Biochimica et Biophysica Acta, 327 (1973) 82–91

BBA 67061

SYNTHESIS OF GLUCOSE 6-PHOSPHATE BY A 1,3-DIPHOSPHOGLYCERATE–GLUCOSE PHOSPHOTRANSFERASE ACTIVITY OF LIVER MICROSOMES

MARJORIE R. STETTEN AND PAUL K. GOLDSMITH

National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health Bethesda, Md. 20014 (U.S.A.)

(Received July 3rd, 1973)

SUMMARY

1. Glucose 6-phosphate can be synthesized directly from 1,3-diphosphoglycerate and glucose in a reaction catalyzed by a liver and kidney microsomal enzyme whose properties resemble those of glucose-6-phosphatase (EC 3.1.3.9) with its accompanying inorganic pyrophosphate–glucose phosphotransferase activity.

2. Specifically labeled radioactive 1,3-[1-^{32}P]diphosphoglycerate has been prepared and used to show that it is exclusively the acid anhydride phosphate on C-1 of that compound that is transferred to glucose.

3. The possibilities that, in the system used, the glucose 6-phosphate synthesis occurs indirectly from 1,3-diphosphoglycerate by way of 3-phosphoglycerate kinase-catalyzed ATP formation or by way of phosphoglucomutase phosphorylation have been eliminated.

4. Enzymatically catalyzed hydrolysis of 1,3-diphosphoglycerate occurs at 30 °C and pH 6.0 in addition to the appreciable spontaneously occurring hydrolysis of the compound.

5. The enzyme activity, like that of glucose-6-phosphatase and related enzyme activities, is greatly increased by pretreatment of the microsomes at about pH 10 or by detergent pretreatment.

6. In activated preparations the pH optimum of the phosphotransferase reaction was 5.2–5.3 and the K_m = 1.4 mM for 1,3-diphosphoglycerate and 0.08 M for glucose.

7. Under comparable conditions, 1,3-diphosphoglycerate was 30–50% as effective a donor as PP_i in the synthesis of sugar phosphates. Other sugars and sugar alcohols, as well as glycerol can be enzymatically phosphorylated by 1,3-diphosphoglycerate, the relative extent of phosphorylation of each acceptor compound being approximately the same as is observed with PP_i as phosphate donor. The enzyme was unable to catalyze the phosphorylation of creatine with either PP_i or 1,3-diphosphoglycerate as donor.

8. PP_i–glucose (glycerol) phosphotransferase was found to be unable to

catalyze the introduction of a second phosphate group into glycerol 3-phosphate or glucose 1-phosphate.

INTRODUCTION

The synthetic phosphotransferase activity of liver and kidney microsomal membrane glucose-6-phosphatase has been the subject of a number of recent studies. Inorganic pyrophosphate[1–3], nucleoside di- and triphosphates[4] and carbamyl phosphate[5] as well as glucose-6-*P* (refs 6, 7) itself and other sugar phosphates[3] can serve as phosphoryl donors. In addition to glucose[1], glycerol and a wide variety of sugars and sugar alcohols[8–10] can be phosphorylated in this way. In the present study we have investigated the possibility that 1,3-P_2-glycerate, a compound which is synthesized in very large amounts[11] as a major intermediate in glycolysis, can, under suitable conditions, donate phosphate to glucose in the enzymatic synthesis of glucose-6-*P* by direct phosphotransferase action without the intervention of ATP formation.

METHODS

The microsomal enzyme used was prepared from the livers of male Sprague–Dawley rats which had been fasted overnight[2]. Livers were homogenized in 0.25 M sucrose with 1 mM EDTA at pH 7.2. After removal of cellular debris, nuclei and mitochondria by two centrifugations for 10 min at 10 000 × *g*, microsomes were separated from the supernatant fluid by 1 h centrifugation at 105 000 × *g*. The microsomes, further freed of any supernatant by resuspension and recentrifugation in sucrose–EDTA, were again resuspended at a concentration of about 10 mg of protein per ml and stored frozen in small aliquots.

Enzymes were assayed by methods previously described[2,12] or given in the captions to Tables and Figures. Activated enzyme was prepared by the addition of 1 vol. of 1 M NH_4OH to 9 vol. of microsomal suspension followed by warming of the resulting suspension at pH 9.6–10.0, for 20 min at 30 °C (ref. 13).

