THE AMINO ACID COMPOSITION OF HORSE LIVER ALCOHOL DEHYDROGENASE*

Donald J. Cannon and Robert H. McKay

Department of Biochemistry and Biophysics, School of Medicine

University of Hawaii, Honolulu, Hawaii 96822

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SUMMARY

A detailed amino acid analysis of horse liver alcohol dehydrogenase is presented, along with a re-evaluation of the extinction coefficient at 280 nm, and a determination of the partial specific volume from the amino acid data.

Horse liver alcohol dehydrogenase (alcohol:NAD oxidoreductase, E.C. 1.1.1.1) catalyzes the reversible oxidation of primary and secondary alcohols to their corresponding aldehydes. The enzyme has a molecular weight of 79,000 (1-3), and contains 4 gram-atoms of zinc per molecule (4,5). The enzyme contains two subunits of approximately 40,000 molecular weight (1,2, 6-8). In addition, various preparations of the enzyme are electrophoretically heterogeneous (9-13) as a result of hybrid formation of the enzyme subunits. Little is known of the primary structure of the enzyme, amino acid analyses have been few (2,13) and have been performed on disparate enzyme preparations. The published extinction coefficients of the enzyme at 280 nm vary from 0.42-0.46 ml mg^{-1} cm^{-1} (2,14-16). This study re-evaluates the extinction coefficient, and presents an amino acid composition of horse liver alcohol dehydrogenase.

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MATERIALS AND METHODS

Horse liver alcohol dehydrogenase (Lots #6048431, 6079131, 6198431, 6427231) and NAD (Lot #06497415) were obtained from Boehringer Mannheim Corp., San Francisco, California. This source of the enzyme is prepared by a modification of the method of Dalziel (17) and we consider it to be better than 97% pure (1). All other chemicals were reagent grade, distilled deionized water was used in all experiments and had a maximum conductivity of 1 μmho.

Enzyme Preparation. Horse liver alcohol dehydrogenase (100 mg) was dialyzed against 3 x 500 ml of 0.05 M Na_2HPO_4-NaH_2PO_4 buffer, pH 7.5 for 24 hours at 4°. Dialysis tubing was prepared according to Hughes and Klotz (18). The enzyme solution was then filtered through a Millipore filter, 0.45 μ pore size. Concentration was determined at 280 nm using an absorptivity of 0.455 ml mg^{-1} cm^{-1}. Activity and zinc content of the enzyme were determined as previously described (4).

Amino Acid Analysis. Hydrolysis of the enzyme (2-3 mg) was carried out in 5.7N constant boiling HCl in vacuo at 105° in an oven. All hydrolyzates contained a crystal of phenol to minimize the formation of chlorotyrosine. Hydrolysis was for 24, 48, and 72 hours, excess acid was immediately removed and the samples were dissolved in 0.2N sodium citrate buffer (pH 2.2). Analyses were performed on a Phoenix Model 6800 amino acid analyzer according to Moore and Stein (19). Detection was with ninhydrin at 570 and 440 nm in a 1.0 cm flow cell adapted to a Zeiss PMQ II spectrophotometer. Absorbancy values were recorded on a Hewlett-Packard Model 7127A recorder via a Zeiss T-E converter. Full scale deflection is equal to an absorbancy of 0.5, and approximately 0.1 μmoles of amino acid. Accuracy is ± 2-3%. Cysteine and methionine were analyzed as cysteic acid and methionine sulfone, respectively, after performic acid oxidation (20). The methods of Boyer (21) and Ellman (22) were employed for the determination of total sulfhydryl groups. The latter assay was performed in the presence of either 8M urea

or 0.3% sodium dodecyl sulfate. Both gave the same results. Tryptophan was determined by the methods of Spies and Chambers (23), Goodwin and Morton (24), Bencze and Schmid(25), Spande and Witkop (26), and Edelhoch (27). Disulfide was assayed by the method of Zahler and Cleland (28), and microkjeldahl nitrogens were done using SeO_2 and $CuSO_4$ as catalysts for digestion, followed by steam distillation of the NH_3 into 2% boric acid and titration.

RESULTS AND DISCUSSION

From the same preparation of enzyme, carefully measured aliquots were analyzed for absorption at 280 nm (Cary Model 14), total nitrogen determination, and amino acid analysis. The results are presented in Table I. Table I indicates, assuming the protein consists only of amino acids and neglecting the weight contribution of zinc atoms (0.3%), that the absorptivity of the enzyme is 0.455 ml mg^{-1} cm^{-1} (E_M=35,900).

