Biochemical Pharmacology, Vol. 36, No. 21, pp. 3715–3722, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

CHARACTERIZATION OF HUMAN ERYTHROCYTE ALDEHYDE DEHYDROGENASE

JAMES W. RAWLES, DEBORAH L. RHODES, JAMES J. POTTER and ESTEBAN MEZEY*
Department of Medicine of the Johns Hopkins University School of Medicine, Baltimore, MD 21205, U.S.A.

(*Received* 29 *December* 1986; *accepted* 6 *April* 1987)

Abstract—Human erythrocyte aldehyde dehydrogenase was purified to homogeneity. The enzyme exhibited a single band of activity on starch gel electrophoresis and on isoelectric focusing. It was a tetramer with an estimated molecular weight of 230,000 daltons and an isoelectric point of 5.0. Its pH optimum of 8.5, Michaelis–Menten constant for acetaldehyde of 46 μM, and high sensitivity to noncompetitive inhibition by disulfiram resembled human liver cytosolic aldehyde dehydrogenase. Low concentrations of magnesium (5–10 μM) resulted in enhancement of erythrocyte aldehyde dehydrogenase activity, whereas higher physiological concentrations of magnesium resulted in uncompetitive inhibition of enzyme activity. Magnesium inhibited the enzyme activity by increasing the binding of NADH to the enzyme as had been found to be the case for the inhibitory effect of magnesium on the human liver cytosolic enzyme. Erythrocyte aldehyde dehydrogenase may metabolize small amounts of acetaldehyde escaping the liver during ethanol metabolism and protect extrahepatic tissues from acetaldehyde toxicity.

Aldehyde dehydrogenase activity (EC 1.2.1.3) is present in human erythrocytes and in many other extrahepatic tissues [1]. The bulk of acetaldehyde produced in ethanol metabolism, however, is oxidized in the liver [2]. The role of erythrocyte aldehyde dehydrogenase is unclear. Previous studies have shown that it is quite similar to the liver cytosolic enzyme in its physico-chemical and catalytic properties [3]. Chronic alcoholism results in similar decreases in the activity of both erythrocyte and liver cytosolic aldehyde dehydrogenase [4, 5]. The decrement in activity of the hepatic enzyme could lead to accumulation of toxic levels of acetaldehyde and subsequent hepatocellular injury. Erythrocyte aldehyde dehydrogenase may, in turn, lower blood acetaldehyde levels, and hence protect peripheral tissues from the effects of the small amounts of acetaldehyde released from the liver.

Magnesium has been shown to have marked and divergent effects on the liver cytosolic and mitochondrial aldehyde dehydrogenase enzymes [6]. Hypomagnesemia is common in chronic alcoholics [7]. However, the effect of magnesium on the erythrocyte enzyme has not been determined. Disulfiram which is used in the therapy of alcoholism also has differential effects on the cytosolic and mitochondrial enzymes with the cytosolic being quite sensitive to, and the mitochondrial relatively resistant to, inhibition [8].

The purpose of the present study was to determine the properties of purified erythrocyte aldehyde dehydrogenase and the effects of magnesium and disulfiram on its catalytic activity.

* Address all correspondence to: Esteban Mezey, M.D., Blalock 903, The Johns Hopkins Hospital, 600 North Wolfe St., Baltimore, MD 21205.

MATERIALS AND METHODS

Materials. NAD^+, NADH, propionaldehyde, disulfiram, horse liver alcohol dehydrogenase, ferritin, bovine serum albumin, nitroblue tetrazolium, and phenazine methosulfate were purchased from the Sigma Chemical Co., St. Louis, MI. Blue Dextran 2000, aldolase, ovalbumin, an electrophoresis calibration kit for low molecular weight proteins, CM-Sephadex C-50, N^6-(6-aminohexyl)-5′-AMP-Sepharose 4B, polybuffer 74, PBE-94 polybuffer exchanger for chromatofocusing, and Sephacryl S-300 were obtained from Pharmacia Laboratories, Piscataway, NJ. Acetaldehyde was purchased from the Aldrich Chemical Co., Milwaukee, WI. Coomassie brilliant blue R-250 and sodium dodecyl sulfate (SDS) were obtained from Bio-Rad Laboratories, Richmond, CA. 2-Mercaptoethanol was purchased from the Fischer Scientific Co., Fairlawn, NJ.

Purification of aldehyde dehydrogenase. Venous blood was collected in 2.7 mM EDTA from a single healthy donor, the first author (J.W.R.). The plasma was separated by centrifugation at 2000 *g* for 15 min at 4° in a Sorvall GSA rotor. The red cells were washed twice in 2 vol. of 145 mM NaCl with 10 mM Tris buffer, pH 7.4, followed by centrifugation at 2000 *g* for 15 min. The cells were then lysed in 4 vol. of distilled water at 4°. The pH was then adjusted to 6.0 by drop-wise addition of 1 M HCl (about 3 ml/250 ml), resulting in a precipitate that was separated by a fourth centrifugation. The supernatant fraction was decanted and adjusted to 1 mM EDTA and 0.1% 2-mercaptoethanol.

