Molecular Identification of Mammalian Phosphopentomutase and Glucose-1,6-bisphosphate Synthase, Two Members of the α-d-Phosphohexomutase Family

Pushpa Maliekel, Tatiana Sokolova, Didier Vertommen, Maria Veiga-da-Cunha, and Emile Van Schaftingen

The molecular identity of mammalian phosphopentomutase has not yet been established unequivocally. That of glucose-1,6-bisphosphate synthase, the enzyme that synthesizes a cofactor for phosphomutases and putative regulator of glycolysis, is completely unknown. In the present work, we have purified phosphopentomutase from human erythrocytes and found it to copurify with a 68-kDa polypeptide that was identified by mass spectrometry as phosphoglucomutase 2 (PGM2), a protein of the α-d-phosphohexomutase family and sharing about 20% identity with mammalian phosphoglucomutase 1. Data base searches indicated that vertebrate genomes contained, in addition to PGM2, a homologue (PGM2L1, for PGM2-like 1) sharing about 60% sequence identity with this protein. Both PGM2 and PGM2L1 were overexpressed in Escherichia coli, purified, and, their properties were studied. Using catalytic efficiency as a criterion, PGM2 acted more than 10-fold better as a phosphopentomutase (both on deoxyribose 1-phosphate and on ribose 1-phosphate) than as a phosphoglucomutase. PGM2L1 showed only low (<=5%) phosphopentomutase and phosphoglucomutase activities compared with PGM2, but was about 5–20-fold better than the latter enzyme in catalyzing the 1,3-bisphosphoglycerate-dependent synthesis of glucose 1,6-bisphosphate and other aldose-bisphosphates. Furthermore, quantitative real-time PCR analysis indicated that PGM2L1 was mainly expressed in brain where glucose-1,6-bisphosphate synthase activity was previously shown to be particularly high. We conclude that mammalian phosphopentomutase and glucose-1,6-bisphosphate synthase correspond to two closely related proteins, PGM2 and PGM2L1, encoded by two genes that separated early in vertebrate evolution.

Phosphopentomutase catalyzes the conversion of the nucleoside breakdown products ribose 1-phosphate and deoxyribose 1-phosphate to the corresponding 5-phosphophosphates. Most bacterial phosphopentomutases characterized so far belong to the same protein family as alkaline phosphatases, sulfatases, and cofactor-independent bisphosphoglycerate mutases (1), though the enzyme from Thermococcus kodakaraensis belongs to the same protein family as mammalian phosphoglucomutase 1 (PGM1), the enzyme that catalyzes the interconversion of glucose 1-phosphate and glucose 6-phosphate (2).

The molecular identity of the mammalian phosphopentomutase is still not ascertained. Biochemical characterization of phosphoglucomutase (PGM) isoforms indicates that one of them, designated PGM2 in man (PGM1 in mouse) was more active as a phosphopentomutase than as a phosphoglucomutase, whereas mammalian PGM1 (equivalent to PGM2 in mouse) has a phosphopentomutase activity representing only about 0.2% of its phosphoglucomutase activity (3). Phosphopentomutase has been purified from rat liver to near homogeneity and shown to coelute with a polypeptide of 32.5 kDa (4), the sequence of which was not determined. Analysis of cell hybrids indicated that the PGM2 locus is on human chromosome 4p14-q12 (5). A putative protein sharing ~20% identity with PGM1 is encoded by a gene present on human chromosome 4p14. It is designated PGM2 in the databases presumably because its chromosomal localization fits with the PGM2 locus but it has never been shown to act as a phosphopentomutase. Furthermore, its size (612 amino acid residues) is about twice that reported for rat liver phosphopentomutase (4). In view of these contradictory findings, we decided to reinvestigate the identity of human phosphopentomutase, which we formally identify in this report as the protein designated PGM2 in the data bases.

We also show that a closely related protein, PGM2-like 1 (PGM2L1), which shares ~60% sequence identity with PGM2, actually corresponds to glucose-1,6-bisphosphate synthase. This enzyme, which had not yet been molecularly identified, catalyzes the 1,3-bisphosphoglycerate-dependent synthesis of glucose 1,6-bisphosphate and other aldose-bisphosphates that serve as cofactors for several sugar phosphomutases and possibly also as regulators of glycolytic enzymes.

