Chemical Reactivity at the Catalytic Sites of Aspartic β-Semialdehyde Dehydrogenase and Glyceraldehyde-3-Phosphate Dehydrogenase†

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ABSTRACT: Aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase from yeast are compared on the basis of sulfhydryl reactivity, amino acid composition, electrophoretic mobility, isoelectric points, and their ability to hydrolyze *p*-nitrophenyl acetate. Inactivation of aspartic β-semialdehyde dehydrogenase by iodoacetate, iodoacetamide, and *N*-ethylmaleimide at pH 6.5 in the presence and absence of pyridine nucleotides is quantitatively similar to the results obtained for glyceraldehyde-3-phosphate dehydrogenase (Racker, E. (1965), Mechanisms in Bioenergetics, New York, N. Y., Academic Press, pp 17–47; Macquarrie, R. A. (1969), Ph.D. Thesis, University Microfilms, Ann Arbor, Mich. The second-order rate constants for *N*-ethylmaleimide inactivation of aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase are 2.1×10^{-5} and 2.3×10^{-5} min^{-1}, respectively. Tetrameric glyceraldehyde-3-phosphate dehydrogenase is fully inactivated by only 2 mol of *N*-ethylmaleimide, in contrast to aspartic β-semialdehyde dehydrogenase which requires only 1 mol of *N*-ethylmaleimide per subunit for complete inactivation. Examination of the amino acid compositions of the two dehydrogenases for identical, chemically related, and aliphatic side-chain amino acid compositions shows some limited similarities between the two enzymes. Aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase have identical electrophoretic mobilities at pH 8.3 and their isoelectric points are 6.17 and 6.25, respectively. It is also shown that aspartic β-semialdehyde dehydrogenase has the ability to hydrolyze *p*-nitrophenyl acetate at half the rate shown by glyceraldehyde-3-phosphate dehydrogenase.

The comparative enzymology of dehydrogenases has long been a subject of great interest. Early studies of dehydrogenases with widely differing physiological roles suggested that sulfhydryl groups were commonly involved in catalysis, subunit molecular weights might generally be about 35,000, and that active-site peptides were at least partially homologous (Kaplan, 1965). More recent findings, however, do not seem to support these premises. Analysis of the primary structures of horse liver alcohol dehydrogenase (Jörnvall and Harris, 1970; Jörnval, 1970a,b), bovine glutamate dehydrogenase (Smith *et al.*, 1970), and the glyceraldehyde-3-phosphate dehydrogenases from lobster and swine (Davidson *et al.*, 1967; Harris and Perham, 1968) reveals a very limited amount of sequence homology as well as quite different molecular sizes among these three enzymes.

Studies of the active sites of glutamate dehydrogenase (Holbrook *et al.*, 1970) and lactate dehydrogenase (Pfleiderer, 1970) show that residues other than sulfhydryls are involved in substrate binding. On the other hand, the active-site sulfhydryl of glyceraldehyde-3-phosphate dehydrogenase does appear to be involved in substrate binding, since stable acyl derivatives of this essential thiol can be obtained under certain conditions with *p*-nitrophenyl acetate and acetyl phosphate (Harris *et al.*, 1963).

The limited sequence similarity among the dehydrogenases most extensively studied shows that the understanding of homology is a more complex problem than anticipated. The pursuit of this study is, however, an important route to the understanding of structure–function relationships. For this reason, we have chosen to study the properties of aspartic β-semialdehyde dehydrogenase, since its reaction mechanism appears to be very similar to that found for glyceraldehyde-3-phosphate dehydrogenase. In the two preceding papers, the preparation and properties of homogeneous aspartic β-semialdehyde dehydrogenase as well as the interaction of the enzyme with adenosine 5′-triphosphate at low temperatures are described. In this paper the properties of the essential thiol groups of the enzyme are examined with sulfhydryl reagents and compared with results obtained for glyceraldehyde-3-phosphate dehydrogenase. The amino acid compositions, electrophoretic mobilities, isoelectric points, and esterase activities of these two enzymes are also compared.