The lysate (about 200 ml) was applied to a 45 × 5 cm CM-Sephadex C-50 column which was equilibrated with 30 mM histidine buffer, pH 6.0. The column was eluted with the same buffer, fractions containing aldehyde dehydrogenase activity

were pooled, and the pH was adjusted to 5.8 with 1 M HCl. The resulting hemoglobin free crude aldehyde dehydrogenase preparation was applied to a 19 × 1.55 cm PBE 94 polybuffer exchanger chromatofocusing column equilibrated to pH 5.8 with 25 mM histidine buffer. The enzyme was eluted with polybuffer 74 (1:8 dilution, pH 4.5), and the active fractions were pooled and applied to a 20 × 1.5 cm column of N^6-(6-aminohexyl)-5′-AMP-Sepharose 4B equilibrated with 30 mM sodium phosphate buffer, pH 6.0. The column was then washed stepwise, first with equilibration buffer and then with 90 mM sodium phosphate, pH 7.3, changing buffers when the absorbance at 280 nm returned to base line. The column was then washed with 30 ml of 90 mM sodium phosphate, pH 7.6, and the enzyme eluted with 100 mM sodium phosphate, pH 8.0. The active fractions were pooled and stored under nitrogen at 0° until used. All buffers were degassed prior to use and contained 0.1% 2-mercaptoethanol. Where necessary, aldehyde dehydrogenase was concentrated by ultrafiltration under nitrogen at 2–4 psi. Higher pressures resulted in substantial loss of enzyme activity. For use in experiments with disulfiram, the purified preparation was dialyzed against three changes of sodium phosphate buffer, pH 7.4, with 1 mM EDTA at 4° under nitrogen.

Aldehyde dehydrogenase assay. Aldehyde dehydrogenase activity was measured spectrophotometrically at 340 nm and at 37° in 50 mM sodium pyrophosphate, pH 7.4, containing 10 mM propionaldehyde and 1 mM NAD^+. A blank reaction without substrate was run in each case. The aldehyde dehydrogenase activities were then calculated from the molar extinction coefficient of 6.2 mM^{-1} cm^{-1} for NADH. One unit of enzyme activity is defined as the formation of 1 μmole NADH/min. Protein concentration was determined by the method of Lowry *et al.* [9] with bovine serum albumin as a standard. Kinetic studies were performed at various concentrations of NAD^+ or substrate (propionaldehyde or acetaldehyde). Studies involving magnesium were performed in 50 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes) buffer, pH 7.4, to avoid the complexing effects of pyrophosphate. The kinetic constants were determined by using Cleland's weighted linear regression model [10]. Where appropriate, other data were fitted to a straight line using standard linear regression analysis. The pH optimum for the enzyme activity was determined in the following buffers: 0.1 M sodium phosphate, pH 5.0 to 7.5; 0.1 M Hepes buffer, pH 6.5 to 8.0; 0.1 M Tris and 0.05 M sodium pyrophosphate, pH 7.5 to 9.5; and 0.1 M glycylglycine, pH 9.0 to 10.0. Marked nonenzymatic increases in the absorbance at 340 nm occurred in sodium pyrophosphate and glycylglycine buffers at pH above 9.0 with propionaldehyde and NAD^+ in the absence of the enzyme. This blank activity was subtracted from the total activity in the determination of the pH optimum.

Binding of NAD^+ to the enzyme. The effect of magnesium on NAD^+ binding to the enzyme was determined using an Aminco–Bowman spectrofluorometer. NAD^+ was added stepwise to the enzyme. Fluorescence was measured at an emission of 346 nm with an excitation at 286 nm. The fluorescence was corrected by the dilution factors. The dissociation constant for NAD^+ was calculated by converting the data obtained on the quenching of fluorescence by NAD^+ to a Scatchard plot [11]. Free NAD^+ concentration was calculated by assuming the binding of 4 molecules of NAD^+ per enzyme molecule.

Electrophoresis. Starch gel electrophoresis was carried out at 40° on horizontal gels containing 10.4% starch, 4 mM NAD^+ and 0.025 M Tris–HCl buffer, pH 8.6. The red cell lysate and partially purified aldehyde dehydrogenase were concentrated approximately 10-fold in an Amicon stirred cell ultrafiltration unit. A sample of 25 μl was added to each well and the gel was run at 15 V/cm for 6 hr. The gels were sliced and then stained for enzyme activity at 37° in 0.025 M Tris buffer, pH 8.6, containing 1.1 mM NAD^+, 0.5 mM nitroblue tetrazolium, 0.03 mM phenazine methosulfate and 10 mM propionaldehyde. Other slices were stained in the presence of 15 mM sodium lactate or absence of substrate as blanks.

Polyacrylamide gel electrophoresis was carried out as described by Laemmli [12] with a 4% stacking gel and a 7.5% separating gel 0.8% cross-linked containing 0.1% SDS. The gels were stained in Coomassie blue.

