POLYMORPHISM OF HORSE LIVER ALCOHOL DEHYDROGENASE

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Summary: The properties of the most cathodal component of horse liver alcohol dehydrogenase (isozyme SS) have been found to vary. The variability is dependent on the livers from which the enzyme is isolated rather than on the purification procedure. Two distinct preparations, differing in catalytic properties, have been obtained and named S-type and A-type preparations. The preparations can be distinguished from each other by the ratio of activity with acetaldehyde to activity with the steroidal ketone 5β-dihydrotestosterone. This ratio is about one for the S-type and twenty for the A-type preparations.

Alcohol dehydrogenase from horse liver (HLADH) separates on gel electrophoresis into at least nine protein bands (shown diagrammatically in Fig. 1). It has been generally accepted that three of these bands are formed by dimerization of two distinct subunits E and S to form three isozymes: EE, ES and SS (1). Isozyme SS, the fastest migrating and most cathodal component, was shown previously to be active with the classical substrates of HLADH (1) as well as with steroids. The results presented in this paper show that the catalytic properties of this component are variable and are dependent on the livers from which the enzyme is isolated.

Horse livers (fresh or frozen) were ground and extracted in 0.05M phosphate buffer, pH 7.5 for 1 hour at room temperature. The slurry was then filtered through cheese cloth, the filtrate was collected and the protein precipitated with ammonium sulphate. The fraction precipitating between 50 to 80% saturated

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The following abbreviations are used: horse liver alcohol dehydrogenase, HLADH; 5β-dihydrotestosterone, 5βDHT.

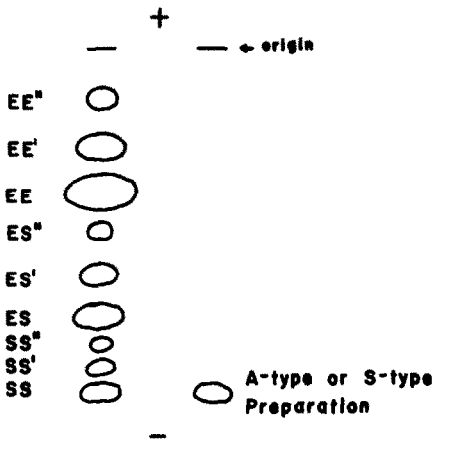

Fig.1. Schematic representation of the electrophoretic separation pattern of HLADH on starch gel in tris-HCl buffer, pH 8.5. Nomenclature as in reference 1.

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ammonium sulphate was collected, redissolved in 0.02M tris-HCl buffer pH 8.8 and dialyzed exhaustively against 0.02M tris-HCl buffer (at least four changes of the buffer are required). The enzyme was purified further by chromatography on DE11 (Whatman) cellulose in 0.02M tris-HCl buffer pH 8.8. In these conditions all but the fastest migrating isozyme of HLADH are attached or retarded by DE11; the fastest migrating component together with some inert protein passes through. The purity of the preparation with respect to other isozymes of HLADH was determined by electrophoresis on starch gel at pH 8.5 in tris-HCl buffer. The preparations contained only the fastest migrating component (Fig.1) of HLADH, previously named SS (1). The total protein was determined by the Lowry procedure (2) employing bovine serum albumin as a primary standard. The concentration of HLADH active sites was determined by titration with NAD in the presence of excess pyrazole (3) and by titration with NADH in the presence of excess isobutyramide (4). The amount of total HLADH protein was calculated using a MW of 40,000 (5) per catalytic site. In all

preparations described in this paper the results obtained by titration with NAD in the presence of pyrazole were identical, within experimental error, with the results obtained by titration with NADH in the presence of isobutyramide. Although preparations were only 10-17% pure with respect to total protein the fact that there was a complete agreement between titrations with NAD and NADH in the presence of two distinct classical HLADH inhibitors indicates that HLADH active sites were accurately determined. The preparations had no reducing NAD or oxidizing NADH activity in the absence of substrates nor did they have detectable aldehyde dehydrogenase activity.

