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THE MECHANISM OF ACTION OF RABBIT MUSCLE α-GLYCEROPHOSPHATE DEHYDROGENASE

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SUMMARY

1. It was demonstrated enzymatically, chemically, as well as with paper chromatographic methods that α-glycerophosphate dehydrogenase catalyzes the formation of both dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate from L-α-glycerophosphate. The pathway of the reaction is pH dependent.

2. The oxidation of L-α-glycerophosphate has a double pH optimum in Tris buffer (pH 8.6 and 9.4).

3. The possible mechanisms of oxidation of L-α-glycerophosphate, the participation of an optically inactive intermediate or the consecutive formation of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate, are discussed.

INTRODUCTION

MEYERHOF[1] as well as VON EULER *et al.*[2] demonstrated that α-glycerophosphate dehydrogenase (L-glycerol-3-phosphate:NAD^+ oxidoreductase, EC 1.1.1.8) catalyzes the oxidation of α-glycero-*P* and the product of this process is D-glyceraldehyde-3-*P*. The experiments were performed with purified muscle homogenate at pH 7.

BARANOWSKI[3] isolated the first crystalline α-glycero-*P* dehydrogenase and demonstrated that the crystalline enzyme catalyzes the reduction of dihydroxyacetone-*P* at pH 7. BARANOWSKI[3] described the following reaction as the specific one catalyzed by α-glycero-*P* dehydrogenase.

$$\alpha\text{-Glycero-}P + NAD^+ \rightleftharpoons \text{dihydroxyacetone-}P + NADH + H^+ \quad (1)$$

No mention has been made concerning the contradiction between the findings of BARANOWSKI[3] and MEYERHOF[1] or VON EULER *et al.*[2]. Since their reports were published only Reaction 1 has been studied and the confirmation or denial of MEYERHOF's results remained an unsettled question.

Our experiments show that α-glycero-*P* dehydrogenase catalyzes the reaction between α-glycero-*P* and both dihydroxyacetone-*P* and glyceraldehyde-3-*P*. The pathway of the reaction depends on pH. In agreement with MEYERHOF[1] and VON EULER *et al.*[2], we found that at about pH 7 the following reaction dominates:

$$\alpha\text{-Glycero-}P + NAD^+ \rightleftharpoons \text{glyceraldehyde-3-}P + NADH + H^+ \quad (2)$$

At about pH 10, Reaction 2 cannot be shown and only dihydroxyacetone-*P* is formed from α-glycero-*P*. Between pH 7 and 10 both reactions can be demonstrated.

MATERIALS AND METHODS

L-α-*Glycerophosphate dehydrogenase* was isolated from rabbit skeletal muscle and purified as described earlier[4]. The molecular weight was taken to be 78 000. Each preparation was controlled carefully in regard to possible isomerase contamination of α-glycero-*P* dehydrogenase. In order to test isomerase activity in the enzyme preparations, solutions of known concentration of glyceraldehyde-3-*P* were incubated for 15 min with 7 to 350 times the amount of α-glycero-*P* dehydrogenase used for the rate measurements, without addition of coenzyme. After incubation the solutions were boiled for 5 min. The concentration of glyceraldehyde-3-*P* in the supernatants was then measured with D-glyceraldehyde-3-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate:NAD^+ oxidoreductase (phosphorylating), EC 1.2.1.12) in the presence of arsenate (Table I).

TABLE I

EXAMINATION OF ISOMERASE CONTAMINATION OF α-GLYCERO-*P* DEHYDROGENASE PREPARATIONS

Preincubation in 0.1 M glycine buffer (pH 8.5), at room temperature for 15 min. After heat treatment the mixtures were centrifuged and the glyceraldehyde-3-*P* content of the supernatants was determined with glyceraldehyde-3-*P* dehydrogenase in the following reaction mixture: NAD^+ = 1.7 μmoles/ml; Na_2HAsO_4 = 0.1 μmole/ml; glyceraldehyde-3-*P* dehydrogenase = $2.1 \cdot 10^{-1}$ μmole/ml, in 0.1 M glycine buffer (pH 8.5).

