Eur. J. Biochem. *42*, 511—517 (1974)

A Kinetic Study of the Isozymes Determined by the Three Human Phosphoglucomutase Loci PGM_1, PGM_2 and PGM_3

Christine B. Quick, Rachel A. Fisher, and Harry Harris

Medical Research Council Human Biochemical Genetics Unit, Galton Laboratory, University College, London

(Received September 1/November 13, 1973)

The isozyme products (PGM_1, PGM_2 and PGM_3) of the three human phosphoglucomutase genes (PGM_1, PGM_2 and PGM_3) were separated and compared as phosphoglucomutases, phosphoribomutases and glucose 1,6-bisphosphatases.

The K_m values for glucose 1-phosphate (Glc-1-*P*) were estimated at various glucose 1,6-bisphosphate concentrations. When the glucose 1,6-bisphosphate concentration was less than 10 μM, PGM_1 was seen to be much more effective at catalysing the phosphoglucomutase reaction than either PGM_2 or PGM_3. The effect of the coenzyme (glucose 1,6-bisphosphate) concentration was studied and was found to affect the K_m for Glc-1-*P* of PGM_2. As the glucose 1,6-bisphosphate concentration was increased the K_m decreased; this effect was particularly noticeable over the concentration range 100 μM to 400 μM where a small alteration in glucose 1,6-bisphosphate concentration produced a marked fall in the value of the K_m for Glc-1-*P* of PGM_2. The concentration of glucose 1,6-bisphosphate in a range of human tissues was estimated and was found to be within the range 10 μM to 600 μM in most tissues.

PGM_2 was found to be more effective at catalysing the phosphoribomutase reaction than PGM_1. The K_m for ribose 1-phosphate for neither the PGM_1 nor the PGM_2 isoenzymes was affected by the concentration of glucose 1,6-bisphosphate. The possibility of glucose 1,6-bisphosphate acting on PGM_2 and thereby directing whether it functions as a phosphoribomutase or a phosphoglucomutase has been discussed. Ribose 1-phosphate inhibited the phosphoglucomutase activity of the products of all three loci. The ribose 1-phosphate appeared, from Dixon plots, to be competing with glucose 1-phosphate.

The effect of 2,3-bisphosphoglycerate on the phosphoglucomutase activity of the PGM_1 and PGM_2 isozymes was examined; this compound was found to inhibit by competing with glucose 1,6-bisphosphate in both cases.

The ability of the isoenzymes of all three genes to catalyse a glucose 1,6-bisphosphatase reaction has been reported. The K_m values for glucose 1,6-bisphosphate for this reaction were estimated as being of the order of 1000 μM.

Phosphoglucomutase (α-D-glucose-1,6-bisphosphate-α-D-glucose-1-phosphate phosphotransferase) catalyses the apparent isomerisation of α-glucose-1-phosphate (Glc-1-*P*) and α-glucose-6-phosphate (Glc-6-*P*). Leloir *et al.* [1,2] found that α-glucose 1-6-bisphosphate (Glc-1,6-P_2) played an important part in the reaction which was therefore expressed as:

$$\text{Glc-1-}P + \text{Glc-1,6-}P_2 \rightleftharpoons \text{Glc-1,6-}P_2 + \text{Glc-6-}P.$$

It was suggested that the transfer of the phosphate group occurred *via* a phosphoenzyme intermediate [3].

Klenow and Larsen [4] and Klenow [5] found that phosphoglucomutase preparations from muscle and yeast would catalyse the conversion of α-D-ribose 1-phosphate (Rib-1-*P*) to α-D-ribose 5-phosphate (Rib-5-*P*), and that α-D-glucose 1,6-bisphosphate activated this reaction. It was suggested that α-D-

Abbreviations. PGM_1, PGM_2 and PGM_3, the three human phosphoglucomutase gene loci; PGM_1, PGM_2 and PGM_3, the isozyme products of the three gene loci; Glc-1-*P*, glucose 1-phosphate; Glc-6-*P*, glucose 6-phosphate; Glc-1,6-P_2, glucose 1,6-bisphosphate; Rib-1-*P*, ribose 1-phosphate; Rib-5-*P*, ribose 5-phosphate; Rib-1,5-P_2, ribose 1,5-bisphosphate.

