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MERCURY-INDUCED REVERSIBLE INCREASE IN 2,3-DIPHOSPHOGLYCERATE PHOSPHATASE AND CONCOMITANT DECREASE IN MUTASE ACTIVITY OF ANIMAL PHOSPHOGLYCERATE MUTASES

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SUMMARY

A reversible transformation induced by $HgCl_2$ of phosphoglycerate mutase (EC 2.7.5.3) into 2,3-diphosphoglycerate phosphatase (EC 3.1.3.13) has been discovered and is documented. Maximum effects are obtained at pH 7 and above and at 3 mM Hg^{2+}.

The interconversion occurs with all animal phosphoglycerate mutases tested, *i.e.* chicken breast, rabbit muscle and pig heart. All these phosphoglycerate mutases contain SH groups and require 2,3-diphosphoglycerate. Interconversion does not occur with yeast phosphoglycerate mutase which contains no SH groups. Also the phosphoglycerate mutases which are non-2,3-diphosphoglycerate dependent (EC 5.4.2.1) are neither inhibited nor the 2,3-diphosphoglycerate phosphatase activity increased with SH reagents.

This reversible Hg^{2+}-induced phosphoglycerate mutase–2,3-diphosphoglycerate phosphatase interconversion is not carried out by other heavy metals such as Cd^{2+} and Pb^{2+} nor by organic mercurials such as *p*-hydroxymercuribenzoate and *p*-chloromercuribenzene sulfonate which are believed to be more specific for mercaptide formation than $HgCl_2$; indeed *N*-ethylmaleimide cannot convert the phosphoglycerate mutase into a 2,3-diphosphoglycerate phosphatase.

The Hg^{2+}-induced reversible phosphoglycerate mutase–2,3-diphosphoglycerate phosphatase transformation does not entail changes in quaternary structure of the protein as shown by ultracentrifuge measurements. However, Hg^{2+} seems to modify the tertiary structure as shown by spectropolarimetric and fluorimetric measurements.

Mercury modifies the active site sufficiently so that the Michaelis constant for 2,3-diphosphoglycerate decreases for the phosphoglycerate mutase and increases for the 2,3-diphosphoglycerate phosphatase.

INTRODUCTION

The phosphoglycerate mutases, which catalyze the reversible conversion of D-3-phosphoglycerate to D-2-phosphoglycerate, are of two types: the 2,3-diphosphoglycerate-dependent (2,3-diphospho-D-glycerate:2-phospho-D-glycerate phosphotransferase, EC 2.7.5.3) and the D-2,3-diphosphoglycerate-independent enzymes (D-phosphoglycerate 2,3-phosphomutase, EC 5.4.2.1). We found, and it has been confirmed by others, that the crystalline and/or highly purified phosphoglycerate mutases possess 2,3-diphosphoglycerate phosphatase (2,3-diphospho-D-glycerate 2-phosphohydrolase, EC 3.1.3.13) activity[2]. Various methods of purification do not separate the 2,3-diphosphoglycerate phosphatase from the phosphoglycerate mutase. Conversely, the 2,3-diphosphoglycerate phosphatase, which had been purified extensively in our laboratory, was shown to have phosphoglycerate mutase activity at all stages of purity[3]. Further, ZANCAN *et al.*[4] have been unable to separate phosphoglycerate mutase from the 2,3-diphosphoglycerate phosphatase with three different types of 2,3-diphosphoglycerate phosphatase.

One of the steps of purification of the 2,3-diphosphoglycerate phosphatase from chicken-breast muscle requires mercuric ions[3]. Hg^{2+} are known to activate the 2,3-diphosphoglycerate phosphatase and to remain firmly bound to the protein[3]. It was found during immunological studies when attempting to remove enzyme-bound Hg^{2+} with dithioerythritol, that the phosphoglycerate mutase increased while the 2,3-diphosphoglycerate phosphatase activity decreased. This effect is reversible.

We have found that this reversible transformation can be accomplished with all animal 2,3-diphosphoglycerate dependent phosphoglycerate mutases tested but not with the yeast enzyme. A comparative study of the reversible changes induced by Hg^{2+} on phosphoglycerate mutases and 2,3-diphosphoglycerate phosphatase is presented in this paper.

EXPERIMENTAL

Materials and methods

Crystalline 2,3-diphosphoglycerate pentacyclohexyl ammonium salt was obtained from Boehringer Mannheim Corporation. 3-Phosphoglycerate (free of 2,3-diphosphoglycerate) and enolase (free of mutase) were prepared as previously described[5]. Dithioerythritol was obtained from Cyclo Chemical Corporation; *p*-hydroxymercuribenzoate and *p*-chloromercuribenzene sulfonate from Sigma Chemical Company and *N*-ethylmaleimide from Schwartz Bioresearch Corporation. The ammonium sulfate used was enzyme grade and supplied by Mann Research Laboratories. Phosphoglycerate mutases from baker's yeast, wheat germ, chicken-breast muscle and rabbit muscle were prepared as previously described[6–9].