Gel filtration chromatography. The molecular weight of the native enzyme was determined by gel filtration chromatography on a 1.5 × 50 cm column of Sephacryl S-300 equilibrated and eluted with 50 mM Hepes buffer, pH 7.4, and 50 mM NaCl at 4°. Blue Dextran 2000 and ferritin, aldolase, horse liver alcohol dehydrogenase, and ovalbumin with molecular weights of 440,000, 158,000, 82,000, and 45,000, respectively, served as standards to calibrate the column.

Isoelectric focusing. Isoelectric focusing was performed according to the method of Harada *et al.* [13] in an LKB Multiphor apparatus with a pH gradient of 3.0 to 11.0. Partially purified enzyme was concentrated and dialyzed against 1% glycine by ultrafiltration prior to application to the gel. The gels were run at 20 W constant power for 4 hr. They were stained for aldehyde dehydrogenase activity as described for starch gel electrophoresis.

RESULTS

The details of the purification of human erythrocyte aldehyde dehydrogenase are summarized in Table 1. A hemoglobin-free aldehyde dehydrogenase preparation was obtained following the initial cation exchange step in the purification sequence. The removal of hemoglobin by this step permitted spectrophotometric assay of the enzyme. The purified enzyme was eluted from the N^6-(6-aminohexyl)-5′-AMP-Sepharose 4B column as a single activity and protein peak after the addition of 100 mM sodium phosphate, pH 8.0. The eluted enzyme was homogeneous, exhibiting a single protein band of 52,500 molecular weight on SDS-polyacrylamide gel electrophoresis. The molecular weight of the native enzyme was estimated at 230,000 by gel filtration on Sephacryl S-300.

Table 1. Purification of human erythrocyte aldehyde dehydrogenase

Fraction	Total activity (units)	Specific activity (units/mg protein)	Purification (fold)	Yield (%)
Erythrocyte lysate	2.59*	0.00023*		
CM-Sephadex C-50	2.59	0.018	78	100*
Chromatofocusing with polybuffer exchanger PBE 94	1.38	0.067	291	53
N^6-(6-Aminohexyl)-5'-AMP Sepharose 4B	0.19	1.04	4522	7

* Estimated assuming 100% recovery of the enzyme from CM-Sephadex C-50 chromatography.

Starch gel electrophoresis of a 10-fold concentrate of the red cell lysate, and of the partially purified hemoglobin free enzyme preparation after CM-Sephadex chromatography, revealed a single band of aldehyde dehydrogenase activity. A second band, more anodic in migration, was present with undiminished intensity in a blank stain without aldehyde. This band was enhanced by the addition of 15 mM lactate to the blank staining solution and therefore probably represents contaminating red cell lactate dehydrogenase. Isoelectric focusing of the partially purified enzyme resulted in a single narrow band of enzyme activity at an isoelectric pH of 5.0. Chromatofocusing column chromatography of partially purified lysate confirmed a single peak of enzyme activity eluting at pH 5.0.

The pH optimum of the enzyme was 8.5 in both pyrophosphate and Hepes buffer. In Tris buffer the enzyme activity continued to increase up to pH 9.5. Kinetic studies with purified aldehyde dehydrogenase showed an apparent K_m of 9 μM for propionaldehyde and 46 μM for acetaldehyde. In some preparations, a coincident higher K_m of 121 μM for propionaldehyde and 357 μM for acetaldehyde was found. The low K_m for substrate was consistently present in all preparations studied. When the purified enzyme preparations lacked the high K_m activity, the high K_m activity was also absent in less purified preparations from the same experiment. The K_m for NAD^+ was 23 μM.

The effect of magnesium on the activity of aldehyde dehydrogenase was determined in 50 mM Hepes buffer, pH 7.4, to avoid the complexing of magnesium by sodium pyrophosphate. There was enhanced activity with the addition of low concentrations (5–10 μM) of magnesium. At higher concentrations, there was increasing inhibition of enzyme activity (Fig. 1). Parallel studies in 50 mM sodium pyrophosphate, pH 7.4, showed similar effects, but at higher magnesium concentration.