α-Glycero-P dehydrogenase preparations	*Glyceraldehyde-3-P solutions (10^2 μmoles/ml added)*	*Glyceraldehyde-3-P content found after heat treatment (10^2 μmoles/ml)*			
		No addition	*α-Glycero-P dehydrogenase 60 μg/ml added*	*Aldolase 60 μg/ml added*	*Isomerase 0.01 μg/ml added*
No. 1	3.85	1.96	1.90	—	—
	3.05	1.19	1.01	1.02	—
No. 2	7.70	4.10	3.95	—	0.22
	5.00	2.99	2.78	—	0.16

The heat treatment itself caused a lowering of the concentration of glyceraldehyde-3-*P* by about 50%. The concentration of the solutions incubated with α-glycero-*P* dehydrogenase did not differ appreciably from that of the heat-treated control. If the mixtures contained added isomerase, a great loss of glyceraldehyde-3-*P* was found, it having been converted into dihydroxyacetone-*P* according to the equilibrium concentrations, *i.e.* the ratio of glyceraldehyde-3-*P* to dihydroxyacetone-*P* is 4:96. This result shows that neither α-glycero-*P* dehydrogenase nor glyceraldehyde-3-*P* dehydrogenase contained detectable isomerase impurity.

D-*Glyceraldehyde-3-phosphate dehydrogenase* was isolated from pig muscle and recrystallized 4 times according the method of ELÖDI AND SZÖRÉNYI[5].

Aldolase was isolated from rabbit muscle and recrystallized 3 times as described by TAYLOR, GREEN AND CORI[6].

Triosephosphate isomerase was isolated from calf muscle and recrystallized 2 times according to the technique of BEISENHERZ[7].

D-*Glyceraldehyde 3-phosphate* was prepared from fructose 1,6-diphosphate (Reanal), according to the procedure of SZEWCZUK *et al.*[8]. The preparations were of 55 to 95% purity. Inorganic phosphate was shown to be an impurity; no detectable amount of dihydroxyacetone-*P* was present in the preparations.

Dihydroxyacetone phosphate was prepared from glyceraldehyde-3-*P* with triosephosphate isomerase. Glyceraldehyde-3-*P* was incubated with isomerase (pH 7.5) for 30 min, then the pH of the mixture was adjusted to pH 3 and the denatured protein was removed by centrifugation.

NAD^+ and NADH were Boehringer and Reanal preparations, respectively. *2,4-Dinitrophenylhydrazin* and *hydrazinsulfate* were reagent grade.

Methods used for the determination of enzymic activity of glycero-*P* dehydrogenase, glyceraldehyde-3-*P* dehydrogenase and aldolase were those of TELEGDI[4], KELETI AND TELEGDI[9], and SWENSON AND BOYER[10], respectively.

Paper chromatography of triosephosphates was performed in an ethylacetate–acetic acid–water (3 : 3 : 1, by vol.) solvent mixture, on Whatman 3 MM paper. Ascending chromatography was performed for 16 h. The spots were developed with ammonium molybdate at 50° during 30 min.

RESULTS

Glyceraldehyde-3-P demonstrated as the substrate of α-glycero-P dehydrogenase

As shown in Fig. 1, the oxidation of NADH could be observed in the presence of isomerase-free α-glycero-*P* dehydrogenase using glyceraldehyde-3-*P* as a substrate as well as dihydroxyacetone-*P* (Fig. 1).

The reaction with glyceraldehyde-3-*P* was supposed to be a reduction, similar to the reduction of dihydroxyacetone-*P* (Eqn. 1), resulting in the formation of α-glycero-*P* (Eqn. 2).

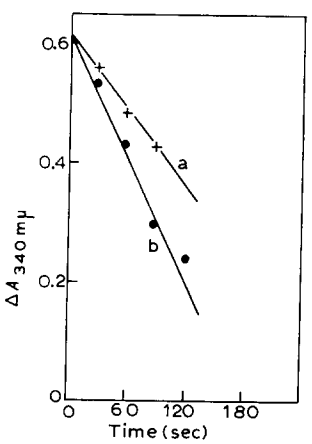
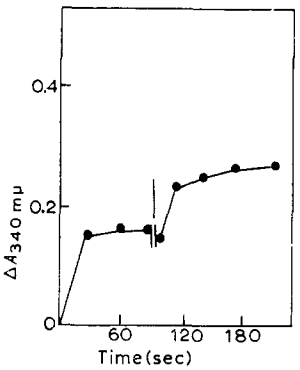


