Glucose 1,6-bisphosphate (Glc-1,6-P₂) concentration in brain is much higher than what is required for the functioning of phosphoglucomutase, suggesting that this compound has a role other than as a cofactor of phosphomutases. In cell-free systems, Glc-1,6-P₂ is formed from 1,3-bisphosphoglycerate and Glc-6-P by two related enzymes: PGM2L1 (phosphoglucomutase 2-like 1) and, to a lesser extent, PGM2 (phosphoglucomutase 2). It is hydrolyzed by the IMP-stimulated brain Glc-1,6-bisphosphatase of still unknown identity. Our aim was to test whether Glc-1,6-bisphosphatase corresponds to the phosphomannomutase PMM1, an enzyme of mysterious physiological function sharing several properties with Glc-1,6-bisphosphatase. We show that IMP, but not other nucleotides, stimulated by >100-fold (Kₐ ≈ 20 μM) the intrinsic Glc-1,6-bisphosphatase activity of recombinant PMM1 while inhibiting its phosphoglucomutase activity. No such effects were observed with PMM2, an enzyme paralogous to PMM1 that physiologically acts as a phosphomannomutase in mammals. Transfection of HEK293T cells with PGM2L1, but not the related enzyme PGM2, caused an ≈20-fold increase in the concentration of Glc-1,6-P₂. Transfection with PMM1 caused a profound decrease (>5-fold) in Glc-1,6-P₂ in cells that were or were not cotransfected with PGM2L1. Furthermore, the concentration of Glc-1,6-P₂ in wild-type mouse brain decreased with time after ischemia, whereas it did not change in PMM1-deficient mouse brain. Taken together, these data show that PMM1 corresponds to the IMP-stimulated Glc-1,6-bisphosphatase and that this enzyme is responsible for the degradation of Glc-1,6-P₂ in brain. In addition, the role of PGM2L1 as the enzyme responsible for the synthesis of the elevated concentrations of Glc-1,6-P₂ in brain is established.

Glucose 1,6-bisphosphate (Glc-1,6-P₂),² a well known cofactor for phosphoglucomutase and other sugar phosphomutases (1), is ubiquitously present in tissues. Its concentration is particularly elevated in brain, where it reaches values of >100 μM (2), i.e. >1000-fold higher than the concentrations required to stimulate phosphoglucomutase. Glc-1,6-P₂ has been proposed to act as an effector for several enzymes. Phosphofructokinase (3, 4) and liver pyruvate kinase (5) are both stimulated by this compound, whereas low Kₐ hexokinases (6–8), 6-phosphogluconate dehydrogenase (9), and fructose-1,6-bisphosphatase (10) are inhibited. These effects have been demonstrated in vitro, but under conditions that are not necessarily physiologically relevant. In addition, the occurrence of this regulation in intact cells has not been demonstrated.

Glc-1,6-P₂ is synthesized from 1,3-bisphosphoglycerate and glucose 1-phosphate or glucose 6-phosphate by Glc-1,6-P₂ synthase, an enzyme particularly abundant in brain (11) and recently identified as PGM2L1 (phosphoglucomutase 2-like 1) (12). In vitro, the related enzyme PGM2 (phosphoglucomutase 2), which shares ~60% identity with PGM2L1 and acts mainly as a phosphofructomutase, also catalyzes the synthesis of Glc-1,6-P₂, although with a lower Vₘₐₓ than PGM2L1 and a much stronger inhibition by the reaction product Glc-1,6-P₂. In comparison with PGM2, PGM2L1 is therefore better suited to provide cells with elevated concentrations of Glc-1,6-P₂, although this still needs to be demonstrated in intact cells.

Glc-1,6-P₂ is degraded by glucose-1,6-bisphosphatase. The brain enzyme, which has been best characterized (13, 14), is dependent for its activity on the presence of IMP, the concentration of which increases in anoxia. This effect is presumably responsible for the decrease in Glc-1,6-P₂ concentration in brain during anoxia (2).

Brain glucose-1,6-bisphosphatase, although not yet molecularly identified, has several characteristics (13, 14) that may help to identify its sequence. It catalyzes an exchange reaction between glucose 6-phosphate and Glc-1,6-P₂, indicating that the reaction mechanism involves the formation of a phosphoenzyme. It also acts as a mannose-1,6-bisphosphatase and displays some phosphoglucomutase activity. Finally, the molecular mass of this enzyme as determined by gel filtration is ~87 kDa.