EXPERIMENTAL PROCEDURES

Materials—Reagents, of analytical grade whenever possible, were from Sigma, Acros (Geel, Belgium), Roche Applied Sci-

5 The abbreviations used are: PGM, phosphoglucomutase; DEAE, diethylaminoethyl; SP, sulfo propyl; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPRT, hypoxanthine phosphoribosyltransferase; MES, 4-morpholineethanesulfonic acid; DTT, dithiothreitol.
Purification of Phosphopentomutase—Phosphopentomutase was purified from human erythrocytes. 80 ml of packed erythrocytes were washed three times with 150 mM NaCl and diluted in 400 ml of a buffer containing 10 mM Tris, pH 8, 1 mM dithiothreitol (DTT), 1 µg/ml leupeptin, and 1 µg/ml antipain. The hemolysate was centrifuged for 20 min at 11,000 × g. The supernatant (300 ml) was diluted twice in buffer A (20 mM Tris, pH 8, 1 mM DTT, 1 µg/ml leupeptin, and 1 µg/ml antipain) and applied to a DEAE-Sepharose column (200 cm²) equilibrated with the same buffer. The column was washed with 400 ml of buffer A, and protein was eluted with a 0–0.25 M NaCl gradient in 1000 ml of buffer A. The most active fractions were pooled (75 ml), brought to 300 ml with buffer B (25 mM MES, pH 6, 1 mM DTT, 25 mM KCl, 5 µg/ml leupeptin, and 5 µg/ml antipain), and applied to a SP-Sepharose column (25 cm²) equilibrated with buffer B. The column was washed with 100 ml of equilibration buffer. Phosphopentomutase was recovered in the flow-through and washing fractions, which were concentrated to 37.5 ml by ultrafiltration in a 200-ml Amicon cell equipped with a PM-10 membrane. This sample was brought to 180 ml with buffer C (25 mM glycine, pH 9, 1 mM DTT, 25 mM KCl, 5 µg/ml leupeptin, and 5 µg/ml antipain) and applied to a Q-Sepharose column (20 cm²) equilibrated with the same buffer. The column was washed with 80 ml of buffer C, and the retained proteins were eluted with a 0–0.75 M NaCl gradient in 300 ml of buffer C. The most active fractions (6.6 ml) were concentrated to 0.5 ml (with Vivaspin-2) and gel-filtered on a Superdex-200 10/300 GL column equilibrated with buffer D (25 mM Hepes, pH 7.4, 1 mM DTT, 5 µg/ml leupeptin, 5 µg/ml antipain, and 100 mM NaCl). Fractions of 0.5 ml were collected. Protein was assayed as described (6) using bovine serum albumin as a standard. The bands co-eluting with the phosphopentomutase activity were cut out from a 12% (w/v) polyacrylamide-SDS gel and digested with trypsin. Peptides were analyzed by nanoelectrospray-ionization tandem mass spectrometry (7) in a LCQ Deca XP Plus ion trap mass spectrometer (ThermoFinnigan, San Jose, CA) fitted with a nanoelectrospray probe. The data were analyzed with the X-calibur software (ThermoFinnigan), and the proteins were identified with TurboSEQUEST in the BioWorks software suite (ThermoFinnigan).

Overexpression and Purification of Human PGM2 and PGM2L1—The open reading frame of human PGM2 and PGM2L1 (GenBank™ accession numbers NP_060760 and NP_775853) were PCR-amplified using a mixture of Taq and Pfu DNA polymerases and human white blood cells cDNA as a template. For PGM2, a 5’ primer containing the initiator codon (CAAGCCTACHGGCTCGCTCCAGAAGGC) in an Ndel site (in bold) and a 3’ primer containing the putative stop codon (GGCTGGAGATCTTTAGTCAGTTTGAGCTGCAAGAT) flanked by a BglII site (in bold) were used. The 1840-bp PCR-product was digested with Ndel-BglII and cloned into pET-15b expression vector. The insert was checked by sequencing. This expression vector was used to transform E. coli BL21(DE3). For PGM2L1, a 5’ primer containing the initiator codon (CCCATATGGCTGAAAACACAGAGGGGG) in an Ndel site (in bold) and a 3’ primer containing the putative stop codon (TGATCCTAAACAGACCCAGATCA) flanked by a BamHI site (in bold) were used. A 1870-bp product was obtained, which was subcloned in pBlueScript and checked by sequencing. A Ndel-BamHI fragment was removed from the pBlueScript plasmid and ligated in pET-15b expression vector. This vector was used to transform E. coli BL21(DE3) pLysS. Protein expression and preparation of bacterial extracts were performed as described previously (8). Both His-tagged proteins were purified on HisTrap columns (Ni²⁺ form) as previously described (9). Both proteins were eluted with ~150 mM imidazole (as indicated by SDS-PAGE analysis). Both proteins were desalted on PD-10 columns equilibrated with 25 mM Hepes pH 7.4 and 50 mM KCl. Protein concentration was estimated by measuring A₂₈₀ assuming extinction coefficients of 83,810 and 97,260 M⁻¹ cm⁻¹ for PGM2 and PGM2L1, respectively; 22 mg of pure PGM2 and 3 mg of pure PGM2L1 were obtained per liter of culture. The purified proteins were supplemented with 10% (w/v) glycerol and stored at −70 °C.