Enzymes. Phosphoglucomutase or α-D-glucose-1,6-bisphosphate: α-D-glucose-1-phosphate phosphotransferase (EC 2.7.5.1).

ribose 1,5-bisphosphate (Rib-1,5-P_2) was formed from glucose 1,6-bisphosphate, *i.e.*:

$$\text{Rib-1-}P_2 + \text{Glc-1,6-}P_2 \rightleftharpoons \text{Rib-1,5-}P_2 + \text{Glc-6-}P$$

and that ribose 1,5-bisphosphate then functioned coenzymatically for the phosphoribomutase reaction.

Guarino and Sable [6] isolated phosphoribomutase from yeast, human blood, and human, rabbit and bovine uterus. They reported that the enzyme with phosphoribomutase activity was more thermolabile than phosphoglucomutase isolated from the same source. Subsequently, Kammen and Koo [7], using ion-exchange chromatography, separated two enzymes both with phosphoribomutase and phosphoglucomutase activities. One of these appeared to be primarily a phosphoglucomutase with some phosphoribomutase activity, whereas the other appeared to be primarily a phosphoribomutase with some phosphoglucomutase activity. These two proteins were found to differ not only in their catalytic properties, but also in their molecular size, the phosphoribomutase being about 25% larger than the phosphoglucomutase.

On examination of human tissues by starch gel electrophoresis and a specific staining procedure, many distinct zones of phosphoglucomutase activity may be detected. These have been shown to be determined by three separate and not closely linked gene loci designated *PGM_1*, *PGM_2* and *PGM_3* [8—12]. At each locus more than one allele has been identified and each allele gives rise to a set of two or three isozymes. It is thought that the least anodal isozyme of each set is the "primary" isozyme and that the others are "secondary" isozymes derived from it [13, 14].

Comparisons of the molecular sizes of the isozymes by gel filtration [15, 16] and ultracentrifugation [17] indicate that the isozymes within a set determined by a single *PGM* allele, as well as sets determined by other alleles at the same locus, have the same molecular weight. However, products of different loci have different molecular sizes, those of *PGM_2* being larger than those of *PGM_1* and *PGM_3*. Investigation of the relative thermostabilities of the isozymes shows that those determined by the *PGM_2* locus are much more stable than those determined by *PGM_1* or *PGM_3* [18].

A previous investigation using qualitative methods [19] suggested that all the detectable phosphoribomutase activity of human tissue could be attributed to the *PGM_2* locus isozymes. This activity could be demonstrated using ribose 1-phosphate, 2-deoxyribose 1-phosphate or ribose 5-phosphate as the substrate.

The work reported here is concerned with the kinetic properties of the isozymes determined by the three phosphoglucomutase loci with respect to glucose 1-phosphate, ribose 1-phosphate and glucose 1,6-bisphosphate.

METHODS

Preparation of Samples

Red-Cell Lysates. Whole blood was collected into heparinised tubes and centrifuged at $2000 \times g$ for 10 min at + 4 °C. The plasma and white cells were removed by suction and the red cells were washed three times with 0.9% saline. They were lysed either by sonication or by freezing and thawing.

Tissue Extracts. A washed portion of the appropriate tissue was homogenised with an approximately equal volume of distilled water, followed by centrifugation at $2000 \times g$ for 20 min at + 4°C. The supernatant was removed and the precipitate discarded.

Separation of the Products of the Three Loci

Starch-Gel Electrophoresis. This was carried out according to the method of Spencer *et al.* [8].

Agarose-Acrylamide-Gel Electrophoresis. The patterns of phosphoglucomutase isozymes produced in this medium were identical to those produced by electrophoresis in starch gel. To prepare agarose acrylamide gels, 8 g Cyanogum 41 (BDH) were dissolved in 90 ml distilled water, and the solution was kept at + 40 °C. 3.2 g agarose were dissolved in 100 ml distilled water in an autoclave at 15 lb/in²; this solution was kept at + 55 °C. Just prior to use, 80 mg ammonium persulphate were dissolved in 10 ml distilled water and this was added to the solution together with 1.28 ml 3-dimethylaminoproprionitrile. This solution was added immediately to the Cyanogum 41 solution, mixed and poured into a mould ($29 \times 10.2 \times 0.4$ cm) formed by two glass plates and spacers. The gel was allowed to cool and set, removed from the mould and steeped in a buffer containing 0.01 M Tris, 0.01 M maleate, 0.001 M EDTA and 0.001 M magnesium chloride pH 7.4. After at least 24 h, the gel was placed on a sheet of Melinex (ICI) and a slot ($7.5 \times 0.2 \times 0.4$ cm) was cut out of the gel approximately 8 cm from its prospective cathodal end. Equal volumes of tissue extract and 3.2% molten agarose were mixed and pipetted into the slot. The mixture was allowed to set and the gel was covered with another sheet of Melinex. The gel was set up using the cooling plate system of McAlpine *et al.* [18] at + 4 °C. The bridge buffer contained 0.1 M Tris, 0.1 M maleate, 0.01 M EDTA and 0.01 M magnesium chloride pH 7.4. Electrophoresis was carried out with a voltage of 200 V across the gel for 18 h, the ends of the gel being dipped directly into the electrode buffer. After electrophoresis, strips (2.5 cm wide) were cut from each side of the gel and placed on a glass plate. Using the specific staining mixture of Spencer *et al.* [8] in an agar overlay, these

