AUGMENTATION OF ALDOLASE ACTIVITY BY GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE*

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Recently, quantitative changes in the activity of certain enzymes by interaction with other proteins have been reported (Litvinenko, 1960; Gulyi, 1960; Gulyi et al., 1962; Sereda, 1963). In particular, the aldolase activity of myogen A was increased in the presence of added glyceraldehyde-3-phosphate (GAP) dehydrogenase (EC 1·2·1·12; D-glyceraldehyde-3-phosphate: NAD oxidoreductase (phosphorylating)). We have now observed an enhanced activity of aldolase (EC 4·1·2·7; Ketose-1-phosphate aldehyde-lyase) with added GAP dehydrogenase preparations under conditions in which the latter was not enzymatically active.

Aldolases were prepared from rabbit and yellowfin tuna (Neothunnus macropterus) muscle following the method described by Taylor et al. (1948). Both preparations gave a single peak during boundary electrophoresis and during ultracentrifugation (Kwon and Olcott, 1965). GAP dehydrogenases from rabbit muscle (Sigma Chemical Co.) and from tuna muscle, prepared according to Velick (1955), were used. Fructose-1,6-diphosphate sodium salt (FDP) and β-nicotinamide adenine dinucleotide (NAD) were obtained from Sigma Chemical Co. Protein concentrations were determined by the optical method of Warburg and Christian (1941) for the dehydrogenases and of Baranowski and Niederland

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(1949) for the aldolases. Aldolase activity was determined by two different methods as described below.

The activity of tuna aldolase was determined by the hydrazine method (Jagannathan et al., 1956). Upon the addition of rabbit GAP dehydrogenase to the reaction mixture, the absorbance at 240 mμ increased at a faster rate, indicating enhanced formation of triose hydrazone (Fig. 1).

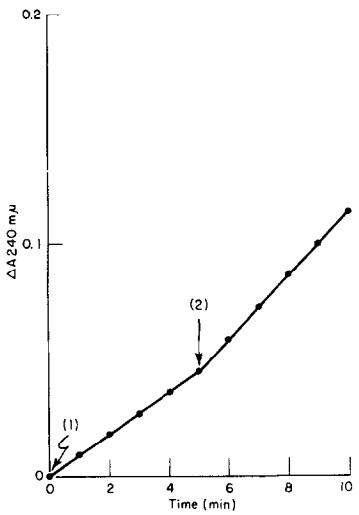

Fig. 1. Effect of rabbit GAP dehydrogenase on tuna aldolase activity. The test solution containing 1.5 ml of 10^{-3} M EDTA, 1 ml of 1.2 x 10^{-2} M FDP, and 0.5 ml of 1.4 x 10^{-2} M hydrazine in a 3 ml cuvette was incubated for 5 min at 25° and pH 7.4. Eleven μg of tuna aldolase was added at ↓(1) and 15 μg of rabbit GAP dehydrogenase at ↓(2). Readings were made with a Beckman DB Spectrophotometer in a 1 cm cell. Corrections were made for absorbances of the components of the reaction mixture at 240 mμ.

In other experiments the aldolase activity of the mixture was 1.6 times as active as that of aldolase alone immediately after mixing but double at 90 min at room temp. There was no additional enhancement of activity after 5 hrs. The rabbit GAP dehydrogenase had no aldolase activity.

The activity was also determined by measuring the amounts of alkali-labile phosphate formed without KCN trapping. 0.3 ml 10^{-2} M FDP and 0.1 ml tuna aldolase (40 μg) in 0.1 M glycine buffer, pH 9,

were incubated at 30° for 2 min, with or without tuna GAP dehydrogenase preparation and other additives. Alkali-labile phosphate was determined by the method of Fiske and Subbarow (1925). Under these conditions the amounts of alkali-labile phosphate produced were linear with time and with the amount of enzyme added. This method is not sensitive but it allows true equilibrium of the aldolase-catalyzed reaction. With different amounts of tuna GAP dehydrogenase preparation in the reaction mixtures, increased activities were again obtained (Fig. 2). The dehydrogenase preparation (350 µg) alone had no aldolase activity.

