Differential Regulation of Phosphoglucose Isomerase/Autocrine Motility Factor Activities by Protein Kinase CK2 Phosphorylation*

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Phosphoglucose isomerase (PGI; EC 5.3.1.9) is a cytosolic housekeeping enzyme of the sugar metabolism pathways that plays a key role in both glycolysis and gluconeogenesis. PGI is a multifunctional dimeric protein that extracellularly acts as a cytokine with properties that include autocrine motility factor (AMF)-eliciting mitogenic, motogenic, and differentiation functions, and PGI has been implicated in tumor progression and metastasis. Little is known of the biochemical regulation of PGI/AMF activities, although it is known that human PGI/AMF is phosphorylated at Ser185 by protein kinase CK2 (CK2); however, the physiological significance of this phosphorylation is unknown. Thus, by site-directed mutagenesis, we substituted Ser185 with aspartic acid (S185D) or glutamic acid (S185E), which introduces a negative charge and conformational changes that mimic phosphorylation. A Ser-to-Ala mutant protein (S185A) was generated to abolish phosphorylation. Biochemical analyses revealed that the phosphorylation mutant proteins of PGI exhibited decreased enzymatic activity, whereas the S185A mutant PGI protein retained full enzymatic activity. PGI phosphorylation by CK2 also led to down-regulation of enzymatic activity. Furthermore, CK2 knockdown by RNA interference was associated with up-regulation of cellular PGI enzymatic activity. The three recombinant mutant proteins exhibited indistinguishable cytokine activity and receptor-binding affinities compared with the wild-type protein. In both in vitro and in vivo assays, the wild-type and S185A mutant proteins underwent active species dimerization, whereas both the S185D and S185E mutant proteins also formed tetramers. These results demonstrate that phosphorylation affects the allosteric kinetic properties of the enzyme, resulting in a less active form of PGI, whereas non-phosphorylated protein species retain cytokine activity. The process by which phosphorylation modulates the enzymatic activity of PGI thus has an important implication for the understanding of the biological regulation of this key glucose metabolism-regulating enzyme.

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Phosphoglucose isomerase (PGI) is a multifunctional protein that plays an important intracellular role in both the glycolytic and gluconeogenesis pathways, catalyzing the interconversion of glucose 6-phosphate and fructose 6-phosphate (1) while extracellularly behaving as a cytokine. Molecular cloning and sequencing have characterized PGI as an autocrine motility factor (AMF), involved in angiogenesis and metastasis (2–4); a T cell lymphokine, e.g. neuroleukin, which supports the survival of spinal and sensory neurons (5, 6); a maturation factor, which differentiates myeloid leukemic cells to terminal monocytic cells (7); sperm antigen-36 (8); and a myofibril-bound serine proteinase inhibitor (9). Aberrations in the expression or activities of PGI due to mutations or deletions are of significant clinical importance since, in humans, they are associated with hereditary non-spherocytic hemolytic anemias diseases (10, 11). In addition, PGI/AMF is an antigen in arthritis disease (12), and its presence in serum and urine is of prognostic value associated with cancer progression (13–15).

Once secreted from tumor cells, PGI behaves as an AMF, which promotes cancer cell invasion and metastasis by stimulating cell motility via the autocrine loop following binding to its seven-transmembrane glycoprotein receptor (AMF receptor) (16, 17). Hypoxia, a phenomenon associated with cancer growth, stimulates its expression (18, 19). PGI/AMF may act as a paracrine factor during cancer progression, as its binding to the endothelial cell AMF receptor promotes angiogenesis and vessel leakiness (3, 4, 20).

To elucidate the catalytic function of PGI, several crystal structure analyses of this protein have been performed (21–24). They revealed that active PGI is a dimer composed of a large and a small domain and that its sugar-binding site, which is responsible for its enzymatic activity, is located in the junction where the large and small domains and the C-terminal tail come together (24, 25). Mutagenesis of the sugar-binding motif of PGI results in abrogation of cytokine activity, suggesting that the active site of the enzyme overlaps with cytokine function (24). These data are consistent with the fact that the specific active-site sugar inhibitors erythrose 4-phosphate and 5-phosphoarabinonate also inhibit its cytokine activity (2, 21). However, the regulation mechanisms of the enzymatic/cytokine activity of PGI are not fully understood.