1,3-P_2-glycerate was prepared from DL-glyceraldehyde-3-*P* (obtained as its diethyl acetal from Sigma Chemical Co.) by oxidative phosphorylation in the presence of NAD^+, inorganic phosphate and glyceraldehyde-3-*P* dehydrogenase with acetaldehyde and alcohol dehydrogenase to reoxidize NADH, as described by Negelein[14]. The product, under all known conditions, decomposes spontaneously to 3-*P*-glycerate and P_i (ref. 14). In order to retard hydrolysis the compound was stored frozen in dilute solution at pH 7.6 in small aliquots. Each freshly thawed sample was assayed for 1,3-P_2-glycerate before use[14].

PP_i labeled with ^{32}P was prepared from $^{32}PO_4^{3-}$ (ref. 15). The same preparation of $^{32}P_i$ was used to synthesize 1,3-[1-^{32}P]-P_2-glycerate from DL-glyceraldehyde-3-*P*. Thus the acid anhydride phosphate of 1,3-P_2-glycerate was labeled with the same concentration of ^{32}P as was present in each phosphate of the $^{32}PP_i$.

Phosphorylated sugars or alcohols formed from labeled precursors in the enzymatic reactions were separated from quantitative aliquots (2 or 4 µl) of reaction mixtures by descending paper chromatography with methanol–NH_4OH–water (6:1:3, by vol.). Radioactivity, measured in the clearly defined product area, was

converted into quantity of phosphorylated product by a parallel enzymatic determination of glucose-6-*P* when glucose was the acceptor[9,12].

RESULTS

Source of the P in glucose-6-P formed by phosphorylation of glucose by 1,3-P_2-glycerate

In order to determine which P of 1,3-P_2-glycerate is transferred to glucose in our enzymatic reaction, the reaction of that compound labeled with ^{32}P exclusively in the acid anhydride P on C-1 was compared with that of inorganic pyrophosphate containing the same concentration of radioactive phosphorus.

$$*P_i{*}P_i + \text{glucose} \rightarrow \text{glucose-6-}{*}P + {*}P_i \quad (1)$$

$$H_2O_3{*}P\text{–}O\text{–}\underset{\underset{O}{\|}}{C}\text{–}CH_2OH\text{–}CH_2\text{–}O\text{–}PO_3H_2 + \text{glucose} \rightarrow \text{glucose-6-}{*}P + \text{3-}P\text{-glycerate} \quad (2)$$

diphosphoglycerate

A series of enzymatic phosphotransferase reactions was carried out with several different concentrations of the labeled compounds and the reactions were stopped after 10 or 20 min (Table I). In each case the concentration of glucose-6-*P* was measured enzymatically and the radioactivity in the glucose-6-*P* was determined in the same solution. If only the acid anhydride phosphate of C-1 of the 1,3-P_2-glycerate were transferred to glucose, the radioactivity per unit quantity of the glucose-6-*P* formed would be identical for Reaction 1 as for Reaction 2. Insofar as the ester phosphate of C-3 contributes to the glucose-6-*P* formed, the ^{32}P of glucose-6-*P* would be diminished relative to that derived from $^{32}PP_i$. In all cases the specific activity of the glucose-6-*P* formed was constant, showing that it is exclusively the P of C-1 of 1,3-P_2-glycerate that is transferred.

TABLE I

SOURCE OF THE PHOSPHATE IN GLUCOSE-6-*P* FORMED BY PHOSPHORYLATION OF GLUCOSE BY 1,3-DIPHOSPHOGLYCERATE

Reaction mixtures, with 0.4 M glucose and the indicated concentration of radioactive donor compounds in acetate buffer at pH 5.2, were incubated at 30 °C with activated microsomal enzyme containing about 0.8 mg of protein per ml. Enzymatic reactions were stopped by heating at 100 °C for 3 min and the quantity and radioactivity of the glucose-6-*P* formed determined as described under Methods.