Measurement of Enzymatic Activities—The enzymatic activities were assayed spectrophotometrically at 30 °C. All assay media (reaction mixture of 600 µl) contained 25 mM Hepes pH 7.4, 25 mM KCl, 1 mM MgCl₂, 1 mM DTT, and 100 µM EGTA (assay buffer). Phosphoribomutase was assayed in assay buffer containing 1 mM MnCl₂, 50 µM CTP-Mg, 0.15 mM NADPH, 0.5 µM glucose 1,6-bisphosphate, different concentrations of ribose 1-phosphate, ribose-5-phosphate isomerase from spinach (1.7 units/ml), Haemophilus influenzae ribulose-5-phosphate reductase (0.3 units/ml) (10) and PGM2 (0.03 µg/ml) or PGM2L1 (2 µg/ml). One unit of enzyme is the amount that converts 1 µmol of substrate per min under these conditions. Phosphodeoxyribomutase was assayed in assay buffer containing 0.15 mM NADH, 5 µM glucose 1,6-bisphosphate, different concentrations of deoxyribose 1-phosphate, deoxyribose-5-phosphate aldolase (6 µg/ml, from Thermus thermophilus), overexpressed in E. coli and purified to homogeneity,6 rabbit muscle triose phosphate isomerase (3.5 units/ml) and glycerol-3-phosphate dehydrogenase (1 units/ml), as well as PGM2 (0.03 µg/ml) or PGM2L1 (5 µg/ml). Phosphoglucomutase was assayed in assay buffer containing 0.25 mM NADP, 5 µM glucose 1,6-bisphosphate, different concentrations of glucose 1-phosphate, yeast glucose-6-phosphate dehydrogenase (3 units/ml) and PGM2 (0.15 µg/ml) or PGM2L1 (2.4 µg/ml).

The glucose-1,6-bisphosphate synthase and aldose-bisphosphate synthase activities were determined in assay buffer containing 500 µM inorganic phosphate, 20 µM glyceraldehyde 3-phosphate, 500 µM NAD, 100 µg/ml bovine serum albumin, different concentrations of aldose monophosphate, rabbit mus-

---

6 T. Sokolova and M. Veiga-da-Cunha, unpublished results.
Phosphopentomutase and Glucose-1,6-bisphosphate Synthase

TABLE 1
Primer sequences for PGM2, PGM2L1, and internal control genes

| Symbol | Name                                      | Forward primer | Reverse primer |
|--------|-------------------------------------------|----------------|----------------|
| PGM2   | Phosphoglucomutase 2                      | GCTGTATAATTGGTGCCAGCTG | CTTCCGCTCATAGTTTCTAG |
| PGM2L1 | Phosphoglucomutase 2-like 1               | GAAGTTAACACTGATGACA | GTGTTACTGCCCCTATAGCA |
| GAPDH  | Glyceraldehyde-3-phosphate dehydrogenase   | GCACCCCAACACGTCTTACCC | GAGACTGAGGATGA70TTCT |
| HPRT   | Hypoxanthine phosphoribosyltransferase     | GTTAAGCGGTACAGCCCACAATGG | AACCCACAAAAGTCGGCTTGA |

### RESULTS

Purification of Human Erythrocyte Phosphopentomutase—During its purification, phosphopentomutase was assayed by measuring the conversion of ribose-1-phosphate to ribose-5-phosphate with a coupled assay using ribose-5-phosphate isomerase and ribulose-5-phosphate reductase. Phosphopentomutase was purified from human erythrocytes about 100-fold by chromatography on DEAE-Sepharose, SP-Sepharose, and Q-Sepharose. Phosphopentomutase (540 milliunits in 0.4 ml) purified by chromatography on a Superdex-200 column. Fractions of 0.5 ml were collected. Phosphopentomutase activity and protein concentration (6) were determined.

