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Influence of Ionic Strength on Apparent Reaction Mechanism of Phosphoglycerate Mutase*

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ABSTRACT: The salt environment markedly affects both the yeast and muscle phosphoglycerate (P-glycerate) mutases which are 2,3-P-glycerate dependent and were believed to catalyze the reaction 3-P-glycerate + 2,3-P-glycerate ⇄ 2,3-P-glycerate + 2-P-glycerate. At low ionic strength 2-P-glycerate is labeled from 3-P-glycerate-^{32}P much faster than 2,3-P-glycerate; this is theoretically impossible according to the reaction outlined above. Increased ionic strength favors ^{32}P fixation on 2,3-P-glycerate. At 0.5–1 ionic strength negligible ^{32}P appears on 2-P-glycerate; the bulk is now on 2,3-P-glycerate. It appears that 2,3-P-glycerate does not function as a *free obligatory intermediate*, but is more likely enzyme bound.

Our findings do not reflect a change in activity but in mechanism, *i.e.*, increased rate for the reaction; enzyme + 2,3-P-glycerate ⇄ enzyme-bound 2,3-P-glycerate, and/or a new enzyme conformation, yielding a mechanism resembling the non-2,3-P-glycerate-dependent P-glycerate mutases. These effects are best demonstrated with cations which likely change the elastoplastic protein behavior. They may also apply to other cases, *e.g.*, some *isotope experiments* using "pools" in metabolic studies with *in vitro* systems in which ionic strength has not been controlled and/or tested may be misleading. The cationic effects do not fit the pattern expected for lytropic series; the optimum pH is 8 which is far from the optimum pH for activity of yeast and muscle P-glycerate mutase (5 and 6), respectively.

There are two types of P-glycerate[1] mutases, 2,3-P-glycerate-independent and -dependent enzymes (Grisolia, 1962), which catalyze reactions 1 and 2, respectively.

$$\text{3-P-glycerate} \rightleftharpoons \text{2-P-glycerate} \quad (1)$$

$$\text{3-P-glycerate} + \text{2,3-P-glycerate} \rightleftharpoons \text{2,3-P-glycerate} + \text{2-P-glycerate} \quad (2)$$

Pizer (1962) pointed out that Sutherland *et al.* (1949) found that in every conversion of 3- to 2-P-glycerate, 2,3-P-glycerate must be simultaneously a donor and an acceptor as depicted by reaction 2. Recent investigations (Torralba and Grisolia, 1966) have demonstrated binding of 2,3-P-glycerate to chicken breast muscle P-glycerate mutase, extending previous findings with rabbit muscle mutase (Grisolia *et al.*, 1961). While these findings together with those of Zwaig and Milstein (1966) indicate coenzyme binding to muscle P-glycerate mutase, similar evidence is lacking for the yeast enzyme (Torralba and Grisolia, 1966; Grisolia *et al.*, 1961). Recent experiments (Grisolia and Cascales, 1966) have demonstrated a change in the extent of incorporation of 3-glycerate-^{32}P into 2,3-glycerate-^{32}P induced by an increase in ionic strength with both muscle and yeast mutases. This finding requires the postulation of such an enzyme cofactor complex for the yeast mutase also, with a change in mechanism *via* an increase in exchange resulting from either a shielding or a buttressing salt effect. Alternatively, these findings may indicate a more complex type of change in mechanism; perhaps the enzyme acquires its

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[1] Abbreviations: 3-PGA, D-3-phosphoglyceric acid; 2-PGA, N-2-phosphoglyceric acid; 2,3-PGA or DPGA, D-2,3-diphosphoglyceric acid; P-glycerate, phosphoglycerate; PEP, phosphoenolpyruvate; GTP, guanosine triphosphate; ADP, adenosine diphosphate.

catalytic conformation upon interaction with 2,3-P-glycerate at low ionic strength and then the mechanism is similar to that of reaction 1. However, it changes to that of reaction 2 at high ionic strength. Although more unlikely, the possibility that the role of 2,3-P-glycerate may be solely in changing the conformation, with the resulting catalytic site also exchanging independently from the kinetic reaction, has not been excluded. Such a mechanism would find support if salt would make the rate of exchange faster than that of the catalytic reaction.