These four properties are reminiscent of those of PMM1 (phosphomannomutase 1), an enzyme belonging to the halocid dehalogenase family of phosphatases/phosphomutases, with a reaction mechanism involving a phosphoenzyme intermediate (15). Interestingly, PMM1 shares 66% sequence identity with PGM2, a specific phosphomannomutase (16) that is deficient in the most frequent form of congenital disorders of
glycosylation, type Iα (17, 18). In contrast to PMM2, PMM1 is less specific: it has nearly equal phosphomannomutase and phosphoglucomutase activities as well as a modest glucose-1,6-bisphosphatase activity corresponding to ~3% of its phosphomannomutase activity (16). However, the effect of IMP on PMM1 activity has never been tested. Like PMM2, PMM1 was once thought to be involved in the in vivo formation of mannose 1-phosphate needed for glycoprotein biosynthesis. However, gene knock-out studies in mice have shown that PMM2 deficiency is early lethal (19), whereas PMM1 deficiency does not lead to any pathological findings (20). As PMM1 is often present in the same cell types as PMM2 (21), these findings indicate that despite its phosphomannomutase activity, PMM1 cannot substitute for PMM2 in PMM2-deficient mice, suggesting that PMM1 has another physiological role. The purpose of this work was to establish whether the enzyme that catalyzes the hydrolysis of Glc-1,6-P₂ in brain indeed corresponds to PMM1 and to identify which of the two enzymes, PGM2L1 and PMM2, is able to make elevated concentrations of Glc-1,6-P₂ in intact cells.

**EXPERIMENTAL PROCEDURES**

**Overexpression and Purification of Mouse PMM1 and PMM2—**

To produce recombinant mouse PMM1, 1 liter of Escherichia coli BL21(DE3) pLysS harboring pET-3d containing full-length mouse PMM1 cDNA was grown, and PMM1 was overexpressed and purified as described (21, 22). After DEAE-Sephrose chromatography (GE Healthcare), we used the most purified fraction (90% purity as indicated by SDS-PAGE analysis) eluted with ~150 mM NaCl for characterization of the kinetic properties of PMM1. For the production of recombinant mouse PMM2, the coding region of PMM2 cDNA was cloned into pET-15b using NdeI (containing the initial ATG site) and BamHI (placed after the stop codon) restriction sites. After confirmation of the sequence by sequencing, the plasmid was used to transform E. coli BL21(DE3) pLysS. The culture was grown in LB-rich medium at 30 °C, and PMM2 with an N-terminal His₆ tag was overexpressed for 18 h after addition of 0.4 mM isopropyl β-D-thiogalactopyranoside. Preparation of the bacterial extract and purification of His₆-tagged PMM2 on a 1-mL HiTrap column (Ni²⁺ form; GE Healthcare) were done as described previously (23). PMM2 was eluted (as indicated by SDS-PAGE analysis) with 20 ml of 150 mM imidazole, concentrated 4-fold on two 15-ml Vivaspin ultrafiltration columns (10 ml/column; Sartorius, Stockport, United Kingdom), and finally desalted on two PD-10 columns (GE Healthcare) equilibrated with 25 mM Hepes (pH 7.2), 30 mM NaCl, 1 mM dithiothreitol, and 2 μg/ml leupeptin and antipain. Protein concentration was estimated by measuring A₂₈₀ assuming a molar absorptivity of 20,775 M⁻¹ cm⁻¹. Purified PMM2 (30 mg of pure protein/liter of culture) was supplemented with 10% glycerol and stored at −70 °C.