Fig. 1. Oxidation of NADH by glyceraldehyde-3-*P* in the presence of α-glycero-*P* dehydrogenase. In 0.1 M phosphate buffer (pH 8.0). Glyceraldehyde-3-*P* = $4.0 \cdot 10^{-1}$ μmole/ml; NADH = $1.76 \cdot 10^{-1}$ μmole/ml; dihydroxyacetone-*P* = $4.0 \cdot 10^{-1}$ μmole/ml. (a) Reduction of glyceraldehyde-3-*P*; α-glycero-*P* dehydrogenase = $2.5 \cdot 10^{-6}$ μmole/ml. (b) Reduction of dihydroxyacetone-*P*; α-glycero-*P* dehydrogenase = $1.5 \cdot 10^{-6}$ μmole/ml.

The reverse reaction was measured indirectly as follows: α-glycero-*P* and NAD^+ were incubated in the presence of α-glycero-*P* dehydrogenase until equilibrium was reached, then glyceraldehyde-3-*P* dehydrogenase was added. Fig. 2 shows that one of the products of α-glycero-*P* oxidation is glyceraldehyde-3-*P*, since the addition of glyceraldehyde-3-*P* dehydrogenase is followed by further reduction of NAD^+.



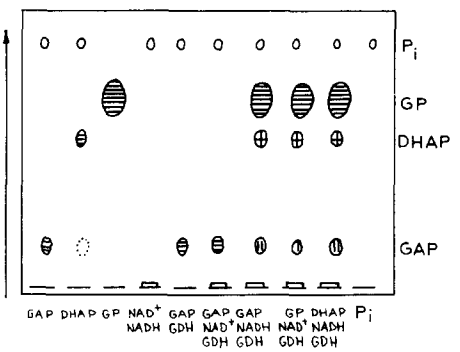


Fig. 2. Glyceraldehyde-3-*P* formation from α-glycero-*P*, on the action of α-glycero-*P* dehydrogenase. In 0.1 M glycine buffer (pH 8.5), in the presence of $1.2 \cdot 10^{-4}$ μmole/ml α-glycero-*P* dehydrogenase. α-Glycero-*P* = $5 \cdot 10^{-1}$ μmole/ml; NAD^+ = 1.7 μmoles/ml. When the equilibrium was reached, glyceraldehyde-3-*P* dehydrogenase and Na_2HAsO_4 were added (see arrow) in a final concentration of $1 \cdot 10^{-3}$ M.

Fig. 3. Demonstration of the reaction products by paper chromatography. The reactions were measured in 0.1 M glycine buffer (pH 8.5). Concentration of components in μmoles/ml: glyceraldehyde-3-*P* (GAP) = $1.3 \cdot 10^2$; dihydroxyacetone-*P* (DHAP) = 8.8; NAD^+ = 20; NADH = 7.8; α-glycero-*P* dehydrogenase (GDH) = $2.5 \cdot 10^{-2}$. GP stands for L-α-glycerophosphate. Aliquots of 0.02 ml were used for chromatography. The equilibrium of the enzymatic reaction was controlled. Chromatography was carried out in ethyl acetate–acetic acid–water (3:3:1, by vol.) for 16 h and developed in ammonium molybdate. The paper was dried at 50° for 30 min.

Chromatography of the products appearing in the reaction catalyzed by α-glycero-P dehydrogenase

An unambiguous proof of the glyceraldehyde-3-*P* $\rightleftharpoons$ α-glycero-*P* reaction catalyzed by α-glycero-*P* dehydrogenase could be given by the indentification of the reaction products.

Dihydroxyacetone-*P*, glyceraldehyde-3-*P* and α-glycero-*P* can be separated by paper chromatography in ethyl acetate–acetic acid–water solvent mixture. This made possible the demonstration of the end-products of the α-glycero-*P* dehydrogenase reaction. Using this method 0.15 μg phosphate per cm^2 gives a visible spot.

Fig. 3 shows the chromatograms of different equilibrium mixtures and of the control solutions.

The following results were obtained:

(a) Each equilibrium mixture where the enzymic reaction was observed contained α-glycero-*P*, dihydroxyacetone-*P* and glyceraldehyde-3-*P*, independently of the substrate used for the reaction. This shows that at least two processes take place simultaneously.