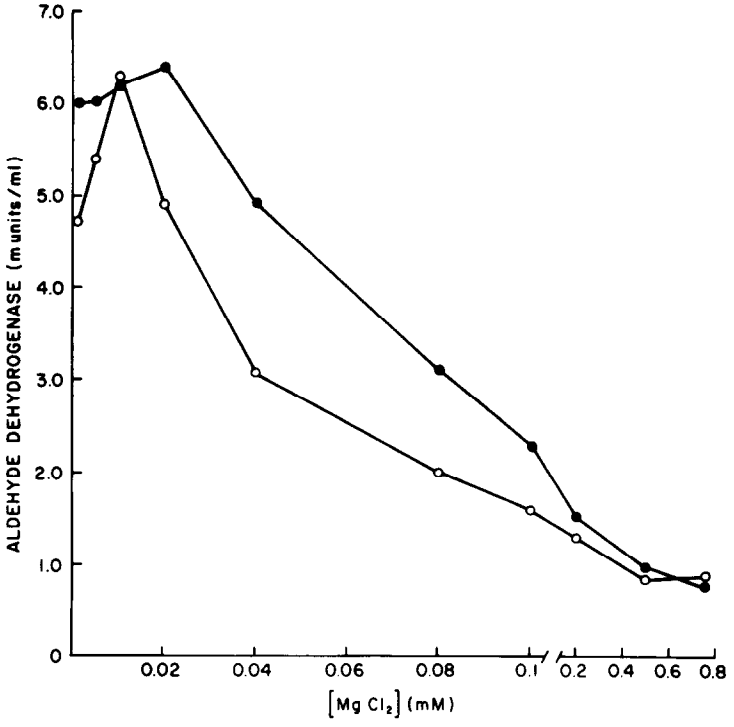

Fig. 1. Effect of $MgCl_2$ on erythrocyte aldehyde dehydrogenase activity. The assay was done with propionaldehyde (10 mM) in the following buffers at pH 7.4: (○) 0.05 M Hepes; and (●) 0.05 M sodium pyrophosphate.

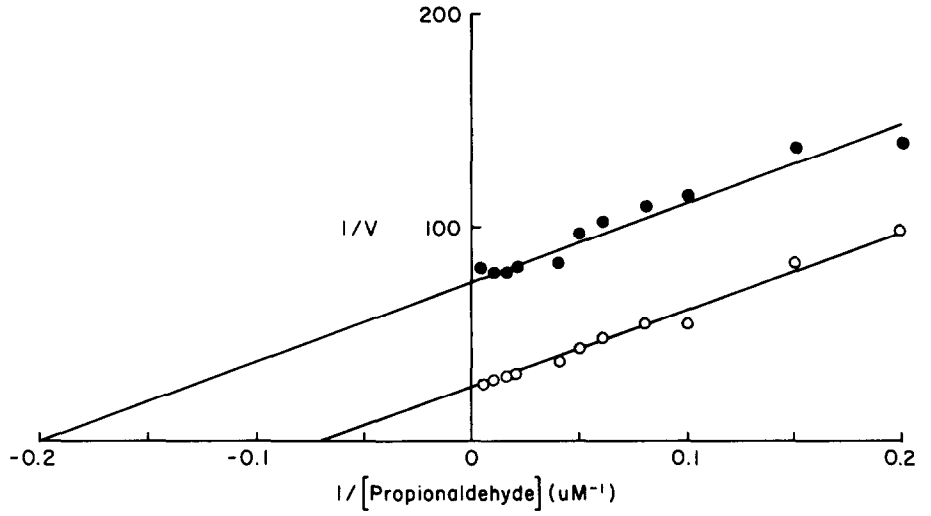

Fig. 2. Lineweaver–Burk plot of the inhibition of erythrocyte aldehyde dehydrogenase by 0.7 mM $MgCl_2$ at various concentrations of propionaldehyde. $MgCl_2$ concentration: (○) 0; and (●) 0.7 mM.

A magnesium concentration of 0.7 mM, which is similar to estimated red cell free magnesium levels [14], inhibited aldehyde dehydrogenase activity uncompetitively with respect to propionaldehyde (Fig. 2). Magnesium inhibition was also uncompetitive with respect to NAD^+.

The K_i for magnesium was derived graphically by plotting V_{max} obtained with various magnesium concentrations versus magnesium concentration as described by Dixon and Webb [15]. The intercept on the horizontal axis estimated the K_i at 20 μM (Fig. 3).

The effects of magnesium on the dissociation constants of the enzyme and substrate were determined by two-dimensional steady-state kinetic analysis as described by Cleland [16]. The Lineweaver–Burk plots for propionaldehyde oxidation at various propionaldehyde and NAD^+ concentrations and the secondary slope and intercept plots in the absence and presence of 160 μM magnesium are shown in Figs. 4 and 5. The effect of magnesium on the dissociation constant (K_i) of NADH was determined from Dixon plots at various NAD^+ concentrations with a constant propionaldehyde concentration (Fig. 6). There was a decrease in both dissociation (K_i) and Michaelis–Menten (K_m) constants in the presence of magnesium (Table 2). Scatchard plots of the quenching of the enzyme fluorescence by the addition of NAD^+ also showed that 1.6 mM magnesium decreased the dissociation constant of the binding sites from 3.45 to 2.33 μM, but did not change the number of binding sites.

Disulfiram was found to be a noncompetitive

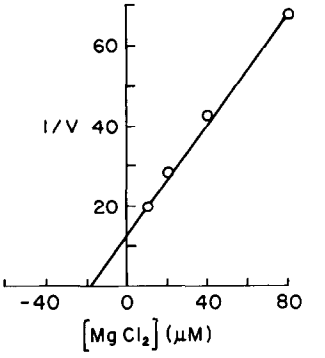

Fig. 3. Graphical determination of the inhibitory constant (K_i) for the noncompetitive inhibition of erythrocyte aldehyde dehydrogenase by $MgCl_2$ [14]. Graph of the intercepts on the vertical axis of a Lineweaver–Burk plot (not shown) of the inhibition of enzyme activity by $MgCl_2$ at various concentrations of propionaldehyde versus the inhibitory $MgCl_2$ concentrations.