Methods and Materials

Inactivations with Sulfhydryl Reagents (Iodoacetic Acid, Iodoacetamide, and N-Ethylmaleimide). Enzyme incubations were carried out at 25° and contained a final concentration of 0.1 mM inhibitor. Aliquots of the incubation mixture were assayed as described previously (Holland and Westhead, 1973a). In order to eliminate dithiothreitol, which reacts instantaneously with all three inhibitors, the enzyme was chromatographed on Sephadex G-25 containing 10 mM potassium phosphate buffer, pH 6.5, prior to use.

Determination of S-Succinylcysteine. The number of moles of *N*-ethylmaleimide[1] (NEM) bound per mol of glyceraldehyde-3-phosphate dehydrogenase was measured by the procedure of Riordan and Vallee (1967). Sulfhydryl groups react

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[1] Abbreviation used is: NEM, *N*-ethylmaleimide.

with NEM to form *S*-(ethylsuccinimido)cysteine, which is converted to *S*-succinylcysteine and ethylamine on acid hydrolysis of the protein. Each incubation was carried out at 25° in 10 mM potassium phosphate buffer, pH 6.5, 0.1 mM NEM, and 27 μM glyceraldehyde-3-phosphate dehydrogenase in a total volume of 1.0 ml. Glyceraldehyde-3-phosphate dehydrogenase used in the experiments was a single isozyme with a specific activity of 120 μmol of NADH per minute per milligram (Holland and Westhead, 1973a) and was free of bound NAD^+ as judged by the ratio of absorbance at 280 nm to that at 260 nm equal to 2.1. The NEM reaction was quenched at determined times with 2 mM thioglycolate, and the protein freed of excess reagents on a Sephadex G-25 column equilibrated with 10 mM potassium phosphate buffer, pH 6.5. Protein was then precipitated with 10% trichloroacetic acid (Cl_3CCOOH) and washed repeatedly with (1:2) ethanol–diethyl ether and finally with ether alone. The dried protein was hydrolyzed and prepared for amino acid analysis by standard procedures. Norleucine was added to each hydrolysate as an internal standard. Hydrolysis was carried out for 72 hr at 110°. A Beckman Model 120C automatic amino acid analyzer was used with the standard 4-hr elution procedure. *S*-Succinylcysteine elutes at 91 ml (relative to 110 ml for aspartic acid) on the 150-cm column and ethylamine elutes at 10 ml (relative to 52 ml for lysine) on the 15-cm column (Guidotti and Konigsberg, 1964).

A standard solution of *S*-(ethylsuccinimido)cysteine (1.0 mM) was prepared quantitatively by titrating 1.0 mM NEM at 25° in 0.1 M potassium phosphate buffer, pH 7.0, with cysteine. The end point was determined by the loss of absorption at 305 nm due to unreacted NEM. Samples of the *S*-(ethylsuccinimido)cysteine were hydrolyzed by the protein used for protein and were used directly for standardization of the amino acid analyzer.

Aspartic acid, valine, and isoleucine show no appreciable decomposition after 72-hr hydrolysis and, based on the known number of each of these residues and the protein concentration, the number of *S*-succinylcysteine residues was calculated.

Polyacrylamide disc gel electrophoresis was carried out at pH 8.3 as previously described (Holland and Westhead, 1973a). After electrophoresis, gels were fixed with 10% Cl_3CCOOH and then scanned at 280 nm with a Gilford Model 2410 scanner attached to a Gilford Model 240 spectrophotometer. Gels were scanned for protein bands at 280 nm and then rescanned at 660 nm as a control for nonprotein "peaks" due to abnormalities in the gels.

Isoelectric Focusing. Isoelectric points were determined with an LKB (120 ml) apparatus using 12.5 mg of protein containing aspartic β-semialdehyde dehydrogenase (specific activity = 8.9) and glyceraldehyde-3-phosphate dehydrogenase (specific activity = 100). The sample was allowed to focus for 72 hr at 1.5 W and eluted in 2-ml fractions, and the enzyme activities and pH of each fraction were determined.

