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ISOENZYMES OF PHOSPHOGLUCOMUTASE FROM HUMAN RED BLOOD CELLS: ISOLATION AND KINETIC PROPERTIES

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ABSTRACT

A procedure has been developed for the purification of phosphoglucomutase from human red cell (phenotype PGM_1 a1 or a3) lysates. It yields homogeneous isoenzyme preparations of the products ("primary" and "secondary") of the two PGM_1 and PGM_2 loci with distinctive pI (from 6.07 to 5.29). There are substantial differences between PGM_1 and PGM_2 isoenzymes, having single polypeptide chains of 58,500 and 69,000 Mr respectively and showing different thermostability. The kinetic properties of all the isoenzymes for the phosphoglucomutase reaction are essentially the same (apart from the specific activity of 1089-1263 units/mg for PGM_1 forms vs 37-42 units/mg for PGM_2 forms), but there are striking differences in substrate specificity. In fact the products of PGM_1 locus are "true" phosphoglucomutases, being specific to mutate glucose monophosphates, whereas the PGM_2 forms also display phosphoribomutase and glucose 1,6-bisphosphate synthetic activities. Some kinetic properties of these "side activities" are also reported.

INTRODUCTION

Phosphoglucomutase (PGM, E.C. 2.7.5.1) plays an important role in glycogen metabolism by catalyzing the interconversion

of glucose 1-phosphate (G1P) and glucose 6-phosphate (G6P), with glucose 1,6-bisphosphate (G1,6P_2) and Mg^{2+} as cofactor[1-4]. The enzyme has been extensively studied in muscle[5-9] and liver tissues[10-12] as well as in yeast[13-15] and bacterial cells[16-18]. It has been reported that PGM from diverse origins has a molecular weight between 60,000 and 65,000 and that the reaction mechanisms of different PGM are not identical. Regarding this, it has been shown that the kinetics of PGM from yeast and bacterial cells proceed via a "random sequential" mechanism[13,14,16,17], although some conflicting results were reported later[15,18], while PGM from other sources exhibited "ping-pong" kinetics[5-12].

Despite the lack of glycogen metabolism, substantial PGM activity has also been found in human erythrocytes[19] where the enzyme displays a polymorphism of genetic origin[20,21]. In fact several enzyme patterns (phenotypes) can be detected by starch gel electrophoresis and/or isoelectric focusing in haemolysates according to the expression of PGM_1 and PGM_2 loci[22,23], the products of which contribute almost equally to the total PGM activity[20,24].

Previously we have partially isolated four PGM forms from human red cell lysate[25] and have found that "primary" and "secondary" PGM_1 isoenzymes are "true" phosphoglucomutases whereas those of PGM_2 exibit multifunctionality, also showing phosphoribomutase (PRM) and G1,6P_2 synthetic ability. By using the partially purified preparations of PGM_1 and PGM_2 "primary" isoenzymes we have also found that they share a "ping-pong" kinetic mechanism, show similar Km for substrate (G1P) and cofactors (G1,6P_2 and Mg^{2+}) and have the same pH-activity profile (optimum close to pH 8.0)[26].

In this paper we describe a procedure for obtaining homogeneous preparations of "primary" and "secondary" PGM_1 and PGM_2 isoenzymes from human erythrocytes (phenotype PGM_1 a_1 or PGM_1 a_3). The comparison of some functional and structural properties of the four isoenzymes is also reported and discussed in relation to the post-translational formation of "secondary" from "primary" isoenzymes[27] and to the "multifunctionality" of the PGM_2 forms.

MATERIALS AND METHODS

Chemicals.

All substrates, coenzymes, auxiliary enzymes were from either Sigma (St. Louis, Mo., U.S.A.) or Boehringer-Mannheim (F.R.G.). [1-^{32}P] -1,3-bisphosphoglycerate (specific radioactivity between 10^7 and 10^8 cpm/μmol) was prepared enzymatically according to Rose[28] by using [^{32}P] -phosphoric acid (Amersham International plc, Amersham, England). The chromatographic matrices DE52, P11 and CM52 celluloses were obtained from Whatman (Maidstone, Kent, U.K.) and Matrex ™ gel green A was from Amicon (Lexington, Mass., U.S.A.). AcA44-Ultragel and Ampholines were purchased from LKB (Bromma, Sweden). Sephadex G-100 from Pharmacia (Uppsala, Sweden). Agarose of low electroendosmosis from FMC (Rockland, Me., U.S.A.). Starch hydrolysed for gel electrophoresis was from Connaught Med. Res. Lab. (Toronto, Canada). Membrane ultrafilter system was supplied by Amicon (Amicon B.V., Oosterhout, Holland). All other reagents employed were of analytical grade.

