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AN ENZYMATICALLY INACTIVE VARIANT OF HUMAN LACTATE DEHYDROGENASE-LDH$_B$GUA-1

STUDY OF SUBUNIT INTERACTION

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The LDH$_B$GUA-1 variant is an electrophoretic variant which is enzymatically inactive. It is only detectable because of its ability to form heterotetramers with A and/or active B subunits and alter the electrophoretic pattern, although all evidence suggests the B-GUA-1 subunits are always enzymatically inactive. All tetrameric combinations of active plus inactive subunits including either an A or active B plus three inactive B subunits possess enzymatic activity. The heterotetramers composed of A and B-GUA-1 subunits are more thermostable than A_4 homotetramers but less thermostable than normal AB heterotetramers. The AB-GUA-1 heterotetramers composed of active A and inactive B subunits have a K_m for pyruvate, for lactate and for NADH which is similar to that observed for normal AB heterotetramers. The substrate specificity of the A plus normal B heterotetramer and the A plus variant B heterotetramer with α-hydroxybutyrate or the acetylpyridine analog of NAD as substrate are similar, while differences between the normal and variant erythrocyte isozymes are observed when glyoxalate is the substrate. Interaction with the B-GUA-1 subunit reduces the sensitivity of the A subunit to urea inhibition (independent of dissociation), as does the active B subunit, but the inactive B subunit does not modulate the inhibition by oxalate. Although a single active subunit in a tetrameric conformation is sufficient for enzymatic activity, many of the kinetic properties of the lactate dehydrogenase molecule reflect the tetrameric structure rather than the sum of independent subunits. Thus communication among the subunits must exist and conformational changes which affect the catalytic properties of the enzyme (L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27) must occur during tetramer formation.

Lactate dehydrogenase (L-lactate:NAD$^+$ oxidoreductase, EC 1.1.1.27) is a tetrameric enzyme ($M_r \approx 140000$) [1,2] catalyzing the interconversion of L-lactate and pyruvate via an ordered mechanism which requires the initial binding of the pyridine nucleotide [3–6]. The lactate dehydrogenase subunits are the products of two loci-M or A (muscle) and H or B (heart) and five different tetramers, separable by electrophoresis are observed in various tissues [2,7]. The relative preponderance of each isozyme is tissue specific within species [8,9] and reflects the relative rates of synthesis and degradation of the subunits in each tissue [10,11].

Extensive sequence and conformation data have been accumulated on the B and A forms of lactate dehydrogenase from many species. Only small differences in tertiary structure are observed, although significant differences in amino acid se-

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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quence between species and isozymes are noted [12,13]. Differences in tertiary structure between the apo-lactate dehydrogenase enzyme and the substrate-cofactor-enzyme complex do exist [12,14], and conformational changes of the later may be associated with the differences in kinetic properties between the heart and muscle isozymes [15].

The catalytic and stability properties of the lactate dehydrogenase tetramer reflect their subunit composition. The B_4 isozyme from mammals has a higher affinity for substrate than the A_4 isozyme [1] and approximately half the turnover number [16]. The B_4 isozyme is also more sensitive to structural modifications at the C_3 position of the pyridine nucleotide cofactor [17,18] and to pyruvate inhibition [1]. The B_4 isozyme is relatively insensitive to changes in chain length of the α keto acid, retaining almost 80% of normal activity when pyruvate is replaced by α-ketobutyrate [18] or glyoxalate [19] as substrate. The B_4 isozyme is more resistant to inhibition by 2 M urea than the A_4 isozyme [20] but is more sensitive to oxalate inhibition [21,22]. These differences in catalytic properties have stimulated much discussion regarding the significance of the tissue specificity of the lactate dehydrogenase isozyme profile [23]. The characteristics of the hybrid tetramer are a property of the subunit composition, with the A_2B_2 heterotetramer having properties intermediate to the A_4 and B_4 isozymes [2]. These differences in characteristics have been utilized to determine the total A to B subunit ratio in various tissues and samples [24]. It has been shown that individual subunits are not enzymetically active [25], but only limited studies have been conducted regarding the nature and the role of subunit interactions in determining the catalytic and stability properties of this enzyme [26,27].