Esterase Assay Procedure. The hydrolysis of *p*-nitrophenyl acetate was followed spectrophotometrically by measuring liberated *p*-nitrophenol at 400 nm. The extinction coefficient used was 16×10^3 (Kezdy and Bender, 1962). Esterase assays were routinely performed at 30° in a total volume of 1 ml containing 10 mM sodium barbital buffer, pH 8.0, 10 mM EDTA and 0.19 μmol of *p*-nitrophenyl acetate. Before assay, the enzymes were chromatographed *twice* on a 1.5 × 15 cm Sephadex G-25 column and equilibrated with 10 mM sodium barbital buffer, pH 8.0, containing 10 mM EDTA. This procedure ensures the complete removal of mercaptans present in the enzyme storage buffers. After chromatography, enzyme was concentrated by ultrafiltration. The enzyme concentrations for aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were 0.9 mg/ml (specific activity = 71) and 1.8 mg/ml (specific activity = 110), respectively.

TABLE I: Inactivation of Aspartic β-Semialdehyde Dehydrogenase by Sulfhydryl Reagents.[a]

	% Inactivation		
	Iodoacetate	Iodoacetamide	*N*-Ethylmaleimide
Glyceraldehyde-3-phosphate dehydrogenase[b]	5	30	97
Glyceraldehyde-3-phosphate dehydrogenase–NAD^+ [b]	94	22	26
Aspartic β-semialdehyde dehydrogenase	3	45.4	91
Aspartic β-semialdehyde dehydrogenase–1 mM $NADP^+$	50	22	12

[a] Inactivations were carried out for 12 min at 25° in 10 mM potassium phosphate buffer, pH 6.5, plus 0.1 mM inhibitor, the same conditions used by Racker (1965). Protein concentration was 1.0 mg/ml (aspartic β-semialdehyde dehydrogenase specific activity, 10.8 units/mg). [b] Data of Racker, 1965.

Reagents. Iodoacetic acid (free acid), iodoacetamide, thioglycolic acid, and *p*-nitrophenyl acetate were obtained from Sigma Chemical Co., St. Louis, Mo. *N*-Ethylmaleimide, L-cysteine, and norleucine were purchased from Mann Research Labs., Orangeburg, N. Y. Ninhydrin and trichloroacetic acid were obtained from Pierce Chemical Co., Rockford, Ill. Ampholine solution (pH 5–8) was obtained from LKB, Sweden. All other reagents were described previously (Holland and Westhead, 1973a).

Results and Discussion

Inactivation of Aspartic β-Semialdehyde Dehydrogenase by Sulfhydryl Reagents. The extent of inactivation of aspartic β-semialdehyde dehydrogenase by iodoacetate, iodoacetamide and *N*-ethylmaleimide (NEM) was measured after 12-min incubations in 10 mM potassium phosphate buffer, pH 6.5. The results obtained for aspartic β-semialdehyde dehydrogenase and $NADP^+$-bound aspartic β-semialdehyde dehydrogenase are shown in Table I, together with the results obtained by Racker (1965) for glyceraldehyde-3-phosphate dehydrogenase under the same experimental conditions.

The patterns of inactivation observed with the two enzymes are very similar. In each case the presence of coenzyme enhances inactivation by iodoacetate and partially protects against inactivation by iodoacetamide and NEM. The observation that 0.1 mM iodoacetate causes no measurable inactivation of aspartic β-semialdehyde dehydrogenase in the absence of $NADP^+$ is consistent with the results reported by Black and Wright (1955) for low concentrations of iodoacetate; however, further experiments carried out in this laboratory show that higher concentrations (*e.g.*, 1 mM) of iodoacetate completely inhibit the enzyme in the presence and absence of $NADP^+$. This result and the observation that 0.1