strips were stained for phosphoglucomutase activity to identify the positions of the isozymes. The central portion of the gel was kept at + 4 °C until the isozymes were clearly visible on the side strips. These were then realigned with the central portion of the gel and pieces corresponding to the positions of the isozymes were cut out, chopped into pieces (approximately 0.5×0.4×0.4 cm) and transferred to small tubes. An approximately equal volume of gel buffer was added to eluate the isozymes. The pieces of gel containing the products of the three gene loci were treated separately and, except where indicated, the "primary" and "secondary" isozymes derived from a single locus were eluted together.

Ion-Exchange Chromatography. A column (45 ×2.5 cm) was packed with DEAE-cellulose (Whatman DE 52) anion-exchange cellulose equilibrated with 0.01 M Tris, 0.01 M maleate buffer pH 6.2. 1.0 ml of an extract of liver was applied to the top of the column. Elution was with a linear gradient of buffered saline produced from 250 ml equilibrating buffer with 250 ml of buffered 0.5 M saline. The column was run at + 4 °C at a flow rate of 100 ml/h and 3-ml fractions were collected. The fractions were examined by starch gel electrophoresis [8] and the isozymes were found to be eluted in an order corresponding to their electrophoretic mobility, *i.e.* the least anodal first and the most anodal last. Ion-exchange chromatography was found to be unsuitable for the isolation of most of the isozymes since there was considerable overlap in their elution. However the "primary" PGM_1 isozyme could be separated, and fractions containing this were pooled and used for kinetic studies.

Enzyme Assays

Phosphoglucomutase. This was assayed using essentially the same reaction sequence as that used for the detection of phosphoglucomutase activity after electrophoresis, except that the reduction of NADP was followed directly by monitoring the change in absorbance at 340 nm. The reaction mixture contained 0.1 M Tris pH 7.6, 5 mM magnesium chloride, 0.02 units/ml glucose-6-phosphate dehydrogenase, 0.2 mM NADP, glucose 1-phosphate containing 1.2% glucose 1,6-bisphosphate and 0.1 ml of suitable diluted sample in a total volume of 1.0 ml. In some experiments, additional glucose 1,6-bisphosphate was included to give higher concentrations of co-enzyme. The reaction was carried out at + 37 °C.

Phosphoribomutase. The reaction sequence is shown in Fig. 1. The reaction mixture contained 0.1 M Tris pH 7.6, 5 mM magnesium chloride, 5 mM NAD, 0.5 mM phosphate pH 7.0, 0.04 mM thiamine pyrophosphate, 0.8 mM xylulose 5-phosphate, 0.08 units/ml transketolase, 0.1 unit/ml glyceraldehyde-3-phosphate dehydrogenase, ribose 1-phosphate containing 0.72% glucose 1,6-bisphosphate and 0.1 ml of a suitably diluted sample in a final volume of 1.0 ml. The blank contained all the components of the reaction mixture except the ribose 1-phosphate. The reaction was carried out at + 37 °C and monitored at 340 nm. In some experiments additional glucose 1,6-bisphosphate was included in the reaction mixture.

Glucose 1,6-bisphosphatase. The glucose 1,6-bisphosphatase assay method was the same as the standard phosphoglucomutase assay except that glucose 1,6-bisphosphate replaced glucose 1-phosphate.

