[Chem. Pharm. Bull. 24(11)2647—2650(1976)]

UDC 577.153.02 : 615.355.015.4

Determination of Aldolase Activity in Serum[1]

Mamoru Sugiura, Kazuyuki Hirano,[2a] Hiroko Yoshimura, Masayasu Sugiyama and Hiroshi Shimizu[2b]

Department of Pharmacy, Tokyo College of Pharmacy[2a] and Research Institute, Ono Pharmaceutical Company[2b]

(Received February 14, 1976)

For the application of glycerol dehydrogenase from a strain of bacterial Erwinia aroideae, a method of measuring serum aldolase activity is determined. This method is possible to measure 0—50 mU/ml aldolase. Until now, spectrophotometric assay with glycerol-1-phosphate dehydrogenase and colorimetric determination with 2,4-dinitrophenylhydrazine are available. The former is possible to be affected with serum alkaline phosphatase, the latter needs much serum.

Correlation between spectrophotometric assay with glycerol-1-phosphate dehydrogenase and this method using rabbit serum experimentally increased aldolase activity is shown.

As a series of study for glycerol dehydrogenase (E.C. 1.1.1.6) from a strain of bacterial Erwinia aroideae, the method for the determination of serum alkaline phosphatase using glycerol dehydrogenase as an indicate enzyme has been already discussed.[3] In the present paper, we discuss the assay method for serum aldolase activity using glycerol dehydrogenase and alkaline phosphatase as indicate enzymes.

Increased serum aldolase activity is found in early phase of myocardial infarction,[4] acute hepatitis,[5,6] malignancy,[7] progressive muscular dystrophy,[8] and also experimental ligation of coronary artery,[4] and intravenus injection of the paraffin to the femoral artery of dog.

A number of assay methods for aldolase activity has been reported. In one of them, triosephosphates (dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate) formed from fructose-1,6-diphosphate by the action of aldolase were trapped with hydrazine and measured as hydrazide.[9] In another method, D-glyceraldehyde-3-phosphate was converted by triosephosphate isomerase to dihydroxyacetone phosphate, which was then reduced by glycerol-1-phosphate dehydrogenase in the presence of nicotineamide adenine dinucleotide (NADH) and the decreased absorbance at 340 nm was measured.[10–12]

The principles of this paper are following; fructose-1,6-diphosphate is cleaved into dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate by aldolase. Dihydroxyacetone phosphate is dephosphorylated by alkaline phosphatase to the free dihydroxyacetone. Dihydroxyacetone is reduced to glycerol by glycerol dehydrogenase in the presence of NADH, and the decrease of absorbance at 340 nm is measured.

1) This forms part CXXI of "Studies on Enzymes" by M. Sugiura.
2) Location: *a) Horinouchi, 1432-1, Hachioji city, Tokyo, 192-03, Japan; b) Sakurai, Simamoto-cho, Mishimagun, Osaka, 618, Japan.*
3) H. Shimizu, F. Hirata, M. Sugiura and K. Hirano, The annual Meeting of the Pharmaceutical Society of Japan, 1974 in Sendai.
4) W.V. Bruno and L. Samuel, *Amer. J. Med. Sci.*, **232**, 38 (1956).
5) F.H. Brunus and W. Puls, *Klin. Wchnschr.*, **32**, 656 (1954).
6) F.H. Brunus, *Clin. Chim. Acta.*, **2**, 257 (1957).
7) T. Shiraishi, K. Tsunematsu, H. Yajima, T. Wada and S. Yokota, *Saishin Igaku*, **23**, 1923 (1968).
8) J.A. Sibley and A.L. Lehninger, *J. Nat. Cancer Inst.*, **9**, 303 (1949).
9) J.A. Sibley and A.L. Lehninger, *J. Biol. Chem.*, **177**, 859 (1949).
10) G. Beisenherz and H.J. Baltz, *Z. Naturforsch*, **86**, 555 (1953).
11) D.S. Pari, C. Richard and S. Weinhouse, *J. Biol. Chem.*, **240**, 1327 (1965).
12) B. Rhoda and J.R. William, *J. Biol. Chem.*, **238**, 3280 (1963).