Table 2. Effect of magnesium on kinetic constants of erythrocyte aldehyde dehydrogenase

	K_i (μM)			K_m (μM)	
	NAD^+	Propionaldehyde	NADH	NAD^+	Propionaldehyde
No Mg^{2+}	206	111	140	50	20
160 μM Mg^{2+}	44	40	30	25	7

Determinations were done in 50 mM Hepes buffer, pH 7.4.

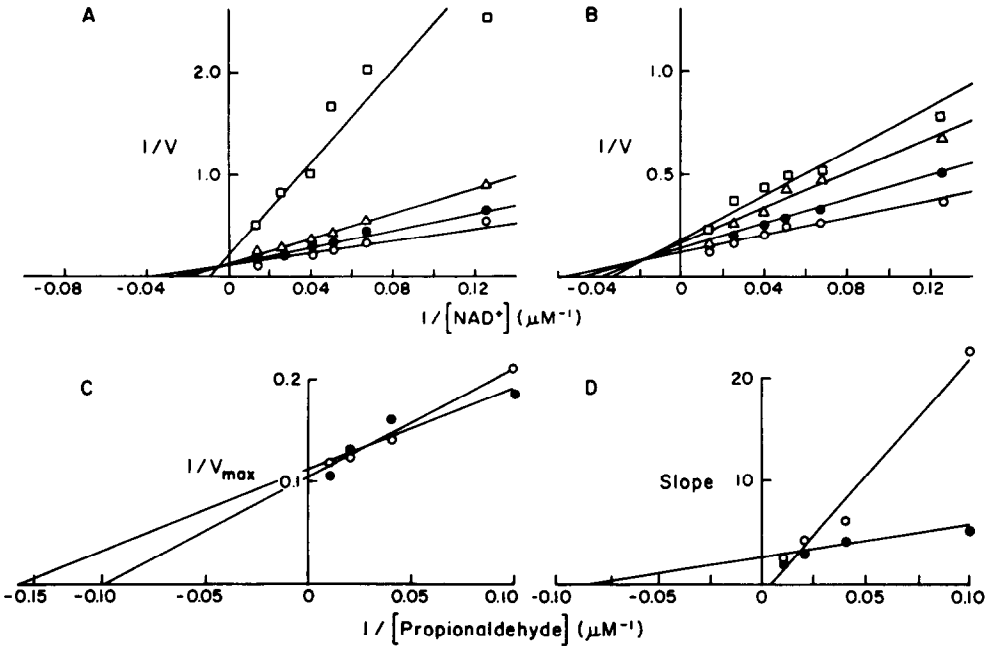

Fig. 4. Two-dimensional steady-state kinetics for erythrocyte aldehyde dehydrogenase activity at varied NAD^+ and changing fixed concentrations of propionaldehyde. (A) Double-reciprocal plot of initial velocity in the absence of magnesium. Propionaldehyde concentrations: (○) 100 μM; (●) 50 μM; (△) 25 μM; and (□) 10 μM. (B) Same plot as (A) but in the presence of 160 μM $MgCl_2$. (C) Intercept plot of the data shown in (A) in the absence of magnesium (○) and shown in (B) in the presence of 160 μM $MgCl_2$ (●). (D) Slope replot of the data shown in (A) in the absence of magnesium (○) and shown in (B) in the presence of 160 μM $MgCl_2$ (●). The constants for the enzyme reaction are defined by the following rate equation for sequential mechanisms described by Cleland [16]: $v = VAB/K_{ia}K_b + K_aB + K_bA + AB$, where K_a and K_b correspond to the Michaelis–Menten constants for substrates A and B, and K_{ia} is the dissociation constant of A. In the intercept replot, slope = K_b/V and intercept = $1/V$, while in the slope replot, slope = $K_{ia}K_b/V$ and intercept = K_a/V. In this figure, substrate A was NAD^+ and substrate B was propionaldehyde.

inhibitor of the enzyme with respect to both propionaldehyde and NAD^+ (Fig. 7). The inhibition was reversed and the activity significantly enhanced relative to control (34%) by 0.2 M 2-mercaptoethanol. The activity of control aldehyde dehydrogenase was also enhanced significantly (47%) by 0.2 M 2-mercaptoethanol. This phenomenon may represent reactivation of enzyme with oxidized sulfhydryl groups at the active site.

DISCUSSION

In this study a single aldehyde dehydrogenase isoenzyme was found in human erythrocytes by starch gel electrophoresis and isoelectric focusing. In the initial description of erythrocyte aldehyde dehydrogenase, four isoenzymes were observed on starch gel electrophoresis [17]. Subsequent studies have demonstrated only a single isoenzyme [18, 19].

Erythrocyte aldehyde dehydrogenase was found to be most similar in its physical, chemical, and kinetic properties to the human liver cytosolic enzyme. The erythrocyte enzyme consisted of four identical subunits with an estimated molecular weight of the intact enzyme of 230,000 daltons and an isoelectric point of 5.0. These values are intermediate between that found for the human liver cytosolic and mitochondrial enzymes which have slightly higher and lower values respectively [8]. The pH optimum of 8.5 for aldehyde oxidation of the erythrocyte enzyme was similar to that reported for the cytosolic enzyme [20], but was lower than that of the mitochondrial enzyme [20].