(b) Reaction with both dihydroxyacetone-*P* and glyceraldehyde-3-*P* in the

presence of α-glycero-*P* dehydrogenase takes place only if NADH is added to the reaction mixture.

(c) The chromatogram confirms that the glyceraldehyde-3-*P* used is free from a detectable amount of dihydroxyacetone-*P* and α-glycero-*P* dehydrogenase is free from a detectable amount of isomerase.

Equilibrium concentrations of the products

The changes in absorption measured at 340 mμ give the amount of NADH formed during the reaction. This is equal to the sum of glyceraldehyde-3-*P* and dihydroxyacetone-*P*. To obtain the individual concentrations of the triosephosphates, independent methods must be used. For this purpose the method of SIBLEY AND LEHNINGER[11], elaborated for the measurement of aldolase activity, was adopted with slight modification and combined with the measurement of NADH concentration at 340 mμ. The original procedure made possible the determination of the dinitrophenylhydrazones of the mixed triose phosphates showing a broad absorption around 500 mμ.

We have found that this method may be used for the separate determination of the triose phosphates from the same reaction mixture.

We determined the molar extinction coefficient of the dinitrophenylhydrazones in the following way.

To a solution of triose phosphate, 0.5 ml hydrazinsulphate (0.5 M) and water up to 2 ml were added. The mixture was incubated for 5 min at room temperature. (Hydrazin increases the intensity of colour making the measurements more accurate.) After incubation 0.5 ml of 0.005 M dinitrophenylhydrazine in 2 M HCl was added and the mixture was incubated for 20 min. Then 4.5 ml NaOH–methylcellosolve mixture (1 M NaOH–methylcellosolve, 2:3, by vol.) were added. After an incubation of 60 min the absorption was measured at 550 mμ, where the products of both dihydroxyacetone-*P* and glyceraldehyde-3-*P* have a wide absorption band. During the incubation there is a slow decrease in the absorption at 550 mμ, however the values measured after 60 min are quite reproducible. The blank is the same mixture without triose phosphate, and is treated in the same manner. The molar extenction coefficients of the dinitrophenylhydrazones at 550 mμ are: for glyceraldehyde-3-*P*, $1.19 \cdot 10^4$ and for dihydroxyacetone-*P*, $7.1 \cdot 10^3$. If dihydroxyacetone-*P* and glyceraldehyde-3-*P* of known concentrations are mixed or are in a reaction mixture, their sum can be measured by Warburg's optical test using α-glycero-*P* dehydrogenase and by the dinitrophenylhydrazine test. The individual concentrations can then be determined with the following equations:

$$[\text{dihydroxyacetone-}P + \text{glyceraldehyde-3-}P] = \frac{\Delta A_{340\ m\mu}}{6.22 \cdot 10^3} \qquad (3)$$

where $6.22 \cdot 10^3$ is the molar extinction coefficient of NADH at 340 mμ, and:

$$7.1 \cdot 10^3[\text{dihydroxyacetone-}P] + 1.19 \cdot 10^4[\text{glyceraldehyde-3-}P] = A_{550\ m\mu} \qquad (4)$$

Table II shows some analyses done with this method.

This method was used for the determination of the equilibrium concentrations of glyceraldehyde-3-*P* and dihydroxyacetone-*P* in reactions catalyzed by α-glycero-*P* dehydrogenase. If the reaction were started with α-glycero-*P*, Eqns. 3 and 4 could be

TABLE II

CONCENTRATION OF DIHYDROXYACETONE-*P* AND GLYCERALDEHYDE-3-*P* DETERMINED IN THEIR MIXTURES

Mixture of glyceraldehyde-3-P and dihydroxyacetone-P	*Determination with dinitrophenylhydrazine test*		*Determination with glyceraldehyde-3-P dehydrogenase and glyceraldehyde-3-P dehydrogenase + isomerase, respectively*	
Glyceraldehyde-3-P + dihydroxyacetone-*P* 10^{-5} *M*	Glyceraldehyde-3-*P* 10^{-5} M	Dihydroxyacetone-*P* 10^{-5} M	Glyceraldehyde-3-*P* 10^{-5} M	Dihydroxyacetone-*P* 10^{-5} M
5.2	3.55	1.67	3.60	1.61
4.75	2.39	2.34	2.42	2.30
4.30	1.07	3.20	1.05	3.30

used. If dihydroxyacetone-*P* or glyceraldehyde-3-*P* was used as substrate, Eqn. 3 was replaced by:

$$[\text{dihydroxyacetone-}P] + [\text{glyceraldehyde-3-}P] = ([\text{dihydroxyacetone-}P]_0 + [\text{glyceraldehyde-3-}P]_0) - \frac{\Delta A_{340\ m\mu}}{6.22 \cdot 10^3} \quad (5)$$

where $[\text{dihydroxyacetone-}P]_0$ and $[\text{glyceraldehyde-3-}P]_0$ are the original concentrations.