Materials and Methods

Enzyme——Alkaline phosphatase from calf intestine, triosephosphate isomerase, aldolase, lactate dehydrogenase were purchased from Boehringer-Mannheim Corp. Glycerol dehydrogenase was prepared in our labolatory.

Substrate——Fructose-1,6-diphosphate was obtained from Boehringer-Mannheim, DL-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate cyclohexylamine salt from Sigma chemical Co. Ltd., and dihydroxyacetone from Tokyo Kasei Kogyo Co. Ltd. Dihydroxyacetone phosphate cyclohexylamine salt was swirled with Dowex 50 (H^+) resin, and incubated at 40° for 4 hours to hydrolyze the ketal. The reaction mixture was subsequently adjusted to pH 4.5 with potassium bicarbonate and filtrate, and then stored in frozen state.

Coenzyme——NADH was obtained from Boehringer-Mannheim Corp.

Substrate Solution——Fructose-1,6-diphosphate (75 mM), dihydroxyacetone (3 mM), dihydroxyacetone phosphate (3 mM), and DL-glyceraldehyde phosphate (2 mM) were dissolved in distilled water.

Coenzyme Solution——NADH was dissolved in distilled water.

High Aldolase Activity Serum——Male rabbits were injected with 0.2 ml/kg of paraffin to the femoral vein and blood was obtained from ear vein daily after 6 days.

Reagent Solution——Five μmoles of NADH, 35 U of alkaline phosphatese, 30 U of trioseisomerase, 75 μmoles of fructose-1,6-diphosphate, 10 μmoles of iodoacetic acid, and 1 mmole of ammonium sulfate were dissolved with 30 ml of 0.1 M Tris-HCl buffer (pH 8.0). This reagent solution was prepared before use.

Standard Assay for Aldolase Activity——Two tenths milliliters of serum was added to 3 ml of the reagent solution, and preincubated for 15 min at 37° and absorbance was measured at 340 nm. Then, 20 μl of glycerol dehydrogenase was added, and after incubation for another 10 min, absorbance was measured again at 340 nm, as shown in chart 1. Aldolase activity was expressed the difference of absorbance at 340 nm.