Erythrocyte aldehyde dehydrogenase, was, without exception, found to have a low K_m for aldehyde. A higher K_m activity was inconsistently observed in various preparations. When absent from the purified enzyme, the high K_m activity was not present in the corresponding partially purified preparation following the first chromatographic step. Thus, it would seem unlikely that the high K_m activity represented a separate isoenzyme removed in varying degrees during purification. It may have been due to an artifact of preparation with alteration or partial denaturation of a portion of the enzyme molecule. Dual K_m values have been similarly observed for the liver cytosolic enzyme [20] and erythrocyte enzyme [3]. This phenomenon has been postulated to be due to inequivalency of subunit binding sites [21]. However, the presence of the high K_m activity should be consistent from preparation to preparation if this is the case. The human liver mitochondrial isoenzyme has a single K_m for aldehyde [20].

The K_m of erythrocyte aldehyde dehydrogenase

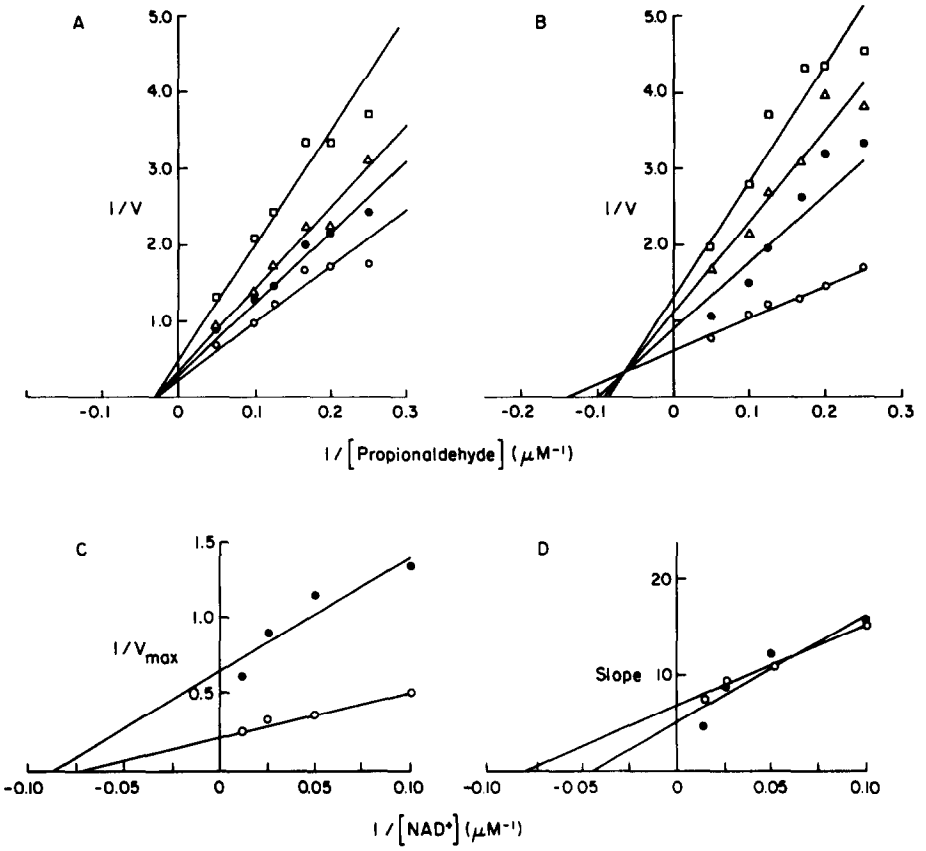

Fig. 5. Two-dimensional steady-state kinetics for erythrocyte aldehyde dehydrogenase activity varied propionaldehyde and changing fixed concentrations of NAD^+. (A) Double-reciprocal plot of initial velocity in the absence of magnesium. NAD^+ concentrations: (○) 80 μM; (●) 40 μM; (△) 20 μM; and (□) 10 μM. (B) Same plot as (A) but in the presence of 160 μM $MgCl_2$. (C) Intercept plot of the data shown in (A) in the absence of magnesium (○) and shown in (B) in the presence of 160 μM $MgCl_2$ (●). (D) Slope replot of the data shown in (A) in the absence of magnesium (○) and shown in (B) in the presence of 160 μM $MgCl_2$ (●). The equations shown in the previous figure apply here, but substrate *A* was propionaldehyde and substrate *B* was NAD^+.