Blood donors.

Blood utilized for the preparation of PGM isoenzymes was drawn, after informed consent was obtained, from normal adult individuals possessing the phosphoglucomutase phenotype PGM_1 a1 or PGM_1 a3 (according to the nomenclature proposed by Kuhnl et al.[29]) as judged by agarose gel electrophoresis[23].

Assay procedures.

(a) Phosphoglucomutase (E.C. 2.7.5.1) activity was assayed by the basic method of Beutler[19]. The assay mixture contained in a total volume of 1 ml a suitable amount of enzyme, 100 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 5 mM $MgCl_2$, 0.15 mM glucose 1,6-bisphosphate, 2 mM glucose 1-phosphate, 0.2 mM $NADP^+$ and 0.05 IU of glucose 6-phosphate dehydrogenase. After 5 min preincubation the increase in absorbance at 340 nm was followed with a spectrophotometer.

(b) Phosphoribomutase (E.C. 2.7.5.6.) activity was as follows: the reaction mixture (0.5ml) contained a suitable amount of enzyme, 100 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 5 mM $MgCl_2$, 0.15 mM glucose 1,6-bisphosphate and 2 mM ribose 1-phosphate. The incubation was performed at 37°C. Aliquots of 0.1 ml were taken at 0,10,20 and 30 min, transferred into tubes containing 0.8 ml of 0.25 mM H_2SO_4 and heated at 100°C for 7 min. The disappearance of the acid-labile phosphate of ribose 1-phosphate signifying the enzyme activity was followed according to Fiske and SubbaRow[30].

One unit of phosphoglucomutase or phosphoribomutase activity is defined as the amount of enzyme which converts 1 µmol of substrate/min at 37°C.

(c) Glucose 1,6-bisphosphate synthesis was measured by the radiochemical method. The reaction mixture (0.5 ml) contained

appropriate enzyme sample, 50 mM Tris-HCl (pH 7.5), 2mM $MgCl_2$, 2 mM 2-mercaptoethanol, 2 mM glucose 1-phosphate and 4 µM [1-^{32}P]-1,3-bisphosphoglycerate. A paired incubation without glucose 1-phosphate was carried out for each enzyme sample. Both reaction mixtures were incubated at 37°C for 5 min. The reaction was stopped by adding 0.25 ml of a solution containing 2M H_2SO_4 and 2 mM NaH_2PO_4. The recovery of the radioactive acid-stable product(s) from the mixture was performed according to Rose at al.[31]. Radioactivity was determined in a Packard Tri-Carb scintillation spectrometer with the scintillant Emulsifier Scintillator 299. The difference between the radioactivity incorporated in the presence and in the absence of glucose 1-phosphate, was used to calculate the glucose 1,6-bisphosphate formation. A unit of synthase activity is that responsible for the tranfer of phosphate at the rate of 1 µmol/min under these conditions.

(d) Protein was determined according to Lowry et al.[32] with bovine serum albumin as a standard, except haemoglobin, which was measured with Drabkin's solution as described by Beutler[19].

Starch gel electrophoresis.

Electrophoresis on starch gel at pH 7.4, 5 V/cm for 17 h at 4°C and the subsequent staining for phosphoglucomutase activity were performed as described by Harris and Hopkinson[20].

Molecular weight determination.