Employing active site titration, turnover numbers of four preparations of "SS isozyme" from four different livers with ethanol, acetaldehyde and 5β-dihydrotestosterone (5βDHT) at a single concentration of these substrates are compared (Table 1). The results show that two kinds of catalytically distinct preparations of the fastest migrating component of HLADH have been obtained: the A-type and the S-type preparations. Preparations of the A-type are characterized by high activity with acetaldehyde, the turnover with this substrate being some ten times more than that of the S-type preparations. While ethanol activity of the A-type preparation is twice as high as that of the S-type preparation, its activity with 5βDHT is somewhat lower than the activity found with the S-type preparation. High activity with acetaldehyde is responsible for the high acetaldehyde to steroid ratio of the A-type preparations.

The differences between preparations are not artefacts of the purification procedure. Several preparations from liver no.1 yielded enzyme with activities characteristic of liver no.1 (A-

Table 1

Turnover numbers with ethanol, acetaldehyde, and 5βDHT of "isozyme SS" prepared from different horse livers.

liver no.	turnover no./active site/min ethanol	acetaldehyde	5βDHT	acetaldehyde 5βDHT	liver type
1	93	1139	54	21.1	A-type
2	45	161	121	1.3	S-type
3	-	1352	58	23.3	A-type
4	-	110	79	1.4	S-type
ref.1		130	95	1.4	SS-isozyme

The assay system contained: NADH, 0.17mM; NAD, 0.5mM; ethanol, 10.8mM; acetaldehyde (freshly distilled), 1.2mM; 5βDHT, 0.115mM added in 10µl dioxane to 3ml volume. In the reductive direction the reaction was carried out at pH 7.0 in 0.1M phosphate buffer, in the oxidative direction the reaction was carried out at pH 10 in 0.062M glycine buffer, in a Beckman DB-GT spectrophotometer at 340nm at 25°.

type preparation) and two separate preparations from liver no.2 gave enzyme with activities characteristic of the S-type preparations. The catalytic properties of the fastest migrating component are clearly dependent on the source of enzyme rather than on the purification procedure.

In Table 2 the Km values of the preparations of A-type and the preparations of S-type with ethanol and acetaldehyde are compared. Although Km values of these two preparations with the steroidal ketone 5βDHT are similar (ca.30uM) the Km values for ethanol and acetaldehyde for the preparations of S-type are some 17-fold greater than those for the A-type preparations. When the effect of lithocholic (3^{α}-hydroxy-5β-cholanoic) acid, a steroid site inhibitor (6), on these two preparations is compared (Fig.2)

Table 2

Kinetic constants with ethanol and acetaldehyde for the A-type and the S-type preparations.

substrate	A-type Km (mM)	A-type V	S-type Km (mM)	S-type V	Boehringer HLADH Km (mM)	Boehringer HLADH V
ethanol	0.71	92	11.2	93	1.2	314
acetaldehyde	0.08	1210	1.4	362	0.23*	5618*

Alcohol oxidation was determined in 0.062M glycine buffer, pH 9.5 at 500μM NAD; aldehyde and ketone reduction was followed in 0.1M phosphate buffer, pH 7.0 at 170μM NADH. All measurements were made in a Beckman DB-GT spectrophotometer at 25°. V = turnover no./active site/min. *from ref. 10.

with the S-type preparation a non-competitive pattern is obtained, with the preparations of the A-type the pattern is uncompetitive (parallel lines) indicating that in the latter case the inhibitor and the substrate react at different catalytic sites. In Table 3 the Ki values with lithocholic acid and with isobutyramide for the A-type and the S-type preparations are compared. Lithocholic acid is a better inhibitor of the S-type preparations while isobutyramide is a better inhibitor of the A-type preparations. The results listed in Tables 2 and 3 and Figure 2 show that the catalytic sites concerned with the interconversion of the classical HLADH substrates in these preparations are distinct.