It seemed, therefore, that the salt effect should be further studied and clarified because of its unusual interest *per se* and in relation to P-glycerate mutase, and also because such salt effects may possibly affect other enzyme reactions in a similar way. For example, salt effects such as those described here may complicate the often used experimental evidence for pathways based on isotopic data. Indeed, it may require the reexamination of some cases where the use of a "pool" has led to postulations and/or exclusions of certain metabolic pathways.

Materials and Methods

Radioactive 3-P-glycerate was prepared as previously described (Grisolia *et al.*, 1961). Pyruvate was determined by the procedure of Friedemann and Haugen (1943). Crystalline P-glycerate mutase was prepared from yeast (Rodwell *et al.*, 1956) and from chicken breast muscle (Torralba and Grisolia, 1966). Enolase free of mutase was obtained as described previously (Rodwell *et al.*, 1957). Pyruvate kinase from rabbit muscle A grade was bought from Calbiochem. 3-P-Glycerate and 2,3-P-glycerate were purchased from C. F. Boehringer and Whatman No. 1 filter paper for chromatography from W. and R. Balston, Ltd. 2,-5-Bis(2,5-*t*-butylbenzoxazolyl)thiophene (scintillation grade) was from Packard Instrument Co., Inc. Deionized, distilled water was used through.

A Gilford Model 2000 spectrophotometer or a Beckman DU spectrophotometer with a SRL Sargent recorder was used to measure absorbancies. The enolase mutase coupled method (Rodwell *et al.*, 1957) was used to determine mutase activity. One enolase unit is the amount of enzyme that causes an increase of 0.1 A at 250 mμ in 1 min. ^{32}P was measured in a Nuclear-Chicago scintillation counter Model 6804 with an efficiency of 87%. Distribution of radioactivity was followed as previously described (Grisolia and Cascales, 1966). Again, since the data are expressed as per cent distribution, calculations of specific activity are not necessary.

All experiments were carried out at 30°. Unless specified otherwise, all reagents were neutralized to pH 7.0. Portions (1.0 ml) of samples were deproteinized by the addition of 0.5 ml of 3 N $HClO_4$ and centrifugation. Portions of the supernatant fluids were assayed for pyruvate. Other portions of deproteinized samples were hydrolyzed by heating for 10 min at 100°. The samples were neutralized with KOH and centrifuged, and then 50-μl aliquots from each sample (to facilitate identification 0.1, 0.2, and 0.3 μmole of nonradioactive 2,3-P-glycerate, 3-P-glycerate, and P_i, respectively, were added) chromatographed on Whatman No. 1 paper (ten sheets of 46 × 56 cm were washed at a time successively with 3 l. of 1 N HCl, 5 l. of H_2O, 3 l. of 0.02% EDTA, and 3 l. of H_2O). The chromatograms were developed as described previously (Grisolia and Cascales, 1966). The concentration of reagents and enzymes were essentially as described previously (Grisolia and Cascales, 1966); that is, in most experiments the enolase and pyruvic kinase were about 30 times higher than the mutase added (0.1 unit). At such levels there was about 50–75% utilization of 3-P-glycerate. On the other hand, at the very high levels of mutase used in some experiments, the exchange rate was so fast that in the presence of the large pool of 2,3-P-glycerate used it did not matter and would not change appreciably the distribution of radioactivity, if a portion of the P-glycerates was converted to PEP.[1]

Results

As illustrated in Table I with muscle mutase, increasing the salt concentration results in a shift in the pattern of labeling so that at the lowest salt concentration the distribution of radioactivity is preferentially into 2-P-glycerate. Increasing the salt concentration to about 0.5 M yields preferential labeling on 2,3-P-glycerate. Again, as seen by the amount of pyruvate formed, the over-all velocity of the reaction changes little. In this experiment, as well as in the ones shown in the other

TABLE I: Effect of Time and of Salt on ^{32}P Distribution into P-Glycerates.[a]