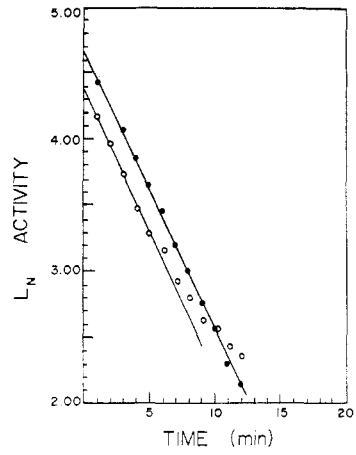

FIGURE 1: Pseudo-first-order rate plot for inactivation of aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase by *N*-ethylmaleimide. The inactivation was carried out at 25° in 10 mM potassium phosphate buffer, pH 6.5, 0.1 mM NEM. The closed circles show the results with 3.2 μM aspartic β-semialdehyde dehydrogenase (specific activity = 73) and the open circles are the data for 3.5 μM glyceraldehyde-3-phosphate dehydrogenase (specific activity = 120). The natural logarithm of activity is plotted *vs.* time.

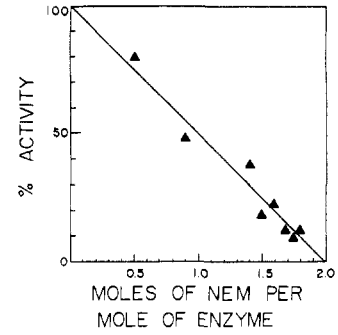

FIGURE 2: Stoichiometry of *N*-ethylmaleimide binding to yeast glyceraldehyde-3-phosphate dehydrogenase. Enzyme was incubated for various times with NEM and the activity and number of moles of bound NEM per mole of enzyme were determined as described in Methods. Inactivations were carried out at 25° in 10 mM potassium phosphate buffer, pH 6.5, 0.1 mM NEM, and 27 μM glyceraldehyde-3-phosphate dehydrogenase (specific activity = 120 units/mg).

mM iodoacetate causes a 50% inactivation of $NADP^+$-bound aspartic β-semialdehyde dehydrogenase indicate that, while this enzyme is less susceptible than glyceraldehyde-3-phosphate dehydrogenase to alkylation with iodoacetate, the difference is not so great as that suggested by Black and Wright (1955).

The accelerated inactivation of glyceraldehyde-3-phosphate dehydrogenase by iodoacetate in the presence of NAD^+ has been confirmed by MacQuarrie (1969). He suggests that the additional positive charge in the active site due to NAD^+ binding causes a stronger attraction for the iodoacetate anion. This argument is supported by the observation that NAD^+ does not enhance inactivation by the neutral inhibitors and the report by Trentham (1968) that NADH, which contains no positive charge, does not accelerate alkylation of glyceraldehyde-3-phosphate dehydrogenase by iodoacetate. The very similar pattern for aspartic β-semialdehyde dehydrogenase inactivation seen in Table I suggests some similarity between the active site of the two proteins.

Kinetics of Inactivation of the Enzymes by N-Ethylmaleimide. Since the rate of inactivation of both dehydrogenases with iodoacetate is complicated by the binding of positively charged coenzymes, the kinetics of inactivation with NEM were determined for each enzyme in the absence of coenzymes in order to compare quantitatively the reactivity of the essential thiol groups. NEM forms thiol esters with reactive sulfhydryl groups quite specifically in the pH range 6.0–7.0 (Riodan and Vallee, 1967).

Figure 1 shows the time course of inactivation of the enzymes with 0.1 mM NEM, plotted as pseudo-first-order reactions. The linear plot obtained up to 90% inactivation of aspartic β-semialdehyde dehydrogenase is consistent with a high degree of specificity for NEM binding and equivalence of the active sites of this enzyme. Protein used in this experiment was not treated with charcoal to remove bound $NADP^+$ since the 280 nm:260 nm absorbance ratio was 1.9. It is unlikely that the enzyme contains bound pyridine nucleotide after purification since Black and Wright (1955) reported that charcoal treatment had no effect on the inactivation of the partially purified enzyme by iodoacetate.