RNA Extraction and Quantitative Real Time PCR Assays—Total RNA was extracted from fresh tissues from 3 male fed mice with TriPure reagent according to the manufacturer’s protocol. RNA integrity was verified by loading RNA onto a 1% agarose gel and evaluating the 28 S and 18 S ribosomal RNA bands, and its purity was assessed by checking $A_{260}/A_{280}$. Approximately 1 μg of RNA from each sample was converted to cDNA by RT-PCR using random hexamers and Superscript II reverse transcriptase. Gene-specific primers (Table 1) were designed to generate PCR products of about 150 bp from PGM2, PGM2L1 and two reference genes: GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and HPRT (hypoxanthine guanine phosphoribosyltransferase). Intron-spanning primers were selected to verify, by agarose gel electrophoresis, that amplification of contaminating genomic DNA did not take place. Real-time PCR was performed using an iCycler IQ™ system (Bio-Rad). The rate of accumulation of amplified DNA was measured by continuous monitoring of SYBR green I fluorescence.

Tree Construction—The sequence of homologues of human PGM2 and PGM2L1, identified with the BLAST program (11), were aligned using Clustal X with the default parameters (12). A neighbor-joining tree was constructed using the tree option of Clustal X after exclusion of positions with gaps and with correction for multiple substitutions. For the bootstrap analysis, 1000 samplings were carried out.

Occurrence of PGM2 Homologues—BLAST searches with human PGM2 indicated that homologues of this protein were found in eukaryotes including vertebrates (>70% identity with the human sequence), insects and Caenorhabditis elegans (both ≈50% identity) and fungi (≈40% identity). No orthologue was detected for PGM2 in plants, fungi and bacteria.

FIGURE 1. Co-elution of human erythrocyte phosphopentomutase with a ~68-kDa polypeptide from a Superdex-200 column. A, a preparation of phosphopentomutase (540 milliunits in 0.4 ml) purified by chromatography on DEAE-Sepharose, SP-Sepharose, and Q-Sepharose was applied on a Superdex-200 column. Fractions of 0.5 ml were collected. Phosphopentomutase activity and protein concentration (6) were determined. B, the indicated fractions were analyzed by SDS-PAGE (15 μl/lane) and the gel stained with Coomassie Blue. The indicated bands were cut out from the gel, submitted to trypsin digestion, and analyzed by mass spectrometry.
Phosphopentomutase and Glucose-1,6-bisphosphate Synthase

| PGM2  | MAAPEQSLGLF-------CADEPQETAQNLDAQNKSLNTLEAVKRLIA | 40 |
|-------|--------------------------------------------------|----|
| PGM2L1 | MAENTGDLNSNLHAPYHTGDQDLTIAQNLQWDKNDNPXQIDNLR | 50 |
| PGM2  | EGNKEELRKCGFARMEFOGTAAGLARAAGPGISRMNDLTIQTTGQFCVRYE | 90 |
| PGM2L1 | NOMKELDRLCRRMTFGATLRASMGAGFVQINDTVIQTSGQMYKVE | 100 |
| PGM2  | KQFSDLKKGIVISRARSHGSRRFRLAATTFIQQIPVLYFS | 140 |
| PGM2L1 | RSDFSDQRFQVQYDTRQGTTSCSSQRLAKFAAIVLAKDVYFLS | 150 |
| PGM2  | ITPTFPVFVFTVSHKLCACTIMAINDQYKVDNQIITHSPDK | 190 |
| PGM2L1 | YVPTFPVFVYQVQLKAVGMAITSHRNQYKVTWQISPITHSPDK | 200 |
| PGM2  | GISQAIEENLEPFWQDSDLiSSSPLHNPSASINNDFPFLKCYFCR | 240 |
| PGM2L1 | EILC1ECCBFVPGN9SN6DNLVTDIPSLRDPFLDQICRYYMEDLKLICPRY | 250 |
| PGM2  | SVNREKTVKFPYTHSVQGHSPQVSAIKAPELDVPROAPKQFQDDEPT | 290 |
| PGM2L1 | ELNKSITKLFVTHSFHHVHDYQVALFVPFGPKIPVPQEDPDDEPT | 300 |
| PGM2  | KQFSDLKQKGIVISFDPARAHPSSGGSSRFAKLVATDPADLAPALAC | 340 |
| PGM2L1 | MAENTEGDLNSNLHAPYHTGDQDLTIAQNLQWDKNDNPXQIDNLR | 40 |
| PGM2  | EGFHFEETLTGFKWMGNRAKQLIDQGKTVLFAFEAIGMCPVFLDQ | 440 |
| PGM2L1 | VFTGNELAALFGWWMFDCWKKNKRNADVKNVLMATVSSQIKLAI | 450 |
| PGM2  | GISQAIEENLEPFWQDSDLiSSSPLHNPSASINNDFPFLKCYFCR | 490 |
| PGM2L1 | VSAAVSNELAALFGWWMFDCWKKNKRNADTSAKLYFC | 500 |
| PGM2  | MAENTEGDLNSNLHAPYHTGDQDLTIAQNLQWDKNDNPXQIDNLR | 50 |
| PGM2L1 | MAAPEQSLGLF-------CADEPQETAQNLDAQNKSLNTLEAVKRLIA | 60 |
| PGM2  | KQFSDLKKGIVISRARSHGSRRFRLAATTFIQQIPVLYFS | 90 |
| PGM2L1 | RSDFSDQRFQVQYDTRQGTTSCSSQRLAKFAAIVLAKDVYFLS | 100 |
| PGM2  | ITPTFPVFVFTVSHKLCACTIMAINDQYKVDNQIITHSPDK | 140 |
| PGM2L1 | YVPTFPVFVYQVQLKAVGMAITSHRNQYKVTWQISPITHSPDK | 150 |
| PGM2  | GISQAIEENLEPFWQDSDLiSSSPLHNPSASINNDFPFLKCYFCR | 190 |
| PGM2L1 | EILC1ECCBFVPGN9SN6DNLVTDIPSLRDPFLDQICRYYMEDLKLICPRY | 200 |
| PGM2  | SVNREKTVKFPYTHSVQGHSPQVSAIKAPELDVPROAPKQFQDDEPT | 240 |
| PGM2L1 | ELNKSITKLFVTHSFHHVHDYQVALFVPFGPKIPVPQEDPDDEPT | 250 |
| PGM2  | MAENTEGDLNSNLHAPYHTGDQDLTIAQNLQWDKNDNPXQIDNLR | 290 |
| PGM2L1 | MAAPEQSLGLF-------CADEPQETAQNLDAQNKSLNTLEAVKRLIA | 300 |