The molecular weights of the purified phosphoglucomutase isoenzymes were determined by polyacrylamide (7.5%) gel electrophoresis in sodium dodecyl sulfate (0.1%) according to the procedure of Weber and Osborn[33]. Molecular weight calibration curves were obtained by using phosphorylase b

(Mr=94,700), phosphoglucomutase (Mr=61,600), 3-phosphoglycerate kinase (Mr=47,000), lactate dehydrogenase (Mr=35,000) and monophosphoglycerate mutase (Mr=29,000) as standards. The molecular weights were also determined by gel filtration on an Ultrogel AcA44 column (1,6 x 45,5 cm) equilibrated with 10 mM Tris-HCl (pH 7.1) containing 2 mM 2-mercaptoethanol, 8% (w/v) glycerol, 0.5 mM EDTA and 100 mM NaCl at 4°C. A 0.5 ml sample was layered on the surface of the gel and eluted with equilibration buffer. The flow rate was 9.6 ml/h and 1 ml fractions were collected. The column was calibrated with lactate dehydrogenase (Mr=140,000), rabbit red cell hexokinase[34] (Mr=105,000), 3-phosphoglycerate kinase (Mr=47,000) and myokinase (Mr=21,000).

Isoelectric focusing in liquid column.

Isoelectric focusing of purified phosphoglucomutase isoenzymes was carried out on LKB 8100 electrofocusing equipment in a glycerol gradient solution and in a pH gradient of 5 to 8 (or 4 to 6) and 3.5 to 10 at 1% (w/v) Ampholine concentration according to the instruction of the manufacturer. The sample was layered after the pH gradient was formed. The focusing was continued for 16 h with a voltage of 400 V and for 2 h at 600 V. Cooling temperature was 4°C, elution flow rate was 60 ml/h and fractions of 1 ml were collected and measured for the phosphoglucomutase activity and the pH value.

RESULTS AND DISCUSSION

The following purification procedures, carried out at 4°C, make it possible to isolate four phosphoglucomutase isoenzymes ("primary" and "secondary" PGM_1 and PGM_2 forms) from human red cell PGM_1 a1 or PGM_1 a3 phenotypes (29) in which a single

allele of the "PGM_1-gene" originally described by Spencer et al.[35] is expressed.

Purification of PGM Isoenzymes

Step 1: Preparation of haemolysate. Blood from a pool of donors was collected into heparin and centrifuged at 1,500g for 10 min to remove plasma and buffy coat. The packed cells were washed twice with cold isotonic solution (160 mM KCl buffered with 10 mM Tris-HCl at pH 7.4) and lysed by adding four volumes of a cold hypotonic solution (1 mM $MgCl_2$ and 1 mM 2-mercaptoethanol). After 30 min the stromata were removed by centrifuging the lysate for 60 min at 20,000g. The resulting supernatant was dialysed overnight against 10mM Tris-HCl (pH 7.1) containing 1 mM $MgCl_2$, 2 mM 2-mercaptoethanol and 8% (w/v) glycerol (Buffer A).

Step 2: Removal of the haemoglobin. The dialysed haemolysate (1,000 ml) was added with glucose 1-phosphate (G1P) to reach 2.5 mM final concentration and treated with 200 g of phosphocellulose P11 moisture (corresponding to 60 g of the original dry powder previously washed with acid and alkali, equilibrated with Buffer A and filtered before use). During the addition with constant stirring, the pH was maintained at 7.3 by adding 2N NaOH. The phosphocellulose was removed by filtration with suction and the treatment was repeated twice more. At this point more than 90% of the haemoglobin was removed from red cells lysate. Solid ammonium sulfate was added to 75% saturation, the precipitate was collected by centrifugation, dissolved in a small volume of Buffer A and dialyzed against 300 volumes of the same buffer without $MgCl_2$ (Buffer B) with two changes.

Step 3: DEAE-cellulose chromatography. The enzyme solution from Step 2 was applied to a column (3.0x40 cm) of DE52 equilibrated with Buffer B. The column was washed with 300 ml of equilibration buffer and developed with a 2,000 ml linear gradient: 0 to 50 mM KCl in the same buffer. A typical elution profile is shown in Fig.1 where four peaks of phosphoglucomutase activity were separated. The first and the second peak correspond to "primary" and "secondary" PGM_1 isoenzymes respectively, the third and the fourth to "primary" and "secondary" PGM_2 isoenzymes respectively, as demonstrated by starch gel electrophoresis (Fig.1, inset) and according to the literature (20). The pooled DE52 cellulose fractions of each peak were added with 100 mM EDTA to obtain 0.5 mM final concentration, and separately concentrated by ultrafiltration through PM-30 Amicon membrane.