Addn (μmoles)	% ^{32}P into 3-PGA	2,3-DPGA	2-PGA	Pyruvate Formed (μmoles)
None	14 (33)	3 (2)	83 (65)	1.0 (0.8)
Tris (35)	27	8	65	0.8
KCl (10)				
KCl (475)	26 (46)	61 (48)	13 (6)	0.8 (0.5)

[a] Each tube contained the following per milliliter: 1.5 μmoles of 3-P-glycerate-^{32}P, 3.3 units of enolase, 3.5 μmoles of ADP, 5 μmoles of $MgSO_4$, 6.6 μmoles of 2,3-P-glycerate, 8 μg of pyruvic kinase, and 0.1 mg of serum albumin. Chicken breast mutase (0.1 unit) was added at zero time. At zero time and at the indicated times 1.0-ml portions were withdrawn, 0.5 ml of 3 N $HClO_4$ was added, the samples were centrifuged, and portions of the supernatant fluids were assayed; 30-min incubation. (The figures in parenthesis refer to samples incubated for 15 min.)

tables, coupling the reaction to pyruvate kinase allows no chance for equilibration of the isotope *via* back reactions. While not illustrated, similar experiments were carried out with the yeast enzyme. All experiments essentially confirm our first findings (Grisolia and Cascales, 1966).

It was of interest to determine the pattern of labeling over a broad range of salt concentrations. As shown in Table II, from negligible to high concentration of KCl,

TABLE II: Effect of Increasing Salt on ^{32}P Distribution into P-Glycerates.[a]

KCl Added (μmoles)	% ^{32}P into 3-PGA	2,3-DPGA	2-PGA	Pyruvate Formed (μmoles)
0	25	4	71	1.1
50	19	10	71	1.1
100	16	18	66	1.1
200	17	37	46	1.1
400	28	49	23	1.0
1000	69	30	1	0.5
1400	82	18	Nil	0.3
1800	83	17	Nil	0.3

[a] The conditions were as described for Table I. All tubes contained 35 μmoles of Tris–Cl^- at pH 7.4.

the incorporation into 2-P-glycerate is larger at low ionic strength, shifting preferentially into 2,3-P-glycerate between 0.2 and 0.4 M KCl. Up to that level there is no inhibition of the over-all reaction. As shown in the table, at 1 M and at higher concentrations of KCl, the incorporation into 2,3-P-glycerate is still very high while the incorporation into 2-P-glycerate becomes negligible.[2] Of course at high salt concentrations there is appreciable inhibition of the over-all reaction. Although not included, this type of experimental data was obtained for a tenfold concentration range with both yeast and muscle P-glyceromutase preparations.

Further experiments tested whether the change in pattern of labeling was really due to an increase in molarity or in ionic strength. As shown in Table III,

[2] The apparently low level of radioactivity in 2-P-glycerate in some experiments at high salt concentration is near the theoretical value for complete exchange. That is, if the radioactivity originally present in 1.5 μmoles of P in 3-P-glycerate is completely exchanged with the 13.2 μmoles of P present in the pool of 2,3-P-glycerate, the theoretical ^{32}P in P will be 6.8% of the starting ^{32}P added. These calculations do not take into consideration the small correction necessary due to spontaneous hydrolysis at zero time (Grisolia and Cascales, 1966), nor any remaining 3-P-glycerate which may not have exchanged, nor small percentages of P_i and P-glycerate which occasionally were present in the radioactive 3-P-glycerate used. Correction for these factors was carried out routinely.

TABLE III: Effect of Increasing Tonicity on ^{32}P Distribution into P-Glycerates.[a]

Addn	% ^{32}P into 3-PGA	2,3-DPGA	2-PGA	Pyruvate Formed (μmoles)
KCl	29	53	18	0.7
NaCl	47	42	11	0.6
LiCl	63	31	6	0.3
Urea	21	3	76	0.95
Glucose	22	3	75	0.7
Mannitol	22	2	76	1.1
Glycerol	20	2	78	1.2
Ethanol	20	3	77	1.1

[a] The conditions were as described for Table II. In all cases 470 μmoles of the indicated reagents was added.