**FIGURE 2. Alignment of human PGM2 and PGM2L1.** Residues conserved are indicated by an asterisk below the alignment. The highly conserved TASHNP motif from the α-phosphohexomutase family is in bold. The peptides identified by mass spectrometry in the protein purified from human erythrocytes are underlined in the PGM2 sequence. Three changes between human PGM2 and PGM2L1 are shown: higher PGM2 (GenBank™ accession number gi-63055049) and PGM2L1 (GenBank™ accession number gi-31377548).
inhibit the phosphoribomutase activity of this enzyme. Interestingly, we found that glycerol 3-phosphate and 2,3-bisphosphoglycerate were strong inhibitors of PGM2L1 though not of PGM2. At a concentration of 200 μM, glycerol 3-phosphate and 2,3-bisphosphoglycerate inhibited the phosphoribomutase activity of PGM2L1 (tested with 20 μM ribose 1-phosphate) by 95 and 99%, respectively, but that of PGM2 by only 50%. This finding suggested that the phosphate ester of a C3 compound could be a substrate for PGM2L1.

Previous work has disclosed the existence of a glucose-1,6-bisphosphate synthase, which uses the high energy compound 1,3-bisphosphoglycerate to convert glucose 1-phosphate or glucose 6-phosphate to glucose 1,6-bisphosphate (17, 18). This enzyme was shown to be particularly active in brain. It was purified from this tissue and identified as a 70-kDa protein, which acted also with low activity, as a phosphoglucomutase, all properties that are similar to those of PGM2L1. To test the ability of the latter to synthesize glucose 1,6-bisphosphate from 1,3-bisphosphoglycerate and glucose 1- or 6-phosphate, we designed a spectrophotometric assay in which the activity of glyceraldehyde-3-phosphate dehydrogenase (monitored by the appearance of NADH) is coupled to the utilization of 1,3-bisphosphoglycerate by the synthase. This coupling is made possible by the fact that the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase (Fig. 5 A) readily reaches its thermodynamic equilibrium in the absence of an enzyme that consumes 1,3-bisphosphoglycerate. However, as illustrated in Fig. 5B, the progression of the reaction (and therefore NADH formation) can be made to resume by adding a 1,3-bisphosphoglycerate consuming system, e.g. 3-phosphoglycerate kinase and ADP. Fig. 5B also shows that NADH formation resumes upon addition of glucose 1-phosphate and PGM2L1 (although not if each of them is added separately; not shown), which indicates that PGM2L1 utilizes 1,3-bisphosphoglycerate to catalyze
The results are the means of three determinations ± S.E. The first three columns indicate the $V_{\text{max}}$, $K_m$, and $k_{\text{cat}}$ (catalytic efficiency) values for the indicated substrates. The last column shows ratios of the catalytic efficiencies of PGM2 and PGM2L1 for each of the substrate.

