reaction, aldehydes formed from putrescine, cadaverine, agmatine, spermidine, and spermine were substrates for E1, E2, and E3 isozymes of aldehyde dehydrogenase (Table II). The oxidation velocities of the aldehydes with E1 and E2 isozymes, when compared with E3 isozyme, were however much lower, and lag periods after initiation of reaction with amine were much longer suggesting that isozymes may differ in K<sub>n</sub> values. This was also confirmed by the observation of considerably higher steady-state level of aldehydes in the enzyme-coupled reaction with E1 and E2 isozymes than with E3 isozyme after specific inhibitors of plasma amine oxidase or diamine oxidase were used in the course of the reaction. The aldehydes generated in the first step of the coupled reaction following removal of plasma amine oxidase or diamine oxidase were, therefore, used for determination of kinetic constants (Table III). Aldehydes arising from diamines and polyamines are excellent substrates for E3 isozyme, which can catalyze their dehydrogenation at low concentrations and with high velocities (Table III). All aldehydes have low K<sub>n</sub> values with E3 isozyme in the μM range (2–18 μM) and high h<sub>cat</sub> values in the range 1.7–1.9 μmol/min/mg for aldehyde arising from spermidine and monoaldehyde arising from spermine. The aldehyde from spermidine was the best substrate giving the highest h<sub>cat</sub>/K<sub>n</sub> ratio. Although K<sub>n</sub> values for the E1 and E2 isozymes with aldehydes arising from diamines and polyamines have not been determined, the results from coupled reaction system suggest that their values are high.

In mammalian organisms putrescine is metabolized via two pathways, one of which leads to γ-aminobutyric acid, the other to polyamines. Both pathways are physiologically important. Conversion of putrescine to γ-aminobutyric acid produces an inhibitory neurotransmitter, and conversion to polyamines produces substrates involved in control of growth.
processes. Increased biosynthesis of polyamines and their rapid accumulation in cells is invariably associated with all rapidly growing systems, either bacterial, plant, or animal, under normal controlled conditions and during neoplastic growth (5). All aldehyde metabolites from both pathways are substrates for aldehyde dehydrogenase (Fig. 2); all are excellent substrates for E3 isozyme (Table III). Comparison of substrate specificity of E1, E2, and E3 isozymes with that of other aldehyde dehydrogenases isolated from human liver and brain, which were identified as glyceraldehyde-3-phosphate dehydrogenase (21, 22), succinic semialdehyde dehydrogenase (21), and glutamic-γ-semialdehyde dehydrogenase (23) showed that 3,4-dihydroxyphenylacetaldehyde (dopamine metabolite) was a substrate for aldehyde dehydrogenase and not a substrate for other aldehyde dehydrogenases. These data were further confirmed by our results (Fig. 1) where gels of human liver homogenates have been developed with various physiological aldehyde metabolites. It can be seen that 2,4-dihydroxyphenylacetaldehyde, γ-aminobutyraldehyde, indole-3-acetaldehyde, and phenylacetaldehyde (Fig. 1) reacted with the same bands at the same gel area as did propionaldehyde at low concentration. These bands correspond to El, E2, and E3 isozymes. Thus, it appears that aldehyde dehydrogenase is the only enzyme in the human liver that has the capability of catalyzing metabolism of aldehydes arising from biogenic amines.