Donor compound	*μmoles/ml in assay*	*Incubation time (min)*	*Glucose-6-P formed (μmoles/ml)*	*Radioactivity (cpm in glucose-6-P)**	*Specific activity (cpm/μmole glucose-6- P formed)*
$^{32}PP_i$ (prep 1)	3.0	20	1.18	1612	1325
$^{32}PP_i$ (prep 2)	38	10	1.84	2476	1379
$^{32}PP_i$ (prep 2)	38	20	3.14	4184	1343
1,3-[1-^{32}P]P_2-glycerate	3.4	10	0.53	702	1366
1,3-[1-^{32}P]P_2-glycerate	6.8	10	0.61	841	1332
1,3-[1-^{32}P]P_2-glycerate	6.8	20	0.93	1249	1346

* cpm in the glucose-6-P separated by paper chromatography from a 2-μl spot of the reaction mixture, all samples counted at the same time.

TABLE II

ACTIVATION OF 1,3-P_2-GLYCERATE-GLUCOSE PHOSPHOTRANSFERASE ACTIVITY BY OH^-

Reaction mixtures, containing 0.4 M glucose and the indicated concentrations of 1,3-P_2-glycerate, were incubated 10 min at 30 °C in acetate buffer at pH 5.2 with microsomal enzyme containing 0.8 mg protein per ml. The enzyme was either untreated, freshly thawed microsomes or the same microsomal preparation pretreated by warming for 20 min at 30 °C in 0.1 M NH_4OH at pH 9.8–10.

1,3-P_2-glycerate (mM)	*Glucose-6-P formed*		*Ratio activated/unactivated*
	(nmoles/min per mg protein)		
	Unactivated enzyme	*Activated enzyme*	
3.6	26	114	4.4
1.8	21	88	4.2
0.9	12	50	4.2

Activation, pH optimum and kinetics

The 1,3-P_2-glycerate–glucose phosphotransferase activity of liver microsomes from fasted adult rats was increased about 4-fold by pretreatment of isolated microsomes with detergents or with alkaline buffer at about pH 10 (Table II) under conditions which were found to cause activation of glucose-6-phosphatase and PP_i–glucose phosphotransferase[16,17]. In most of the studies NH_4OH-activated microsomal preparations were used. The pH optimum for the phosphotransferase was found to be 5.2–5.3 (Fig. 1). At all pH values the quantity of glucose-6-*P* measured by enzymatic analysis was the same as that calculated from the incorporation of ^{32}P from the C-1 phosphate of labeled 1,3-P_2-glycerate, further evidence for the C-1 as the unique source of the P.

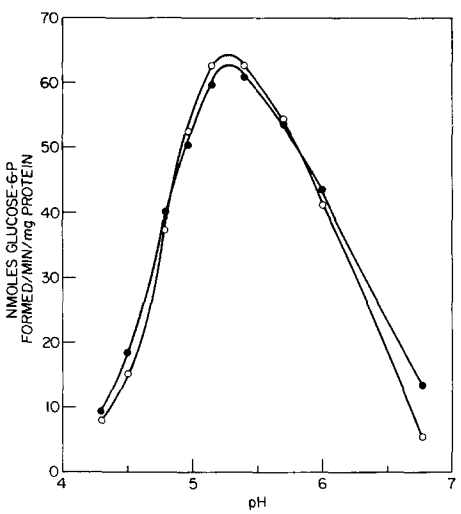


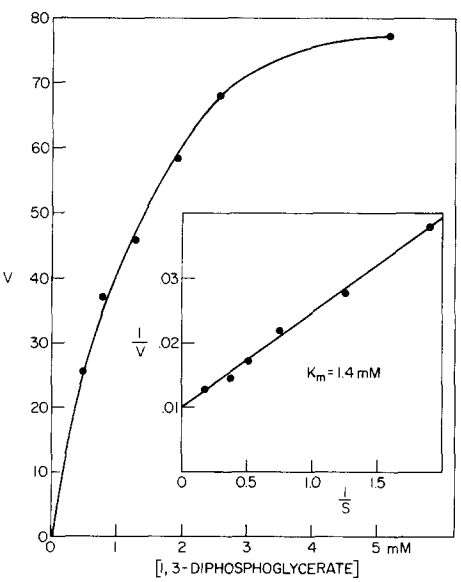
Fig. 1. Effect of pH on microsomal 1,3-P_2-glycerate-glucose phosphotransferase activity. Reaction mixtures contained 0.017 M 1,3-[1-^{32}P]P_2-glycerate, 0.4 M glucose, 0.2 M sodium acetate buffer of the pH indicated, and about 0.8 mg of NH_4OH-activated microsomal enzyme per ml. Incubation was for 10 min at 30 °C and reactions were stopped by heating for 3 min at 100 °C. The pH values were those measured at the end of the enzymatic reactions. Samples were analyzed for glucose-6-*P* content (a) enzymatically by measuring the reduction of NADP at pH 7.5 in the presence of glucose-6-*P* dehydrogenase and (b) isotopically by measuring the incorporation of ^{32}P from 1,3-[1-^{32}P]P_2-glycerate. ○, values from enzymatic analyses; ●, values from isotope analyses.