Estimations of Glucose 1,6-bisphosphate in Substrates

Glucose 1-phosphate. Spencer *et al.* [8] found that glucose 1,6-bisphosphate was present as an impurity

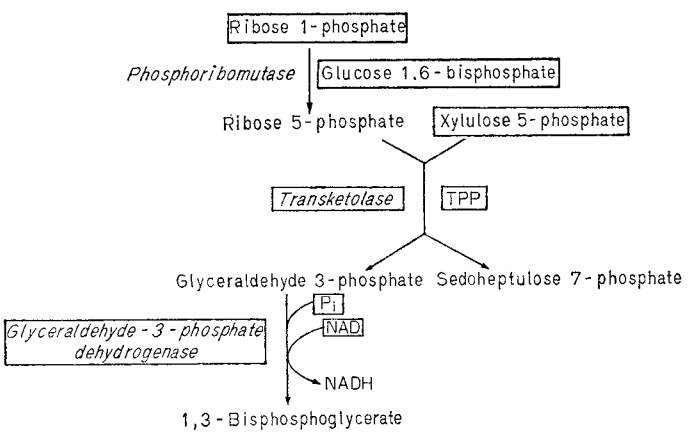

Fig. 1. *Diagram of the reaction sequence for the assay of phosphoribomutase.* The ingredients of the reaction mixture are boxed. TPP = thiamine pyrophosphate

in certain commercial preparations of glucose 1-phosphate. In the present investigations, the level of contamination of glucose 1-phosphate with glucose 1,6-bisphosphate was estimated to be 1.2% by method A of Spencer *et al.* [8].

Ribose 1-phosphate. Previous work [19] demonstrated that commercial preparations of ribose 1-phosphate were contaminated with a co-factor. Using ion-exchange chromatography according to method B of Spencer *et al.* [8], the commercial preparation of ribose 1-phosphate was separable into two peaks of acid-labile phosphate compounds. Paper chromatography of the column fractions indicated that each peak was homogeneous. Material from the first peak had an R_F value similar to that of glucose 1-phosphate, and that from the second peak had an R_F value similar to that of glucose 1,6-bisphosphate. The first peak was therefore assumed to be a sugar monophosphate and the second a sugar bisphosphate. Both were tested for carbohydrate and pentose using the Molisch and Bial's tests, respectively. The first peak contained pentose, but the tests were not sensitive enough to detect either carbohydrate or pentose in the second peak. Material from the second peak was therefore heated with 1 N H_2SO_4 for 10 min in a boiling water bath. It was calculated that this would hydrolyse the phosphate sugar bond in the C-1 position. After neutralisation, the material was assayed using glucose 6-phosphate dehydrogenase, and was found to contain glucose 6-phosphate. This was not present in the unhydrolysed material.

It was therefore concluded that the commercial preparation of ribose 1-phosphate contained a pentose 1-phosphate, presumably ribose 1-phosphate, and a sugar bisphosphate which appeared to be glucose 1,6-bisphosphate. This was present at a level of 0.72%.

Estimation of Glucose 1,6-bisphosphate in Tissues

Tissue extracts were made by homogenising 1.0 g (wet weight) tissue with 5.0 ml distilled water. The supernatant was passed through an ultrafiltration apparatus with an exclusion limit of 10000. The filtrate was used to assay for glucose 1,6-bisphosphate using the method of Spencer *et al.* [8]. The results are shown in Table 1.

Reagents

NAD and NADP were obtained from Boehringer, as were glyceraldehyde-3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase. Transketolase, xylulose 5-phosphate, thiamine pyrophosphate and ribose 1-phosphate were from Sigma. The glucose 1-phosphate and glucose 1,6-bisphosphate were from Wessex Biochemicals Ltd.

Table 1. *Estimated concentration of glucose 1,6-bisphosphate found in tissues*
Figures are based on assays of mol/g (wet weight) and values for water content of tissues from *Documenta Geigy* (1962) 6th Ed

Tissue	Estimated conc. glucose 1,6-bisphosphate
	μM
Liver	76
Brain	467
Spleen	196
Kidney	60
Red cells	588
Uterus	115
Heart	445
Diaphragm	13
Pectoralis	116
Psoas	111
Pyloric sphincter	127

RESULTS

pH-Activity Profiles

Phosphoglucomutase pH activity profiles were obtained for the products of each of the three gene loci. The reaction velocities were determined over the pH range 5.5 to 8.5 in the presence of a large excess of the linking enzyme glucose 6-phosphate dehydrogenase, and using 0.05 M Tris, 0.05 M maleate buffers instead of the 0.1 M Tris buffer of the standard assay system. The concentration of glucose 1-phosphate was 4000 μM and that of glucose 1,6-bisphosphate was 48 μM.