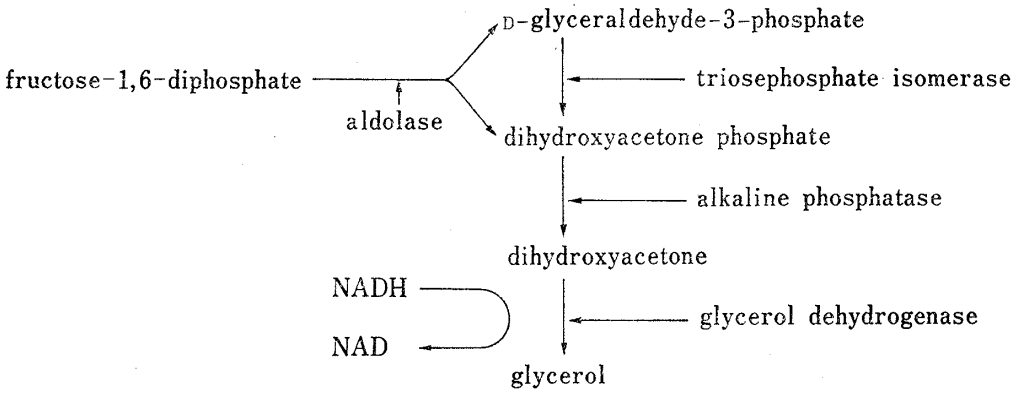

Chart 1. Procedure for the Determination of Aldolase Activity

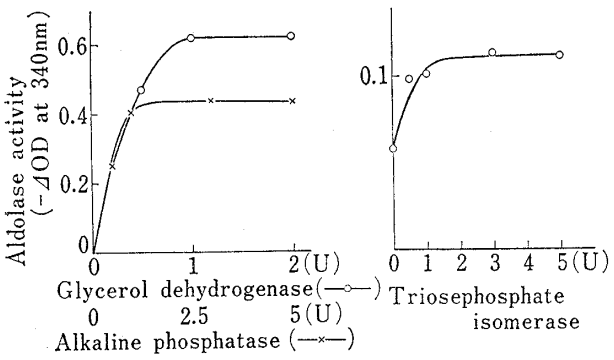

Fig. 1. Optimum Amount of Enzyme

Results

Optimum Amount of Enzymes

Optimum amounts of enzyme, substrate, coenzyme were investigated. After incubation at 37° for 15 min using 0.3 μmoles of dihydroxyacetone as a substrate according to the standard assay system, the absorbance decreased at 340 nm was examined. As shown in Fig. 1, it was found that the amount of glycerol dehydrogenase, alkaline phosphatase, and trioseisomerase were enough to obtain the maximal constant value of aldolase activity above 1U, 3U, 1U, respectively, in the reaction mixture.

Specificity of Glycerol Dehydrogenase for Substrate

As shown in Table I, on the assumption that the relative activity of glycerol dehydrogenase was 100 percent when the substrate was dihydroxyacetone, the activity level of glycerol

dehydrogenase was 9 percent with the substrate of DL-glyceraldehyde phosphate, while the activity was 20 percent under the coexistence of glycerol dehydrogenase and alkaline phosphatase.

TABLE 1. Specificity of Glyceroldehydrogenase for the Substrate in the Reaction Mixture

Enzyme	Relative activity (%)	
	Dihydroxyacetone	DL-Glyceraldehyde phosphate
Glycerol dehydrogenase	100	9
Glycerol dehydrogenase+Alkaline phosphatase	100	20

Effect of Ammonium Sulfate and Iodoacetic Acid on the Aldolase Activity

It was known that glycerol dehydrogenase was activated by ammonium sulfate. It was found that the amount of glycerol dehydrogenase required in our assay system was reduced to only 0.5U in the presence of 0.1 mmole ammonium sulfate. Iodoacetic acid, which was inhibitor of serum glyceraldehyde phosphate dehydrogenase, was added to the standard assay system to avoid glyceraldehyde dehydrogenase action on glyceraldehyde phosphate derived from fructose-1,6-diphosphate. It was found that iodoacetic acid had no effect on the determination of aldolase activity and iodoacetic acid.

Optimum pH

Optimum pH for the determination of aldolase activity was investigated. Aldolase was most active at pH 8.0 in Tris buffer. While optimum pHs of glycerol dehydrogenase, trioseisomerase lie between pH 7—8, and that of alkaline phosphatase lies between 8—10. As shown in Fig. 2, aldolase was more active in colidine, veronal and tris buffers than in borate and phosphate buffers.

Effect of Glycerol on the Assay System

Effect of glycerol on the determination of aldolase activity was investigated, and it was found that with increasing amounts of glycerol the aldolase activity was slightly decreased, but at the normal serum glycerol value, 1 mg/dl, the effect on the aldolase activity was negligible, as shown in Fig. 3.

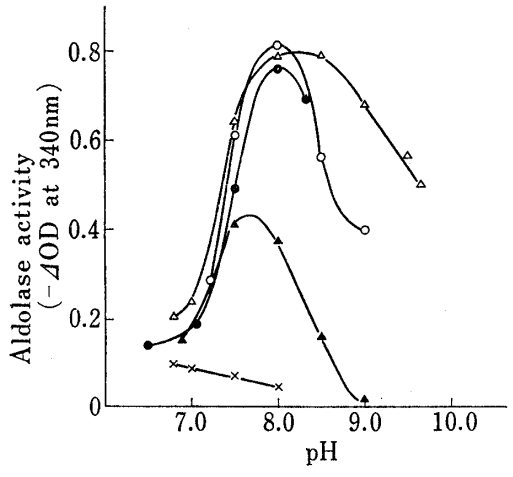

Fig. 2. pH- Activity Curve for Aldolase by Proposed Method

—●—: 0.1M collidine buffer
—△—: 0.1M veronal buffer
—○—: 0.1M Tris buffer
—▲—: 0.1M borate buffer
—×—: 0.1M phosphate buffer