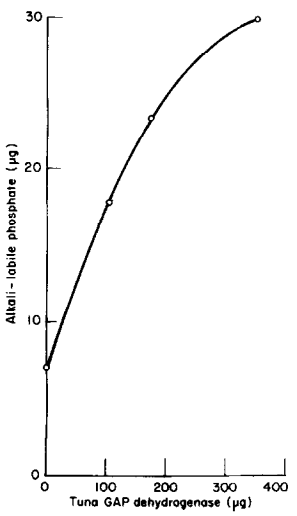

Fig. 2. Effect of amounts of tuna GAP dehydrogenase on tuna aldolase activity of the mixture. Experimental conditions: see text.

When the dehydrogenase was heated for 1 min at 80° before addition to the aldolase assay mixture, the activity, although greatly reduced, was still higher than that of the system containing aldolase alone. After standing for 4 additional hrs at room temp, the heated dehydrogenase no longer enhanced aldolase activity (Table 1). Thus, the native structure of this protein is required for the enhancement, in accord with the suggestion of Gulyi (1960).

Table 1. Effect of heat treatment of GAP dehydrogenase on the aldolase activity of tuna aldolase - GAP dehydrogenase mixture.*

Aldolase (μg)	GAP dehydrogenase (μg)	FDP (μmole)	Alkali-labile P produced (μg)
40	350	3	34.1
40	350**	3	21.7
40	-	3	9.3

* This experiment was conducted after preincubation of the enzyme mixtures at room temp for 15 min.
** Heated for 1 min at 80°; after an additional 4 hrs at room temp, the value was the same as that of the control system containing aldolase alone.

The cofactors required for GAP dehydrogenase activity are NAD and inorganic phosphate or arsenate. In order to obtain further evidence that the enhancement of aldolase activity by GAP dehydrogenase is due to protein-protein interaction rather than removal of one of the end-products, studies were conducted under conditions in which the GAP dehydrogenase was at maximum activity. The system containing NAD and arsenate showed the highest apparent aldolase activity (Table 2). In this particular experiment, the removal of GAP from the equilibrium reaction system by the dehydrogenase enhanced the apparent aldolase activity by an additional 80% of the original value. These data report activities in terms of percentages of a control run at the same time; they are not directly comparable with those of Table 1 since the preparations were not run at the same time. GAP dehydrogenases are unstable in the absence of cysteine and tuna muscle aldolase is more unstable than rabbit muscle aldolase (Kwon and Olcott, 1965).

The Michaelis constant, Km, for rabbit aldolase as reported for different methods varies by a factor of about 100 (Rutter, 1961) (10^{-3} M, by chemical trapping methods; 6×10^{-5} M, by enzymic coupling methods).

Table 2. Effect of NAD and arsenate on the apparent aldolase activity of tuna aldolase - GAP dehydrogenase mixture.

Aldolase (μg)	GAP dehydrogenase (μg)	FDP (μmole)	NAD (μmole)	Arsenate (μmole)	Alkali-labile P produced* (μg)	Apparent Activity (%)
40	350	3	0.5	17	29.5	380
40	350	3	-	-	23.5	300
40	-	3	-	-	7.8	100

* Reaction mixtures were treated with $NaHSO_3$ at the end of the reaction in order to eliminate the interference of arsenate (Pett, 1933) in the phosphate determination.

It may be that some of these differences are due to the presence or absence of secondary proteins such as GAP dehydrogenase and their cofactors, as well as to the presence or absence of trapping agents.

The problem of specificity with regard to aldolase-enhancing activity requires further study. Gulyi (1960) showed that hemoglobin had no such activity. In the present work neither bovine serum albumin nor myoglobin changed aldolase activity when present at ten times the level of the aldolase. However, other proteins may influence aldolase in the same manner as does GAP dehydrogenase. For example, α-glycerophosphate dehydrogenase (EC 1·1·1·6; glycerol: NAD oxidoreductase) is reported to activate aldolase (Sereda, 1963); and Dahlquist and Crane (1964) showed that the removal of triose-phosphate isomerase (EC 5·3·1·1; D-glyceraldehyde-3-phosphate ketol-isomerase) decreased the apparent aldolase activity of a rabbit liver preparation against FDP but did not affect the activity against fructose-1-phosphate. Thus the enhancement phenomenon may be limited to protein-protein interactions in which the proteins are localized favorably in the cell, such as are the enzymes of the glycolytic cycle. The study of the effect of other proteins and enzymes on aldolase activity is being continued.

The precise mechanism for the protein-protein interaction is not

clear. However, the interactions by which enzymatic activities of the participating proteins can be augmented or reduced may provide an important mechanism through which the rates of metabolism of the substrates are regulated and controlled in vivo.

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