The products of PGM_1 and PGM_3 each appeared to have a single optimum at pH 7.5 (Fig. 2A). The profile for the products of PGM_2 was slightly more complex. The main peak was at pH 8.0, but in addition, at approximately pH 6.5, there was a minor peak which was sometimes apparent only as a shoulder to the main curve (Fig. 2A).

Curves were also obtained using 4000 μM glucose 1,6-bisphosphate as substrate (Fig. 2B). These gave optima similar to those found with glucose 1-phosphate.

pH activity profiles were not determined with ribose 1-phosphate because of the high cost of the substrate and of the linking enzymes when used in the concentrations required to provide an excess over the pH range.

K_m *Determinations*

The K_m values for glucose 1-phosphate without added glucose 1,6-bisphosphate were determined for the separate products of the three gene loci. Thus, for each estimate of the reaction velocity within one K_m determination, the ratio of glucose 1,6-bisphosphate to glucose 1-phosphate was constant at the contaminating level of 1.2%. Estimations of the

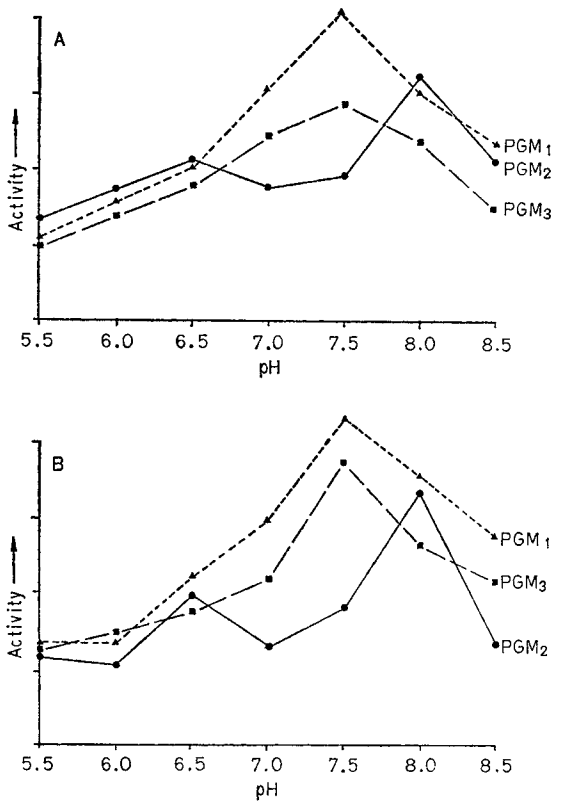

Fig. 2. *pH activity profiles for the products of the three gene loci (A) for the phosphoglucomutase reaction and (B) for the glucose 1,6-bisphosphatase reaction.* (▲-----▲) PGM_1, (●——●), PGM_2, and (■----■) PGM_3

Table 2. *K_m values of PGM_1, PGM_2 and PGM_3 for glucose 1-phosphate and ribose 1-phosphate with increasing concentrations of glucose 1,6-bisphosphate*

K_m values for the products of all three loci for glucose 1,6-bisphosphate are also shown. Where more than one estimation was done, the number of experiments is indicated in brackets. The mean K_m value is then given

Substrate	Concentration of added Glc-1,6-P_2	K_m for PGM_1	PGM_2	PGM_3
	μM			
Glucose 1-phosphate	None	49 (3)	1600 (3)	31000
	190	53	490 (2)	17000[a]
	250	45[b]	110 (2)	36000[a]
	340	34 (2)	19 (2)	18000
Ribose 1-phosphate	None	12000	120 (2)	—
	190	11000	130	—
	340	—	150	—
Glucose 1,6-bisphosphate	—	840 (3)	2600 (2)	1400 (2)

[a] PGM_1 type 2.
[b] PGM_3 type 2—1.

K_m for Glc-1-*P* at glucose 1,6-bisphosphate concentrations of the order of 190 μM and 340 μM were also made. The results are shown in Table 2. Increasing the concentration of glucose 1,6-bisphosphate to 340 μM had no effect on this K_m for PGM_1 or PGM_3. However the estimates of K_m for PGM_2 decreased by approximately 100-fold.