Step 4: Passage through CM-cellulose column. The four isoenzyme preparations from DE52 were differently pinkish due to the presence of traces of haemoglobin. This, and probably other proteins, were removed by passing through small CM-cellulose columns (1.6x8 cm) equilibrated with 10 mM Tris-MES (pH 6.1) containing 8% (w/v) glycerol, 0.5 mM EDTA and 2 mM 2-mercaptoethanol. Before loading, each PGM isoenzyme was brought to pH 6.1 by addition of 0.2 M Tris-MES pH 5.8. The flow rate was 26 ml/h and 3-ml fractions were collected. Under these conditions no appreciable enzyme was adsorbed, thus increases of 1.6-3.0 fold in specific activities could be accomplished with negligible dilution of the samples.

Step 5: Dye-ligand Matrex™-green chromatography. The CM-cellulose fractions of each PGM isoenzyme were pooled and brought to pH 7.0 with a suitable volume of 1 M Tris-HCl,

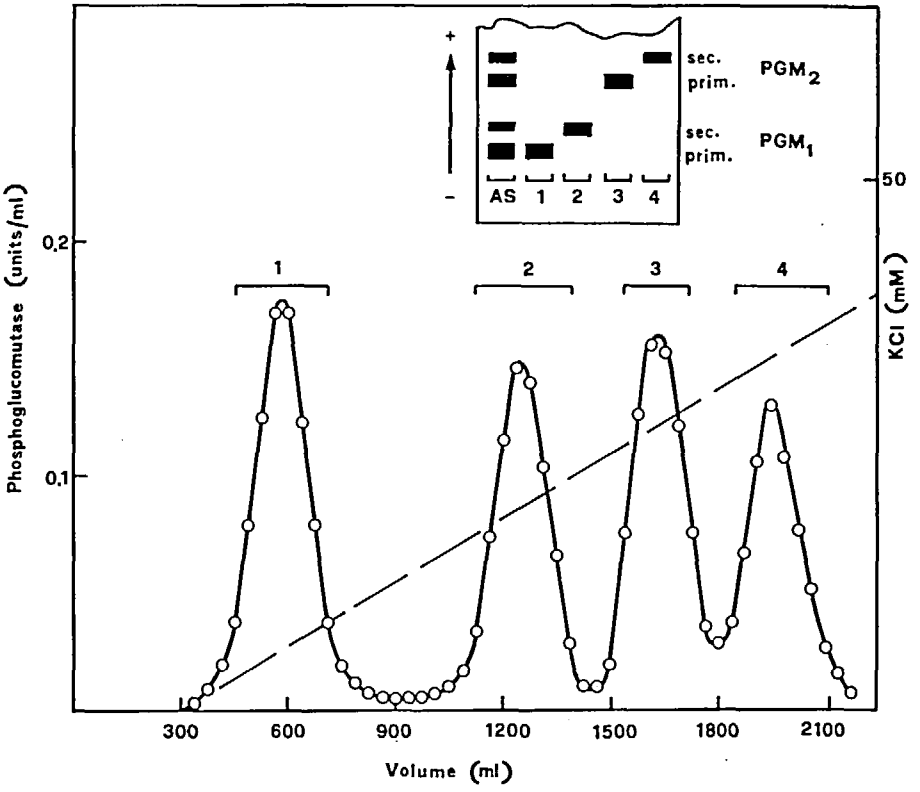

FIGURE 1

DEAE-cellulose column chromatography of phosphoglucomutase activity from human erythrocytes. Phosphoglucomutase preparation from ammonium sulfate precipitation (50 ml, 170 units, 4,000 mg of protein) obtained as described under Results, was dialyzed with two changes against 300 volumes at 10 mM Tris-HCl buffer, pH 7.1, containing 2 mM 2-mercaptoethanol, 8% (w/v) glycerol. After dialysis, the enzyme solution was applied to a DE52 column (3.0 x 40 cm) equilibrated with the same buffer used for the dialysis. After loading, the column was washed with 300 ml of the equilibrating buffer and the isoenzymes were eluted with a 2000 ml, 0 to 50 mM KCl linear gradient (dashed line), made up in the buffer. Ten ml fractions were collected at a flow rate of 1 ml/min. The fractions were analyzed for PGM and pooled as indicated by the bars. Inset: diagram of the analysis by starch gel electrophoresis of phosphoglucomutase isoenzymes. 50-100 µl aliquots of the pooled fractions of column were applied to the gel together with 50 µl of the 10-fold diluted phosphoglucomutase preparation from ammonium sulfate precipitation loaded on the column. Details are given under Methods.