| Substrate       | $V_{\text{max}}$ (µmol min$^{-1}$ mg$^{-1}$) | $K_m$ (µM) | $k_{\text{cat}}/K_m$ (s$^{-1}$ µM$^{-1}$) | Catalytic efficiency ratio (PGM2/PGM2L1) |
|-----------------|---------------------------------------------|------------|-----------------------------------------|-----------------------------------------|
| Ribose-1-P      | 104.3 ± 3.7                                 | 45.7 ± 3.7 | 2.7 ± 0.2                               | 16.5 ± 2.5                              |
| Deoxyribose-1-P | 20.8 ± 1.0                                  | 4.1 ± 1.3  | 6.2 ± 0.7                               | 407 ± 74                                |
| Glucose-1-P     | 22.8 ± 0.6                                  | 114 ± 3.1  | 0.23 ± 0.01                             | 4.7 ± 0.5                               |

**PMGL21**

| Substrate       | $V_{\text{max}}$ (µmol min$^{-1}$ mg$^{-1}$) | $K_m$ (µM) | $k_{\text{cat}}/K_m$ (s$^{-1}$ µM$^{-1}$) | Catalytic efficiency ratio (PGM2/PGM2L1) |
|-----------------|---------------------------------------------|------------|-----------------------------------------|-----------------------------------------|
| Ribose-1-P      | 0.71 ± 0.04                                 | 5.2 ± 0.8  | 0.17 ± 0.02                             | 0.05 ± 0.001                            |
| Deoxyribose-1-P | 0.09 ± 0.01                                 | 6.7 ± 0.7  | 0.015 ± 0.001                           | 0.051 ± 0.003                           |
| Glucose-1-P     | 0.87 ± 0.09                                 | 20.7 ± 1.3 | 0.051 ± 0.003                           | 0.051 ± 0.003                           |

**DISCUSSION**

Identification of Phosphopentomutase as PGM2—Our work demonstrates that phosphopentomutase activity in human and presumably also in other vertebrates is contributed by the protein designated PGM2 in the protein data bases (except in mouse where PGM2 is the orthologue of human PGM1). This conclusion is based on the finding that human erythrocyte phosphopentomutase copurifies with PGM2. Furthermore, recombinant PGM2 displays a higher phosphopentomutase activity than its phosphoglucomutase activity, having therefore properties similar to those of the phosphopentomutase (PGM2) previously characterized in mammalian tissues (20, 21). These findings are at variance with the results of Barksy and Hoffee (4), who purified phosphopentomutase from rat liver to near homogeneity and concluded that it was a dimer consisting of two subunits of 32.5 kDa. However, the SDS-PAGE gel shown by these authors indicates that their most purified fractions also contained a faint ≈70-kDa band in addition to the 32.5-kDa band. Our presumption is that the ≈70-kDa band rather than the 32.5-kDa band corresponds to rat phosphopentomutase. Accordingly the rat genome also encodes a PGM2 orthologue sharing 90% sequence identity with human PGM2.

Our findings indicate that PGM2 comprises the TASHNP motif common to members of the α-β-phosphohexomutase family, which comprises eukaryotic phosphoglucomutases, bacterial phosphoglucomutases and phosphomannomutases, eukaryotic phosphoacetylglucosaminemutase and bacterial phosphoglucosamine mutase (14). The reaction mechanism of phosphoglucomutase has been thoroughly studied (14, 22, 23). To be active, phosphoglucomutase needs to be phosphorylated on the serine present in the TASHNP motif, and this phosphorylation is achieved by transferring one of the phospho-groups on mannose 6-phosphate, ribose 5-phosphate, and deoxyribose 5-phosphate (Table 3). These findings are in good agreement with previous results obtained with glucose-1,6-bisphosphate synthase purified from mouse brain (17, 19). The homologous enzyme PGM2 showed some ability to synthesize aldose bisphosphates but was about 5–20-fold less active than PMGL21 in this respect. The synthase activity was more potently inhibited by glucose 1,6-bisphosphate, the product of the reaction, in the case of PGM2 than of PMGL21: when tested with 1 mM glucose 1-phosphate, 50% inhibition was reached with 10 µM glucose 1,6-bisphosphate in the case of PGM2 as compared with 50 µM in the case of PMGL21.
of glucose 1,6-bisphosphate. To catalyze the net conversion of glucose 1-phosphate to glucose 6-phosphate, the phosphorylated enzyme reacts with glucose 1-phosphate, thus re-forming glucose 1,6-bisphosphate. The latter flips inside the catalytic site and rephosphorylates the enzyme by transferring its 1-phospho group, thus forming glucose 6-phosphate. Because of the conservation of the TASHNP motif in PGM2 and of the fact that this enzyme also requires glucose 1,6-bisphosphate as a cofactor, it is likely that the conserved serine also serves as a transient phosphate acceptor.