Although reports of purification to homogeneity of aldehyde dehydrogenase from various sources have been appearing in the last 20 years, only two purifications of the enzyme that catalyzed dehydrogenation of γ-aminobutyraldehyde from bacteria were ever reported (24, 25). Purification of the enzyme from human liver to homogeneity was reported recently (2). Since then a report has appeared of purification to homogeneity of a similar enzyme from rat brain (26). Characterization of the human enzyme catalyzing dehydrogenation of γ-aminobutyraldehyde showed that the enzyme had low Kₐ values with acetaldehyde and propionaldehyde (2) and in this way resembled the other two human aldehyde dehydrogenases E1 and E2 isozymes purified to homogeneity in our laboratory about 13 years previously (15). The results presented in this paper offer further support for functional relationship of E3 to E1 and E2 isozymes. From overall comparison of data with straight chain-saturated aldehydes of one to six carbon atoms resemblance to both E1 and E2 isozymes in catalytic properties is seen (Table V). Thus, identity of the E3 isozyme is further confirmed as a third low Kₐ human aldehyde dehydrogenase with broad substrate specificity. In fact, its substrate specificity profile is almost identical with that of the cytoplasmic E1 isozyme (Table V). Similar support comes from comparison of substrate specificity with natural substrates (Tables II-IV). 5-Imidazolecetaldehyde (histamine metabolite) is an equally good substrate for all isozymes. Acrolein, the product of β-elimination of oxidized polyamines, is also a good substrate. All three isozymes bind and dehydrogenate biogenic aldehydes arising from monoamines, diamines, and polyamines and appear to be the only enzymes in the human liver homogenate capable of catalyzing this reaction (Fig. 1). Individual specialization of isozymes is also apparent, the most striking of which is adaptation of E3 isozyme to metabolism of aminoaicdehydes. More than two decades ago, Axelrod et al. (27), Erwin and Deitrich (28), Tipton et al. (29), and Alivisatos and Tabakoff (30) postulated involvement of aldehyde dehydrogenase in oxidation of aldehydes arising from biologically active monoamines (serotonin, epinephrine, dopamine) in mammalian brain tissue via the action of monoamine oxidase. Our results (Table IV) demonstrate that 3,4-dihydroxyphenylacetaldehyde (dopamine metabolite) has low Kₐ values for all three isozymes (~2.5 mM or less), but the kcat value with E3 isozymes is also low resulting in lower catalytic efficiency. 5-Hydroxyindoleacetaldehyde, a metabolite of serotonin, is a poor substrate for the E3 isozyme; it is an excellent substrate for both the E1 and E2 isozymes. Thus, it appears more likely that E1 and E2 isozymes may be involved in monoamine metabolism.

Because of its widespread substrate specificity aldehyde dehydrogenase is often regarded as an enzyme of detoxication (31) which functions in removal of various aldehydes ingested in foodstuffs. Our data are not inconsistent with such a role but indicate that the enzyme also has an additional specialized role involving metabolism of biogenic amines, polyamines, and other physiologically important compounds. Thus, the enzyme most likely performs a dual role, that of metabolism of physiological compounds and that of detoxication. Conversion of putrescine to γ-aminobutyric acid is an example of metabolism of a physiologically active compound; the enzyme functions in conversion of a polyamine precursor to an inhibitory metabolite. The enzyme catalyzing dehydrogenation of γ-aminobutyraldehyde is the only enzyme in the human liver that has the capability of catalyzing metabolism of aldehydes arising from biogenic amines.
itary neurotransmitter. The function of detoxication is performed with naturally occurring compounds (such as biogenic amines) as well as with extraneous ingested compounds (such as ethanol). Interaction of serotonin, dopamine, and norepinephrine metabolism with the metabolism of ethanol is well-documented (32–36) and has been claimed to occur via competition between aldehyde metabolites of monoamines and acetaldehyde for aldehyde dehydrogenase. From our results it appears that aldehyde dehydrogenase E3 may be involved in all stages of putrescine and polyamine degradation (Fig. 2). In the presence of acetaldehyde (arising from ethanol metabolism) aldehyde metabolites of putrescine and polyamines might accumulate and contribute to pathogenesis of alcoholism. Complications of alcoholism, such as fetal alcohol syndrome, may be a simple outcome of a competition between acetaldehyde and aldehyde metabolites of putrescine and polyamines.

REFERENCES
1. Pietruszko, R. (1989) in Biochemistry and Physiology of Substance Abuse (Watson, R., ed) Vol. 1, pp. 89–127, CRC Press, Inc., Boca Raton, FL.
2. Kurys, G., Ambroziak, W., and Pietruszko, R. (1989) J. Biol. Chem. 264, 4715–4721.
3. Fogel, W. A. (1986) in Mechanisms in the Mammalian Periphery (Erdo, S. L., and Boloery, N. G., eds) pp. 35–61, Raven Press, New York.