pH 8.0. Subsequently the PGM_1 and PGM_2 isoenzymes were dialyzed at pH 7.2 and 7.8, respectively, against 10 mM Tris-HCl containing 8% (w/v) glycerol, 0.5 mM EDTA, 2 mM 2-mercaptoethanol and 4 mM $MgCl_2$. The enzyme dialyzed solutions were applied to dye-ligand Matrex™ -green columns (2.0x10 cm) equilibrated with the respective dialysis buffer. The flow rate was 26 ml/h and 3-ml fractions were collected. After sample application, the chromatography columns were washed until the protein absorbance at 280 nm was zero. The PGM activity was eluted by adding 1 mM G1P and 0.05 mM $G1,6P_2$ to the equilibrating buffer. The preparations obtained at this stage were about 100,000-fold purified for PGM_1 isoenzymes and 5,000-10,000 fold for PGM_2 isoenzymes.

Step 6: Sephadex G-100. The enzyme solutions from Step 5 were concentrated by ultrafiltration through a PM-30 Amicon membrane. At this stage the four isoenzyme preparations still contained the impurities. These were removed by passing through a column (1.8x90 cm) of Sepharose G-100 previously equilibrated with 10 mM Tris-HCl, pH 7.1, containing 8% (w/v) glycerol, 2 mM 2-mercaptoethanol and 0.5 mM EDTA. Enzyme activity was eluted from the column with the same solution at a flow rate of 8 ml/h and 1.3 ml fractions were collected with an automatic fraction collector. Enzyme activity was measured and those with higher PGM activity were pooled and concentrated by ultrafiltration. The results of the purification of PGM isoenzymes are summarized in Table I. The final specific activities of "primary" and "secondary" PGM_1 isoenzymes were 1263.3 and 1089.2 units/mg of protein respectively, while those of PGM_2 isoenzymes were 42 and 37.5 units/mg of protein for "primary" and "secondary" respectively.

TABLE I

PURIFICATION OF PHOSPHOGLUCOMUTASE (PGM) ISOENZYMES FROM HUMAN RED BLOOD CELLS (phenotype PGM_1 a3)

Enzyme fraction		Volume (ml)	Tot. Prot. (mg)	Tot. Activ. (units)	Spec. Activ. (units/mg)	Purif. (fold)	Rec. (%)
1. Haemolysate		1,000	69,000	216.66	0.0031	--	--
2. P-11 cell.		1,000	4,140	173.33	0.0418	13	80
3. DE-52 (conc.fracts)							
PGM_1	"prim"	4.5	105.0	26.66	0.254	82	56
	"sec"	5.0	75.0	29.16	0.389	125	
PGM_2	"prim"	5.2	45.0	34.00	0.755	243	
	"sec"	4.6	70.0	31.50	0.450	145	
4. CM-52							
PGM_1	"prim"	4.9	63.0	25.83	0.410	132	54
	"sec"	5.5	30.5	27.91	0.915	295	
PGM_2	"prim"	5.3	20.5	33.75	1.646	531	
	"sec"	4.8	23.0	30.83	1.340	432	
5. Matrex™-green (conc.fracts)							
PGM_1	"prim"	1.7	0.060	20.33	338.8	109,290	42
	"sec"	2.0	0.073	22.50	308.2	99,419	
PGM_2	"prim"	1.9	0.786	24.58	31.3	10,097	
	"sec"	2.0	1.454	23.75	16.3	5,258	
6. Sephadex G-100 (conc.fracts)							
PGM_1	"prim"	1.0	0.012	15.16	1263.3	407,516	32
	"sec"	0.9	0.013	14.16	1089.2	351,355	
PGM_2	"prim"	1.5	0.496	20.83	42.0	13,548	
	"sec"	1.0	0.522	19.58	37.5	12,097	