The LDH_BGUA-1 variant, previously identified as a genetic polymorphism among the Guaymi Indians of Panama and Costa Rica, is characterized by an altered electrophoretic mobility for lactate dehydrogenase-2, 3 and 4, while no enzymatic activity is detectable in the lactate dehydrogenase-1 (B_4) region of the gel [28,29]. Characterization of this variant, which is without enzymatic activity, has provided us with a unique opportunity to study the subunit interactions occurring in the heterotetrameric molecule of lactate dehydrogenase, independently of the enzymatic activity of the B subunit.

Materials and Methods

Blood samples were collected from the Guaymi Indians in Costa Rica into acid-citrate-dextrose anticoagulent vacutainers and shipped at 4°C to Ann Arbor [29]. The plasma was removed and the cells repeatedly washed with saline; packed red blood cells and plasma were stored in liquid nitrogen. Human liver samples, obtained at time of autopsy, were washed and stored in liquid nitrogen.

Electrophoresis was performed using a 7% polyacrylamide slab gel in a continuous Tris-glycine buffer system (37 mM Tris/0.182 M glycine, pH 8.5) [30] or the discontinuous system of Davis [31]. Lactate dehydrogenase isozyme bands were visualized as described previously [30].

Red blood cell and liver samples for thermostability, relative enzyme activity and substrate analog studies were prepared as follows: packed red blood cells were diluted 1:6 (v/v) with lysing buffer (10 mM Hepes, 1 mM EDTA, 1 mM dithiothreitol, 0.06% Triton X-100, pH 7.0) and centrifuged at $48000 \times g$ for 20 min at 4°C. Liver samples were thawed, weighed and suspended in 50 mM Tris-HCl buffer, pH 7.5 (1:10, w/v). Soluble proteins were extracted by homogenization with a Brinkmann Polytron. The homogenates were centrifuged at $48000 \times g$ for 30 min at 4°C. The supernatant fractions from hemolysates and liver homogenates were used as described in the text.

Enzymatic activity was determined on a centrifugal analyzer interfaced with a PDP-8e computer [32]. Units of enzyme activity (IU) are μmol product formed per min at 30°C. All chemicals were of reagent grade and used without further purification with the exception of 3-acetylpyridine adenine dinucleotide (Sigma Chem. Co., St. Louis, MO) which was re-chromatographed on a DEAE-52 ion-exchange column developed with a 0–3% NaCl gradient.

Michaelis constants were determined utilizing the unweighted nonlinear regression method of Wilkinson [33] and the computer program de-

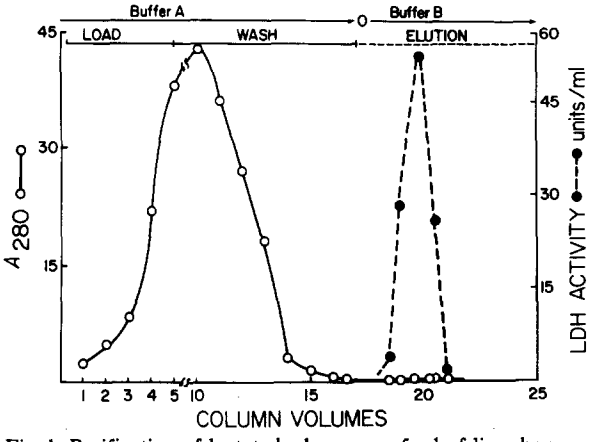

Fig. 1. Purification of lactate hydrogenase. 5 ml of liver homogenate containing approx. 400 IU of lactate dehydrogenase activity in buffer A (20 mM $NaHPO_4$ (pH 7.0), 0.5 M NaCl and 400 μM NADH) was applied to the column. Washing with buffer A was continued until the A_{280} of the eluant was less than 0.005. Lactate dehydrogenase was then eluted with buffer B (20 mM $NaHPO_4$ (pH 7.0), 1 mM pyruvate).