The plot for glyceraldehyde-3-phosphate dehydrogenase is approximately linear to about 65% inactivation but deviates from first-order kinetics beyond that. The enzyme used in this experiment was free of bound NAD^+ as shown by the 280 nm:260 nm absorbance ratio of 2.1. The pseudo-first-order rate constants for NEM inactivation of aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase (based on the linear portion of the curve) are 0.21 and 0.23 min^{-1}, respectively. Although the rate constant for the glyceraldehyde enzyme is less reliable due to nonlinearity of the rate plot, when one considers the wide range of possible rate constants for the titration of thiol groups at the active sites of two different enzymes, the similarity of these two values appears quite significant.

Stoichiometry of N-Ethylmaleimide Binding to Yeast Glyceraldehyde-3-Phosphate Dehydrogenase. An important property of alkyl derivatives formed from NEM is their resistance to acid hydrolysis. Upon acid hydrolysis *S*-(ethylsuccinimido)-cysteine is converted to *S*-succinylcysteine and ethylamine which can be determined accurately with the amino acid analyzer. Yields of 90% or better (Guidotti and Konigsberg, 1964) of *S*-succinylcysteine and ethylamine are obtained when the alkylated protein solutions are evaculated carefully before hydrolysis as described in Methods. The results of *S*-succinylcysteine determinations during the time course of inactivation of glyceraldehyde-3-phosphate dehydrogenase with 0.1 mM NEM are shown in Figure 2. A maximum of 1.7–1.8 cysteines were titrated when 90% of the catalytic activity was lost. Since the specific activity of the enzyme used in this experiment was 120 units/mg and *S*-succinylcysteine for standardization of the amino acid analyzer was prepared by the same method as the protein samples, it appears unlikely that these data contain more than a 5–10% error; in addition, no new species other than *S*-succinylcysteine and ethylamine was observed in the amino acid analysis. These data are surprising in light of the fact that 4 mol of iodoacetate or iodoacetamide is required to completely inactivate rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (MacQuarrie, 1969) and since both rabbit muscle and yeast glyceraldehyde-3-phosphate dehydrogenase are composed of four chemically identical subunits (Perham and Harris, 1963). On the other hand, with another bulky reagent, β-(2-furyl)acryloyl phos-

TABLE II: Amino Acid Analyses for Aspartic β-Semialdehyde Dehydrogenase and One Isozyme of Glyceraldehyde-3-Phosphate Dehydrogenase.[a]

Amino Acid	Glyceraldehyde-3-Phosphate Dehydrogenase		Aspartic β-Semialdehyde Dehydrogenase			
	No. of 24-hr Residues	No. of 48-hr Residues	No. of 24-hr Residues	No. of 48-hr Residues	Mol % GAPDH[b]	Mol % ASADH[c]
Lysine	81	78	85	92	7.9	7.8
Histidine	22	23	26	29	2.1	2.4
Arginine	33	31	44	44	3.2	4.0
Aspartic acid	126	128	126	126	12.2	11.5
Threonine	68	62	52	56	6.6	4.8
Serine	77	63	72	62	7.5	6.6
Glutamic acid	76	64	113	118	7.4	10.4
Proline	45	47	70	64	4.4	6.4
Glycine	82	79	91	98	8.0	8.3
Alanine	108	104	106	103	10.5	9.7
Valine	95	107	75	81	9.2	6.9
Methionine	17				1.6	
Isoleucine	54	57	66	70	5.2	6.0
Leucine	79	76	102	105	7.7	9.3
Tyrosine	33	24	24	22	3.2	2.2
Phenylalanine	34	31	39	38	3.3	3.6
Totals	1030	985	1089	1108		

[a] Reported as nearest integral number of residues per mole of enzyme after 24 and 48 hr of hydrolysis. For each hydrolysis, 1.0 mg of enzyme was hydrolyzed as described in Methods. Calculation of mol % data is described in the text. [b] Glyceraldehyde-3-phosphate dehydrogenase. [c] Aspartic β-semialdehyde dehydrogenase.