The effect of the concentration of 1,3-P_2-glycerate on glucose-6-P formation at pH 5.2 at constant glucose concentration was studied with activated enzyme. A K_m value of 1.4 mM for 1,3-P_2-glycerate was obtained (Fig. 2). The K_m for glucose in the same phosphotransferase reaction was found to be 0.08 M, identical with the K_m for glucose in the PP_i–glucose phosphotransferase reaction[2].

Organ distribution of the enzyme—occurrence in kidney as well as liver

The occurrence in various organs and tissues of the enzyme which utilizes 1,3-P_2-glycerate to phosphorylate glucose was studied using whole homogenates from which cellular debris had been removed by centrifugation for 10 min at 1000 × g. When assays were carried out at pH 5.1 with 4 mM 1,3-P_2-glycerate as donor and 0.4 M glucose as acceptor, liver homogenates produced 38 nmoles of glucose-6-P per min per mg protein while kidney homogenates, correspondingly assayed, formed 40 nmoles of glucose-6-P. In the other preparations tested, *i.e.* spleen, muscle and brain homogenates, no glucose-6-P was formed under the same conditions. The distribution and relative quantities of this enzyme activity are thus the same as glucose-6-phosphatase and PP_i–glucose phosphotransferase activities[2].

Since 1,3-P_2-glycerate is known readily to undergo spontaneous non-enzymatic decomposition to P_i and 3-P-glycerate[14], a comparison was made between the rates of disappearance of 1,3-P_2-glycerate in the presence and absence of microsomal



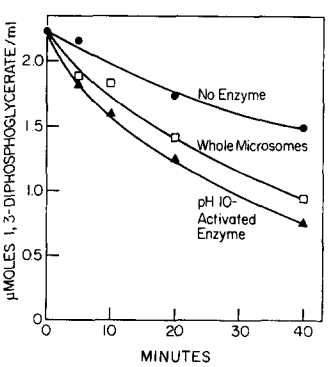


Fig. 2. Effect of concentration of 1,3-P_2-glycerate on rate of formation of glucose-6-P. The concentration of 1,3-[1-^{32}P]P_2-glycerate was varied between 0.5 and 5 mM with glucose constant at 0.4 M in 0.1 M acetate buffer of pH 5.2. Incubation with NH_4OH-activated microsomes was for 10 min at 30 °C and glucose-6-P was determined by the isotope method. v = nmoles of glucose-6-P formed per min per mg protein.

Fig. 3. Enzymatic and non-enzymatic disappearance of 1,3-P_2-glycerate. Reaction mixtures initially contained 2.17 μmoles of 1,3-P_2-glycerate in 0.06 M cacodylate buffer of pH 6.0. Incubation was at 30 °C in the absence of enzyme or with unactivated or NH_4OH-activated microsomal enzyme. Aliquots were removed at the indicated times and analyzed for 1,3-P_2-glycerate by the method of Negelein.

enzyme under conditions optimal for enzymatic glucose-6-*P* hydrolysis (Fig. 3). The spontaneous nonenzymatic loss of 1,3-P_2-glycerate by hydrolysis at 30 °C and pH 6.0 was about 30% in 30 min, which is comparable to the rate observed by Negelein[14] of 2.6% hydrolysis per min or 50% in 27 min at 38 °C and pH 7.2. This rate of hydrolysis is appreciably increased in the presence of microsomal enzyme and still further accelerated in the presence of a pH 10-activated enzyme preparation (Fig. 3). Thus, in addition to the enzymatic synthesis of glucose-6-*P* from 1,3-P_2-glycerate it appears that the enzyme catalyzes the hydrolysis of 1,3-P_2-glycerate in a reaction comparable to the glucose-6-phosphatase activity of the same microsomal preparation.