**Expression of Human PGM2 and PGM2L1 and Mouse PMM1 and PMM2 in HEK293T Cells—**

The open reading frames encoding human PGM2 or PGM2L1 and mouse PMM1 or PMM2 were PCR-amplified (primer sequences available upon request). The template DNAs for the polymerase reactions were the corresponding bacterial expression plasmids described above (PMM1 and PMM2) and in Ref. 12 for PGM2 and PGM2L1. After cloning the PCR-amplified fragments in pBluescript and excluding any PCR errors by sequencing, PGM2 and PGM2L1 inserts were ligated into the eukaryotic expression vector pEF6/Myc-HisA (Invitrogen) using EcoRV and NotI (PGM2) or EcoRI and EcoRV (PGM2L1) restriction sites, allowing the production of a protein with a C-terminal His₆ tag. PMM1 and PMM2 inserts were ligated into the pEF6/HisA expression vector (Invitrogen) using KpnI and NotI restriction sites, allowing the production of proteins with an N-terminal His₆ tag. HEK293T cells were transfected (8 μg of DNA) or cotransfected (2 × 4 μg of DNA) using the jetPEI transfection kit (Polyplus Transfection, Illkirch, France) as described previously (24). We used four culture dishes per condition tested: triplicates to measure Glc-1,6-P₂ concentration and one dish to confirm protein expression. After 48 h of incubation at 37 °C, the medium was completely removed, and the proteins were immediately denatured in three dishes by addition of 400 μl of ice-cold 5% HClO₄. The cells in the fourth dish were washed with phosphate-buffered saline and harvested in 500 μl of 20 mM Hepes (pH 7.1) containing 5 μg/ml leupeptin and antipain. The HClO₄ extracts were prepared by recovering the suspension from the dish and centrifuging at 4 °C for 5 min at 16,000 × g. To quantify the proteins, the pellets were resuspended in 200 μl of 0.2 M NaOH, and protein concentration was measured using γ-globulin as a standard (25). The supernatant was recovered, neutralized with 3 M K₂CO₃, and used to assay Glc-1,6-P₂ after elimination of the salt precipitate by centrifugation. In the fourth dish, proteins were extracted as described (24), and expression of all four His₆-tagged proteins was quantified by Western blot analysis using Penta-His™ monoclonal antibody (Qiagen GmbH, Hilden, Germany).

**Extraction of Glc-1,6-P₂ from Mouse Tissues—**

Tissues were removed from wild-type or PMM1-deficient mice anesthetized by inhalation with sevoflurane and immediately frozen-clamped. When various tissues were taken from the same animal, the brain was always the first one to be removed, followed by kidney, liver, lung, and muscle. When blood was collected, we used a different set of mice. Neutralized HClO₄ extracts were prepared by homogenizing the frozen tissues (or 300 μl of freshly collected whole blood) in 3 volumes (w/v) of ice-cold 5% HClO₄, centrifuging at 16,000 × g for 10 min at 4 °C, neutralizing the supernatant with 3 M K₂CO₃, and eliminating the salt precipitate by centrifugation. PMM1-deficient mice were obtained as described previously (20).

**Assay of Glc-1,6-P₂ in Neutralized HClO₄ Extracts from Mouse Tissues or Transfected HEK293T Cells—**

Glc-1,6-P₂ was assayed through stimulation of the activity of muscle phosphoglucomutase. Phosphoglucomutase activity was measured spectrophotometrically at 30 °C in a cuvette containing 50 mM Tris (pH 7.1), 0.1 mM EGTA, 5 mM MgCl₂, 0.5 mM NADP⁺, 0.5 mM glucose 1-phosphate free of Glc-1,6-P₂ (Merck), 1.75 units/ml yeast glucose-6-phosphate dehydrogenase (Roche Applied Science), and 0–0.5 μM Glc-1,6-P₂ (used as a standard; Roche Applied Science) or 5–20 μl of sample neutralized HClO₄ extracts. The reaction was initiated by addition of 0.05 units/ml desalted rabbit muscle phosphoglucomutase (Roche Applied Science). The concentration of Glc-1,6-P₂ in the
unknown samples was calculated from a standard curve obtained under the same conditions.