The average specific activity of the enzyme was 9.13 ± 0.53 μM mg^{-1} min^{-1} (average molecular activity = 721 ± 42) (340 nm, 25°, and pH 8.8), the zinc content was 4.2 ± 0.1 gram atoms per mole, and the nitrogen content of the enzyme was 16.4 ± 0.3% (A.D., n=9).

TABLE I

Determination of the Extinction Coefficient of Horse Liver Alcohol Dehydrogenase

Total Nitrogen (mg per ml)	
Kjeldahl	0.138 ± 0.003
Amino acid analysis	0.140 ± 0.006
Total amino acids (mg per ml)	0.853
Absorbance at 280 nm	0.388
Absorptivity (ml mg^{-1} cm^{-1})	0.455
Molar extinction coefficient	35,900

The amino acid analysis results are presented in Table II. The results are the average of the 24, 48, and 72 hour hydrolysates except that serine and threonine were extrapolated to zero time of hydrolysis and valine and isoleucine were the average of the 48 and 72 hour hydrolysates. A minimum of 10 analyses were performed at each hydrolysis time. A molecular weight

TABLE II

The Amino Acid Composition of Horse Liver Alcohol Dehydrogenase

Amino Acid	Gm Res/ 10^5 gm Protein	Res/ 79,000 gm	Nearest Integral No. of Res.	Integral No. x M.W. of Res.
Asp	7420	50.9	51	5870
Thr	5734	44.8	45	4549
Ser	5451	49.4	49	4267
Glu	9297	56.9	57	7358
Pro	5100	41.5	42	4078
Gly	5519	76.3	76	4339
Ala	4986	55.4	55	3910
Val	9151	72.9	73	7241
Ile	6384	44.6	45	5094
Leu	6954	48.5	49	5546
Tyr	1662	8.0	8	1305
Phe	6774	36.4	36	5299
Lys	10120	62.4	62	7948
His	2977	17.1	17	2332
Arg	4781	24.2	24	3748
Cys	3732	27.7	28	2889
Met	2930	17.6	18	2361
Trp	1039	4.2	4	744
	100,011			78,878
			+ 4 zinc/mole	260
				79,138

of 79,000 is used in these calculations and in the calculations of molar concentrations of the enzyme. The values for the total sulfhydryl content of the enzyme and the absence of disulfide bonds indicates that the total sulfur amino acid content can be attributed to cysteine and methionine (Table III). The value used in Table II for methionine is based on performic acid oxidation (20). The value for cysteine is the average of three different methods (Table III).

TABLE III

Determination of Sulfur Amino Acids

		Moles/Mole*
Methionine		
Moore (20)		17.6 ± 0.2 A.D.
Amino acid analysis		17.9 ± 1.3
Cysteine		
Moore (20)		27.3 ± 0.4
Ellman (22)		27.8 ± 0.4
Boyer (21)		28.0 ± 0.7
Average	27.7 ± 0.5	
Cystine		
Zahler and Cleland (28)		0.0

*A minimum of 3 determinations were made for each method.

The values for tryptophan are presented in Table IV. The method of Spies and Chambers (23) was inconclusive since maximum absorption appeared at 540-550 nm whereas tryptophan absorbs at 590 nm. Spies (29) also

TABLE IV

Determination of Tryptophan

	Moles/Mole Enzyme*
Goodwin and Morton (24)	4.5 ± 0.2 A.D.
Bencze and Schmid (25)	4.6 ± 0.3
Edelhoch (27)	4.2 ± 0.2
Spande and Witkop (26)	
8M urea-pH 4.0	6.7 ± 0.2
pH 4.0	2.4 ± 0.2

*A minimum of 3 determinations were made for each method.

observed these results, and determined values of 4.2 and 4.3 moles of tryptophan per mole of enzyme (corrected to 79,000) by alkaline hydrolysis. Oxidation with N-bromosuccinimide (26) (2x recrystallized) gave consistently high values. In 8M urea at pH 4.0, 15-16 moles of N-bromosuccinimide were consumed per mole of tryptophan, indicating the oxidation of amino acid residues other than tryptophan. In the absence of urea at pH 4.0 tryptophan was extremely refractory to oxidation, a maximum of 40% of those residues reacting in the presence of urea were oxidized. The possibility exists that some tryptophan residues may be buried in the interior of the protein. The remaining methods were more consistent and since the assay of Edelhoch (27) gave more accurate results on proteins of known tryptophan content we consider the values obtained with this method to be the most reliable. This result is presented in Table II. The determination of the amino acid composition of horse liver alcohol dehydrogenase allows the calculation of the partial specific volume ($\bar{v}$) of the protein. Using the values of Cohn and Edsall (30) for the specific volume of amino acid residues, $\bar{v}$ is 0.746.

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