phate, MacQuarrie found that only 2 mol of acyl group/mol of rabbit muscle enzyme was required to completely inhibit catalytic activity. The data showed slight deviation from pseudo-first-order kinetics and indicate that the binding of the first acyl group diminishes the binding of the second. Titration of phosphoglyceroyl enzyme (native glyceraldehyde-3-phosphate dehydrogenase incubated with excess 3-phosphoglyceroyl phosphate) with [^{14}C]iodoacetate results in the binding of only 2 mol of [^{14}C]carboxymethyl groups per mol of enzyme. Similarly, it has been shown that there are two pairs of NAD^+ binding sites on yeast glyceraldehyde-3-phosphate dehydrogenase, one of which appears to be primarily responsible for catalytic activity, and that acetylation of the yeast enzyme with [^{14}C]acetyl phosphate in the presence of saturating NAD^+ yields 1.5 mol of bound [^{14}C]acetyl groups per mol of enzyme (Chance and Park, 1967).

The results obtained with NEM binding to yeast glyceraldehyde-3-phosphate dehydrogenase are consistent with the proposal that the tetrameric enzyme is arranged so that the active thiols are organized into two nonequivalent pairs, since only 2 mol of NEM is required to inhibit completely. These data are equally consistent with the possibility that the differences between the active sites arise from conformational changes induced by NEM binding. The latter possibility would be in better accord with the nonlinear rate plot in Figure 1.

Amino Acid Composition of the Enzymes. Amino acid compositions were determined for both enzymes after 24- and 48-hr acid hydrolysis as shown in Table II. Glyceraldeyde-3-phosphate dehydrogenase was a single isozyme whose purification had been described previously (Holland and Westhead, 1973a). The results obtained for that isozyme are in good agreement with the results obtained by Allison and Kaplan (1964) for a three times recrystallized mixture of the yeast glyceraldehyde-3-phosphate dehydrogenase isozymes.

The last two columns of Table II show comparison of the amino acid compositions expressed as mole per cent of the average or best value of the 24- and 48-hr hydrolyses. With the exception of threonine, serine, methionine, valine, and isoleucine, the results calculated from the analysis of 24- and 48-hr hydrolysates varied within 5%. Methionine was completely destroyed after a 48-hr hydrolysis of both enzymes and in the 24-hr hydrolysate for aspartic β-semialdehyde dehydrogenase. Serine and threonine showed 10–20% decomposition from 24- to 48-hr hydrolysis times and, since only 24- and 48-hr hydrolyses were performed, these values were not corrected for decomposition. Valine and isoleucine increased with hydrolysis time and the 48-hr hydrolysate values are reported for these residues. While differences do exist between amino acid compositions of the two enzymes, a number of significant similarities are apparent and should be noted. An overall comparison of the two amino acid compositions shows that the mole per cent values for lysine, histidine, aspartic acid, glycine, alanine, and phenylalanine appear to be identical within experimental error. No comparison of the values for methionine, threonine, or serine could be made since the true mole per cents of these residues were not determined; however, the ratios of threonine to serine for aspartic β-semialde-

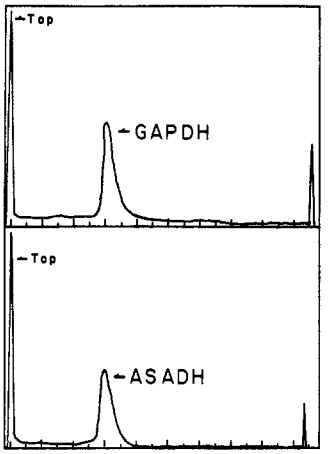

FIGURE 3: Densitometer measurements of the electrophoretic mobilities of aspartic β-semialdehyde dehydrogenase (ASADH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Polyacrylamide disc gel electrophoresis was carried out at pH 8.3 and the gels were scanned as described in Methods.