The role of phosphopentomutase is to utilize ribose 1-phosphate and deoxyribose 1-phosphate, which are formed by purine nucleoside phosphorylase and uridine phosphorylase. The molecular identification of phosphopentomutase might help to solve unexplained cases of congenital immunodeficiency. Both adenosine deaminase and nucleoside phosphorylase deficiencies are indeed known to be causes of immunodeficiency (24, 25). The absence of phosphopentomutase should result in the accumulation of ribose 1-phosphate and deoxyribose 1-phosphate and therefore in a functional block of purine nucleoside phosphorylase.

Identification of PGM2L1 as Glucose-1,6-bisphosphate Synthase—The presence of a human protein sharing about 60% sequence identity with human PGM2 was very intriguing. Our first hypothesis was that this enzyme was a second form of phosphopentomutase/phosphoglucomutase with slightly different kinetic properties. However, this enzyme proved to have a very low phosphomutase activity on the three tested substrates (glucose 1-phosphate, ribose 1-phosphate, and deoxyribose 1-phosphate). The finding that PGM2L1 is expressed at a high level in brain, its molecular mass of 72 kDa and its (low) phosphomutase activity are all properties that PGM2L1 shares with glucose-1,6-bisphosphate synthase (17, 18, 26), an enzyme whose molecular identity had not yet been established.

That PGM2L1 corresponds to glucose 1,6-bisphosphate synthase is indicated by the fact that recombinant PGM2L1 used 1,3-bisphosphoglycerate as a phosphate donor and a series of sugar-monophosphates as acceptors. As for brain glucose 1,6-bisphosphate synthase (17, 19), 1-phosphosugars (glucose 1-phosphate, mannose 1-phosphate, ribose 1-phosphate, and deoxyribose 1-phosphate) were good substrates, whereas 5 or 6-phosphosugars (to the exception of glucose 6-phosphate) were not. This broad substrate specificity makes that PGM2L1 is able to synthesize cofactors for various phosphomutases.

The fact that PGM2L1 also comprises a potentially phosphorylatable serine in a similar motif as found in PGM1-related enzymes agrees with previous findings indicating that glucose-1,6-bisphosphate synthase is phosphorylated on a serine residue by transfer of the 1-phosphogroup of 1,3-bisphosphoglycerate (18). As previously shown, this reaction is the first step of aldose-bisphosphate synthesis. The second step is the transfer of the phosphate group from the phosphorylated serine onto a suitable sugar-phosphate acceptor (18, 26).

As previously reported by others for the enzyme purified from human erythrocytes (21), recombinant PGM2 also displays glucose-1,6-bisphosphate synthase activity. However, as shown in the present study, the synthase activity of PGM2 is lower than that of PGM2L1 and it is more powerfully inhibited by glucose 1,6-bisphosphate. This means that PGM2L1 is more prone to form elevated concentrations of glucose 1,6-bisphosphate than PGM2. This agrees with the finding that the glucose 1,6-bisphosphate concentration is particularly elevated in brain, where values of the order of 75 nmol/g have been reported (27, 28). These concentrations are far above the levels needed to saturate phosphomutases ($K_m = 0.1 \mu M$) and it is therefore thought that glucose 1,6-bisphosphate plays another role, possibly a regulatory one.

Glucose 1,6-bisphosphate is, at least in vitro, an inhibitor of low $K_m$ hexokinases (29, 30), of 6-phosphogluconate dehydrogenase (31) and of fructose-1,6-bisphosphatase (32), and a stimulator of phosphofructokinase (33), though a much poorer one than fructose 2,6-bisphosphate (34), and of liver pyruvate kinase (35). The idea that glucose 1,6-bisphosphate plays a regulatory role is supported by the finding that brain is also rich in glucose-1,6-bisphosphatase, the enzyme that degrades glucose 1,6-bisphosphate. The activity of this enzyme is nearly dependent on inosine-monophosphate, a nucleotide that accumulates in anoxia (36) and this effect may at least partly account for the fact that the glucose 1,6-bisphosphate concentration decreases in brain during ischemia. The decrease in the concentration of glucose 1,6-bisphosphate is likely to deinhibit low $K_m$ hexokinases and favor glycolysis. A complementary role is presumably played by ribose 1,5-bisphosphate, which is also formed by PGM2L1 and is a potent stimulator of phosphofructokinase (37). The concentration of ribose 1,5-bisphosphate was shown to increase in macrophages during hypoxia (38). The molecular identification of the enzyme that synthesizes glucose 1,6-bisphosphate and ribose 1,5-bisphosphate will facilitate inves-
tigations aimed at testing the regulatory role of these molecules in intact cells.