Table III shows the effect of pH on the composition of the equilibrium mixture of α-glycero-*P* dehydrogenase. The composition of the equilibrium mixture depends on pH. Around pH 7 the mixture contains glyceraldehyde-3-*P* and α-glycero-*P* but no detectable amount of dihydroxyacetone-*P* is present. Around pH 10 it contains α-glycero-*P* and dihydroxyacetone-*P*, but glyceraldehyde-3-*P* cannot be detected.

TABLE III

CONCENTRATION OF DIHYDROXYACETONE-*P* AND GLYCERALDEHYDE-3-*P* IN EQUILIBRIUM MIXTURES AT DIFFERENT pH VALUES

α-Glycero-*P* dehydrogenase = $2.0 \cdot 10^{-4}$ μmole/ml; $NAD^+ = 1.2 \cdot 10^{-2}$ M; in 0.1 M glycine buffer. Total volume 3.0 ml. The equilibrium was tested in samples diluted 15 × at 340 mμ. For the dinitrophenylhydrazine reaction 1-ml aliquots were taken from the original mixture.

pH	*Original concns. (M)*	*Concns. in the equilibrium mixtures (M)*		
	α-Glycero-P	*α-Glycero-P*	*Dihydroxyacetone-P*	*Glyceraldehyde-3-P*
7.10	$6.60 \cdot 10^{-3}$	$6.56 \cdot 10^{-3}$	—	$4.20 \cdot 10^{-5}$
7.80*	$2.40 \cdot 10^{-2}$	$2.32 \cdot 10^{-2}$	$3.60 \cdot 10^{-4}$	$2.10 \cdot 10^{-5}$
8.10*	$1.20 \cdot 10^{-2}$	$1.10 \cdot 10^{-2}$	$3.00 \cdot 10^{-4}$	$1.40 \cdot 10^{-5}$
8.90	$1.20 \cdot 10^{-2}$	$1.09 \cdot 10^{-2}$	$2.90 \cdot 10^{-4}$	$1.80 \cdot 10^{-5}$
9.60	$1.20 \cdot 10^{-2}$	$1.14 \cdot 10^{-2}$	$6.10 \cdot 10^{-4}$	—
9.80	$1.20 \cdot 10^{-2}$	$1.15 \cdot 10^{-2}$	$5.00 \cdot 10^{-4}$	—
10.00	$1.20 \cdot 10^{-2}$	$1.16 \cdot 10^{-2}$	$4.10 \cdot 10^{-4}$	—

* $NAD^+ = 6.6 \cdot 10^{-2}$ M

Between these pH values both triose phosphates are found in the equilibrium mixture. The equilibrium is far to the side of α-glycero-*P* formation at any pH value.

Double pH optimum of oxidation of α-glycero-P

The pH dependence of the oxidation of α-glycero-*P* was measured in 0.1 M glycine buffer and in 0.1 M Tris buffer (Fig. 4a). While the optimum pH in glycine buffer