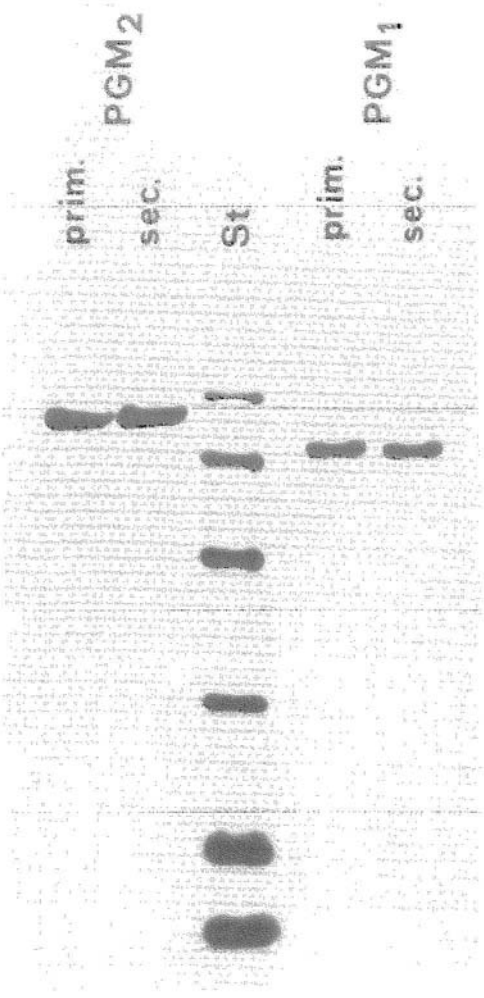

FIGURE 2

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of "primary" and "secondary" phosphoglucomutase 1 (PGM_1) and 2 (PGM_2) purified from human red cells. The isoenzymes were run in a 12% polyacrylamide gel with standards of Mr 97,400 (phosphorylase b), 66,200 (bovine serum albumin), 42,700 (ovalbumin), 31,000 (carbonic anhydrase), 21,500 (trypsin inhibitor) and 14,400 (lysozyme).

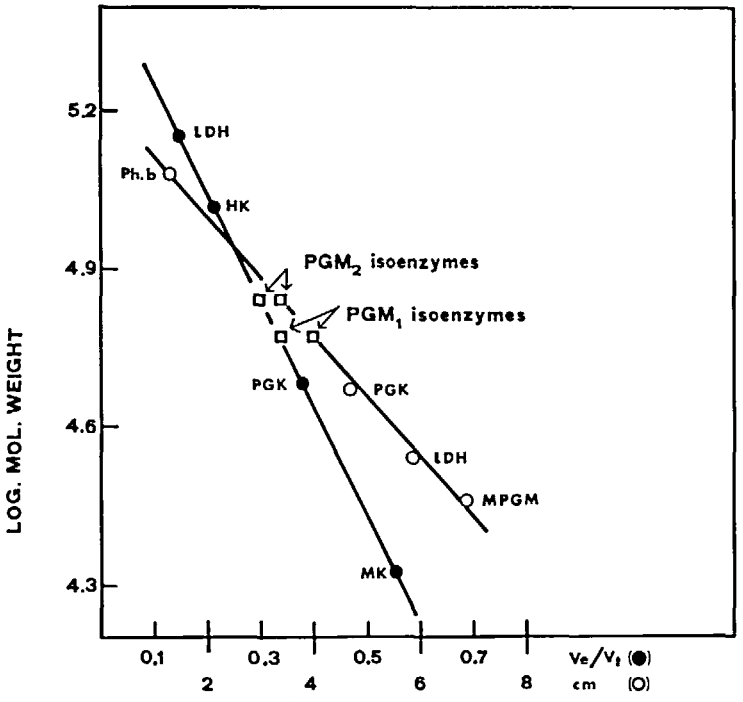

FIGURE 3

Determination of molecular weights of PGM_1 and PGM_2 isoenzymes ("primary" and "secondary") from human erytrocytes by gel filtration on an Ultrogel AcA44 column (●) and sodium dodecyl sulfate-(SDS) polyacrylamide gel electrophoresis (O). LDH, lactate dehydrogenase; HK, hexokinase; PGK, 3-phosphoglycerate kinase; MK, miokinase; MPGM, phosphoglycerate mutase; Ph.b, phosphorylase b.