Other possible routes of synthesis of glucose-6-P from 1,3-diphosphoglycerate

Since 1,3-P_2-glycerate is an important intermediate in glycolysis and a major source of the ATP which, in the presence of a suitable enzyme system, produces glucose-6-*P*, it was necessary for us to establish that, under our experimental conditions, the glucose-6-*P* which accumulated had not been formed by this route. The enzymes necessary for this synthetic route, 3-*P*-glycerate kinase and hexokinase, are soluble enzymes requiring divalent cations and a neutral or alkaline pH for activity. Since our insoluble enzyme system consisted of washed microsomes, free of supernatant and of soluble nucleotides, prepared in the presence of added EDTA to remove cations and has a low pH optimum, our conditions were highly unfavorable for the formation of any glucose-6-*P* from ATP by kinase action. In addition a comparison was made of the effect of added adenine nucleotides, with and without Mg^{2+}, on the phosphorylation of glucose by 1,3-P_2-glycerate and PP_i with microsomal enzyme (Table III). Added ADP did not increase the formation of glucose-6-*P* from either 1,3-P_2-glycerate or PP_i. The small amount of glucose phosphorylation occurring with ADP or ATP, less than with 1,3-P_2-glycerate as donor, has been shown to be due to phosphotransferase catalyzed by the same microsomal enzyme system[4]. Mg^{2+} had a slight inhibitory effect on the reaction with 1,3-P_2-glycerate as it does on the other phosphotransferase reactions[2,9]. Therefore the glucose-6-*P* formation in our system from 1,3-P_2-glycerate was due to direct phosphotransferase activity rather than by way of ADP phosphorylation.

TABLE III

EFFECT OF NUCLEOTIDES, WITH AND WITHOUT Mg^{2+}, ON PHOSPHORYLATION OF GLUCOSE BY 1,3-P_2-GLYCERATE AND PP_i

Reactions were run for 10 min at 30 °C with 0.4 M glucose as acceptor and 2 mM donor compound in acetate buffer at pH 5.1 with activated microsomal enzyme.

Donor substrates (2 mM)	*Glucose-6-P formed (nmoles/min per mg protein)*	
	No Mg^{2+}	*2 mM Mg^{2+}*
1,3-P_2-glycerate	63	61
1,3-P_2-glycerate + ADP	64	55
ADP	22	18
ATP	43	28
PP_i	132	120
PP_i + ADP	135	128

TABLE IV

ABSENCE OF PHOSPHOGLUCOMUTASE ACTIVITY

Enzymatic reactions were run for 20 min at 30 °C in acetate buffer of pH 5.2 with activated microsomal enzyme containing 0.7 mg of protein per ml. The glucose-1-*P* used (Sigma) was free of contaminating glucose-1,6-P_2.

Substrates	*glucose 0.4 M*	*Glucose-6-P formed (nmoles/min permg protein)*		
		no Mg^{2+}	*with Mg^{2+}*	
			2 mM	*20 mM*
Glucose-1-*P* (20 mM)	+	0		0
Glucose-1-*P* (20 mM)	0	0		0
Glucose-1-*P* (20 mM) + 1,3-P_2-glycerate (2 mM)	0	0		0
Glucose-1-*P* (2 mM) + 1,3-P_2-glycerate (2 mM)	0	0	0	
1,3-P_2-glycerate (2 mM)	+	80	79	
Glucose-1-*P* (20 mM) + 1,3-P_2-glycerate (2 mM)	+	69		58
Glucose-1-*P* (2 mM) + 1,3-P_2-glycerate (2 mM)	+	74	74	

The more remote possibility of glucose-6-*P* formation occurring *via* phosphorylation of phosphoglucomutase could also readily be eliminated. Alpers[18,19] has shown that 1,3-P_2-glycerate is capable of phosphorylating phosphoglucomutase in the enzymatic formation of glucose-6-*P* from glucose-1-*P*. The experiment reported in Table IV establishes the absence of phosphoglucomutase activity in our glucose-6-*P*-synthesizing system. No glucose-6-*P* was formed from glucose-1-*P* by our microsomal enzyme preparation either in the presence or absence of Mg^{2+} or of 1,3-P_2-glycerate. Hydrolysis of these reaction mixtures with 0.1 M HCl for 10 min at 100 °C produced no glucose-6-*P*, showing that no glucose-1,6-P_2 had been formed. Added glucose-1-*P* did not increase the quantity of glucose-6-*P* formed from glucose and 1,3-P_2-glycerate either with or without added Mg^{2+} in our complete system.