The enolase–mutase-coupled assay was used to determine the phosphoglycerate mutase activity[9]. 2,3-Diphosphoglycerate phosphatase was measured as described before[3] except that the total volume of the incubation mixture was 1 ml. P_i was determined by GOMORI's method[10]. Protein was estimated by the modified biuret method of MOKRASCH, DAVIDSON AND MCGILVERY[11].

An enzyme unit of mutase caused an increase in absorbance at 240 mμ of 0.100 per min. This equals 0.307 μmole of 3-phosphoglycerate converted to 2-phospho-

glycerate under the conditions of the enolase-coupled method[9]. An enzyme unit of 2,3-diphosphoglycerate phosphatase is defined as 1 μmole of P_i liberated in 30 min under the conditions of the assay[3]. Specific activity is the ratio of enzyme units per mg of protein.

All operations were carried out at 0°, unless specified otherwise. Centrifugation at 2000 $\times$ g for 10 min usually gave a clear separation. The $HgCl_2$, $CdSO_4$, lead acetate and *N*-ethylmaleimide used were 0.01 M. The *p*-hydroxymercuribenzoate solution was 0.002 M in 0.01 M Tris buffer (pH 7.8). The buffers used were sodium acetate (pH 5.4), Tris–acetate (pH 6.4), phosphate (pH 7) and Tris–Cl^- (pH 7.3–8). The 2,3-diphosphoglycerate used was 0.02 M.

For treatment of the enzyme with heavy metals and other –SH reagents, unless specified otherwise, 1 ml of the enzyme containing 8–10 mg of protein per ml was added to 0.5 ml of a solution containing 75 μmoles of Tris–Cl^- buffer (pH 7.3), 3 μmoles of 2,3-diphosphoglycerate (when used) and 1.5 μmoles of $HgCl_2$ or other heavy metals and –SH reagents. The mixtures were incubated at 37–38° for 5 min, cooled at 0°, centrifuged and the supernatant fluids were collected.

The bulk of the reagents used were separated from the enzyme by precipitation of the protein with ammonium sulfate or ethanol. For ammonium sulfate precipitation 2.5–3.0 vol. of saturated ammonium sulfate (pH 7) were added to each volume of the enzyme solution. For ethanol precipitation 2 ml 95% ethanol were added to each ml of the enzyme. The precipitate was taken in water and recentrifuged to remove any denatured protein.

For reactivation of –SH inhibition 1 vol. of 0.001 M freshly prepared dithioerythritol was added to each sample containing 1 mg of protein per ml. The mixtures were allowed to stand at room temperature for 5 min before assaying for either phosphoglycerate mutase or 2,3-diphosphoglycerate phosphatase activity.

In all the physical measurements reported here, chicken-breast muscle phosphoglycerate mutase of specific activity approx. 2500 was used.

A Gilford model 2000 spectrophotometer was used to measure absorbances. The sedimentation-velocity experiments were performed in the model E Spinco ultracentrifuge. The speed was 60 000 rev./min at 23° and at a bar angle of 70°. Optical rotatory dispersion measurements were carried out at 20° with a standard model D Keston polarimetric unit attached to a Beckman DU.

Fluorescence studies were carried out in an Aminco Bowman spectrophotofluorimeter using a 10-mm light path cell. The protein concentration was 1 mg/ml in 1.6 mM Tris–Cl^- buffer (pH 7.0).

RESULTS AND DISCUSSION

As shown in Table I, Expt. 1, treatment of chicken-breast muscle extracts with Hg^{2+} caused an increase in specific activity of the 2,3-diphosphoglycerate phosphatase and a decrease in the specific activity of the phosphoglycerate mutase. Subsequent treatment with dithioerythritol reversed the effect of Hg^{2+} on both activities. The initial total activity of both enzymes before Hg^{2+} treatment was not recovered fully after dithioerythritol treatment, because of the extensive protein denaturation during the Hg^{2+} and ethanol fractionation. Nevertheless, it is clear that Hg^{2+} did activate extensively the 2,3-diphosphoglycerate phosphatase while inhibiting the phosphoglycerate mutase.