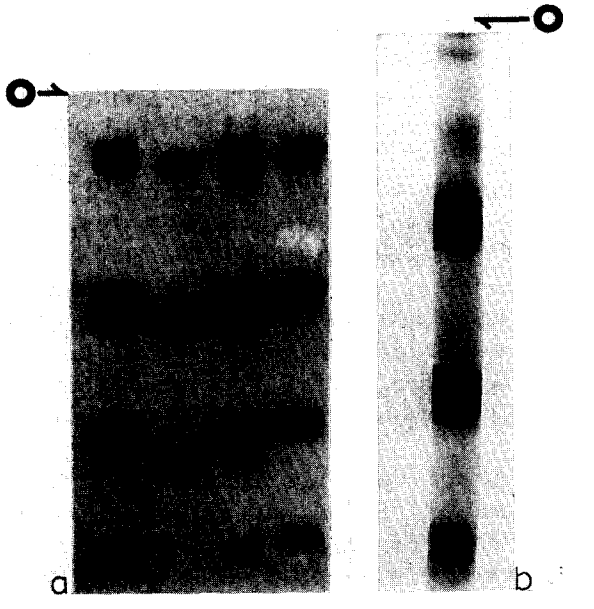

Fig. 2. Electrophoretic pattern of normal and variant lactate dehydrogenase. (a) Electrophoresis of lactate dehydrogenase from plasma from a normal (well 4), a heterozygous (wells 1 and 3) and a homozygous (well 2) individual was conducted as described in Materials and Methods utilizing a continuous buffer system. O is the origin. The bands were visualized by staining for enzyme activity. (b) Electrophoresis of plasma from a heterozygous variant individual as described in Materials and Methods as the Davis method. The bands were visualized by staining for enzyme activity.

scribed by Tiffany et al. [34] with purified lactate dehydrogenase samples from red blood cells and liver. Protein concentration was determined as described by Sedmak and Grossberg [35] and also by the A_{280} extinction coefficient [36].

The enzyme was purified utilizing a modification of the oxamate affinity chromatographic technique [37]. (Oxalic acid was bound to ω-aminohexyl-Sepharose from Sigma Chem. Co., St. Louis, MO). A column 0.8 × 3 cm was used, which was of sufficient size to bind 275 IU of lactate dehydrogenase. After loading the sample, either liver homogenate or red cell hemolysate, and extensive washing to remove unbound protein, the lactate dehydrogenase was eluted in the presence of 1 mM pyruvate (Fig. 1). The inclusion of pyruvate in the elution buffer sharpened the peak of lactate dehydrogenase activity considerably. Purified lactate dehydrogenase fractions were judged homogeneous by the presence of only two protein bands corresponding to dissociated lactate dehy-

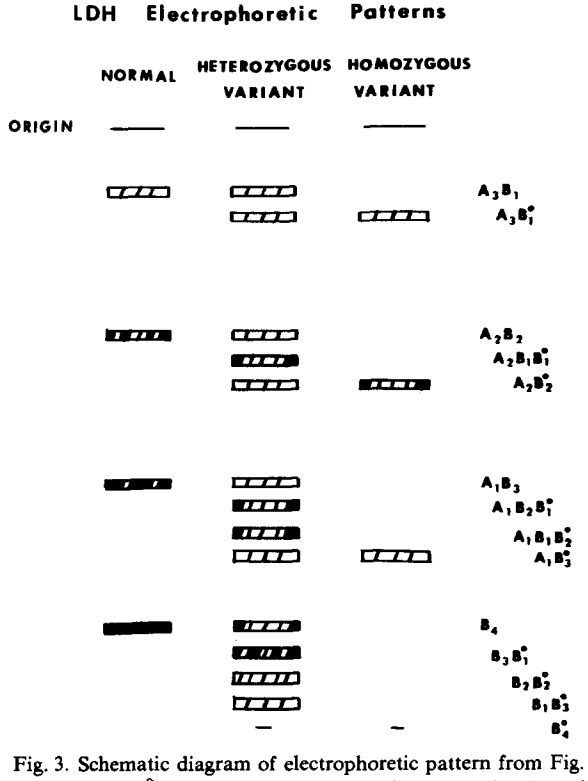

Fig. 3. Schematic diagram of electrophoretic pattern from Fig. 2a and 2b. B^0 designates the inactive B-GUA subunit and —indicates the expected position of the B_4 tetramer.