Comparison of alternative donor and acceptor compounds

The other known mono- and diphosphorylated glyceric acid compounds were

TABLE V

COMPARISON OF PHOSPHOTRANSFERASE DONOR COMPOUNDS IN THE SYNTHESIS OF GLUCOSE-6-*P*

Reaction mixtures, containing 0.4 M glucose and 10 mM donor compound and either acetate buffer at pH 5.3 or Tris–cacodylate buffer at pH 7.2, were incubated for 10 min at 30 °C with NH_4OH-activated microsomes containing 0.8 mg protein per ml of reaction mixture.

Donor	*Glucose-6-P formed (nmoles/min per mg protein)*	
	At pH 5.3	*At pH 7.2*
2-*P*-glycerate	0	0
3-*P*-glycerate	0	0
2,3-P_2-glycerate	0	0
1,3-P_2-glycerate	104	5
PP_i	218	27
Carbamyl-*P*	259	100

compared with 1,3-P_2-glycerate as donors in the phosphorylation of glucose catalyzed by the microsomal enzyme system and were found to be totally ineffective (Table V). This is in accord with the observation that only the acid anhydride phosphate on C-1 is so transferred. When compared at the same concentrations of donor (10 mM) and acceptor (0.4 M glucose) compounds and at the optimum pH of about 5.3, PP_i and carbamyl-P were found to be more efficient donors than 1,3-P_2-glycerate. At pH 7.2, 1,3-P_2-glycerate resembles PP_i in having slight phosphotransferase activity, in contrast to carbamyl-P which retains appreciable activity at the higher pH[5].

TABLE VI

COMPARISON OF PHOSPHOTRANSFERASE ACCEPTOR COMPOUNDS WITH PP_i OR 1,3-P_2-GLYCERATE AS DONORS

Reaction mixtures, containing either 2 mM $^{32}PP_i$ or 1,3-[1-^{32}P]P_2-glycerate and the indicated concentration of acceptor compound in acetate buffer of pH 5.3 were incubated for 10 min at 30 °C with NH_4OH-activated microsomes containing 0.8 mg protein per ml of reaction mixture. Enzymatic reactions were stopped by heating at 100 °C for 3 min and the extent of incorporation of ^{32}P into the phosphorylated product was determined as described under Methods. Controls were carried out with the radioactive compounds in the absence of active enzyme and, where necessary, the isotope concentration of the phosphorylated product was corrected for the blank values. Formation of glucose-6-P was determined enzymatically as well as isotopically on identical samples as the basis for quantitative estimations of the products formed. Creatine phosphate was determined enzymatically[20].

Acceptor	*Concn (M)*	*Phosphorylated product formed (nmoles/min per mg protein)*	
		From 1,3-P_2-glycerate	*From PP_i*
D-Glucose	0.4	56	130
D-Mannose	1.0	50	148
D-Arabitol	1.0	37	107
Glycerol	2.0	32	92
Glycerol	1.0	25	60
D-Galactose	1.0	24	72
Ribitol	1.0	27	55
D-Ribose	1.0	14	33
D-Xylose	1.0	0	0
Creatine	0.1	0	0

Microsomal fractions of liver can utilize PP_i to phosphorylate glucose, glycerol and a wide variety of sugars and sugar alcohols[8]. Table VI records the results of an experiment in which 1,3-[1-^{32}P]P_2-glycerate and $^{32}PP_i$ were compared as donors in the phosphorylation of a number of these acceptor compounds. The relative extent of phosphorylation of the various acceptors was roughly comparable for the two phosphoryl donor compounds. For example, mannose was about as good an acceptor as glucose while the other hexoses, pentoses and sugar alcohols were somewhat less efficiently phosphorylated and xylose was not enzymatically phosphorylated by either donor. These results give support to the assumption that the same phosphotransferase enzyme is catalyzing the reactions with 1,3-P_2-glycerate as with PP_i.