TABLE I

THE EFFECT OF $HgCl_2$ AND DITHIOERYLTHRITOL ON PHOSPHOGLYCERATE MUTASE AND 2,3-DIPHOSPHOGLYCERATE PHOSPHATASE PREPARATIONS FROM CHICKEN-BREAST MUSCLE

For Expt. 1 chicken-breast muscle crude acetone powder extract[3] was used. The crude 2,3-diphosphoglycerate phosphatase was treated with $HgCl_2$ in the usual manner. The enzyme was then fractionated with 95% ethanol. The fraction obtained after the addition of ethanol up to 40% final concentration was discarded. The second fraction obtained after further addition of ethanol to a final concentration of 70% was used. For Expt. 2 partially purified chicken-breast phosphoglycerate mutase was used.

Expt.	*Treatment*	*Protein (mg)*	*Total activity (units)*		*Specific activity*	
			Phosphoglycerate mutase	*2,3-Diphosphoglycerate phosphatase*	*Phosphoglycerate mutase*	*2,3-Diphosphoglycerate phosphatase*
1	None	560	47 000	90	84	0.16
1	$HgCl_2$–2,3-diphosphoglycerate + ethanol*	29	570	87	20	2.98
1	As above + dithioerythritol	29	32 000	26	1100	0.88
2	None	80	76 800	84	960	1.05
2	$HgCl_2$–2,3-diphosphoglycerate + ethanol	10	90	26	9	2.60
2	As above + dithioerythritol	10	7 400	6	740	0.60

* This treatment is a standard purification step for the phosphoglycerate phosphatase.

A similar effect of Hg^{2+} and dithioerythritol treatment was observed with the phosphoglycerate preparation from chicken-breast muscle (Table I, Expt. 2). Approx. 100-fold decrease in phosphoglycerate mutase specific activity resulted after addition of Hg^{2+} while the specific activity of the 2,3-diphosphoglycerate phosphatase doubled. Again, after the addition of dithioerythritol to the Hg^{2+}-treated enzyme the effects of Hg^{2+} are reversed; the phosphoglycerate mutase regained almost completely

TABLE II

THE INFLUENCE OF 2,3-DIPHOSPHOGLYCERATE ON $HgCl_2$ AND DITHIOERYTHRITOL EFFECTS ON CHICKEN-BREAST MUSCLE PHOSPHOGLYCERATE MUTASE

The conditions were as described in the text. 2,3-Diphosphoglycerate was added to the samples of Expt. 1.

Expt.	*Treatment*	*Total protein (mg)*	*Total activity (units)*		*Specific activity*	
			Phosphoglycerate mutase	*2,3-Diphosphoglycerate phosphatase*	*Phosphoglycerate mutase*	*2,3-Diphosphoglycerate phosphatase*
1	None	80	76 800	84	960	1.05
1	$HgCl_2$, $(NH_4)_2SO_4$	75	720	130	9	1.78
1	As above + dithioerythritol	75	106 000	48	1400	0.64
2	None	16	16 300	20	1020	1.21
2	$HgCl_2$, $(NH_4)_2SO_4$	13	114	30	9	2.32
2	As above + dithioerythritol	13	14 800	9	1140	0.69

its initial specific activity while the 2,3-diphosphoglycerate phosphatase activity decreased. It should be noted again that the decrease in total activity was due to protein denaturation during the ethanol precipitation.

Since precipitation of the protein with ethanol to remove the excess Hg^{2+} caused considerable denaturation of the protein, it was of interest to test ammonium sulfate as the protein precipitant.

As shown in Table II, the recovery of protein from ammonium sulfate precipitation was practically complete and the phosphoglycerate mutase remained fully active after dithioerythritol treatment. It should be noted that only about half of the initial total 2,3-diphosphoglycerate phosphatase appears to be recovered after the addition of dithioerythritol. This is an artifact due to SO_4^{2-} inhibition of the 2,3-diphosphoglycerate phosphatase. Table II, Expt. 2, also illustrates that the addition of 2,3-diphosphoglycerate is not necessary to protect the phosphoglycerate mutase or the 2,3-diphosphoglycerate nor does it influence the effect of Hg^{2+} and dithioerythritol on the enzyme.

It was of interest next to establish the optimum conditions necessary to achieve this reversible change of the two enzyme activities.

Effect of concentration of Hg^{2+} on phosphoglycerate mutase–2,3-diphosphoglycerate phosphatase interconversion

As shown in Table III, there is some effect of Hg^{2+} at the lowest level tested. However, by doubling the concentration (0.34 mM) nearly maximal effects are obtained. Beyond this concentration up to the highest level tested (5–10 mM), no marked changes on both activities are observed. Inasmuch as the enzyme contains –SH groups and in addition may bind some Hg^{2+} non-specifically, other experiments were carried out to test the effect of concentration of Hg^{2+} with twice the concentration of enzyme. Again 10–20% effects were noted at the lowest concentration of Hg^{2+} (~0.3 mM) at

TABLE III

THE EFFECT OF $HgCl_2$ ON CHICKEN-BREAST MUSCLE PHOSPHOGLYCERATE MUTASE WITH AND WITHOUT DITHIOERYTHRITOL

1-ml samples containing 8 mg of protein were mixed with 0.5 ml containing varying amounts of $HgCl_2$ (0, 0.25, 0.5, 1.0, 1.5, 2.5 and 7.5 μmoles) and 75 μmoles of Tris buffer (pH 7.3) (with 7.5 μmoles of $HgCl_2$, 375 μmoles of buffer were used). The mixtures were incubated at 37–38° for 5 min, cooled, centrifuged when turbid and the supernatant fluids were collected and assayed.