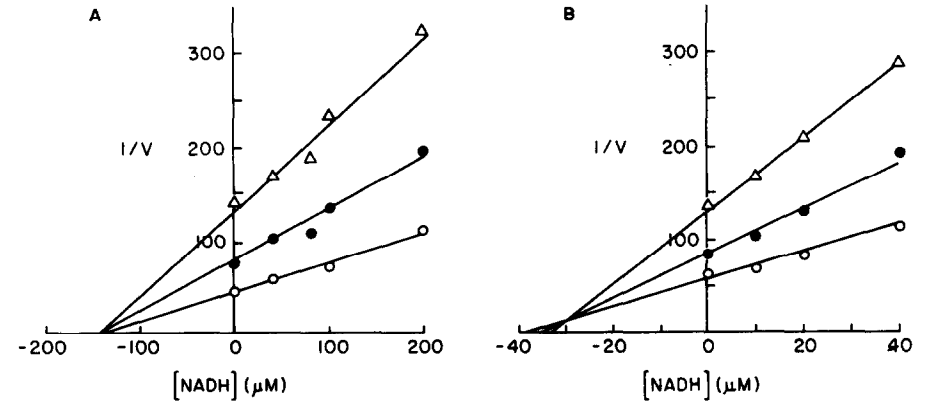

Fig. 6. Dixon plots of NADH inhibition with respect to NAD^+. (A) Magnesium absent in the reaction mixture. (B) $MgCl_2$ (160 μM) present in the reaction mixture. The NAD^+ concentrations were: (○) 40 μM; (●) 20 μM; and (△) 10 μM.

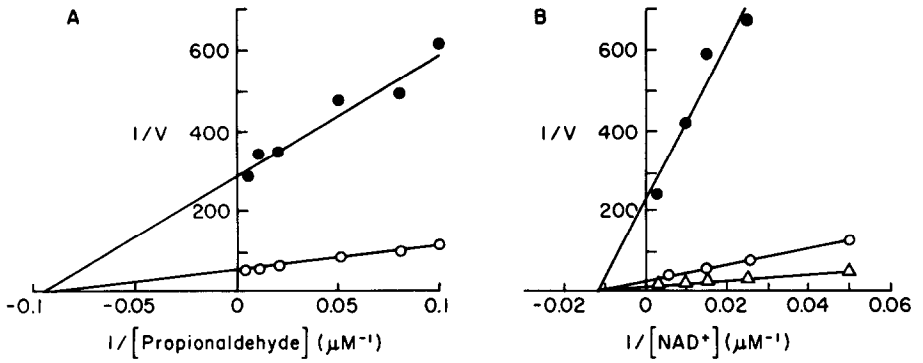

Fig. 7. Lineweaver–Burk plots of the inhibition of erythrocyte aldehyde dehydrogenase by disulfiram. (A) Inhibition at various concentrations of propionaldehyde and (B) various concentrations of NAD^+. The concentrations of disulfiram for panel A were: (○) 0; and (●) 0.14 μM; and for panel B, (○) 0; (●) 0.25 μM; and (△) 0.25 μM plus 0.2 M 2-mercaptoethanol.

for NAD^+ is in the range previously reported for the liver cytosolic enzyme [8] and for the purified erythrocyte enzyme [3]. The mitochondrial enzyme is reported to have a higher K_m for NAD^+ [20].

The physiological function of erythrocyte aldehyde dehydrogenase is not known. A significant role in aldehyde metabolism is suggested by a number of observations. Orientals with disulfiram-like flushing attacks in response to alcohol ingestion have elevated blood levels of acetaldehyde and moderately depressed erythrocyte aldehyde dehydrogenase activity [22]. Chronic alcoholics have similarly lowered activity of erythrocyte aldehyde dehydrogenase and elevated circulating acetaldehyde in response to alcohol ingestion [4, 23]. However, Orientals with alcohol-induced flushing have been shown to lack the liver mitochondrial aldehyde dehydrogenase [24], and chronic alcoholism also reduces the hepatic cytosolic enzyme activity [5]. Hence, it is difficult to ascertain the importance of erythrocyte aldehyde dehydrogenase in overall acetaldehyde metabolism. It is accepted that most acetaldehyde metabolism occurs in the liver [25]. The observation of a substantial gradient between hepatic vein (2–20 μM) and peripheral venous (< 2 μM) acetaldehyde levels suggests that extrahepatic metabolism occurs [2]. This could be mediated by aldehyde dehydrogenase found in blood and many other tissues [1] and may serve to protect these tissues from the toxic effects of the small amounts of acetaldehyde escaping the liver or generated by non-hepatic ethanol oxidation [25]. The observed substrate affinities are not inconsistent with this hypothesis. The estimated potential aldehyde oxidizing capacity of the blood in a 70 kg man approximates 6.8 μmol/min based on an enzyme activity of 1.31 nmol/ml blood/min [18], and a blood volume of 75 ml/kg of body weight. However, the significance of erythrocyte aldehyde dehydrogenase activity must be interpreted in the light of the intra-erythrocyte milieu.