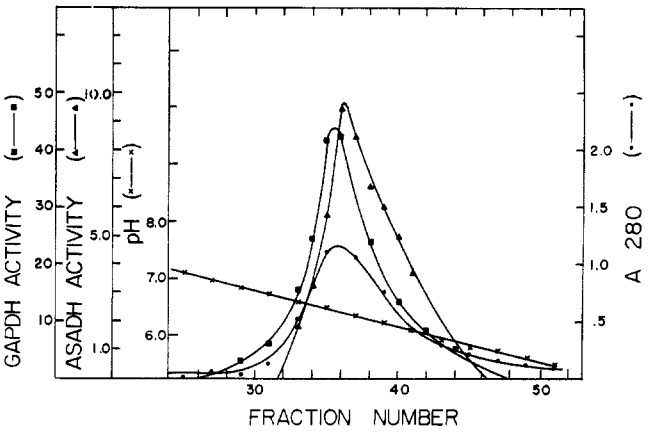

FIGURE 4: Isoelectric focusing pattern for aspartic β-semialdehyde dehydrogenase (ASADH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Isoelectric focusing was carried out with 12.5 mg of a protein sample containing aspartic β-semialdehyde dehydrogenase (specific activity = 8.9 units/mg) and glyceraldehyde-3-phosphate dehydrogenase (specific activity = 100 units/mg), using a pH 5–8 ampholine solution.

hyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase are 0.73 and 0.88, respectively. The values for proline, valine, isoleucine, leucine, arginine, and tyrosine are significantly different for the two enzymes; however, Allison and Kaplan (1964) note that glyceraldehyde-3-phosphate dehydrogenase contains a high proportion of aliphatic side-chain amino acids and it is significant that the mole per cents of the sum of proline, alanine, valine, isoleucine, and leucine for glyceraldehyde-3-phosphate dehydrogenase and aspartic β-semialdehyde dehydrogenase are 37 and 38%, respectively.

Electrophoresis and Isoelectric Focusing. Measurements of the electrophoretic mobilities and isoelectric points of the enzymes were made in order to determine the effect of the differences in amino acid composition of the two enzymes on the overall charge of the proteins. Since asparagine and glutamine were not determined, the differences in polar amino acids between the two enzymes cannot be determined from the amino acid composition.

Figure 3 shows densitometer measurements of the electrophoretic mobilities of the dehydrogenases at pH 8.3. Slight variations in mobilities were observed for both enzymes from experiment to experiment; however, statistically no differences in mobility could be detected between the two enzymes.

Isoelectric points were determined by isoelectric focusing of a mixture of both enzymes with a pH 5–8 ampholine solution. As shown in Figure 4 the isoelectric points for aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase are 6.17 and 6.25, respectively. These values and the values for electrophoretic mobility at pH 8.3 suggest that the composition of charged amino acids for each enzyme is very similar, lending further support for significant similarity between the amino acid compositions of the two dehydrogenases.

Esterase Activities of Aspartic β-Semialdehyde Dehydrogenase and Glyceraldehyde-3-Phosphate Dehydrogenase. The initial velocities of *p*-nitrophenyl acetate hydrolysis catalyzed by the enzymes were compared at pH 8.0 in 10 mM sodium barbital buffer containing 10 mM EDTA and 0.19 μmol of *p*-nitrophenyl acetate as described in Methods. In a typical comparison with glyceraldehyde-3-phosphate dehydrogenase at 0.54 mg/ml and aspartic β-semialdehyde dehydrogenase at 0.68 mg/ml the rates of *p*-nitrophenol release were 9.1 and 5.3 nmol/min, respectively. The spontaneous rate of hydrolysis under these conditions was 1.2 nmol/min. When this value is subtracted from that observed in the presence of enzyme, the net rate of hydrolysis by aspartic β-semialdehyde dehydrogenase is 49% of that catalyzed by glyceraldehyde-3-phosphate dehydrogenase on an equimolar enzyme concentration basis. It is unlikely that hydrolysis due to contaminating protein or nonprotein catalysts contributes to the rates reported since the enzyme preparations employed in these studies were homogeneous and were chromatographed twice on Sephadex G-25 equilibrated with esterase assay buffer.