$HgCl_2$ present (mM)	*Total activity (units)*				*Specific activity*			
	Phosphoglycerate mutase		*2,3-Diphosphoglycerate phosphatase*		*Phosphoglycerate mutase*		*2,3-Diphosphoglycerate phosphatase*	
	No DTE	*DTE*	*No DTE*	*DTE*	*No DTE*	*DTE*	*No DTE*	*DTE*
0	6600	6600	3.2	3.3	1800	1800	0.87	0.91
0.17	2800	6300	3.7	3.0	780	1750	1.07	0.84
0.34	54	6600	7.3	3.5	19	2300	2.57	1.24
0.68	60	7200	7.5	3.5	18	2200	2.30	1.07
1.02	57	6300	6.8	3.0	18	2100	2.28	1.01
1.70	60	7500	7.0	3.5	18	2270	2.15	1.09
5.10	78	6300	7.2	3.0	23	1900	2.20	0.93

TABLE IV

THE EFFECT OF $HgCl_2$, DITHIOERYTHRITOL AND pH ON CHICKEN-BREAST MUSCLE PHOSPHOGLYCERATE MUTASE

Each tube contained in a final volume of 1.5 ml, 8 mg of the enzyme, 0.5 μmole of $HgCl_2$ and 120 μmoles of buffer at the indicated pH values. The mixtures were incubated at 37–38° for 5 min, cooled, centrifuged when necessary and assayed. DTE, dithioerythritol.

pH	*Specific activity*							
	Phosphoglycerate mutase				*2,3-Diphosphoglycerate phosphatase*			
	No Hg^{2+}		*Hg^{2+}*		*No Hg^{2+}*		*Hg^{2+}*	
	No DTE	*DTE*	*No DTE*	*DTE*	*No DTE*	*DTE*	*No DTE*	*DTE*
5.4	1670	1840	0	0	0.78	0.87	0	0
6.4	1840	2000	1	880	0.81	0.85	0.56	0.57
7.3	1800	1800	19	2300	0.87	0.91	2.57	1.24
7.5	1840	1840	17	1840	0.84	0.80	1.74	0.82
8.0	1750	1840	13	1750	0.83	0.78	1.70	0.86
pH	*Total activity (units)*							
	Phosphoglycerate mutase				*2,3-Diphosphoglycerate phosphatase*			
	No Hg^{2+}		*Hg^{2+}*		*No Hg^{2+}*		*Hg^{2+}*	
	No DTE	*DTE*	*No DTE*	*DTE*	*No DTE*	*DTE*	*No DTE*	*DTE*
5.4	6000	6900	0	0	2.8	3.1	0	0
6.4	6600	7200	2	1860	2.9	3.0	1.2	1.2
7.3	6600	6600	54	6600	3.2	3.3	7.3	3.5
7.5	6600	6600	60	6600	3.0	2.9	6.3	3.0
8.0	6300	6600	48	6300	3.0	2.8	6.1	3.1

0.6 mM the phosphoglycerate mutase activity had decreased to 30% while the 2,3-diphosphoglycerate phosphatase activity increased nearly 2-fold. At 1.2 mM level near maximum effects were obtained and remained essentially unchanged up to 9 mM. In other words, once non-specific binding of Hg^{2+} is taken into consideration[3] the optimum effects occur at about 10^{-4}–10^{-3} M and remained unchanged up to 10^{-2} M. The residual phosphoglycerate mutase activity in the presence of Hg^{2+} *i.e.* about 1% could indicate a non-2,3-diphosphoglycerate dependent activity[12]. However, this was shown experimentally not to be the case.

Effect of pH

Table IV demonstrates that the optimum pH is 7.3. The effect of Hg^{2+} remained essentially unchanged between pH 7 and pH 8. This is to be expected if –SH groups are involved[13]. It is of interest that both the phosphoglycerate mutase and the 2,3-diphosphoglycerate phosphatase became less stable to pH in the presence of Hg^{2+}. Indeed as shown in Table IV at pH 5.4 both activities were completely destroyed.