Other glycolytic enzymes of vertebrates, including phosphofructokinase (26–28), glyceraldehyde-3-phosphate dehydrogenase (26, 29), phosphoglycerate mutase (26, 30), enolase (26, 31,
Phosphorylation of PGI/AMF Regulates Its Enzymatic Activity

32, pyruvate kinase (33), and lactate dehydrogenase (31), are also phosphoproteins like PGI. Phosphofructokinase and pyruvate kinase are phosphorylated by cAMP-dependent kinase, resulting in down-regulation of activity (27, 33). Protein kinase C phosphorylation of phosphofructokinase induces up-regulation of enzyme activity (28), and phosphorylation of enolase precedes glycolysis (34). It is still unknown whether the expression or activities of the rest of the glycolytic enzymes are also regulated by phosphorylation. Previously, it was reported that PGI is phosphorylated at Ser^{185} by protein kinase CK2 (CK2) (35), although the physiological significance of such phosphorylation remained to be determined. Thus, to study the function(s) of phosphorylated PGI, we generated, by site-directed mutagenesis, non-phosphorylation- and phosphorylation-mimicking species of recombinant PGI proteins and analyzed their structures and enzymatic and cytotoxic activities.

**EXPERIMENTAL PROCEDURES**

**Cells and Monolayer Culture Conditions**—The human fibrosarcoma cell line HT1080 (CCL121) was obtained from American Type Culture Collection (Manassas, VA). Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, nonessential amino acids, and antibiotics and maintained at 37 °C in a humidified atmosphere of 5% CO2 and 95% air. Site-Directed Mutagenesis—A Ser-to- Ala substitution at residue 185 (S185A) to abolish phosphorylation and Ser-to-Asp (S185D) and Ser-to-Glu (S185E) substitutions to mimic phosphorylation were generated using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The pGEX6P vector containing human PGI/AMF protein was used as a template for PCR. The sequences of the primers used to generate point mutations were GGTATGCCCAACATGGAGAAC, GGTATGCGCAACATTGTGGGAAC, and GGTATGCGCAACATTGTGGGAAC for S185A, S185D, and S185E, respectively. The PCR conditions used for all mutants were as follows: 95 °C for 0.5 min and 16 cycles of 95 °C for 1 min, 55 °C for 1 min, and 68 °C for 1 min. After the template DNA was digested with the DpnI restriction enzyme, the PCR product was transferred into One Shot® TOP10 competent cells (Invitrogen). Recombinant plasmids including the mutants were purified and sequenced. We have previously established the crystal structure of human PGI (24); and based on these data, the structural changes between the wild-type (WT) and mutant PGI proteins were examined using the program MODELLER (36). We constructed an energy-minimized model of human PGI phosphorylated at Ser^{185} using the program REFMAC (37).

**Transfection with Mutant PGI Genes and RNA Interference**—WT and mutant PGI genes were ligated into the mammalian expression vector pcDNA3.1-His (Invitrogen). Recombinant plasmids including the mutants were purified and sequenced. Parental HT1080 cells were transfected with Lipofectamine™ 2000 (Invitrogen) according to the manufacturer’s instructions. The medium was changed to serum-containing Dulbecco’s modified Eagle’s medium supplemented with or without unlabeled PGI (2 mg) and differing concentrations of unlabeled WT and mutant PGI proteins (0.1 μM NaCl (pH 7.2)) were incubated with 10 μg of N-hydroxysuccinimide fluorescein (photomultiplier) on ice for 2 h. Labelled proteins were dialyzed to remove free N-hydroxysuccinimide fluorescein, and their concentrations were determined. HT1080 cells treated with trypan blue after three washes with PBS were diluted at 3 x 10^6 cells/sample and incubated in PBS containing 0.1% BSA with or without unlabeled PGI (2 mg) and differing concentrations of labeled WT and mutant PGI proteins (0.1 μM NaCl (pH 7.2)) were incubated at 24 °C for 1 h. Flow cytometric analysis was performed using a FACSCalibur (BD Biosciences, San Jose, CA).