All of the K_m determinations for PGM_2 were done on phenotype-1 samples, but for the products of the other two loci two phenotypes were compared and were found to have very similar K_m values in each case. Furthermore, it was found that the "primary" PGM_1 isozymes separated either by ion-exchange chromatography or by electrophoresis in agarose-acrylamide had K_m values comparable to the K_m obtained for a mixture of the "primary" and "secondary" isozymes.

Estimates of the K_m values for ribose 1-phosphate were made for PGM_1 and PGM_2 (Table 2). Estimates for PGM_3 were not made because of the very small samount of these isozymes available, and because of their low activity towards ribose 1-phosphate. The K_m for Rib-1-*P* for PGM_1 was approximately 100 times greater than that obtained for PGM_2. The K_m values were not significantly altered by further addition of glucose 1,6-bisphosphate. This suggests that the amount of co-enzyme contaminating the ribose 1-phosphate was sufficient to saturate both enzymes.

Estimates of the K_m values for glucose 1,6-bisphosphate acting as substrate rather than coenzyme were made (Table 2). These values were similar for the products of all three loci, *i.e.* approximately 1000 μM. Since the concentrations of glucose 1,6-bisphosphate when used as a coenzyme were considerably lower than this (maximum 340 μM) the effects of its competing as a substrate in its own right must be minimal.

K_i *Determinations*

In order to determine whether the substrates, glucose 1-phosphate and ribose 1-phosphate, were binding at the same active site, inhibition studies were done. Examination of the Dixon plots (Fig. 3) indicated that for the products of all three loci, ribose 1-phosphate was competitively inhibiting the phosphoglucomutase reaction. Similarly, for PGM_1 and PGM_2, glucose 1-phosphate competitively inhibited the phosphoribomutase reaction. Estimates of the K_i values are given in Table 3.

2,3-Bisphosphoglycerate, present in high concentrations in red cells [20—25], has been shown to inhibit the phosphoglucomutase reaction, competing with the coenzyme glucose 1,6-bisphosphate [25]. The K_i values for 2,3-bisphosphoglycerate were estimated for the PGM_1 and PGM_2 isozymes. PGM_3 cannot be demonstrated in red cells. Examination

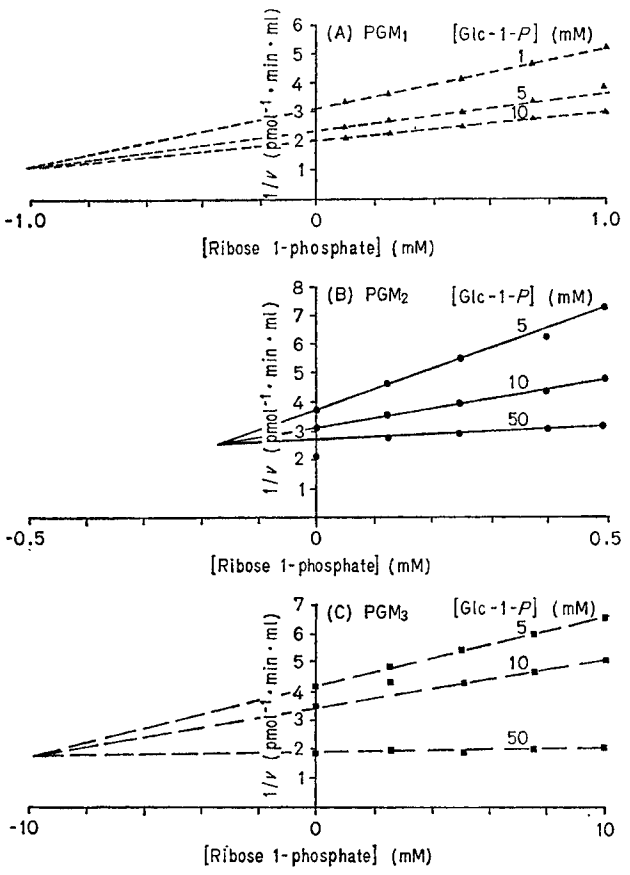

Fig.3. *Dixon plots for the inhibition of the phosphoglucomutase reaction by ribose 1-phosphate for (A) the PGM_1 (B) the PGM_2 and (C) the PGM_3 isozymes*