In Table 2 the Km values and turnover numbers at Vmax with ethanol and acetaldehyde for Boehringer HLADH (80% EE isozyme (7)) are included for comparison. The Km values for the A-type preparations resemble those of Boehringer HLADH, the Km values

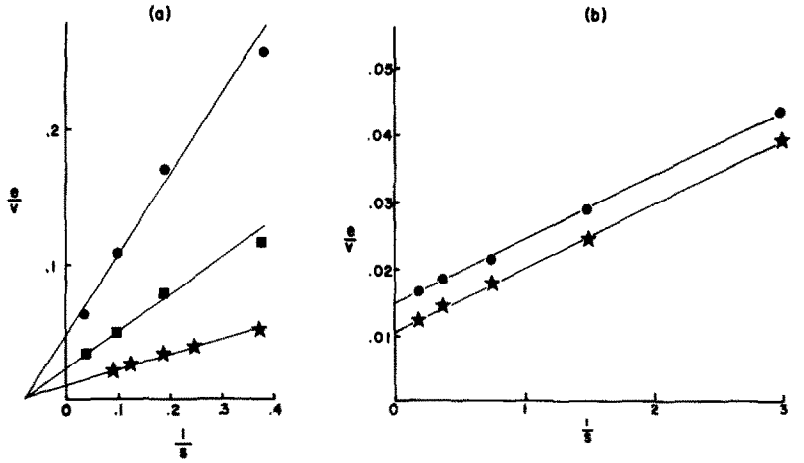

Fig.2. Effect of lithocholic acid on the oxidation of ethanol by the S-type (a) and the A-type (b) preparations. ★-no inhibitor; ■-lithocholic acid, 8.8μM; ● -lithocholic acid, 44.2μM. e = enzyme concentration, μN; V = NADH formed, μM/min; S = ethanol, mM.

Table 3

Effect of lithocholic acid and isobutyramide on ethanol and acetaldehyde activity of the A-type and the S-type preparations.

Inhibitor	substrate	A-type Ki (μM)	A-type Inhibition type	S-type Ki (μM)	S-type Inhibition type	Boehringer ADH Ki (μM)	Boehringer ADH Inhibition type
lithocholic acid	ethanol	120	UC	8	NC		
isobutyramide	acetaldehyde	70	C	810	C	9*	C*

C = competitive; UC = un-competitive; NC = non-competitive. Experimental conditions as in Table 2. *from ref. 8.

with ethanol and acetaldehyde for the S-type preparations are an order of magnitude greater. The Ki values with isobutyramide for both preparations (Table 3) are higher than that determined for the classical HLADH (8). In Km values the preparations of the

A-type resemble Boehringer HLADH; in Ki values the A-type and the S-type preparations differ from each other and from Boehringer HLADH.

Even in the cases where the SS band was not easily detectable, HLADH prepared from horse livers in our laboratory showed a general electrophoretic pattern depicted in Fig. 1 and was always steroid active. The homogenates from either the A-type or the S-type livers showed high activity with 5βDHT, and this activity was due to HLADH as indicated by its sensitivity to pyrazole and isobutyramide. This indicates that the S subunit as well as the E subunit are universally distributed and are probably coded for by distinct genetic loci.

Both the A-type and the S-type preparations migrate on starch gels as single bands of similar electrophoretic mobility (Fig. 1) which corresponds to the electrophoretic mobility of the previously described SS isozyme (1) and not to the electrophoretic bands designated SS' and SS" nomenclature. Both preparations are active with steroids, ethanol and acetaldehyde (Table 1). Although electrophoretic mobility and steroid activity characteristics are the same the catalytic constants with ethanol and acetaldehyde differ; the preparations of S-type resemble the previously described SS isozyme more than the A-type preparations (Table 1). The differences appear to be related to the liver from which the enzyme is prepared, suggesting that preparations of the A-type are a polymorphic form of the SS isozyme. The fact that two of the livers contained the S-type and the other two the A-type enzyme is probably coincidental and does not necessarily reflect natural distribution.

Further work providing proof for heterogeneity of the A-type preparations and for the relationship between the S-type and the

A-type preparations (9) has been completed and is being prepared for publication.

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