RESULTS

Effect of Ser^{185} Substitution or Phosphorylation of PGI on Its Enzymatic Activity and Secretion—It was previously reported that PGI undergoes post-translational modification by phosphorylation of Ser^{185} by CK2 (35). Based on the evidence that phosphorylation of glycolytic enzymes alters their enzymatic activities (27, 28, 33), we questioned whether phosphorylation of PGI is of physiological significance. To address this, we constructed mutants by site-directed mutagenesis whereby we constructed mutants by site-directed mutagenesis whereby we substituted Ser^{185} with aspartic acid (S185D) or glutamic acid (S185E), which introduces a negative charge and conformational changes that mimic phosphorylation (43) and Ser^{185} with alanine (S185A), which abolishes phosphorylation. As shown in Fig. 1A (inset), the WT and S185A, S185D, and S185E mutant
Phosphorylation of PGI/AMF Regulates Its Enzymatic Activity

FIG. 1. Enzymatic activity of WT and mutant PGI/AMF proteins. A, the in vitro enzymatic activity of recombinant WT and mutant PGI/AMF proteins was monitored for 10 min. The enzymatic activity of S185A was similar to that of the WT protein, whereas the enzymatic activity of S185D and S185E diminished to 20% or less of that of the WT protein. ○, WT; ●, S185A; ◊, S185D; ▲, S185E. Inset, 1 μg of each sample was subjected to SDS-PAGE, stained with Coomassie Brilliant Blue, and detected as the same molecular mass band. The data shown are representative of five independent experiments with similar results. B, parental HT1080 cells were transfected with WT and mutant PGI genes. The cytosolic and secreted PGI/AMF proteins were analyzed by Western blotting of samples (30 μg/protein) from cell lysates and conditioned medium and probed with anti-Xpress™ antibody. The WT and mutant PGI proteins were detected in both cell lysates and conditioned medium (inset). The cell lysates were also used for measuring in vitro PGI protein enzymatic activity. The PGI activities of WT and S185A PGI gene-transfected cells were >2-fold higher than that of the parental cells, whereas transfections with the S185D and S185E PGI genes hardly affected their PGI enzymatic activities. Lane a and ●, parental HT1080 cells; lane b and ×, null vector-transfected HT1080 cells; lane c and ◊, WT PGI gene-transfected HT1080 cells; lane d and ●, S185A PGI gene-transfected HT1080 cells; lane e and ◊, S185D PGI gene-transfected HT1080 cells; lane f and ▲, S185E PGI gene-transfected HT1080 cells. The data shown are representative of three independent experiments with similar results.

FIG. 2. Enzymatic activity of phosphorylated PGI/AMF. The enzymatic activity of phosphorylated PGI/AMF (●) was 50% of that of non-phosphorylated PGI/AMF (○) in the linear phase, although it was higher than that of the S185D and S185E mutants, which mimic phosphorylation. Inset, detection of PGI/AMF phosphorylation by autoradiography. WT PGI/AMF was phosphorylated by CK2 (lane a) and was not phosphorylated without CK2 (lane b), whereas S185D was not phosphorylated even with CK2 (lane c). Comparison between Coomassie Brilliant Blue (CB) and autoradiography (32P) suggests incomplete phosphorylation of PGI/AMF. The data shown are representative of three independent experiments with similar results.

S185D and S185E mutants showed markedly depressed PGI activities (Fig. 1A). To analyze mutant PGI functions in vivo, we used the pcDNA3.1-His mammalian expression vector, which can produce proteins with a unique antigen, the Xpress™ epitope, because expressed mutant proteins should be differentiated from the PGI protein that HT1080 cells originally express. As shown in Fig. 1B, the cells transfected with WT and all mutant PGI genes expressed and secreted PGI/AMF. Previously, we hypothesized that PGI phosphorylation provokes PGI secretion because PGI/AMF secreted to conditioned medium is phosphorylated (35). The present study shows, however, that not only phosphorylation-mimicking mutant PGI proteins (S185D and S185E), but also WT and phosphorylation-abolishing (S185A) PGI proteins were secreted. As shown in Fig. 1, WT and S185A mutant PGI gene-transfected cells appeared to augment PGI activity, whereas null vector-, S185D, and S185E-transfected cells did not.