Creatine phosphorylation by direct phosphoryl transfer from 1,3-P_2-glycerate, supposedly catalyzed by a single soluble muscle enzyme, has been proposed[21]. This phosphorylation was later shown to be dependent upon the intermediate formation of ATP from assay ingredients, followed by the synthetic activity of creatine phos-

phokinase in the muscle enzyme preparations used[22,23]. Our insoluble liver microsomal enzyme is also unable to catalyze phosphoryl transfer from either 1,3-P_2-glycerate or PP_i to creatine (Table VI).

Inability of the phosphotransferase enzyme to catalyze the introduction of a second phosphate group

The possible synthesis of diphosphate compounds by PP_i-phosphotransferase action on monophosphorylated compounds having free primary alcohol groups was investigated. Phosphorylation of glycerol, catalyzed by microsomal glucose-6-phosphatase, specifically produces *sn*-glycerol 1-phosphate[9]. Thus the free primary alcohol group of the biologically significant isomer *sn*-glycerol 3-phosphate might be available for phosphorylation by phosphotransferase action. A method using gas chromatography of silyl derivatives[24] was devised by Dr Frank Eisenberg, to whom the authors are indebted, by means of which very small amounts of glycerol-1,3-P_2 could be separated from P_i, PP_i and glycerol-3-P. No trace of glycerol-1,3-P_2 could be detected among the products of the reaction between PP_i and glycerol-3-P with microsomal enzyme under conditions which were optimal for phosphorylation of free glycerol[9].

Attempts to synthesize glucose-1,6-P_2 by phosphorylation of the primary alcohol group of glucose-1-P were also unsuccessful. Glucose-1-P, free of all contaminating glucose-6-P or glucose-1,6-P_2 (Sigma), was used as the acceptor compound with PP_i and microsomal enzymes under conditions optimal for phosphorylation of free glucose[2]. The absence of glucose-1,6-P_2 among the products was established by the finding of no glucose-6-P after heating the products with 1 M HCl for 10 min at 100 °C to hydrolyze the hemiacetal phosphate from C-1. Thus the presence of a

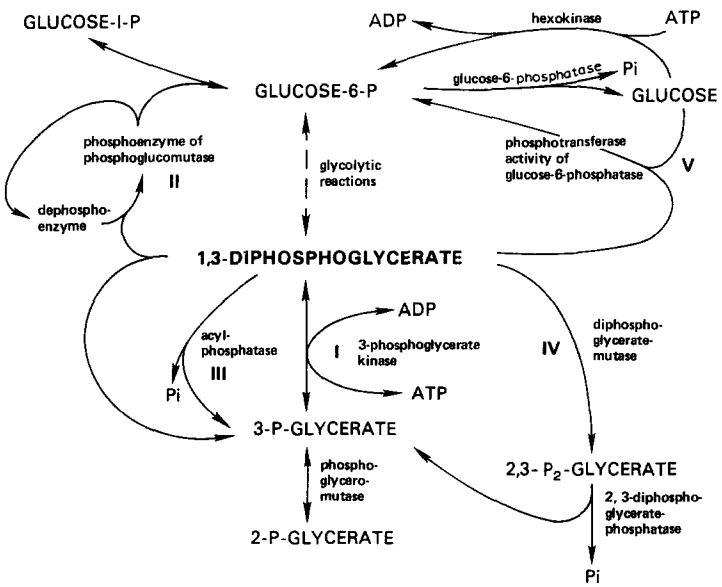


Fig. 4. Pathways of 1,3-P_2-glycerate metabolism. Adapted from Grisolia[25].

phosphate in a sugar or alcohol molecule effectively prevents the phosphorylation of the primary alcohol group by PP_i-phosphotransferase activity.