4. Tabor, C. W., and Tabor, H. (1984) Annu Rev. Biochem. 53, 749–790.
5. Tabor, C. W., and Tabor, H. (1984) Annu Rev. Biochem. 53, 749–790.
6. Hirsch, J. G. (1953) J. Exp. Med. 97, 327–331.
7. Tabor, C. W., Tabor, H., and Rosenthal, S. M. (1954) J. Biol. Chem. 208, 645–661.
8. Tabor, C. W., Tabor, H., and Bachrach, U. (1964) J. Biol. Chem. 239, 2194–2203.
9. Seiler, N., Knodgen, B., and Gittos, W. (1981) Biochem. J. 200, 123–132.
10. Nakajima, T. (1972) J. Neurochem. 20, 735–742.
11. Hempel, J. D., Reed, D. M., and Pietruszko, R. (1982) Alcohol. Clin. Exp. Res. 6, 417–425.
12. Ambroziak, W., and Pietruszko, R. (1987) Alcohol. Clin. Exp. Res. 6, 528–532.
13. Fellman, J. H. (1958) Nature 182, 311–312.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
15. Greenfield, N. J., and Pietruszko, R. (1977) Biochim. Biophys. Acta 438, 35–45.
16. MacKerell, A. D., Jr., and Pietruszko, R. (1987) Biochim. Biophys. Acta 911, 306–317.
17. Yun, S. L., and Suelter, C. H. (1977) Biochim. Biophys. Acta 480, 324–337.
18. Lineweaver, H., and Burk, D. (1934) J. Am. Chem. Soc. 56, 668–670.
19. Kimes, B. W., and Morris, D. R. (1971) Biochim. Biophys. Acta 228, 223–234.
20. MacKerell, A. D., Jr., Blatter, E. E., and Pietruszko, R. (1986) Alcohol. Clin. Exp. Res. 10, 266–270.
21. Ryzlak, M. T., and Pietruszko, R. (1989) Alcohol. Clin. Exp. Res. 13, 755–761.
22. Ryzlak, M. T., and Pietruszko, R. (1988) Biochim. Biophys. Acta 954, 309–324.
23. Forte-McRobbie, C. M., and Pietruszko, R. (1985) J. Biol. Chem. 261, 2154–2165.
24. Yorifuji, T., Koike, K., Sakurai, T., and Yokoyama, K. (1986) Agric. Biol. Chem. 50, 2009–2016.
25. Jakoby, W. B., and Fredericks, J. (1959) J. Biol. Chem. 234, 2145–2150.
26. Abe, T., Takada, K., Okhawa, K., and Matsuda, M. (1990) Biochem. J. 269, 29–29.
27. Axelrod, J., Albers, W., and Clemente, C. D. (1959) J. Neurochem. 5, 68–72.
28. Erwin, V. G., and Dietrich, R. A. (1966) J. Biol. Chem. 241, 3533–3539.
29. Tipton, K. F., Housley, M. D., and Turner, A. J. (1977) Essays Neurochem. & Neuropharmacol. 1, 103–130.
30. Alvisatos, S. G., and Tabakoff, B. (1973) in Chemical Modulation of Brain Function (Sabelli, H., ed) pp. 41–66, Raven Press, New York.
31. Jakoby, W. B., and Ziegler, D. M. (1990) J. Biol. Chem. 265, 20715–20718.
32. Feldstein, A., Hoagland, H., Freeman, H., and Williamson, O. (1967) Life Sci. 6, 53–61.
33. Davis, V. E., Brown, H., Huff, J. A., and Cashaw, J. (1967) J. Lab. Clin. Med. 69, 132–140.
34. Davis, V. E., Brown, H., Huff, J. A., and Cashaw, J. (1967) J. Lab. Clin. Med. 69, 787–799.
35. Tanka, A. W., and Weiner, H. (1979) Biochem. Pharmacol. Exp. Ther. 174, 401–412.
36. Deitrich, R. A., and Erwin, V. V. (1980) Annu. Rev. Pharmacol. Toxicol. 20, 55–80.