The preparations obtained at this stage gave a single band in sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig.2).

Properties of the Purified PGM Isoenzymes

Molecular weight. The molecular weights of the pure "primary" and "secondary" PGM_1 and PGM_2 isoenzymes were determined by gel filtration on Ultragel AcA44 for five

TABLE II

SOME PROPERTIES OF PURIFIED RED CELL PHOSPHOGLUCOMUTASE (PGM) ISOENZYMES (phenotype PGM_1 a1 or PGM_1 a3)

	PGM_1		PGM_2	
	"prim"	"sec"	"prim"	"sec"
pH optima	8.0	8.0	8.0	8.0
Km G1P (μM)	83.0	80.5	57.0	60.8
Km G1,6P_2 (μM)	0.77	1.05	1.43	1.57
Km Mg^{2+} (μM)	20	20	20	20
Ki 2,3DPG (μM)	80	90	30	45
Ki F1,6P_2 (μM)	92	95	70	65
PRM-activity	no	no	yes	yes
G1,6P_2-synthesis	no	no	yes	yes
Thermal inactivation (% res. act. after 15 min at 52°C)	10	10	45	45
Molecular weight (Mr)	58,500	58,500	69,000	69,000
pI	6.07	5.79	5.47	5.29

different preparations each and were found to be about 58,500 and 69,000 for PGM_1 and PGM_2 isoenzymes respectively, without any appreciable difference between the "primary" and "secondary" isoenzyme (Fig 3). The molecular weights were also estimated by sodium dodecyl sulfate - polyacrylamide gel electrophoresis, and they were found to be similar to those obtained by gel filtration (Fig 3). These values are quite different from those of 51,000 and 62,000 for PGM_1 and PGM_2 isoenzymes, respectively, reported by Mc Alpine et al.[36].

General properties. (Table II) The kinetic properties of pure "primary" and "secondary" PGM_1 and PGM_2

isoenzymes, ascertained as previously reported for their less purified preparations, are similar to those found for the latter[26]. Apart from the striking difference between the specific activities of PGM_1 and PGM_2 forms, all four isoenzymes display an optimal pH of 8, require Mg^{2+} for activity, show "ping-pong" kinetic mechanism and have similar Km for substrate (G1P) and cofactor (G1,6P_2). Micromolar concentrations of fructose 1,6-bisphosphate and glycerate 2,3-bisphosphate yield inhibitions of comparable extent. The pIs of the pure isoenzymes, measured by isoelectric focusing in liquid column under native conditions are 6.07 and 5.79 for "primary" and "secondary" PGM_1 isoenzymes respectively, 5.47 and 5.29 for "primary" and "secondary" PGM_2 isoenzymes respectively. As we have previously reported[37], these data are in agreement with those referred by others[38-40] only for the pIs of the PGM_1 forms and not with those of PGM_2 forms. This discrepancy seems to be due to the different techniques employed rather than to charge modification of PGM_2 isoenzymes induced by the purification procedure.

Distinctive Properties. Although the four purified isoenzymes show similar properties regarding the phosphoglucomutase reaction it is possible to distinguish the products of PGM_1 locus from those of PGM_2 locus because the former are "true" phosphoglucomutases, being specific to mutate glucose monophosphates, whereas the latter also display phosphoribomutase (PRM) activity and glucose 1,6-bisphosphate synthesis. This derives from data of other[41] and from our previous reports in which we found that red cell PRM and G1,6P_2-synthetic activities remained strictly associated to phosphoglucomutase activity of PGM_2 isoenzymes after partial

TABLE III

SOME KINETIC PROPERTIES OF THE PHOSPHORIBOMUTASE (PRM) ACTIVITY RETAINED BY PURIFIED PHOSPHOGLUCOMUTASE PGM_2 ISOENZYMES

	PGM_2 "prim"	PGM_2 "sec"
[a] Specific activity (units/mg)	441.0	412.5
PRM[a] /PGM ratio	10.5	11.0
[a] pH optima	8.0	8.0
[b] Km R1P (mM)	0.8	0.95
[b] Km R5P (mM)	1.17	1.05
[b] Km G1,6P_2 (μM)	1.50	1.30
[b] Km Mg^{2+} (μM)	15.0	18.0

[a] Determined by the assay procedure reported under Materials and Methods. The buffers (100 mM) used to determine the pH dependence of PRM activity were 2-(N-morpholino)ethane sulfonate (MES) at pH 6.5, N-tri(hydroxymethyl)methyl-2 aminoethanesulfonic acid (TES) at pH 7.0, and tris (hydroxymethyl)aminomethan (TRIS) at pH 7.5, 8.0, 8.5, 9.0 and 9.5.