the changes are clearly due to ionic strength; neither glucose, urea, manitol, glycerol, nor ethanol had any effect on exchange rate at the concentrations tested. Nevertheless, as will be exemplified below, at least one of the uncharged materials tested, imidazole, can change the pattern of distribution. It should be further noted that KCl is more effective than NaCl or LiCl. On the other hand, the latter two reagents inhibit somewhat the over-all reaction. While the effect is primarily a cationic effect as shown in Table IV (which also shows

TABLE IV: Effect of Cl^- and of Urea on ^{32}P Distribution into P-Glycerate.[a]

Addn (μmoles)	% ^{32}P into 3-PGA	2,3-DPGA	2-PGA	Pyruvate Formed (μmoles)
None	24	3	73	1.14
Cl^- (47)	25	5	70	1.17
Cl^- (94)	22	5	73	1.12
Cl^- (282)	27	5	68	1.04
Cl^- (440)	31	8	61	1.00
Urea (1000)	20	4	76	1.06
Urea (1880)	43	5	52	0.74

[a] The conditions were as described for Table I. Tris–Cl at pH 7.4 was added when indicated.

that high urea concentrations affect little the distribution of radioactivity), the cations do not increase or decrease the effects as expected if they were to follow a Hofmeister series (see also Table VIII).

Since it has been known that extensive labeling and

equilibration of phosphate among P-glycerates could be obtained, the effect of enzyme concentration on ^{32}P distribution into phosphoglycerates was checked. As illustrated in Table V, with over a 10,000-fold change in mutase concentration, as the concentration of mutase increases the distribution of ^{32}P goes preferentially into 2,3-P-glycerate. This is further exemplified in Table VI where the distribution of label is compared at low and at high salt concentrations and at low and at 100 times higher enzyme concentration. As shown with low enzyme and high salt or with high enzyme concentration, the pattern of distribution is preferentially into 2,3-P-glycerate. Finally, with high enzyme and salt concentrations, essentially no radioactivity is found in 2-P-glycerate. Since it appeared from these and similar experiments that the change in the pattern of labeling may be due to salt-induced increased rate of exchange with the cofactor, 2,3-P-glycerate, it would be possible to study the salt effect without the need of a coupling system, particularly since, from data such as shown in the table, it appeared that the exchange reaction 3-P-glycerate ⟷ 2,3-P-glycerate at low salt is 100–1000 times slower than the catalytic reaction *per se*. As illustrated in Table VII the incorporation of ^{32}P from P-glycerate into 2,3-P-glycerate (from here on 3- and 2-P-glycerates were not separated) increases in the now predictable manner whether high salt, enzyme, or both are used.

TABLE V: Effect of Increasing Mutase on ^{32}P Distribution into Phosphoglycerates.[a]

Expt	Mutase Added (units)	% ^{32}P into 3-PGA	2,3-DPGA	2-PGA	Pyruvate Formed (μmoles)
1	0.2	25	8	67	1.1
	1	9	17	74	1.3
	2	8	20	71	1.3
	4	7	46	47	0.8
2[b]	0.1	35	6	59	1.0
	1	8	12	80	1.2
	10	4	37	59	1.0
	100	4	80	16	1.0
	1000	4	87	9	1.0
3[b]	0.025	97	Nil	3	Nil
	0.25	88	Nil	12	0.3
	10	3	20	77	1.2
	100	4	59	37	1.0
	1000	5	84	11	1.0

[a] The conditions were as described for Table II except as indicated. Incubation 30 min for expt 1 and 15 min for expt 2 and 3. Chicken breast mutase was used for expt 1 and 2 and yeast mutase for expt 3. [b] No Tris–Cl$^-$ or KCl was added to samples of expt 2 and 3.

TABLE VI: ^{32}P Distribution at Several Levels of Chicken Breast Mutase.[a]

KCl Added (μmoles)	Mutase Added (units)	% ^{32}P into 3-PGA	2,3-DPGA	2-PGA	Pyruvate Formed (μmole)
None	0.1	34 (63)	2 (2)	64 (35)	0.7 (0.5)
None	10	14 (14)	55 (51)	31 (35)	0.9 (0.9)
490	0.1	23 (54)	73 (43)	4 (3)	0.7 (0.4)
490	10	8 (8)	92 (92)	Nil	0.9 (1)

[a] The conditions were as per the experiments of Table I except as indicated. The figures in parentheses refer to incubations carried out for 15 min.

