THE FORMATION OF A STABLE ALDOLASE-DIHYDROXYACETONE PHOSPHATE COMPLEX

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Evidence for the existence of a covalent complex between the enzyme fructose 1,6-diphosphate aldolase and one of its substrates, dihydroxyacetone phosphate (DHAP), has been presented from a number of laboratories (1-5). Recently we have found (6) that reduction with borohydride stabilizes the complex formed between transaldolase and dihydroxyacetone; a similar result has now been obtained with aldolase and DHAP.

Stabilization of the complex was followed in two ways: (1) formation of radioactive protein with DHAP^{32} as substrate, and (2) loss of enzyme activity. In the second procedure the specific activity of the enzyme was determined before and after treatment with borohydride and compared with controls treated with borohydride in the absence of DHAP. The enzyme activity proved to be stable to borohydride treatment in the absence of DHAP (Table I), provided that pH control was effective during the addition of borohydride.

Calculation of the molar ratio of aldolase to P^{32} in the precipitated protein yielded values which ranged from 0.66 to 1.27 (Table II),

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Table I

Formation of the Reduced Aldolase-Dihydroxyacetone Complex

Experiment	Addition	Reduction with $NaBH_4$	Aldolase Recovered	P^{32} in Protein
			units/mg	cpm/mg
1	$DHAP^{32}$ + aldolase	-	180	2,160
2	aldolase alone	+	174	--
3	$DHAP^{32}$ + aldolase	+	51	23,000

The reaction mixture (2.0 ml) contained 1.2 mg per ml of crystalline muscle aldolase (7), 0.06 M phosphate buffer, pH 6.0, and 3.1×10^{-3} M $DHAP^{32}$ (2.8×10^{6} cpm/μmole) where indicated. Reduction with borohydride was carried out at 0-2° over a period of 30 minutes. Borohydride was added in small aliquots; the total was equivalent to a final concentration of 0.14 M. The solution during this time was maintained at pH 6.0 by the addition of 2 M acetic acid. The total acetic acid added was equivalent to a final concentration of 0.14 M. After reduction the proteins were precipitated with ammonium sulfate (600 mg per ml of reaction mixture), washed twice by centrifugation with 1 ml portions of 80% saturated ammonium sulfate, and dissolved in water. Aliquots were taken for measurement of aldolase activity and for determination of radioactivity.

Aldolase activity was measured at 22-23° in the test system described by Racker (8) with α-glycerophosphate dehydrogenase, triose phosphate isomerase, and DPNH. A unit of aldolase is the quantity giving an optical density change of 1.0 per minute at 340 mμ.

based on a molecular weight of 149,000 (9). This calculation assumes that all of the protein present in the crystalline preparation was enzymatically active aldolase and that no inactivation of aldolase occurred except through reduction of the DHAP complex. Provided that these assumptions are correct, it may be concluded that about one mole of $DHAP^{32}$ is fixed per mole of enzyme. This is of interest since aldolase is considered to contain at least three peptide chains (10).

The use of borohydride reduction to stabilize labile groups on a protein was first reported by Fischer and Krebs (11) and enabled

Table II

Equivalence of P^{32}-Labeling and Loss of Aldolase Activity

Total Protein (1)	Total Protein (2)	Active Aldolase (3)	Reduced Aldolase (4) = (2)-(3)	P^{32}-labeled* Protein (5)	Equivalence** (5)/(4)
mg/ml	μmoles/ml***	μmoles/ml	μmoles/ml	μmoles/ml	
1.97	1.34×10^{-2}	0.05×10^{-2}	1.29×10^{-2}	0.85×10^{-2}	0.66
1.85	0.26×10^{-2}	0.06×10^{-2}	1.20×10^{-2}	0.09×10^{-2}	0.91
1.36	0.92×10^{-2}	0.17×10^{-2}	0.75×10^{-2}	0.70×10^{-2}	0.93
1.86	1.27×10^{-2}	0.19×10^{-2}	1.08×10^{-2}	1.37×10^{-2}	1.27

*Corrected for the radioactivity precipitated in the control experiment without borohydride (see Table I).

**Moles of P^{32} per 149,000 g of reduced aldolase.

***Based on MW = 149,000. The reaction mixtures were as in Table I except that in the first experiment the concentration of $DHAP^{32}$ was 1.55×10^{-3} M.

them to demonstrate that pyridoxal phosphate in phosphorylase was bound as a Schiff's base with an ε-amino group of lysine. The results with dihydroxyacetone and DHAP suggest that similar reducible linkages may be formed with aliphatic carbonyl groups. Our experiments with transaldolase and aldolase demonstrate that the borohydride technique can be extended to other enzymes in order to bring about stabilization of complexes involving the active site of the enzyme.

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