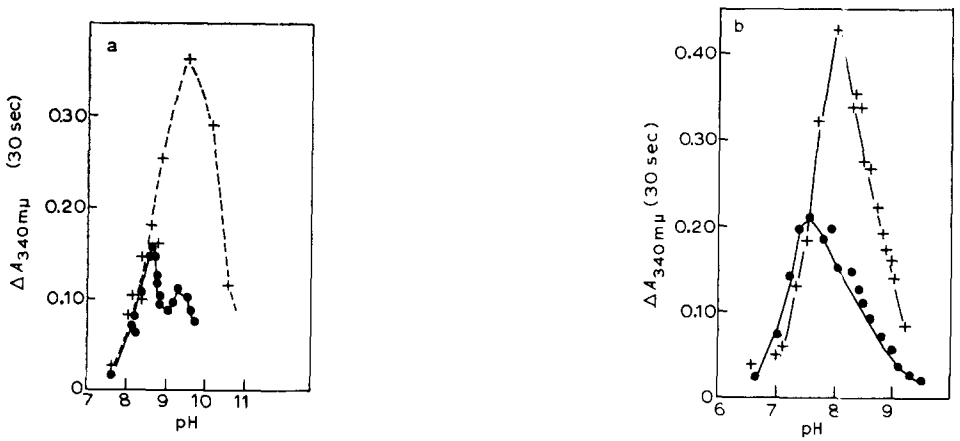


Fig. 4. pH dependence of α-glycero-*P* dehydrogenase action. (a) α-glycero-*P* oxidation. α-glycero-*P* dehydrogenase = $6.4 \cdot 10^{-6}$ μmole/ml. + — — — +, in 0.1 M glycine buffer; ●—●, in 0.1 M Tris buffer. α-glycero-*P* = 5.8 μmoles/ml; NAD^+ = 1.7 μmoles/ml. (b) Dihydroxyacetone-*P* and glyceraldehyde-3-*P* reduction. α-Glycero-*P* dehydrogenase = $6.4 \cdot 10^{-6}$ μmole/ml; dihydroxyacetone-*P*, or glyceraldehyde-3-*P* = $3.0 \cdot 10^{-1}$ μmole/ml; NADH = $8.8 \cdot 10^{-2}$ μmole/ml; in 0.1 M Tris buffer. +—+, dihydroxyacetone-*P* reduction; ●—●, glyceraldehyde-3-*P* reduction.

is pH 9.6 there are 2 maxima in Tris buffer, at pH 8.6 and 9.4. It is reasonable to suppose that the 2 peaks represent the different pH optima of the formation of dihydroxyacetone-*P* and glyceraldehyde-3-*P*. Since the formation of dihydroxyacetone-*P* is favoured at higher pH values and glyceraldehyde-3-*P* is formed at lower pH's, the pH optima for the formation of dihydroxyacetone-*P* and glyceraldehyde-3-*P* are supposed to be 9.4 and 8.6, respectively. Accordingly, the pH optimum of the α-glycero-*P* $\rightleftharpoons$ glyceraldehyde-3-*P* reaction is at 9.6 in glycine buffer.

As is seen from Fig. 4 there are great differences at higher pH values in the activity depending on the buffer used. While the same velocities were obtained at pH 8.6 in glycine or Tris buffers, the activity at pH 9.6 in 0.1 M Tris buffer is only 30% of the activity measured in 0.1 M glycine buffer. It may be supposed that Tris inhibits the rate of dihydroxyacetone-*P* formation but it has no effect on the formation of glyceraldehyde-3-*P*. This effect may account for the first maximum observed in Tris buffer.

The pH optimum of reduction of dihydroxyacetone-*P* and glyceraldehyde-3-*P* is shown in Fig. 4b. The reduction of dihydroxyacetone-*P* has an optimum at pH 8.3, and the optimum of reduction of glyceraldehyde-3-*P* is pH 7.6.

The pH dependence of the equilibrium constant

The equilibrium mixtures of α-glycero-*P* dehydrogenase reaction contain 90–99% α-glycero-*P* (Table IV). Therefore, the equilibrium constant can be determined

only by measuring the oxidation of α-glycero-*P*, *i.e.* the formation of NADH, the experimental error being too high if it is approached from the side of triose phosphate reduction.

Since the sum of the concentration of the oxidation products is equal to NADH, Eqn. 6 was used for the determination of the equilibrium constant.

$$\frac{[\text{NADH}]^2[\text{H}^+]}{[\alpha\text{-glycero-}P][\text{NAD}^+]} = K \qquad (6)$$

where $[\text{NADH}]^2$ = [dihydroxyacetone-*P* + glyceraldehyde-3-*P*] [NADH] calculated from the change in absorption at 340 mμ.

The equilibrium constant of a reaction is independent of pH if the concentration of H^+ is included in its value[12]. On this basis, the different pathways of α-glycero-*P* dehydrogenase reaction must be reflected in the change of the constant at certain pH values (Fig. 5).