TABLE VII: ^{32}P Distribution at Several Levels of Chicken Breast Muscle.[a]

KCl Added (μmoles)	Mutase Added (units)	% ^{32}P into 3-PGA + 2-PGA 15 min	3-PGA + 2-PGA 30 min	2,3-DPGA 15 min	2,3-DPGA 30 min
None	0.1	96	92	2	8
None	10	26	15	74	84
500	0.1	51	50	44	50
500	10	18	20	82	80

[a] The conditions were as per the experiments of Table I except that no enolase, ADP, or pyruvic kinase was added.

The pH profile for the exchange reaction at low and at high salt concentration shows in Figures 1 and 2 with yeast and muscle enzymes, respectively, that the maximum rate of exchange induced by salt into 2,3-P-glycerate occurs at pH between 7 and 8. At both extremes the incorporation is decreased and at these extremes salt does not increase the rate. It is of interest that with the muscle mutase there are two small peaks of increased activity at low salt concentration at pH 5 and 9. The pH profile does not agree with the pH profile of the catalytic reaction *per se* (Rodwell *et al.*, 1957), where the optimum pH is from 1 to 3 pH units lower (muscle and yeast, respectively), but resembles that of the non-2,3-dependent mutases (Grisolia and Joyce, 1959).

As exemplified in Table VIII, no evidence for a Hofmeister series type of effect was noted. On the other hand, as shown, imidazole increases the exchange rate into 2,3-P-glycerate.

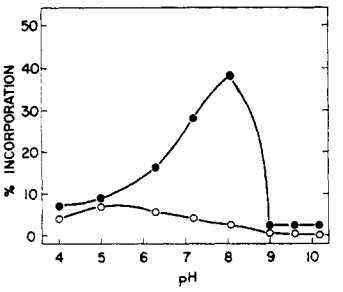

FIGURE 1: Effect of pH and KCl on the extent of ^{32}P incorporation into 2,3-P-glycerate with yeast mutase. The conditions were as described for the experiments of Table I except that no enolase, ADP, or pyruvic kinase was added; mutase 0.18 unit/ml. Open signs, without salt addition; black signs, 400 μmoles of KCl/ml added.

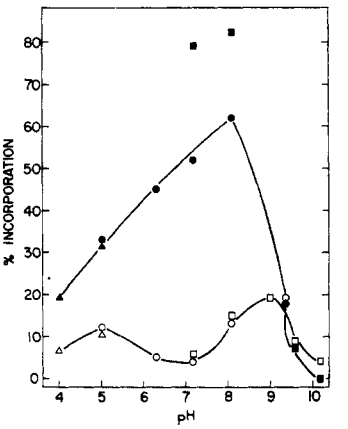

FIGURE 2: Effect of pH and KCl on the extent of ^{32}P incorporation into 2,3-P-glycerate with muscle mutase. The conditions were as described for the experiments of Table I except that no enolase, ADP, or pyruvic kinase was added; mutase 0.22 unit/ml. □, Δ, or O signs refer to experimental points for three separated experiments. Open signs, without salt addition; black signs, 400 μmoles of KCl/ml added.

TABLE VIII: Effect of Some Reagents on Extent of ^{32}P Incorporation into 2,3-P-glycerate with Yeast and Muscle Mutases.[a]

	% ^{32}P into 2,3-DPGA	
Addn (μmoles)	Yeast Mutase	Muscle Mutase
None	8 (71)	7 (89)
LiCl (400)	43 (83)	38 (90)
NaCl (400)	48 (81)	33 (90)
KCl (400)	49 (83)	53 (90)
RbCl (400)	48 (82)	— —
CsCl (400)	34 (82)	41 (91)
Imidazole (350)	— —	45 (83)
KSCN (80)	— —	40 (86)

[a] The conditions were as for the experiments of Table I except that no enolase, ADP, or pyruvic kinase was added. Mutase (0.1 unit) was added to each tube. The figures in parenthesis were obtained with 10 units of mutase and thus served as "controls" to test for essentially complete equilibration of the isotope.