[b] Determined by the coupled optical assay of Tozzi et al.(42) at 37°C, pH 7.9. Km for R1P, R5P were measured at 0.15 mM G1,6P_2 and 5 mM $MgCl_2$. Km for G1,6P_2 at 4mM R5P, 5 mM $MgCl_2$. Km for Mg^{2+} at 4 mM R5P, 0.15 mM G1,6P_2. Km for R1P was measured in reaction mixtures (0.5 ml) containing a suitable amount of enzyme, 100 mM Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 0.15 mM G1,6P_2 and different concentrations of R1P. Those incubations were performed at 37°C. Aliquots of 0.15 ml were taken at 0, 5 and 10 min, heated at 100°C for 5 min and used to determine the rate of the enzyme activity by assaying R1P disappearance with end point coupled optical assay method (42).

purification[25], and we provided evidence that these three enzymatic activities follow the same decay during cell aging[27]. In the present study we have purified to homogeneity erythrocytic PGM isoenzymes and have observed that PGM_2 forms still display PRM and G1,6P_2-synthetic abilities.

In Tab.III some kinetic properties of the PRM reaction catalyzed by purified PGM_2 isoenzymes are reported.

TABLE IV

SOME KINETIC PROPERTIES OF THE GLUCOSE 1,6 BISPHOSPHATE SYNTHETIC ACTIVITY RETAINED BY PURIFIED PHOSPHOGLUCOMUTASE PGM_2 ISOENZYMES

	PGM_2 "prim"	PGM_2 "sec"
Specific activity (units/mg)	0.76	0.60
G1,6P_2-synthesis/PGM ratio	0.018	0.016
pH optima	8.0	8.0
Km 1,3 PGA (µM)	1.25	1.66
Km G1P (mM)	0.15	0.17
Km G6P (mM)	0.22	0.30
Km Mg^{2+} (mM)	0.16	0.15

All values were determined by the assay procedures reported under Materials and Methods. The buffers (50 mM) used to determine the pH dependence of G1,6P_2 synthesis were the same as reported in legend to Table III. Km for 1,3 PGA was measured at 2 mM G1P and 2 mM $MgCl_2$. Km for G1P and G6P at 4 µM 1,3 PGA and 2 mM $MgCl_2$. Km for Mg^{2+} at 4 µM 1,3 PGA and 2 mM G1P.

It is interesting to note that ribose monophosphates mutation is 10-fold higher than that of glucose monophosphates.

Tab.IV shows that G1,6P_2-synthesis from 1,3PGA and G1P, is only about 1.7% of the phosphoglucomutase activity of purified PGM_2 isoenzymes and some kinetic properties of the synthetic reaction are also reported.

We would like to stress that the majority of the PRM activity present in human red cell lysates is recovered in the purified PGM_2 forms. Similarly, there is no evidence for the existence in human erythrocytes of G1,6P_2-synthesis performed by proteins other than PGM_2 isoenzymes[28]. According to the data

herein reported, we can conclude that i) although the expression of the PGM_2 locus in erythrocytes is unusually high (approximately equal to that of PGM_1 locus) when compared with other human cells, there are no substantial differences in the kinetic properties of the various isoenzymes regarding the phosphoglucomutase reaction; ii) the product of PGM_2 locus in human red cells, also displaying PRM and G1,6P_2-synthetic activities, seems to meet the need of the cell to possess these abilities not present as unique enzymes; iii) the conversion of "primary" into "secondary" isoenzymes, apart from evident modification of the electric charge, does not involve variations in the kinetic properties of the catalyzed reactions and in the mass of the protein molecule.

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