Effect of Hg^{2+} on phosphoglycerate mutase from other sources

Table V shows that the rabbit-muscle and the pig-heart phosphoglycerate mutase were affected in the same manner as the chicken-muscle phosphoglycerate mutase by Hg^{2+}, ammonium sulfate precipitation and dithioerythritol treatment. With the

TABLE V

THE EFFECT OF $HgCl_2$ AND DITHIOERYTHRITOL ON RABBIT-MUSCLE, PIG-HEART, YEAST AND WHEAT-GERM PHOSPHOGLYCERATE MUTASE

The methods of treatment were described in the text. With the wheat-germ phosphoglycerate mutase twice the usual amount of $HgCl_2$ was used. The 2,3-diphosphoglycerate phosphatase activity of the wheat germ is given as the μmoles of P_i liberated per 30 min at pH 8.5 under the condition of the assay[3].

Enzyme source	*Treatment*	*Total activity (units)*		*Specific activity*	
		Phosphoglycerate mutase	*2,3-Diphosphoglycerate phosphatase*	*Phosphoglycerate mutase*	*2,3-Diphosphoglycerate phosphatase*
Rabbit muscle	None	10 800	10.0	680	0.62
	$HgCl_2$–2,3-diphosphoglycerate + $(NH_4)_2SO_4$	75	9.0	14	1.62
	As above + dithioerythritol	6 600	2.0	1200	0.38
Pig heart	$(NH_4)_2SO_4$	3 750	1.4	480	0.18
	$HgCl_2$–2,3-diphosphoglycerate + $(NH_4)_2SO_4$	605	2.9	84	0.40
	As above + dithioerythritol	3 320	0.6	460	0.09
Yeast	None	18 800	30.0	1800	2.80
	$HgCl_2$–2,3-diphosphoglycerate + $(NH_4)_2SO_4$	22 000	23.0	2250	2.30
	As above + dithioerythritol	21 000	23.0	2150	2.30
Wheat germ	None	400	5.0	20	0.24
	$HgCl_2$, $(NH_4)_2SO_4$	75	0.8	14	0.14
	As above + dithioerythritol	119	1.6	22	0.26

TABLE VI

THE EFFECT OF OTHER –SH REAGENTS AND DITHIOERYTHRITOL ON CHICKEN-BREAST MUSCLE AND YEAST PHOSPHOGLYCERATE MUTASE

Expt.	*Enzyme source*	*Treatment*	*Total activity (units)*		*Specific activity*	
			Phosphoglycerate mutase	*2,3-Diphosphoglycerate phosphatase*	*Phosphoglycerate mutase*	*2,3-Diphosphoglycerate phosphatase*
1	Chicken-breast muscle	$(NH_4)_2SO_4$	44 800	11	2800	0.70
		p-Chloromercuribenzene sulfonate	12	12	1	0.81
		As above + dithioerythritol	32 500	10	2200	0.66
	Yeast	None	10 900	13	680	0.83
		p-Chloromercuribenzene sulfonate –2,3-diphosphoglycerate+$(NH_4)_2SO_4$	6 750	6	560	0.53
		As above + dithioerythritol	7 200	7	600	0.55
2	Chicken-breast muscle	$(NH_4)_2SO_4$	44 800	11	2800	0.70
		p-Hydroxymercuribenzoate, $(NH_4)_2SO_4$	24	10	2	0.70
		As above + dithioerythritol	33 900	11	2300	0.77
	Yeast	None	10 900	13	680	0.83
		p-Hydroxymercuribenzoate–2,3-diphosphoglycerate + $(NH_4)_2SO_4$	7 700	6	640	0.54
		As above + dithioerythritol	7 700	6	640	0.51
3	Chicken-breast muscle	$(NH_4)_2SO_4$	22 400	6	2800	0.70
		N-Ethylmaleimide, $(NH_4)_2SO_4$	2 640	1	400	0.13
		As above + dithioerythritol	1 850	1	280	0.13

yeast enzyme the treatment with Hg^{2+} produced no effect on either its 2,3-diphosphoglycerate phosphatase or phosphoglycerate mutase activity. The wheat-germ phosphoglycerate mutase, a 2,3-diphosphoglycerate-independent enzyme, was practically unaffected by the Hg^{2+} treatment.

Effect of other –SH reagents

Inasmuch as the effects thus far described with $HgCl_2$ could be interpreted as due to –SH groups, we tested other organic mecurials and *N*-ethylmaleimide. As illustrated in Table VI, Expts. 1 and 2, both *p*-hydroxymercuribenzoate and *p*-chloromercuribenzene sulfonate inhibits extensively the phosphoglycerate mutase from chicken muscle. However, the 2,3-diphosphoglycerate phosphatase activity remained practically unaffected. The yeast phosphoglycerate mutase and 2,3-diphosphoglycerate phosphatase were not affected by similar treatment. *N*-Ethylmaleimide, on the other hand, caused decreased activity of both the phosphoglycerate mutase and the 2,3-diphosphoglycerate phosphatase.