The Effect of Salt on Initial Velocity. Mutase activity was measured as previously described (Rodwell *et al.*, 1957) except that to approximate the conditions of the exchange experiments the volume was reduced to 1 ml and the components of the assay were as follows, expressed in micromoles: 3-P-glycerate, 1.5; 2,3-DPGA, 6.6; and $MgSO_4$, 5. With 0.1, 0.2, 0.3, 0.4, 0.6, and 1 unit of mutase the initial velocities were, expressed as absorbancy change per minute, 0.018, 0.030, 0.040, 0.054, 0.080, and 0.115. In the presence of 500 μmoles of KCl/ml the optical density changes per minute were 0.016, 0.032, 0.041, 0.050, 0.078, and 0.114, with 0.1, 0.2, 0.3, 0.4, 0.6, and 1 unit of mutase, respectively. Velocities were also measured under several other conditions and in no case was there inhibition of mutase activity by salt. However, it should be pointed out that salt does influence the mutase reaction at much lower levels (*e.g.*, micromolar) of 2,3-P-glycerate. However, these salt effects are not germain to the present paper since in the experiments reported here 2,3-P-glycerate was in the millimolar range. It should be noted that 2,3-P-glycerate in the millimolar range is present in erythrocyte of many species (Towne *et al.*, 1957). On the other hand, the marked inhibitory effects of salt at lower concentration, *e.g.*, millimicromolar 2,3-P-glycerate, and its reversal by high 2,3-P-glycerate concentrations will require extensive documentation. At any rate, the apparent change in affinity for 2,3-P-glycerate induced by salt, together with the fact that the substrates 3-P-glycerate or 2-P-glycerate at relatively low concentration are marked inhibitors of the reaction, makes essentially uninterpretable Cleland's (1963) kinetic analysis for P-glycerate mutase.

It should be noted that the findings reported here do not reflect changes in phosphatase activity. The phosphatase activity of the chicken breast P-glycerate mutase is less than $^{1}/_{10,000}$ the mutase activity (Torralba and Grisolia, 1966) and the phosphatase activity of the yeast mutase is $^{1}/_{100,0000}$ the mutase activity (Rodwell *et al.*, 1957). Also, while not documented, similar results were obtained in the presence of added inorganic phosphate which is a well-known inhibitor of the phosphatase activity. Further the 3-P-glycerate present is also a good inhibitor of the phosphatase (Grisolia, 1962).

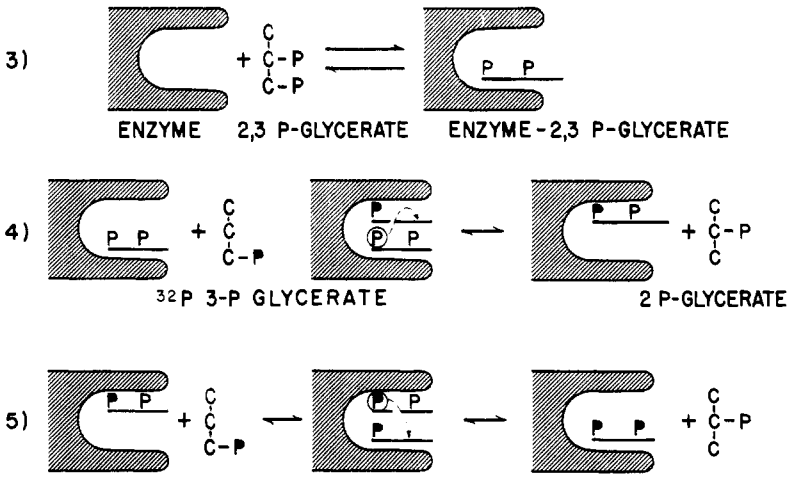

FIGURE 3: A scheme for exchange reactions of P-glycerate mutases.