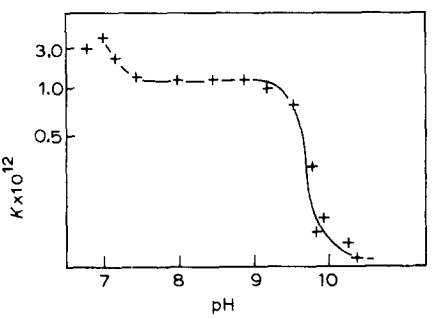


Fig. 5. pH dependence of the equilibrium constant of α-glycero-*P* dehydrogenase reaction. In 0.1 M Tris buffer. α-Glycero-*P* dehydrogenase = $2.0 \cdot 10^{-4}$ μmole/ml; α-glycero-*P* = $1.9 \cdot 10^{-3}$ M; NAD^+ = $1.7 \cdot 10^{-4}$ M and $5.1 \cdot 10^{-3}$ M (both concentrations resulted in the same value of constant).

As Fig. 5 shows, the constant does not change in a wide pH range, its value between pH 7.5 and 9.5 is about $1 \cdot 10^{-12}$. Above pH 9.5 a continuous decrease and, below pH 7.5, a slight increase of *K* can be observed. The middle part of the curve represents the reactions in which both dihydroxyacetone-*P* and glyceraldehyde-3-*P* are formed. The changes of *K* at higher and lower pH values show that it approaches the constant of α-glycero-*P* ⇌ dihydroxyacetone-*P* or α-glycero-*P* ⇌ glyceraldehyde-3-*P* reactions. The exact values of these constants could not be determined because of the denaturation of α-glycero-*P* dehydrogenase at extreme pH values.

DISCUSSION

Participation of both dihydroxyacetone-*P* and glyceraldehyde-3-*P* as oxidized substrates in α-glycero-*P* dehydrogenase reaction was demonstrated. At certain pH values the equilibrium reaction mixture of α-glycero-*P* dehydrogenase contains both triose phosphates and α-glycero-*P*.

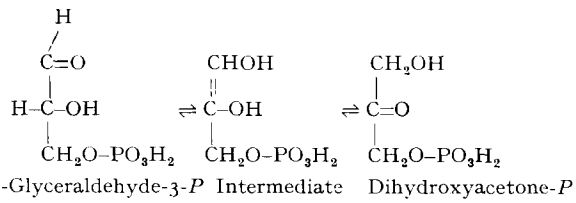
The number of possible mechanisms is limited by the fact that the D isomer of glyceraldehyde-3-*P* is formed from L-α-glycero-*P*. The formation of D-glyceraldehyde-

3-*P* was shown by the effect of glyceraldehyde-3-*P* dehydrogenase which is specific for the D-glyceraldehyde-3-*P*.

The conversion of L-α-glycero-*P* into D-glyceraldehyde-3-*P* can be supposed to take place through the optically inactive dihydroxyacetone-*P*, or through an optically inactive intermediate compound. In the first case the formation of dihydroxyacetone-*P* and D-glyceraldehyde-3-*P* from L-α-glycero-*P* is a consecutive reaction, while in the second mechanism both triose phosphates can be formed, simultaneously.

In the consecutive reaction the reaction mixture must definitely contain dihydroxyacetone-*P*, independently of D-glyceraldehyde-3-*P* formation during the reaction. The results obtained with the dinitrophenylhydrazine test show that at pH 7 α-glycero-*P* and D-glyceraldehyde-3-*P* are present in the equilibrium mixture but no dihydroxyacetone-*P* is detectable under the experimental conditions. If only D-glyceraldehyde-3-*P* is formed from the 2 triose phosphates, the first pathway may be excluded.

In the second possible mechanism the existence of an optically inactive intermediate may be assumed. From the intermediate both dihydroxyacetone-*P* and D-glyceraldehyde-3-*P* can be produced depending on the conditions:



D-Glyceraldehyde-3-*P* Intermediate Dihydroxyacetone-*P*

In this mechanism the formation of the 2 triose phosphates is not a consecutive reaction, as in the formal case, therefore dihydroxyacetone-*P* or D-glyceraldehyde-3-*P* can be formed from L-α-glycero-*P* independently or simultaneously.

It may be assumed that in addition to triose phosphate isomerase, α-glycero-*P* dehydrogenase takes part in the physiological regulation of the glyceraldehyde-3-*P* to dihydroxyacetone-*P* ratio.

ACKNOWLEDGEMENTS

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