Comparison of these rates with those reported for other enzymes capable of hydrolyzing *p*-nitrophenyl acetate shows a marked similarity in the ability of both enzymes to carry out this hydrolysis reaction. At equal molar concentrations of enzyme, the relative rates of *p*-nitrophenyl acetate hydrolysis are: chymotrypsin, 1.0; aspartic β-semialdehyde dehydrogenase, 5.0; rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, 6.5; yeast glyceraldehyde-3-phosphate dehydrogenase, 10; horse liver aldehyde dehydrogenase, 250. Data on chymotrypsin and rabbit muscle glyceraldehyde-3-phosphate dehydrogenase are from Park *et al.* (1961) and those on liver aldehyde dehydrogenase from Feldman and Weiner (1972). The fact that all three dehydrogenases are capable of catalyzing the hydrolysis of *p*-nitrophenyl acetate at significant rates relative to chymotrypsin supports the suggestion by Racker (1955) that aldehyde dehydrogenases form a thiohemiacetal as a reactive intermediate. The similarity of the rates of hydrolysis of *p*-nitrophenyl acetate catalyzed by aspartic β-semialdehyde dehydrogenase and yeast and rabbit muscle glyceraldehyde-3-phosphate dehydrogenase further suggests a similarity in the conformation and electronic environment of the intermediate formed by these two enzymes. These data, taken together with the data on sulfhydryl reactivity of the two enzymes reported earlier in this paper, further enhance the argument that aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase might indeed be structurally related enzymes.

Summary

In this paper and the two preceding papers[2] some of the properties of aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase from yeast are described. Although the molecular weights and amino acid compositions of these two dehydrogenases are significantly different, many of their properties suggest that the two enzymes may be closely related. The following is a summary of the properties of aspartic β-semialdehyde dehydrogenase compared with those of glyceraldehyde-3-phosphate dehydrogenase obtained in our laboratory or reported by other laboratories. (a) The enzymes copurify through six purification steps and are only resolved in our scheme on hydroxylapatite with a sulfate gradient. (b) Both enzymes are composed of four identical subunits and the molecular weights of native tetrameric aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate are 156,000 and 144,000, respectively. (c) The amino acid compositions of the two dehydrogenases show only a limited degree of similarity. (d) The migration patterns of the two enzymes on pH 8.3 polyacrylamide gels appear to be identical. The isoelectric points of aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase are 6.17 and 6.25, respectively. (e) Both glyceraldehyde-3-phosphate dehydrogenase (Racker, 1965) and aspartic β-semialdehyde dehydrogenase (Black and Wright, 1955) catalyze an arsenolysis reaction which is inhibited by iodoacetate. (f) Both dehydrogenases undergo a cold-induced ATP-dependent reversible inactivation. (g) Inactivation of both enzymes by the sulfhydryl reagents, iodoacetate, iodoacetamide, and *N*-ethylmaleimide, is strikingly similar. Both enzymes show increased inhibition by iodoacetate in the presence of their respective pyridine nucleotide cofactors. The second-order rate constants for aspartic β-semialdehyde dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase by *N*-ethylmaleimide are 2.1×10^{-5} and 2.3×10^{-3} min^{-1} respectively. (h) Aspartic β-semialdehyde dehydrogenase catalyzes the hydrolysis of *p*-nitrophenyl acetate at a rate equal to 50% that observed for yeast glyceraldehyde-3-phosphate dehydrogenase and 77% that observed for rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (Park *et al.*, 1961).

We think the extensive similarities listed above, in addition to the similarity of the two complex catalytic reactions, show that these enzymes are homologous molecules. The determination of the sequence or crystallographic structure of aspartic β-semialdehyde dehydrogenase should be especially valuable for relating details of enzymic structure to catalytic function.

[2] Holland and Westhead, 1973a,b.

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