**Enzymatic Assays**—The phosphoglucomutase and phosphomannomutase activities of PMM1 and PMM2 were assayed in 25 mM Mes (pH 6.5), 25 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol, 0.5 mg/ml bovine serum albumin, 0.5 mM NADP⁺, 1 μM Glc-1,6-P₂, 1.75 units/ml desalted yeast glucose-6-phosphate dehydrogenase, and 25 μM glucose 1-phosphate (phosphoglucomutase activity) or mannose 1-phosphate (phosphomannomutase activity). When the substrate was mannose 1-phosphate (Sigma), we added 2 units/ml yeast phosphomannose isomerase (Sigma) and 3 units/ml desalted yeast phosphoglucoisomerase (Roche Applied Science) to convert the product of the reaction, mannose 6-phosphate, to glucose 6-phosphate. The reaction was started by addition of purified PMM1 (0.1 μg/ml) or PMM2 (1.8 μg/ml) to assay phosphoglucomutase activity and 0.18 μg/ml to assay phosphomannomutase activity. Glucose-1,6-bisphosphatase activity was measured in 50 mM Tris (pH 7.1), 0.1 mM EGTA, 2.5 mM MgCl₂, 1 mM dithiothreitol, 0.5 mg/ml bovine serum albumin, 0.5 mM NADP⁺, and 0.15 mM Glc-1,6-P₂, with or without IMP or other nucleotides, 0.2 units/ml rabbit muscle phosphoglucomutase, and 1.75 units/ml yeast glucose-6-phosphate dehydrogenase. The reaction was started by addition of purified PMM1 (0.02 μg/ml) or PMM2 (18 μg/ml). In the assay conditions specified, one unit is the amount of enzyme that catalyzes the conversion of 1 μmol of substrate that is coupled to the reduction of 1 μmol of NADP⁺/min.

**RESULTS**

**IMP and GMP Stimulate PMM1 (but Not PMM2) Glucose-1,6-bisphosphatase Activity**—To investigate whether PMM1 is the glucose-1,6-bisphosphatase described in mouse brain by Guha and Rose (13, 14), we overexpressed recombinant mouse PMM1 in E. coli and partially purified it on an anion exchange column. The most purified fraction (~90% purity) was used to investigate the glucose-1,6-bisphosphatase activity of PMM1. This activity amounted to 2.1 ± 0.25 μmol/min/mg of protein in the presence of 150 μM Glc-1,6-P₂. Both IMP and GMP (Fig. 1), but not AMP (data not shown), increased the glucose-1,6-bisphosphatase activity of PMM1 up to 110-fold with a Kₘ of 3 μM in the case of IMP and up to 30-fold with a Kₘ of 80 μM in the case of GMP. The Kₘ of PMM1 for Glc-1,6-P₂ was 17 and 40 μM in the presence of 1 and 20 μM IMP, respectively. In contrast to PMM1, the glucose-1,6-bisphosphatase activity of PMM2 was extremely low (0.01 ± 0.0005 μmol/min/mg of protein, i.e. 0.05% of the basal glucose-1,6-bisphosphatase activity of PMM1) and was insensitive to IMP and GMP (both tested at up to 1 mM). Furthermore, other nucleotides (AMP, ADP, ATP, UMP, UDP, UTP, CTP, GMP, and GTP, all tested at 100 μM) did not stimulate the glucose-1,6-bisphosphatase activity of PMM1 or PMM2 (data not shown).

Because IMP activated the glucose-1,6-bisphosphatase activity of PMM1, we were interested to find out whether IMP inhibits the phosphoglucomutase and phosphomannomutase activities of this enzyme. Both mutase activities, measured with glucose 1-phosphate (Fig. 2a) or mannose 1-phosphate (Fig. 2b) as substrate, were inhibited by increasing IMP concentrations.

**FIGURE 1.** Stimulation of the glucose-1,6-bisphosphatase activity of PMM1 by IMP and GMP. The enzymatic activities (μmol/min/mg of protein) were assayed as described under “Experimental Procedures” with 150 μM Glc-1,6-P₂, and the indicated concentrations of IMP or GMP. The results shown are the means ± S.E. of three determinations with the same enzyme preparation.

**FIGURE 2.** Inhibition by IMP of the phosphoglucomutase (a) and phosphomannomutase (b) activities of PMM1. Both enzymatic activities were assayed as described under “Experimental Procedures” with 1 μM Glc-1,6-P₂, increasing concentrations of IMP, and 25 μM glucose 1-phosphate (a) or mannose 1-phosphate (b). The results shown are the means ± S.E. of three determinations with the same enzyme preparation. PGM, phosphoglucomutase; PMM, phosphomannomutase; m, mouse.