Magnesium is the major intracellular divalent cation and, as such, may have a significant influence on aldehyde dehydrogenase activity *in vivo*. The current study showed a slight activation of erythrocyte aldehyde dehydrogenase by very low (< 10 μM), non-physiologic levels of magnesium. Above these levels the enzyme activity was uncompetitively inhibited by magnesium with respect to both substrate and coenzyme. Two-dimensional kinetic analysis demonstrated increased enzyme affinity for aldehyde, NAD^+, and NADH in the presence of inhibitory levels of magnesium. In addition, magnesium was also shown to increase binding of NAD^+ to the enzyme binding sites by fluorescence quenching. With the exception of the slight activation at very low magnesium levels, qualitatively identical effects on overall rate and kinetic constants were demonstrated for the human liver cytosolic enzyme [6]. It has been shown that NADH dissociation is the rate-determining step for the liver cytosolic enzyme and that the increased binding affinity for NADH caused by magnesium results in decreased enzyme catalytic rate. The behavior of the human liver mitochondrial enzyme is different in that magnesium activates the catalytic rate probably by increasing hydrolysis of the enzyme·product·NADH·Mg^{2+} intermediate [6]. The findings of an inhibitory effect of magnesium on erythrocyte aldehyde dehydrogenase indicate that decreases in the enzyme activity observed in alcoholics [4] are not explained by changes in erythrocyte magnesium concentration which is decreased after chronic ethanol consumption [7].

Disulfiram strongly inhibited erythrocyte aldehyde dehydrogenase in a noncompetitive manner. The inhibition was reversed by 2-mercaptoethanol which also activated the control enzyme, probably by regenerating catalytically important free sulfhydryl groups which are oxidized by disulfiram as well as by intrinsic oxidants. These observations confirm those of Inoui *et al.* [3] and are similar to those with the liver cytosolic aldehyde dehydrogenase [8].

Acknowledgements—This work was supported by Grant AA 00 626 from the United States Public Health Service and by the Alcoholic Beverage Medical Research Foundation.

REFERENCES

1. S. Harada, D. P. Agarwal and H. W. Goedde, *Life Sci.* **26**, 1773 (1980).

2. H. U. Nuutinen, M. P. Salaspuro, M. Valle and K. O. Lindros, *Eur. J. clin. Invest.* **14**, 306 (1984).
3. K. Inoue, N. Nishimukai and K. Yamasawa, *Biochim. biophys. Acta* **569**, 117 (1979).
4. C. Lin, J. J. Potter and E. Mezey, *Alcoholism: Clin. expl Res.* **8**, 539 (1984).
5. W. J. Jenkins, K. Cakebread and K. R. Palmer, *Lancet* **1**, 1048 (1984).
6. R. C. Vallari and R. Pietruszko, *J. biol. Chem.* **259**, 4927 (1984).
7. J. D. Hines, in *Erythrocyte Structure and Function* (Ed. G. J. Brewer), p. 621. Alan R. Liss, New York (1975).
8. R. Pietruszko, in *Isozymes. Cellular Localization, Metabolism, and Physiology* (Eds. M. C. Rattazzi, J. E. Scandalios and G. S. Whitt), p. 195. Alan R. Liss, New York (1983).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. W. W. Cleland, *Adv. Enzymol.* **29**, 1 (1967).
11. K. Takahashi, H. Weiner and J. H. J. Hu, *Archs Biochem. Biophys.* **205**, 571 (1980).
12. U. K. Laemmli, *Nature, Lond.* **227**, 680 (1970).
13. S. Harada, D. P. Agarwal and H. W. Goedde, *Hum. Genet.* **40**, 215 (1978).
14. H. F. Bunn, B. J. Ransil and A. Chao, *J. biol. Chem.* **246**, 5273 (1971).
15. M. Dixon and E. C. Webb, *Enzymes*, 3rd Edn, p. 245. Academic Press, New York (1979).
16. W. W. Cleland, in *Enzymes. Kinetics and Mechanism* (Ed. P. D. Boyer), 3rd Edn, Vol. 2, p. 1. Academic Press, New York (1970).
17. R. Pietruszko and R. C. Vallari, *Fedn Eur. Biochem. Soc. Lett.* **92**, 89 (1978).
18. P. Agarwal, L. Tobar-Rojas, S. Harada and H. Goedde, *Pharmac. Biochem. Behav.* **18** (Suppl. 1), 89 (1983).
19. A. Helander and O. Tottmar, *Alcoholism: Clin. expl. Res.* **10**, 71 (1986).
20. N. J. Greenfield and R. Pietruszko, *Biochim. biophys. Acta* **483**, 35 (1977).
21. R. Pietruszko, K. Ferencz-Biro and A. D. Mackerell, in *Enzymology of Carbonyl Metabolism 2: Aldehyde Dehydrogenase, Aldo-Keto Reductase, and Alcohol Dehydrogenase* (Eds. T. G. Flynn and H. Weiner), p. 29. Alan R. Liss, New York (1985).
22. K. Inoue, M. Fukanaga and K. Yanasawa, *Pharmac. Biochem. Behav.* **13**, 295 (1980).
23. K. R. Palmer and W. J. Jenkins, *Gut* **23**, 729 (1982).
24. S. Harada, D. P. Agarwal and H. W. Goedde, *Lancet* **2**, 982 (1981).
25. J. P. Von Wartburg and R. Buhler, *Lab. Invest.* **50**, 5 (1984).