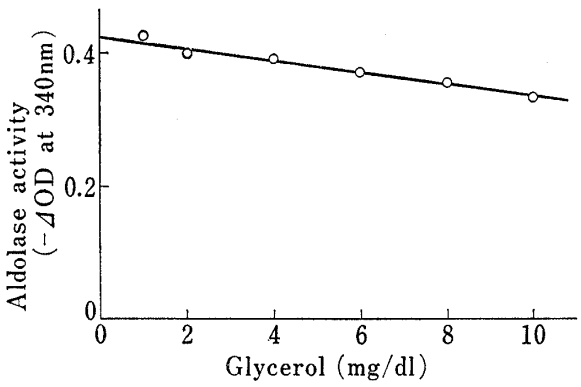

Fig. 3. Effect of Glycerol on the Standard Assay System

Calibration Curve of Aldolase

Calibration curve of aldolase was drew up under the condition of existence of aldolase. It gave a straight line to the extent of 50 mU/ml and also obtained a similar straight line in case of coexistence of human serum. It seems that a value of human serum at the 0 mU/ml point of aldolase activity was serum aldolase activity as shown in Fig. 4.

Correlation

The correlation between glyceraldehyde phosphate dehydrogenase method and the proposed method was examined using 6 cases of normal rabbit sera and 10 rabbit sera with rather high aldolase activities. The results is present in Fig. 5, the regression line calculated is shown ($Y=0.94X+19.3$). The correlation coefficient was 0.874.

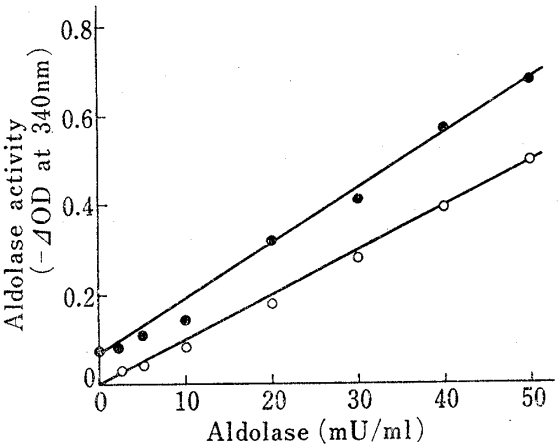

Fig. 4. Calibration Curve for Aldolase Activity

—○—: standard aldolase
—●—: standard aldolase+human serum

$r=0.874$
$Y=0.94X+19.3$
$N=16$
$X=17.8$
$Y=36.1$

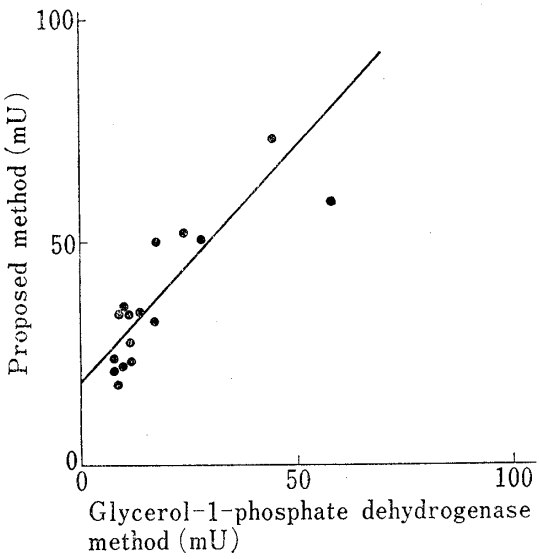

Fig. 5. Correlation between Glycerol-1-phoshate Dehydrogenase Method and Proposed Method in Rabbit Serum

Discussion

The standard curve was linear for the value of aldolase up to the 50 mU/ml. This value was well above the upper range of normality. Since alkaline phosphatase and glycerol dehydrogenase act on DL-glyceraldehyde phosphate and it was resulted in decreasing NADH, it seems that glycerol dehydrogenase has less specificity for substrate.

The colorimetric method with 2,4-dinitrophenylhydrazine will need 1.0 ml of the serum, and glycerol-1-phosphate dehydrogenase method will make serum alkaline phosphatase act on dihydroxyacetone phosphate derived from fructose-1,6-diphosphate. The correlation between glycerol-1-phosphate dehydrogenase and glycerol dehydrogenase method was examined using rabbit sera. The value from glycerol dehydrogenase method is apt to be higher than that from glycerol-1-phosphate dehydrogenase method.