REFERENCES

1. Galperin, M. Y., Bairoch, A., and Koonin, E. V. (1998) Protein Sci. 7, 1829–1835
2. Rashid, N., Imanaka, H., Fukui, T., Atomi, H., and Imanaka, T. (2004) J. Bacteriol. 186, 4185–4191
3. Quick, C. B., Fisher, R. A., and Harris, H. (1972) Ann. Hum. Genet. 35, 445–454
4. Barsky, D. L., and Hoffee, P. A. (1983) Biochim. Biophys. Acta 743, 162–171
5. Wijnen, L. M., Grzeschik, K. H., Pearson, P. L., and Meera Khan, P. (1977) Hum. Genet. 37, 271–278
6. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
7. Achouri, Y., Noel, G., Vertommen, D., Rider, M. H., Veiga-Da-Cunha, M., and Van Schaftingen, E. (2004) Biochem. J. 381, 35–42
8. Fortpied, J., Gemayel, R., Stroobant, V., and Van Schaftingen, E. (2005) Biochem. J. 388, 795–802
9. Follens, A., Veiga-da-Cunha, M., Mercck, R., van Schaftingen, E., and van Eldere, J. (1999) J. Bacteriol. 181, 2001–2007
10. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
11. Thompson, I. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) Nucleic Acids Res. 25, 4876–4882
12. Ray, W. J., Jr., Hermodson, M. A., Puvathingal, J. M., and Mahoney, W. C. (1983) J. Biol. Chem. 258, 9166–9174
13. Shackelford, G. S., Regni, C. A., and Beamer, L. J. (2004) Protein Sci. 13, 2130–2138
14. Horton, P., and Nakai, K. (1997) Proc. Int. Conf. Intell. Syst. Mol. Biol. 5, 147–152
15. Rose, I. A., and Warms, J. V., and Kaldij, G. (1975) J. Biol. Chem. 250, 3466–3470
16. Wong, L. J., and Rose, I. A. (1976) J. Biol. Chem. 251, 5431–5439
17. Guha, S. K., and Rose, Z. B. (1986) Arch. Biochem. Biophys. 250, 513–518
18. Dadi, H., and Koo, R. (1969) J. Biol. Chem. 244, 4888–4893
19. Regni, C., Schramm, A. M., and Beamer, L. J. (2006) J. Biol. Chem. 281, 15564–15571
20. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B., and Meuwissen, H. J. (1972) Lancet 2, 1067–1069
21. Giblett, E. R. (1985) Ann. N. Y. Acad. Sci. 451, 1–8
22. Rose, I. A., Warms, J. V., and Kaklij, G. (1975) J. Biol. Chem. 250, 3466–3470
23. Wong, L. J., and Rose, I. A. (1976) J. Biol. Chem. 251, 5431–5439
24. Rose, I. A., and Warms, J. V. (1974) Biochem. Biophys. Res. Commun. 59, 1333–1340
25. Van Schaftingen, E., Jett, M. F., Hue, L., and Hers, H. G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3483–3486
26. Koster, J. F., Slee, R. G., Staal, G. E., and van Berkel, T. I. (1972) Biochim. Biophys. Acta 258, 763–768
27. Ishikawa, E., Ogushi, S., Ishikawa, T., and Uyeda, K. (1982) J. Biol. Chem. 257, 6634–6637
28. Ishikawa, E., Ogushi, S., Ishikawa, T., and Uyeda, K. (1990) J. Biol. Chem. 265, 18875–18878
29. Kawaguchi, T., Veech, R. L., and Uyeda, K. (2001) J. Biol. Chem. 276, 28554–28561
31. Beitner, R., and Nordenberg, J. (1979) Biochim. Biophys. Acta 583, 266–269
32. Marcus, C. J. (1976) J. Biol. Chem. 251, 2963–2966
33. Rose, I. A., and Warms, J. V. (1974) Biochem. Biophys. Res. Commun. 59, 1333–1340
34. Van Schaftingen, E., Jett, M. F., Hue, L., and Hers, H. G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3483–3486
35. Koster, J. F., Slee, R. G., Staal, G. E., and van Berkel, T. I. (1972) Biochim. Biophys. Acta 258, 763–768
36. Guha, S. K., and Rose, Z. B. (1982) J. Biol. Chem. 257, 6634–6637
37. Ishikawa, E., Ogushi, S., Ishikawa, T., and Uyeda, K. (1990) J. Biol. Chem. 265, 18875–18878
38. Kawaguchi, T., Veech, R. L., and Uyeda, K. (2001) J. Biol. Chem. 276, 28554–28561