drogenase A and B [11] subunits after Coomassie blue staining of samples electrophoresed on sodium dodecyl sulfate (SDS) polyacrylamide gels [38].

Results

Electrophoretic analysis

The electrophoretic pattern of lactate dehydrogenase from presumptive heterozygous (normal + variant) and homozygous variant individuals is shown in Fig. 2. The A_4 (M_4 or lactate dehydrogenase-5) band migrates cathodally in this polyacrylamide gel electrophoresis buffer system but is observed as a single band on starch gel electrophoresis [28]. The band patterns corresponding to the A_3B_1, A_2B_2 and A_1B_3 tetramers in both heterozygous and homozygous variant individuals are consistent with the existence of an electrophoretic variant of the B locus of lactate dehydrogenase (Fig. 2a). The absence of a band corresponding to the B_4 tetramer in the homozygous individual and, in this same region of the gel, a four-banded rather than the expected five-banded pattern in the heterozygous individual, again without the B_4 band (Fig. 2b), suggested that the variant subunit responsible for the altered electrophoretic pattern was enzymatically inactive, at least in a tetramer composed of four variant subunits. A similar alteration in electrophoretic pattern, again with the B_4 band missing, was observed when erythrocytes rather than plasma were the source of enzyme. The decreased staining intensity of the various bands containing 1–3 variant B subunits suggested that the B-GUA-1 subunit was not only inactive when combined with itself but was probably inactive when combined with normal A or B subunits. A schematic representation of the electrophoretic pattern (activity stain) is found in Fig. 3.

Enzyme activity

The level of total enzyme activity in both erythrocytes and plasma is consistent with the interpretation that the B-GUA-1 subunit is enzymatically inactive (Table I). The 22% of expected activity in erythrocytes from homozygous variant individuals agrees well with the estimation that 20% of the lactate dehydrogenase subunits in erythrocytes are products of the A locus [9]. It is estimated that approximately 30% of the lactate dehydrogenase subunits in plasma are of the A type, consistent with the 36% of normal activity in plasma from homozygous variant individuals. The level of enzyme activity in plasma and erythrocytes from heterozygous individuals is as expected if all of the A and 50% of the B subunits are active. The specific activity of the lactate dehydrogenase purified from erythrocytes from a homozygous variant individual was 66 IU/mg, compared to a specific activity of 272 IU/mg for lactate dehydrogenase from normal erythrocytes, which is similar to previous reports for lactate dehydrogenase B [36]. Approximately 60 IU/mg is the specific activity expected if the lactate dehydrogenase B-GUA-1 subunits were present in normal quantity but were enzymatically inactive.

TABLE I

THE LEVEL OF LACTATE DEHYDROGENASE ACTIVITY IN ERYTHROCYTES AND PLASMA FROM NORMAL AND VARIANT INDIVIDUALS

Assays were as described in Materials and Methods. *N* equals 6 in each group for erythrocytes and 5 for plasma.

	Erythrocytes	Plasma
	(I.U./g Hgb ± S.D.)	(I.U./ml ± S.D)
Normal	172 ± 10.6	0.25 ± 0.007
Heterozygous variant	85 ± 7.8 (50%) [a]	0.20 ± 0.03 (80%)
Homozygous	38 ± 9.6 (22%)	0.09 ± 0.02 (36%)