Next, we questioned whether the reduced enzymatic activity of the S185D and S185E mutant PGI proteins results from mimicked phosphorylation status or amino acid substitutions. Thus, recombinant human CK2 (500 units) was used to phosphorylate affinity-purified human PGI (10 μg), and phosphorylated PGI was detected by autoradiography after SDS-PAGE (Fig. 2, inset, lower panel, lane a). The S185D mutant PGI protein we used for a control was not phosphorylated, as expected (Fig. 2, inset, lower panel, lane b), and PGI did not undergo autophosphorylation in the absence of CK2 (lane c). The enzymatic activity of phosphorylated PGI was measured (Fig. 2) and found to be reduced by ~50% at the linear portion of the enzymatic curve. This significantly reduced activity was not as dramatic as that observed in the phosphorylation-mimicking mutant proteins (Fig. 1A), which could be attributed to the fact that not all of the PGI molecules in the phosphorylation reaction mixture were phosphorylated and gives credence to the use of the phosphorylation-mimicking mutant proteins.
In addition, we transfected HT1080 cells with siRNA against CK2 to examine CK2 involvement with PGI activity. 2 days after transfection, CK2 expression was suppressed by <50% by siRNA, and the PGI activity of transfected cells increased 1.6-fold more compared with controls (Fig. 3).

Effect of Ser185 Substitution of PGI on Its Cytokine Activity—To study the possible relationship between PGI phosphorylation and cytokine activity, we resorted to two independent motility assays: 1) invasion assay utilizing Transwell® for measuring direct motility (Fig. 4A) and 2) phagokinetic track assay for measuring overall random motility capabilities (Fig. 4B). The WT and S185A, S185D, and S185E mutant proteins at 100 pg/ml increased HT1080 cell invasion (directional motility) compared with controls in the invasion assay (analysis of variance; $F_{3,50} = 8.156$, $p < 0.0001$ and $p = 0.0005$, 0.0033, and 0.0026, respectively) (Fig. 4A). Similarly, in the phagokinetic track motility assay, the mutant proteins stimulated the motility of HT1080 cells to the same level as the WT protein relative to unstimulated control cells (analysis of variance; $F_{3,245} = 3.622$, $p = 0.0007$ (WT protein), 0.0041 (S185A), 0.008 (S185D), and 0.0074 (S185E) (Fig. 4B). Next, to confirm that PGI phosphorylation does not affect cytokine activity, we tested binding to HT1080 cell surfaces and found that all of the Ser185 mutant PGI proteins exhibited cell-surface saturable binding kinetics similar to those of the WT protein (Fig. 4C).

Structures of Cross-linked WT and Mutant PGI Proteins—Next, we questioned whether PGI phosphorylation leads to conformational changes, which might explain, in part, the observed reduction in enzymatic activity. PGI is a dimeric protein, and homodimerization is essential for its enzymatic activity. Thus, to study the behavior of the molecule in suspension, we tested the molecular size of the proteins in the presence or absence of the cross-linker DSP and examined whether these mutations affect the dimerization of PGI. Under reducing conditions and in the absence of the cross-linker DSP, the in vitro WT (Fig. 5A, lane 1–), S185A (lane 2–), S185D (lane 3–), and S185E (lane 4–) proteins comigrated upon SDS-PAGE as a single 55-kDa monomeric protein band. However, the presence of the cross-linker revealed that both the WT and S185A protein species exist as dimers in solution (Fig. 5A, lanes 1+ and 2+, respectively), whereas the S185D (lane 3+) and S185E (lane 4+) proteins formed both dimers and tetramers in solution. Phosphorylated PGI also formed a tetramer in the presence of the cross-linker, as did the S185D and S185E mutants (Fig. 5B, lane 2+), whereas non-phosphorylated PGI did not, even with the cross-linker (lane 1+). To examine the conformation of in vivo PGI mutants, we incubated the transfected cells with the cross-linker at 24 °C for 30 min and then analyzed them by Western blotting. As in the in vitro assay, an augmented band expected to be a tetramer of PGI was detected with the S185D and S185E proteins (Fig. 5C, lanes 5 and 6, respectively), but not with the WT and S185A proteins (lanes 3 and 4, respectively). This suggests that the introduced negative charge and conformational changes that mimic phosphorylation induce structural changes that allow the molecules to be in a monomer-dimer-tetramer equilibrium, reflecting, in part, the loss of enzymatic activity.