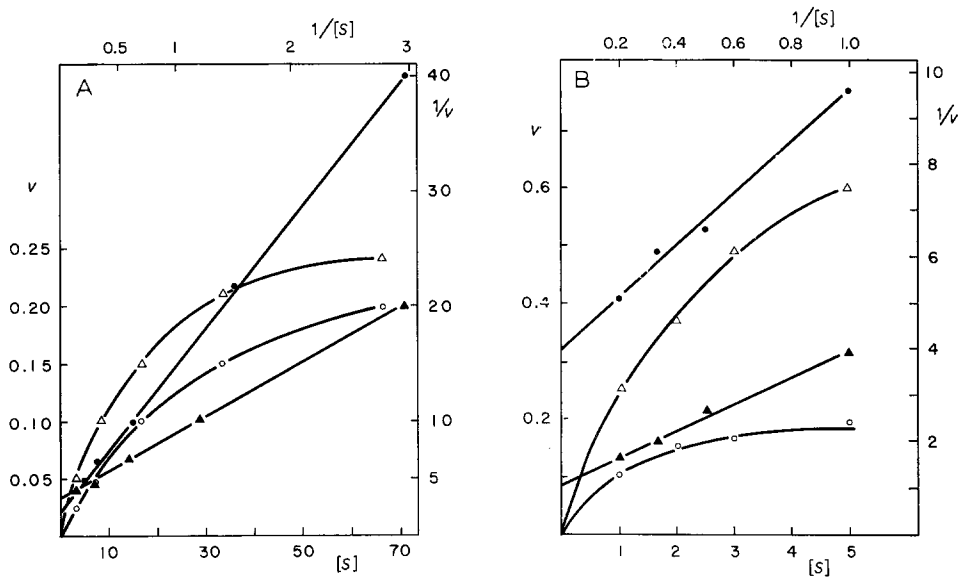

Fig. 1. Kinetic measurements on the chicken-breast phosphoglycerate mutase. A. Effect of coenzyme concentration on the mutase activity. The incubation volume was 3.0 ml and the temperature of the reaction 30°. The components were as follows: D-3-phosphoglycerate (2,3-diphosphoglycerate free)50 μmoles; $MgSO_4$, 10 μmoles; Tris (pH 7.3), 100 μmoles; 2,3-diphosphoglycerate, from 10 to 200 mμmoles; mutase-free enolase, 15 units; and 0.072 μg of chicken-breast mutase or 30 μg of Hg^{2+}-treated mutase. [*S*] is expressed as mμmoles of 2,3-diphosphoglycerate per ml, *v* as mutase units. ○—○, non-treated enzyme; △—△, Hg^{2+}-treated enzyme; ●—● and ▲—▲, the same data expressed as reciprocals of velocity and coenzyme concentration. B. The influence of substrate concentration on the velocity of the 2,3-diphosphoglycerate phosphatase action. The standard conditions of assay adapted to 1 ml total incubation volume[3] were used except for the variation of substrate concn. [*S*] as indicated in the abscissa of the figure, expressed in μmoles per ml. About 0.30 mg of enzymes were used per 1 ml of incubation mixture. Velocity (*v*) is expressed as μmoles of P_i liberated during the incubation. ○—○, untreated enzyme; △—△, Hg^{2+}-treated enzyme; ●—● and ▲—▲, the same data expressed as reciprocals of velocity and substrate concentration.

Effect of other heavy metals

Cd^{2+} and Pb^{2+} have essentially no effect when replacing $HgCl_2$ on the phosphoglycerate mutase–2,3-diphosphoglycerate phosphatase interconversion.

Kinetic measurements

Experiments were carried out with the untreated and the Hg^{2+}-treated chicken-breast muscle phosphoglycerate mutase. Using the phosphoglycerate mutase–enolase-coupled method the K_m for 2,3 diphosphoglycerate was calculated from LINEWEAVER AND BURK plots[14] to be $1.7 \cdot 10^{-6}$ M with the Hg^{2+}-treated enzyme and $5 \cdot 10^{-6}$ M with the untreated enzyme as illustrated in Fig. 1A. The K_m for the 2,3-diphosphoglycerate phosphatase of the untreated and Hg^{2+}-treated enzyme were 1.5 and $2.3 \cdot 10^{-3}$ M respectively (see Fig. 1B).

Physical measurements

The absorbances of untreated, Hg^{2+}-treated and Hg^{2+}–dithioerythritol-treated enzyme samples were measured between 240 and 340 mμ. As shown in Fig. 2 there were only small differences between the three samples in the region of 300 to 340 mμ.