[a] % of normal

Thermostability

The thermostability of the lactate dehydrogenase from erythrocytes of homozygous and heterozygous variant individuals was compared to lactate dehydrogenase with a normal electrophoretic pattern from erythrocytes (composed of 80% B subunits) or liver (composed of 80% A subunits) (Fig. 4). As has been previously reported, the A subunits are less resistant to thermodenaturation than are B subunits [39]. The thermodenaturation profile of lactate dehydrogenase from erythrocytes of a homozygous variant individual is intermediate to the stability of lactate dehydrogenase from normal erythrocytes and liver indicating that the enzymatically inactive B-GUA-1 sub-

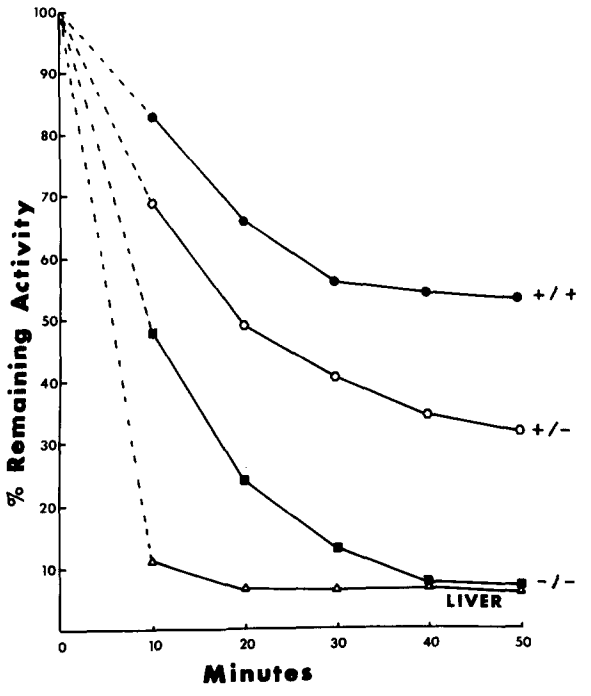

Fig. 4. Thermostability profile of erythrocyte lactate dehydrogenase from normal and variant individuals. Aliquots were incubated at 61°C for the indicated time, transferred to a 4°C bath and centrifuged at 48000×*g* to remove precipitated protein. Assays were conducted as described in Materials and Methods and the activity remaining was expressed as a percent of the original activity. Samples are (●) normal erythrocyte, (○) heterozygous variant erythrocyte, (■) homozygous variant erythrocyte and (△) normal liver.

units confer some thermostability to the A subunits. The rate of loss of enzyme activity of the homozygous variant should have been more rapid relative to that observed for lactate dehydrogenase from liver, which has 20% B subunits, if the rate of thermodenaturation were only a property of the active subunits.

Kinetic properties

The kinetic properties of the lactate dehydrogenase A_4 and B_4 tetramers are very different, thus the kinetic properties of lactate dehydrogenase from four sources (liver (80% A subunits), erythrocytes from a normal individual (80% B subunits), erythrocytes from a heterozygous variant individual (20% A, 40% B, 40% inactive B) and erythrocytes from a homozygous variant individual (20% A, 80% inactive B) were determined (Table II). THe K_m values for pyruvate for lactate dehydrogenase from liver and normal erythrocytes were $3.6 \cdot 10^{-4}$ M and $1.4 \cdot 10^{-4}$ M, respectively, consistent with previous reports for the mammalian A_4 isozymes [1] and the human B_4 isozyme [36]. The K_m for pyruvate for erythrocyte lactate dehydrogenase from the homozygous variant individual (all activity due to the A subunits) was $1.0 \cdot 10^{-4}$ M and similar to normal erythryocyte lactate dehydrogenase, which is composed of 80% B subunits. The K_m value obtained for the lactate dehydrogenase from the heterozygous variant individual ($1.3 \cdot 10^{-4}$ M) was also nearly identical to the value obtained for the normal erythrocyte enzyme. Similarly, the enzyme from normal, heterozygous variant and homozygous variant red cells each had similar K_m values for lactate and NADH ($(0.5–0.8) \cdot 10^{-2}$ M and $(1.2–2.3) \cdot 10^{-5}$ M, respectively) and thus different from the $2.2 \cdot 10^{-2}$ and

TABLE II

MICHAELIS CONSTANTS FOR LACTATE DEHYDROGENASE TETRAMERS COMPOSED OF A PLUS NORMAL AND/OR VARIANT B SUBUNITS

All assays are described in Materials and Methods.