In contrast, neither the phosphoglucomutase (0.85 ± 0.006 μmol/min/mg of protein) nor the phosphomannomutase (16 ± 0.15 μmol/min/mg of protein) activity of PMM2 decreased by >5% in the presence of 1 mM IMP (data not shown).
Intracellular Glc-1,6-P₂ in HEK293T Cells Transfected with Human PGM2 and PGM2L1 and Mouse PMM1 and PMM2—

Even though PGM2L1 is particularly suited to synthesize elevated concentrations of Glc-1,6-P₂, PGM2 is also able, at least in vitro, to form Glc-1,6-P₂ from the same substrates (12). Furthermore, human erythrocyte Glc-1,6-P₂ synthase was shown to copurify with phosphopentomutase, i.e. PGM2 (26, 27). On the other hand, PPM1 was never shown to hydrolyze Glc-1,6-P₂, in vivo. To investigate the synthesis and degradation of Glc-1,6-P₂ under more physiological conditions, we transfected HEK293T cells with plasmids driving the expression of human PGM2 or PGM2L1 and/or mouse PMM1 or PMM2 (Fig. 3). Glc-1,6-P₂ concentration was determined in cell extracts with a highly sensitive assay based on the activation of rabbit muscle phosphoglucomutase by Glc-1,6-P₂. Neutralized HClO₄ extracts from cell cultures transfected with PGM2L1 or PMM2 showed either a 20-fold (p < 0.0001; n = 3) or a non-significant 1.5-fold (p = 0.076; n = 3) increase in the concentration of Glc-1,6-P₂ compared with control cultures transfected with the empty plasmid (51.7 ± 5.2 nmol/g of protein), confirming that PGM2L1 is indeed the enzyme responsible for Glc-1,6-P₂ synthesis. Remarkably, transfection of PMM1 alone or in cells cotransfected with PGM2L1 almost depleted the cells of Glc-1,6-P₂ (3.7 ± 3.7 nmol/g of protein, i.e. 14-fold lower than the control (p = 0.0016; n = 3)) in the first case or lowered its concentration to levels close to control values in the second case. On the other hand, when PMM2 was cotransfected with PGM2L1, intracellular Glc-1,6-P₂ levels were decreased by only 25% (p = 0.0025; n = 3) despite the fact that PMM2 was 6-fold better expressed than PPM1 (see below). Similarly, overexpression of PMM2 alone did not significantly lower the intracellular concentration of Glc-1,6-P₂ (22.3 ± 11.2 nmol/g of protein) compared with control cells, confirming that the role of PPM1 in intact cells is indeed to hydrolyze Glc-1,6-P₂.

As all constructs encoded His₆-tagged proteins, we could verify the expression of the transfected enzymes by Western blotting (data not shown). Interestingly, PGM2 was >2-fold better expressed than PGM2L1, and PPM2 was 6–7-fold better expressed than PPM1. When proteins were cotransfected, expression of PGM2L1 and of each of the PMM proteins was lowered by 2–4-fold (data not shown).

Deletion of PPM1 Blocks Glc-1,6-P₂ Hydrolysis in PMM1-deficient Mouse Brain—To confirm that PPM1 is the physiological IMP-activated glucose-1,6-bisphosphatase described in mouse brain (13, 14) and is indeed responsible for the Glc-1,6-P₂ hydrolysis observed in ischemic brain (2), we measured Glc-1,6-P₂ concentration in neutralized HClO₄ extracts from brains of wild-type and PPM1-deficient mice. For this purpose, the brains of deeply anesthetized mice were removed. One hemisphere was frozen as fast as possible (~30 s) after removal from the mouse skull (t = 0 min), whereas the other was frozen after 5 min at room temperature (t = 5 min). Fig. 4a shows that the Glc-1,6-P₂ concentration in t = 0 min brains was about two times higher in the brains of PPM1-deficient mice in comparison with those of wild-type mice. In addition, we observed that the Glc-1,6-P₂ concentration decreased with time in wild-type mouse brain, but not in PPM1-deficient mouse brain.

Although PPM1 is highly expressed in adult mouse brain, some authors have also reported low PPM1 expression in other adult tissues such as lung and liver (21). Furthermore, Passonneau et al. (2) reported Glc-1,6-P₂ concentrations in muscle and red blood cells that are
similar to the ~100 μm measured in brain. In view of these results, we decided to measure Glc-1,6-P₂ in neutralized HClO₄ extracts of various tissues from wild-type and PMM1-deficient mice. Fig. 4b shows that the absence of PMM1 failed to affect significantly the concentration of Glc-1,6-P₂ in liver, lung, blood, and skeletal muscle, suggesting that PMM1 is absent from these tissues and/or that the concentration of IMP is too low to stimulate its activity.