DISCUSSION

The major fate of 1,3-P_2-glycerate in glycolysis involves the transfer of the 1-phosphate group to ADP to form ATP and 3-*P*-glycerate, a reaction catalyzed by 3-*P*-glycerate kinase (Fig. 4, Reaction I). Grisolia[25] has pointed out that, at the level of 1,3-P_2-glycerate in the glycolytic scheme, a multi-shunt of this reaction may be operating, involving at least three pathways. Fig. 4 is a modification of Grisolia's diagram of his 1,3-P_2-glycerate trident shunt. 1,3-P_2-glycerate can serve in place of glucose-1,6-P_2 in the phosphorylation of phosphoglucomutase (Reaction II)[18,19], or it can be hydrolyzed to 3-*P*-glycerate and P_i by the action of acyl phosphatase (Reaction III)[26]. Alternatively P_2-glycerate mutase can catalyze the conversion (Reaction IV) of 1,3- to 2,3-P_2-glycerate[27], which may then undergo enzymatic hydrolysis to 3-*P*-glycerate.

The present results indicate a fourth possible route, *i.e.* direct phosphorylation of glucose (Reaction V), by which 1,3-P_2-glycerate may be shunted away from the important pathway of ATP synthesis catalyzed by *P*-glycerate kinase. Optimal conditions *in vitro* for direct enzymatic phosphorylation of glucose by 1,3-P_2-glycerate are such that a major role for such a reaction *in vivo* appears unlikely. To the extent that it occurs, it may be pictured as serving, along with the other shunt pathways in controlling levels of glycolytic intermediates and of ATP formation.

REFERENCES

1 Rafter, G. N. (1960) *J. Biol. Chem.* 235, 2475–2477
2 Stetten, M. R. (1964) *J. Biol. Chem.* 239, 3576–3583
3 Nordlie, R. C. and Arion, W. J. (1964) *J. Biol. Chem.* 239, 1680–1685
4 Nordlie, R. C. and Arion, W. J. (1965) *J. Biol. Chem.* 240, 2155–2164
5 Lueck, J. D. and Nordlie, R. C. (1970) *Biochem. Biophys. Res. Commun.* 39, 190–196
6 Hass, L. F. and Byrne, W. L. (1960) *J. Am. Chem. Soc.* 82, 947–955
7 Segal, H. L. (1959) *J. Am. Chem. Soc.* 81, 4047–4050
8 Stetten, M. R. (1965) *J. Biol. Chem.* 240, 2248–2253
9 Stetten, M. R. and Rounbehler, D. (1968) *J. Biol. Chem.* 243, 1823–1832
10 Stetten, M. R. and Kehoe, J. J. (1971) *Biochim. Biophys. Acta* 250, 501–513
11 Ramponi, G. and Grisolia, S. (1970) *Biochem. Biophys. Res. Commun.* 38, 1056–1063
12 Stetten, M. R. (1970) *Biochim. Biophys. Acta* 208, 394–403
13 Stetten, M. R., Malamed, S. and Federman, M. (1969) *Biochim. Biophys. Acta* 193, 260–267
14 Negelein, E. (1957) in *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O. eds), Vol. III, pp. 216–220, Academic Press, New York
15 Malowan, J. E. (1950) in *Inorganic Syntheses* (Audrieth, ed.), Vol. 3, pp. 96–110, McGraw-Hill, New York
16 Stetten, M. R. and Burnett, F. F. (1966) *Biochim. Biophys. Acta* 128, 344–350
17 Stetten, M. R. and Burnett, F. F. (1967) *Biochim. Biophys. Acta* 139, 138–147
18 Alpers, J. B. (1968) *J. Biol. Chem.* 243, 1698–1704
19 Alpers, J. B. and Lam, G. K. H. (1969) *J. Biol. Chem.* 244, 200–204
20 Lamprecht, W. and Stein, P. (1963) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 610–616, Academic Press, New York
21 Cori, O., Abarca, F., Frenkel, R. and Traverso-Cori, A. (1956) *Nature* 178, 1231–1233
22 Morrison, J. F. and Doherty, M. D. (1961) *Biochem. J.* 79, 433–446
23 Cori, O., Marcus, F. and Traverso-Cori, A. (1962) *Nature* 194, 476
24 Hashizume, T. and Saski, Y. (1966) *Anal. Biochem.* 15, 346–350
25 Grisolia, S. (1970) in *Metabolic Regulation and Enzyme Action* (Sols, A. and Grisolia, S., eds), FEBS, Vol. 19, pp. 245–247, Academic Press, Few York
26 Harary, I. (1957) *Biochim. Biophys. Acta* 26, 434–436
27 Rose, Z. B. (1968) *J. Biol. Chem.* 243, 4810–4820