	K_m (M)			
	Liver	Heterozygous variant	Homozygous variant	Normal Erythrocyte
Pyruvate	$3.6 \cdot 10^{-4}$	$1.3 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$	$1.4 \cdot 10^{-4}$
Lactate	$2.2 \cdot 10^{-2}$	$0.8 \cdot 10^{-2}$	$0.5 \cdot 10^{-2}$	$0.7 \cdot 10^{-2}$
NADH	$16 \cdot 10^{-5}$	$2.3 \cdot 10^{-5}$	$1.8 \cdot 10^{-5}$	$1.2 \cdot 10^{-5}$

$16 \cdot 10^{-5}$ M values obtained with the liver enzyme. The standard deviation for the K_m determinations is approx. 8–14% of the K_m value, thus the $K_m$values for the different substrates obtained with lactate dehydrogenase from liver and normal erythrocytes differ by some 30-times the standard deviation.

Substrate specificity

The heart (B) type and muscle (A) type enzymes exhibit quantitative differences in substrate specificity with the heart type enzyme, having higher activity with α hydroxybutyrate and glyoxylate as substrate. The erythrocyte lactate dehydrogenase, 80% B type, has 83% of normal activity with α-hydroxybutyrate as substrate while the liver sample (20% B) has only 34% of the activity obtained with pyruvate as substrate (Table III). The enzyme from erythrocytes of homozygous and heterozygous variant individuals retains 90 and 75% of the activity with the alternate substrate relative to the normal assay with 0.7 mM pyruvate as substrate. Glyoxylate is also an alternative substrate, but with a chain length one carbon shorter than pyruvate. In contrast to the results with the higher carbon chain homolog, the activity, with glyoxalate as substrate relative to pyruvate, of the homozygous variant is 0.23 and similar to the ratio observed for the liver enzyme (0.24). Thus the glyoxalate activity reflects the ratio of active subunits in the heterotetramer. Specific differences in substrate specificity between the A and B enzyme are also noted when the acetylpyridine analog of NAD replaces NAD in the enzyme reaction with the B type enzyme being more sensitive to the substitution of structural analogs. As observed in Table III, the erythrocyte lactate dehydrogenase retained less activity than did the liver enzyme when NAD was replaced by 3-acetylpyridine adenine dinucleotide. The heterotetramers from the homozygous variant individuals retained essentially the same activity as the normal erythrocyte enzyme, thus the response reflected the ratio of A to total B type subunits present in the sample.

Inhibitor studies

Urea inhibits the enzyme reaction via a mechanism involving conformational alterations of the NAD binding sites [22]. The A type enzyme is more sensitive to inhibition, with only 15% of the activity remaining after 20 min, than is the B type enzyme which retains 70% of the initial activity. The lactate dehydrogenase of erythrocytes from heterozygous and homozygous variant individuals has the same sensitivity to inhibition (68% and 72% remaining) as the erythrocyte enzyme from normal individuals (Table IV).

Oxalate is a non-competitive inhibitor of lactate dehydrogenase (assayed in the direction of pyruvate to lactate) with the B type enzyme being more sensitive than the A type enzyme [18]. The liver enzyme, assayed in the presence of 0.1 mM oxalate,

TABLE III

SUBSTRATE SPECIFICITY OF LACTATE DEHYDROGENASE TETRAMERS COMPOSED OF A PLUS NORMAL AND/OR VARIANT B SUBUNITS

All assays are described in Materials and Methods

	Relative activity			
	Liver	Heterozygous variant	Homozygous variant	Normal Erythrocyte
α-Hydroxybutyrate/pyruvate [a]	0.34	0.75	0.90	0.83
Glyoxalate/pyruvate [b]	0.24	0.31	0.23	0.40
Ac Pyr NAD/NAD [c]	0.57	0.25	0.29	0.22

[a] Activity with 0.7 mM α-hydroxybutyrate as substrate divided by activity with 0.3 mM pyruvate as substrate.
[b] Activity with 0.7 mM glyoxalate as substrate divided by activity with 0.3 mM pyruvate as substrate.
[c] Activity with 1.5 mM acetylpyridine NAD as substrate divided by activity with 1 mM NAD as substrate.