DISCUSSION

PMM1 Is the IMP-sensitive Glucose-1,6-bisphosphatase—Our results show that PMM1 actually corresponds to the enzyme described by Guha and Rose (13, 14) as glucose-1,6-bisphosphatase. PMM1 and glucose-1,6-bisphosphatase have similar molecular masses (87 kDa by gel filtration for glucose-1,6-bisphosphatase; PMM1 is known to be a 2 × 28-kDa dimer) (28). Furthermore, they both have the ability to catalyze phosphomutase reactions in the absence of IMP, and they both are Mg²⁺-dependent and act predominantly as glucose-1,6-bisphosphatases in the presence of IMP. Both PMM1 and brain glucose-1,6-bisphosphatase are exquisitely sensitive to this nucleotide, with $K_v$ values in the micromolar range. In both cases, GMP is the only nucleotide that mimics the effect of IMP. Finally, both enzymes show a particularly high level of expression in brain. The identity of PMM1 as brain glucose-1,6-bisphosphatase is therefore warranted.

This work leads moreover to the conclusion that PMM1 is the enzyme responsible for Glc-1,6-P₂ degradation in ischemic brain (2). This role was initially suggested by the finding that glucose-1,6-bisphosphatase is stimulated by a metabolite (IMP) whose concentration is elevated in anoxia (29). We now show that the decrease in the concentration of Glc-1,6-P₂ observed in ischemic brain does not occur in PMM1-deficient mice.

That PMM1 acts as a glucose-1,6-bisphosphatase in the physiological environment of the cytosol is further indicated by transfection studies. The level of Glc-1,6-P₂ was markedly decreased by PMM1 overexpression both in cells that had control levels of Glc-1,6-P₂ to start with and in cells in which this level had been raised by overexpression of PGM2L1. On the contrary, PGM2 had only a minor effect on the Glc-1,6-P₂ level, despite its higher levels of overexpression in HEK293T cells compared with PMM1. Thus, despite their close structural similarity, PMM1 and PGM2 have distinctly different functions. This accounts for the apparent inability of PMM1 to ensure sufficient phosphomannomutase activity in PGM2-deficient mice (20).

Reaction Mechanism of Glucose-1,6-bisphosphatase—To account for the multiple activities of glucose-1,6-bisphosphatase, Guha and Rose (13, 14) proposed that this enzyme uses Glc-1,6-P₂ to form a phosphoenzyme, thus releasing glucose 1-phosphate (or glucose 6-phosphate). PMM1 has indeed been shown to form an aspartyl phosphate at Asp¹⁹⁹ (15). The phosphomutase activity results from subsequent binding of glucose 6-phosphate (or glucose 1-phosphate) to the phosphoenzyme, followed by the transfer of the phosphoryl group to re-form Glc-1,6-P₂ (30, 31). The phosphomannomutase activity of this enzyme can be explained in a similar manner. The phosphatase activity involves IMP, which presumably binds to the same site as hexose monophosphates. This prevents the re-formation of the biphosphate cofactor (thereby inhibiting the phosphomutase activity), but more importantly stimulates the phosphatase activity of glucose-1,6-bisphosphatase/PMM1, which exceeds the mutase activity by >5-fold. The observation that this type of effect takes place with IMP and, to a lesser extent, with GMP, but not with AMP, underlines the importance of the presence of an oxygen atom bound to C-6 and/or of a hydrogen atom bound to N-1 on the purine base. Furthermore, PGM2 does not become a phosphatase in the presence of IMP (or any other nucleotide that we tested), and its mutase activity is unaffected by IMP, despite its close structural similarity to PMM1. This indicates that IMP is unable to bind to PGM2.