Sedimentation-velocity experiments were also carried out. The sedimentation coefficient of the samples with and without Hg^{2+} were respectively, $4.083 \cdot 10^{-13}$ and $3.917 \cdot 10^{-13}$. Fig. 3 shows no differences in the sedimentation patterns.

Optical-rotatory dispersion studies were carried out with Hg^{2+} and Hg^{2+}–dithioerythritol-treated enzyme samples. The results shown in Fig. 4 indicate a marked change in conformation induced by Hg^{2+}. Further, the rotatory power of the enzyme

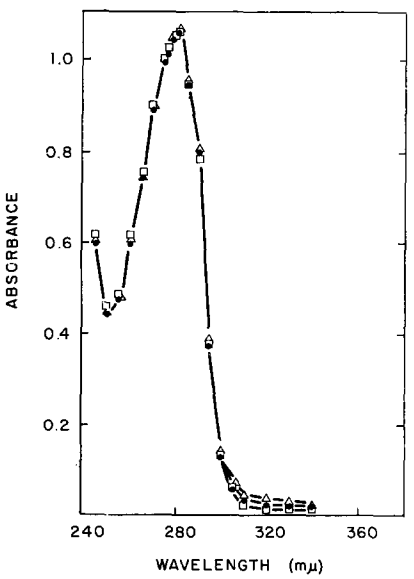

Fig. 2. Absorption spectrum of chicken-breast phosphoglycerate mutase. ●—●, enzyme; □—□, enzyme + Hg^{2+} (0.38 μmole Hg^{2+} per mg enzyme) precipitated from saturated ammonium sulfate (pH 7); △—△, the same Hg^{2+}-treated enzyme + dithioerythritol (1 μmole dithioerythritol per mg enzyme). The protein concentration was 1 mg/ml. 0.2 ml of saturated ammonium sulfate (pH 7.5) was added (to prevent turbidity). Final volume 3 ml.

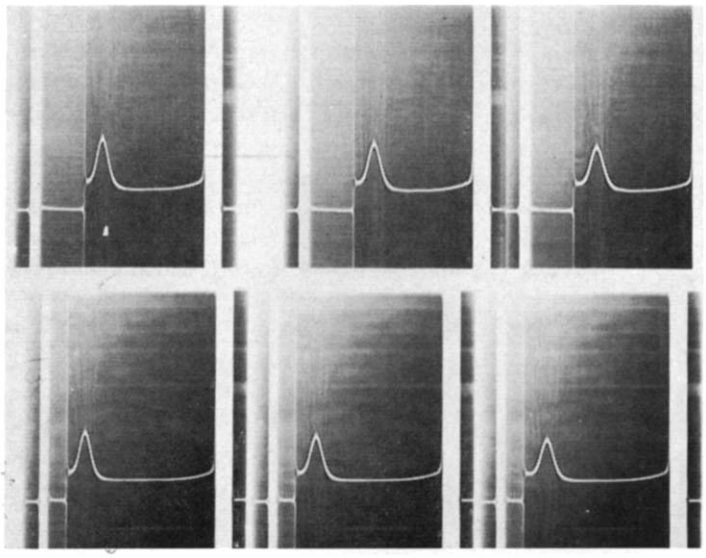

Fig. 3. Ultracentrifugal pattern of chicken-muscle phosphoglycerate mutase. Protein 10 mg/ml in 0.1 M NaCl–0.1 M phosphate buffer (pH 7.0). The exposures shown from left to right were taken at 28, 32, and 36 min after speed equilibration. Top, sedimentation pattern of the untreated enzyme. Bottom, sedimentation pattern of the Hg^{2+}-treated enzyme (0.08 μmole Hg^{2+} per mg enzyme.)

before Hg^{2+} treatment is partially regained with dithioerythritol. It should be noted that only one fourth of the usual amount of dithioerythritol used was added in this experiment (due to resulting cloudiness of the solution more dithioerythritol could

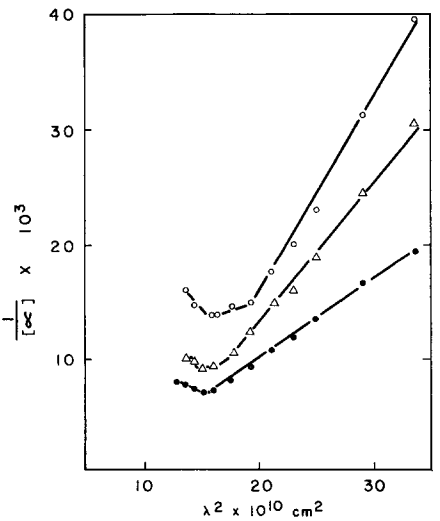

Fig. 4. Optical rotatory dispersion of chicken-muscle phosphoglycerate mutase. ●—●, untreated enzyme; ○—○, enzyme + Hg^{2+} (0.104 μmole Hg^{2+} per mg enzyme); △—△, the same Hg^{2+}-treated enzyme + dithioerythritol (0.25 μmole dithioerythritol per mg enzyme). Enzyme solutions containing 12 mg of protein per ml in a 10-cm cell were used.