TABLE IV

INHIBITOR STUDIES OF LACTATE DEHYDROGENASE TETRAMERS COMPOSED OF A PLUS NORMAL AND/OR VARIANT B SUBUNITS

All assays are described in Materials and Methods

	Relative activity			
	Liver	Heterozygous variant	Homozygous variant	Normal Erythrocyte
Urea inhibition [a]	15	68	72	70
Oxalate inhibition [b]	37	30	41	20

[a] Activity remaining after 20-min preincubation in presence of 2 M urea and 0.3 mM NADH $\times 100$.
[b] Activity in presence of 0.1 mM oxalate and 0.7 mM pyruvate divided by activity without oxalate $\times 100$.

retains 27% of the activity measured in the presence of 0.7 mM pyruvate but without oxalate. The normal erythrocyte enzyme retains only 20% activity when assayed under the same set of conditions. The erythrocyte lactate dehydrogenase from the heterozygous individual retains 30% of normal activity and the enzyme from the homozygous variant individual retains 41% of normal activity (Table IV). The response to oxalate addition to the enzyme reaction reflects the ratio of active B to active A subunits rather than the total B to A ratio as observed for other kinetic properties.

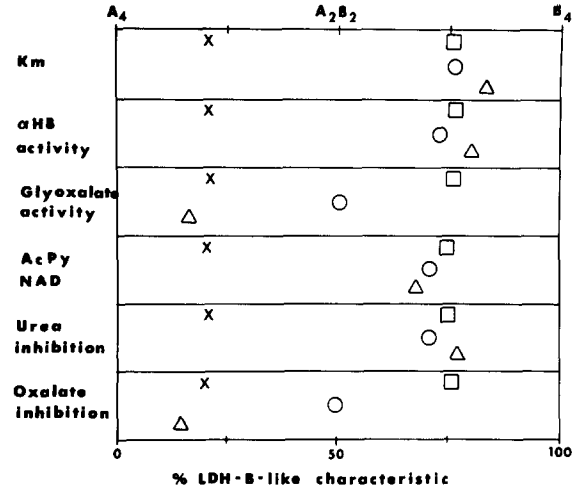

Fig. 5. Summary of kinetic properties of lactate dehydrogenase isozymes. The samples are (□) normal erythrocyte, (○) heterozygous variant erythrocyte, (△) homozygous variant erythrocyte and (×) normal liver. The values obtained when lactate dehydrogenase from normal erythrocytes and liver were studied were assigned positions at 80% and 20% B like characteristics, respectively. The positions for the heterozygous and homozygous variants were calculated from the data presented in Tables II–IV.

As seen in Fig. 5, for four of six kinetic properties which are useful for discriminating between lactate dehydrogenase A and B, the characteristics of the mixed tetramers are not dependent upon the B subunit's being enzymatically active. However, for two other characteristics, subunit interactions do not appear to alter the conformation of the active sites sufficiently to make all of the subunits catalytically similar.

Discussion

The LDH_BGUA-1 variant appears to be a classic example of a catalytically inactive, CRM^+ variant. The altered electrophoretic pattern of enzyme from erythrocytes and plasma is consistent with the presence of a variant at the B locus of lactate dehydrogenase, except that the B-variant homotetramer is not detectable with enzyme-specific staining. The level of enzymatic activity in erythrocytes and plasma from homo- and heterozygous individuals and the reduced specific activity of the enzyme purified from erythrocytes and plasma of affected individuals suggests that the B-GUA-1 subunit is enzymatically inactive even when combined with normal A or B subunits.