Discussion

The paper, in confirmation of preliminary experiments (Grisolia and Cascales, 1966), shows a change in extent of ^{32}P-labeling from 3-P-glycerate into the P-glycerate mutase cofactor 2,3-P-glycerate. The changing pattern is essentially a cationic effect and not a primary salt effect (Maron and Prutton, 1965), and it does not follow a Hofmeister type of behavior. While the pH profile would possibly indicate the participation of a histidine site, and indeed the stimulation by imidazole could be thus interpreted, a great deal of work needs to be done before a clear understanding of the effects described here can be achieved. Regardless of the intimate mechanism, two possibilities are apparent. At high ionic strength the 2,3-P-glycerate-dependent mutases behave as previously thought (Sutherland *et al.*, 1949), that is to say essentially catalyzing reaction 2. At low ionic strength 2.3-P-glycerate yields a new conformation of the enzyme protein (Grisolia, 1964) which would then appear to catalyze reaction 1 without participation of the cofactor, as in the case of the non-2,3-P-glycerate-dependent enzymes (Grisolia and Joyce, 1959). A more likely possibility is that our findings represent an internal shift of phosphoglycerate involving an enzyme–cofactor complex. Such a complex rather than an enzyme–phosphate entity was thought to occur for the muscle mutase (Grisolia *et al.*, 1961) and further documented recently (Torralba and Grisolia, 1966). A mechanism explaining our findings is depicted by the scheme of Figure 3. Reaction 3 represents the reversible interaction of 2,3-P-glycerate and enzyme. The enzyme–cofactor is necessary for the catalytic reaction to proceed, as per reactions 4 and 5. Since we have not been able to detect kinetically any induction period, the velocity of reaction 3 from left to right is very rapid at least as much as the conversion of 3-P-glycerate to 2-P-glycerate.

Presumably then, at low salt concentrations, the enzyme–2,3-P-glycerate is formed from the nonlabile 2,3-P-glycerate, reaction 3, then the first molecule of 3-P-glycerate (^{32}P, indicated by solid P) is incorporated into 2,3-P-glycerate with the dissociation of a molecule of unlabeled 2-P-glycerate, reaction 4; in a successive reaction another mole of 3-P-glycerate will label entirely the bound 2,3-P-glycerate, reaction 5. From here on successive reactions will result in formation of 2-P-glycerate with the same or essentially as high specific activity as the starting 3-P-glycerate provided that the bound 2,3-P-glycerate remains bound and/or dissociates very slowly and thus does not exchange extensively with the pool of 2,3-P-glycerate. However, at high salt concentration, the rate of reaction 3 should increase from right to left to the point that the 2,3-P-glycerate-^{32}P dissociates rapidly and mixes with the pool of 2,3-P-glycerate. Nevertheless in this case it appears that any 2-P-glycerate formed will become progressively labeled even if by the small amounts, *i.e.*, 6% or less, expected due to equilibrium of ^{32}P under the conditions used. Since at the high mutase and salt concentrations no radiation was noted on 2-P-glycerate, some of the lower values, *e.g.*, 0–3%, may not be reliable. However, this does not change the over-all aspects of the findings reported here.

Regardless of the mechanism, our findings may possibly apply to other cases, particularly when an enzyme–cofactor or substrate may be intermediates. For example, such may be the explanation for the discrepancy between the findings of Ray and Roscelli (1964) and Harshman *et al.* (1965) regarding the role of glucose 1,6-phosphate in the phosphoglucomutase reaction, and for the report of Conway (1964) showing that at high salt levels the need for GTP in amino acid polymerization can be partially by-passed.

There has been a great deal of interest recently in reactions using P-glycerates, probably because of the regulatory roles of such reagents in several systems. Ghosh and Preiss (1965), for example, have shown

that 3-P-glycerate increases the biosynthesis of starch using spinach chloroplasts (Dr. Arnon kindly called this to our attention); Levy and Alpers (1965) found that P-glycerate stimulates the biosynthesis of glucose 1,6-phosphate from glucose monophosphates, and Zancan *et al.* (1965) reported transferase reactions between adenoyl-P-glycerates and P-glycerates. Our new findings (Grisolia and Cascales, 1966) extends the implication of other possible roles of 2,3-P-glycerate, a strong chelating agent, perhaps for maintaining conformation of hemoglobin (Grisolia, 1959).

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