not be added). The excitation and fluorescence maxima of the chicken-breast muscle enzyme were at 290 and 350 mμ respectively. There was 17% quenching when $HgCl_2$ (10 μl in 3 ml) was added to a final concentration of $3.3 \cdot 10^{-5}$ M. Doubling the amount of $HgCl_2$ added resulted in additional quenching of the excitation and fluorescence maxima to 75% of the original. Further addition of $HgCl_2$ caused precipitation. With the yeast phosphoglycerate mutase under the same conditions $3.3 \cdot 10^{-5}$ M $HgCl_2$ caused 4% quenching of the fluorescence, and 10% quenching resulted when the $HgCl_2$ concentration was doubled. The excitation and fluorescence maxima of the yeast enzyme were similar to those of the chicken-breast muscle phosphoglycerate mutase.

Mechanism of the phosphoglycerate mutase–2,3-diphosphoglycerate phosphatase interconversion

The phosphoglycerate mutase–2,3-diphosphoglycerate phosphatase interconversion could be attributed to the masking and reactivation of –SH groups of the enzyme. However, the effects seem to be very specific for Hg^{2+}. As shown here, other –SH reagents commonly believed to be more specific than Hg^{2+} did not produce the reversible phosphoglycerate mutase–2,3-diphosphoglycerate phosphatase changes. Furthermore, as shown in Table VII, the effect of $HgCl_2$ was not influenced by *p*-

TABLE VII

THE INFLUENCE OF *p*-HYDROXYMERCURIBENZOATE ON $HgCl_2$ AND DITHIOERYTHRITOL EFFECTS ON CHICKEN-BREAST MUSCLE PHOSPHOGLYCERATE MUTASE

Treatment	*Total activity (units)*		*Specific activity*	
	Phospho-glycerate mutase	*2,3-Di-phospho-glycerate phospha-tase*	*Phospho-glycerate mutase*	*2,3-Di-phospho-glycerate phospha-tase*
$(NH_4)_2SO_4$	19 200	5	2400	0.57
$HgCl_2$ 10 min, $(NH_4)_2SO_4$	116	10	18	1.54
As above + dithioerythritol	13 200	3	2000	0.43
p-Hydroxymercuribenzoate	11	4	2	0.59
As above + dithioerythritol	13 000	3	1800	0.45
$HgCl_2$ 5 min, *p*-hydroxymercuribenzoate	100	10	14	1.38
As above + dithioerythritol	12 600	2	1800	0.30
p-Hydroxymercuribenzoate 5 min, $HgCl_2$	115	10	16	1.41
As above + dithioerythritol	14 400	2	2000	0.32

hydroxymercuribenzoate whether it was added before or after Hg^{2+}. The possibility that the enzyme dissociated or aggregated with the Hg^{2+} treatment was eliminated by the ultracentrifugation studies. On the other hand, optical-rotatory measurements showed a marked change. It seems, therefore, most likely that the enzyme is undergoing change in its tertiary structure during the process. $HgCl_2$, a relatively small molecule as compared with the organic mercurials used, may fit or penetrate better in an enzyme site whether or not acting *via* mercaptide formation. At any rate the

Hg^{2+} change the protein conformation; the initial conformation favors phosphoglycerate mutase action while the Hg^{2+}-induced conformation favors 2,3-diphosphoglycerate phosphatase action.

Aside from its theoretical interest, the reversible transformation of the phosphoglycerate mutase into a 2,3-diphosphoglycerate phosphatase may be of practical interest since the transformation of an enzyme, involved in energy-yielding reactions, to a 2,3-diphosphoglycerate phosphatase could perhaps serve to explain certain aspects of mercury poisoning, *e.g.* lower nephron nephrosis.

Another practical application has been the facilitation of the purification of the heart phosphoglycerate mutase. Numerous attempts to purify the heart phosphoglycerate mutase in this laboratory met with moderate success[15]. However, we have now found that the solubility and other chemical characteristics of the heart phosphoglycerate mutase change sufficiently in the presence of mercury so that extensive purification is possible *via* fractionation with solvents and other reagents. At the end of the fractionation steps, the mercury is removed with dithioerythritol (A. TORRALBA AND S. GRISOLIA, unpublished experiments). The findings presented here strengthen the idea that phosphoglycerate mutase and 2,3-diphosphoglycerate phosphatase are related activities on the same protein[16].

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