Previous studies with enzyme bound to inert resins indicated that the individual subunits of lactate dehydrogenase are not enzymatically active

[25]. The existence of bands corresponding to A_1 B-GUA-1_3 and B_1 B-GUA-1_3 tetramers, although not in conflict with the previous observation that subunits are catalytically inactive [25], indicates that a single active subunit in combination with three inactive subunits has catalytic function. This would suggest that the conformational changes which occur during tetramer formation are critical to the realization of catalytic function for this enzyme. Additional evidence for this interaction is obtained from examining other properties of the heterotetramers. The inactive B subunits appear to confer quite normal conformational changes on the active A subunit in that the A subunits in combination with from one to three B-GUA-1 subunits have K_m values for pyruvate, lactate and NADH which are not different from K_m values determined for molecules consisting of A plus active B subunits. These K_m values are different from the values observed for the A_4 tetramer. Similarly, the inactive B subunits influence the substrate specificity of the heterotetramer in terms of activity toward α hydroxybutyrate and acetylpyridine NAD in a manner almost identical to that of the active B subunits. Also the response of the heterotetramer to structural alterations due to urea incubation in the presence of NADH was not affected by the replacement of active B by inactive B subunits. Thus each of these kinetic characteristics reflects the total A to B ratio rather than the ratio of A to active B subunits. This is not in conflict with previous observations that the hybrid tetramers have intermediate properties [2], but does suggest that interactions between nonidentical subunits are instrumental in determining these properties. More important, these results suggest that each subunit in the tetramer has similar characteristics and that the kinetic characteristics of the tetramer are not simply the numerical average of subunits with different properties. At least four properties of the enzyme reflect the interaction of subunits with differing characteristics and are independent of the catalytic function of the subunit.

Two kinetic parameters, the activity with the 2-carbon substrate analog, glyoxalate, and the response to assaying the enzyme in the presence of oxalate, were different between AB and $AB^{GUA\text{-}1}$ heterotetramers and reflect the A to active B ratio rather than A to total B ratio. It is not yet clear if this difference is related to the structural alteration causing the B-GUA-1 molecule to be inactive or indicates that conformation changes associated with tetramer formation have a lesser effect on glyoxalate and oxalate binding.

Many examples of subunit interaction associated with regulatory subunits [40], ordering of substrate binding [41] or binding of regulatory molecules [42] are known. Most of the interactions involving identical subunits act to make the catalytic sites nonequivalent, which appears to be the converse of the situation with the lactate dehydrogenase molecule; that is, interaction between nonidentical subunits alters the active site of each subunit in a manner which apparently makes all four sites catalytically similar. These data suggest that the conformational changes which occur upon tetramer formation are responsible for determining many of the kinetic properties of the lactate dehydrogenase molecule and that within the heterotetramic molecule a high level of communication exists between subunits.

The lactate dehydrogenase B-GUA-1 variant is found among the Guaymi Indians of Panama [28] and Costa Rica [29], occurring with an allele frequency of approximately 0.10. Some 10 individuals have been identified as being homozygous-deficient. This group includes several mature adults, thus the enzyme deficiency would not appear to be associated with severe negative metabolic consequences, which would be selected against in this primitive population. In addition to the erythrocyte, this enzyme deficiency should be most severe in heart tissue where the B subunit is responsible for approximately 80% of the enzymatic activity [9]. Kitamura et al. [43] have described a hereditary deficiency of lactate dehydrogenase, also associated with the B-locus. No indication of the presence of any B type subunit was observed during studies of this variant. The lactate dehydrogenase deficiency in this individual was associated with impaired glucose tolerance and a slightly elevated serum cholesterol and the individual was a marginal diabetic. Similar studies have not been conducted among the Guaymi Indians. Significant metabolic consequences might not be observed in the Guaymi, because the residual enzyme activity has nearly normal kinetic properties. It is still of interest that this enzyme de-

ficiency and a low activity variant of erythrocyte acid phosphatase have both attained high frequencies in this tribe [28,29].

Acknowledgments

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