Multiple alignments of vertebrate PMM1 and PGM2 and PMM proteins from fungi and plants indicate that all residues that putatively contact the substrate are totally conserved in the two proteins. However, a few residues that are highly conserved in vertebrate PGM2 as well as in PMM proteins from primitive organisms (which are most likely all phosphomannomutases) are replaced in eukaryotic PMM1 by residues that appear to be strictly conserved in the PMM1 subfamily. This is particularly the case for Glu²¹⁹ in mouse PMM1. The carboxyl group of this residue, which is at a distance of ~10 Å from the pyranose ring of mannose 1-phosphate in the structure of this enzyme, possibly makes a hydrogen bond with the purine base N-1 hydrogen in IMP and in GMP if these nucleotides bind where mannose 1-phosphate does. No such bond would be made with AMP, explaining the specificity of the stimulatory effect of IMP and GMP. Another residue that potentially plays a role is Met¹⁸⁶, which replaces a highly polar residue, glutamine, in PGM2 and PMM proteins from fungi and plants. The methyl group in Met¹⁸⁶ is at a distance of 6 Å from mannose 1-phosphate in the crystal structure of PMM1. Its hydrophilic character could help PMM1 to bind the purine ring of IMP.

Respective Roles of PGM2 and PGM2L1 in Glc-1,6-P₂ Synthesis—Our results show also that PGM2L1 is able to induce a large increase in the concentration of Glc-1,6-P₂, whereas this is not the case for PGM2. This does not mean that PGM2 cannot make Glc-1,6-P₂ in vitro. The explanation for the lack of rise in intracellular Glc-1,6-P₂ following PGM2 overexpression in HEK293T cells could rather be the intrinsically lower Glc-1,6-P₂ synthase activity of PGM2 in comparison with PGM2L1 and its stronger inhibition by the reaction product Glc-1,6-P₂ (12). Thus, PGM2L1 is tailored to raise the concentration of Glc-1,6-P₂ to high values.

This conclusion may apparently contradict the findings that erythrocytes contain elevated concentrations of Glc-1,6-P₂ (2, 32) and that Glc-1,6-P₂ synthase copurifies with PGM2 in human erythrocyte extracts (26). An explanation for this discrepancy could be that PGM2L1 was lost in the purification reported by Accorsi et al. (26) due to, for instance, denaturation or proteolysis. Alternatively, free Glc-1,6-P₂ may represent only a small fraction of total Glc-1,6-P₂ in erythrocytes due to binding to hemoglobin. Hemoglobin is indeed known to bind avidly multiply charged phosphate esters such as 2,3-bisphosphoglycerate and inositol pentakisphosphate (33). It is therefore likely that it will also bind with great affinity bisphosphate esters such as Glc-1,6-P₂. If so, the free concentration of Glc-1,6-P₂ may be
extremely low, leading to insignificant feedback inhibition of the Glc-1,6-P$_2$ synthase activity of PGM2.

Physiological Role of Glc-1,6-P$_2$—The existence of specialized enzymes able to make high concentrations of Glc-1,6-P$_2$ and to degrade it in a controlled manner indicates that Glc-1,6-P$_2$ plays a role other than as a cofactor of phosphomutases. As mentioned in the Introduction, Glc-1,6-P$_2$ is in vitro an effector of several enzymes that are either inhibited (hexokinases I and II, fructose-1,6-bisphosphatase; 6-phosphogluconate dehydrogenase) or activated (liver pyruvate kinase, phosphofructokinase). Several of these are not relevant for the regulation of brain intermediary metabolism. This is the case for liver-type pyruvate kinase (which is not expressed in brain) and probably also for fructose-1,6-bisphosphatase (the activity for liver pyruvate kinase, which is not expressed in brain) regulated enzymes able to make high concentrations of Glc-1,6-P$_2$ over a 50-fold range in HEK293T cells. Preliminary data indicate that changes in the concentration of Glc-1,6-P$_2$ during the period that it takes to freeze the brain after removal from the physiological removal of brain (PGM2L1) deficiency. The fact that Glc-1,6-P$_2$ degradation is (almost) specifically and markedly stimulated by IMP suggests a link between the Glc-1,6-P$_2$ synthase activity of PGM2.

The availability of the sequences of the enzymes that make and degrade Glc-1,6-P$_2$ will allow one to study the effect of this compound in intact cells. Preliminary data indicate that changing the levels of Glc-1,6-P$_2$ over a 50-fold range in HEK293T cells (by overexpressing PMM1 or PGM2L1) does not significantly affect the rate of [2$^3$H]glucose metabolism (which measures glucose phosphorylation) and of lactate formation. These negative results have to be taken with caution because the effect of Glc-1,6-P$_2$ on hexokinase may be masked by compensatory changes in the concentration of glucose 6-phosphate, an even more powerful regulator of hexokinase activity than Glc-1,6-P$_2$.

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