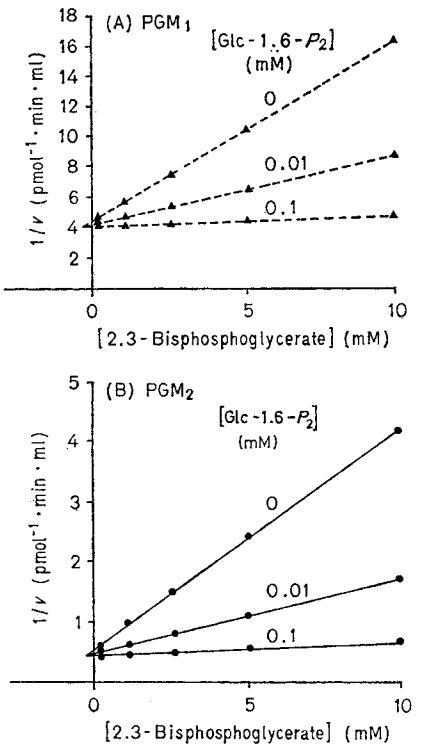

Fig.4. *Dixon plots for the inhibition of the phosphoglucomutase reaction by 2,3-bisphosphoglycerate for (A) the PGM_1 and (B) the PGM_2 isozymes*

of the Dixon plots (Fig.4) suggest that 2,3-bisphosphoglycerate is competing with glucose 1,6-bisphosphate. The K_i values for 2,3-bisphosphoglycerate were similar, 380 μM for PGM_1, and 300 μM for PGM_2.

DISCUSSION

PGM_1 is an efficient phosphoglucomutase having a low K_m for Glc-1-*P* unaffected by increasing the concentration of glucose 1,6-bisphosphate above about 1 μM. Its high K_m for Rib 1-*P* and the low degree of competitive inhibition by ribose 1-phosphate suggest that it is a poor phosphoribomutase compared with PGM_2.

PGM_2 with low concentrations of glucose 1,6-bisphosphate has a high K_m for Glc-1-*P*, but its efficiency as a phosphoglucomutase increases with increasing concentrations of glucose 1,6-bisphosphate. At 340 μM glucose 1,6-bisphosphate it appears to be as effective a phosphoglucomutase as PGM_1. However unlike PGM_1 it has a very high affinity for ribose 1-phosphate and is an efficient phosphoribomutase. In this role it is not dependent on high levels of glucose 1,6-bisphosphate.

PGM_2, therefore, is peculiar in that at low concentrations of glucose 1,6-bisphosphate it functions best as a phosphoribomutase, whereas at high concentrations of the coenzyme it is rather more effective as a phosphoglucomutase than a phosphoribomutase. Thus, the degree to which PGM_2 can be

Table 3. K_i *values for Glc-1-P for PGM_1 and PGM_2 using ribose 1-phosphate as substrate, and K_i values for Rib-1-P for PGM_1, PGM_2 and PGM_3 using glucose 1-phosphate as substrate*

No additional glucose-1,6-bisphosphate was used in any of these determinations. Mean values are given, and the number of determinations is indicated in brackets

	K_i for	
	Glc-1-*P* (Rib-1-*P*, substrate)	Rib-1-*P* (Glc-1-*P*, substrate)
	μM	
PGM_1	38 (2)	25000 (3)
PGM_2	3900 (2)	390 (4)
PGM_3	—	27000 (3)

considered as a phosphoribo or phosphoglucomutase must depend on the concentration of glucose 1,6-bisphosphate.

The concentration of glucose 1,6-bisphosphate in most tissues is between 10 μM and 600 μM (Table 1). Over this range, the K_m for Glc-1-*P* for PGM_2 changes rapidly (Table 2). Thus it seems possible that in any particular tissue the overall level of phosphoglucomutase potential, being dependent on both PGM_1 and PGM_2, will be affected by the concentration of glucose 1,6-bisphosphate.

Beutler reported that 2,4-bisphosphoglycerate inhibits the phosphoglucomutase reaction by competing with glucose 1,6-bisphosphate. Our results confirm this finding and show that PGM_1 and PGM_2 are similarly affected.

PGM_3 is a poor phosphoglucomutase compared with PGM_1 and PGM_2 and its efficiency is not increased by additional glucose 1,6-bisphosphate. The results of the ribose 1-phosphate inhibition experiments suggest that it also has a low afinity for this substance. Possibly it has some other primary role.

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C. B. Quick, R. A. Fisher, and H. Harris, M.R.C. Human Biochemical Genetics Unit, Wolfson House, 4 Stephenson Way, London, Great Britain NW1 2HE