Structure of Mutant PGI and Effects of Ser185 Phosphorylation on the Active Site—To further analyze the possible effect of Ser185 phosphorylation on the structure of PGI, we utilized the program MODELLER for homology modeling calculations using the crystal structure of inhibitor-free PGI as a template (24). Human PGI is a dimer presented as a ribbon diagram with the two monomers in blue and red (Fig. 6A). Bound inhibitor molecules are shown in yellow to localize the enzyme active site, and the position of Ser185 is circled and is located on the reverse side of the active-site cleft (Fig. 6A). To further visualize the possible role of Ser185 phosphorylation, we constructed an energy-minimized model of human PGI to introduce phosphorylated Ser185 into substrate-free human PGI using the energy minimization function of the REFMAC program (Fig. 6, B and C, front view and 20° rotation for better visualization). No significant structural changes were observed for the active site of human PGI phosphorylated at Ser185. Because the side chain of Ser185 is exposed to the inner depression of the dimeric
molecule of PGI, it is possible that no structural hindrance of the surrounding residues is observed following phosphorylation. As shown in Fig. 6D, the phosphate group of Ser(P)185 could be accommodated between the side chains of Asp161 and Asn386 (Asn386 belonging to the other subunit). Although minor rearrangements of the side chain of Asp161 were observed, no significant structural changes were observed for the active-site residues.
DISCUSSION

In this study, we have shown that human PGI/AMF is a phosphoprotein that is phosphorylated at Ser\textsuperscript{185} by CK2 and that phosphorylation inhibits its enzymatic activity. In this respect, PGI is not unique, as it shares similarity with other phosphoglycolytic enzymes such as phosphofructokinase (26–28), glyceraldehyde-3-phosphate dehydrogenase (26, 29), phosphoglycerate mutase (26, 30), enolase (26, 31, 32), pyruvate kinase (33), and lactate dehydrogenase (31) in that phosphorylation regulates enzymatic activity. PGI is nearly ubiquitous in evolution; a cluster of 40 amino acid residues has remained conserved, whereas most amino acid residues outside the enzyme active site have changed (44). However, similar to the active site, the Ser consensus phosphorylation motif of PGI has also remained highly conserved during evolution (Fig. 6). Human PGI Ser\textsuperscript{185} corresponds to Ser\textsuperscript{182}, Ser\textsuperscript{193}, Ser\textsuperscript{189}, and Ser\textsuperscript{185} in bacteria, yeast, Drosophila, and rabbit, respectively (Fig. 7), implying that PGI phosphorylation is a conserved common process regulating its isomerase activity during glycolysis and glycogenesis. Molecular cloning and sequencing have characterized PGI as a leaderless protein that is secreted via the non-classical pathway and designated it as an AMF, which is involved in angiogenesis and metastasis (2–4); a T cell

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\caption{Cross-linked WT and mutant PGI/AMF proteins. A, the WT and mutant proteins formed a monomer without the cross-linker DSP \textit{in vitro}. After incubation with the cross-linker for 30 min, the WT and mutant proteins formed a dimer. In addition, S185D and S185E also formed a small amount of tetramer. Lanes 1— and 1+, WT protein without and with DSP, respectively; lanes 2— and 2+, S185A without and with DSP, respectively; lanes 3— and 3+, S185D without and with DSP, respectively; lanes 4— and 4+, S185E without and with DSP, respectively. B, recombinant human PGI was incubated without or with CK2 for 1 h and cross-linked. Phosphorylated PGI formed a tetramer. Lanes 1— and 1+, incubation without CK2 in the absence and presence of DSP, respectively; lanes 2— and 2+, incubation with CK2 in the absence and presence of DSP, respectively. C, mutant PGI gene-transfected cells were incubated with the cross-linker (1 mM DSP), and the cell lysates were analyzed by immunoblotting. S185D and S185E formed a tetramer \textit{in vivo}. Lane 1, parental HT1080 cells; lane 2, null vector-transfected HT1080 cells; lanes 3—6, WT, S185A, S185D, and S185E PGI gene-transfected HT1080 cells, respectively. The data shown are representative of three independent experiments with similar results.}
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\begin{figure}[h]
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\includegraphics[width=\textwidth]{Figure6.png}
\caption{Structures of WT, mutant, and Ser\textsuperscript{185}-phosphorylated PGI/AMF proteins. A, dimeric structure of PGI/AMF with erythrose 4-phosphate (E4P). There was no significant structural difference between the WT and mutant PGI/AMF proteins. Cytokine and enzyme active sites where erythrose 4-phosphate binds overlap each other (arrow). One subunit is colored blue, and another one is colored red. Erythrose 4-phosphate is colored yellow. Note that Ser\textsuperscript{185} is located on the reverse side of the active site. B and C, ribbon diagrams showing the positions of the active site (dashed circle) and Ser\textsuperscript{185} (ball-and-stick, green) of dimeric human PGI/AMF. Because the location of Ser\textsuperscript{185} is rather unclear from the view in the upper panels, the PGI/AMF molecule was rotated by 20° and is shown in the lower panels. B, human WT PGI/AMF (experimentally obtained crystal structure). C, human PGI/AMF phosphorylated at Ser\textsuperscript{185} (theoretically calculated energy-minimized model). Phosphorylated Ser\textsuperscript{185} is indicated by the thin arrow. There was no significant difference between the WT and Ser\textsuperscript{185}-phosphorylated PGI/AMF proteins. D, ball-and-stick models showing the phosphorylation site of human PGI/AMF. The oxygen and phosphorus atoms of Ser\textsuperscript{185} are shown in red and orange, respectively. There was no significant structural change in the active-site residues, although minor change was detected at Asp\textsuperscript{161}.}
\end{figure}
lymphokine, e.g. neureolukin, which supports the survival of spinal and sensory neurons (5, 6); a maturation factor, which differentiates myeloid leukemic cells to terminal monocyctic cells (7); sperm antigen-36 (8); and a myofibrill-bound serine proteinase inhibitor (9). In addition, extracellular PGI is an antigen in arthritis disease (12), and its presence in serum and urine is of prognostic value associated with cancer progression (13–15). This study has shown that PGI phosphorylation is not associated with its secretion. In addition, an energy-minimized model revealed that Ser185 phosphorylation of human PGI may not affect the topography of the active-site residues, suggesting that phosphorylation may interfere with isomerization, not with binding of the sugar substrates. This notion is strengthened by the fact that phosphorylation alters its homodimerization ability, similar to the serine phosphorylation of the tau protein (43), and that phosphorylation increases the stability of the individual protein helices that should relieve an unfavorable electrostatic interaction in the tetramer (45).

Although the exact mechanism for decreasing PGI enzymatic activity by Ser185 phosphorylation still remains unknown, the location of Ser185 may be an important clue for resolving this mechanism. It is noteworthy that Ser185 is located on the reverse side of the active-site cleft (like a lining). Therefore, its phosphorylation may cause subtle structural changes (which may not be detected by energy minimization) at the active-site cleft. Because enzymatic reaction strictly requires intact geometry of active-site residues, subtle structural changes caused by the phosphorylation might abolish the enzymatic activity of phosphorylated PGI. An electrostatic effect introduced by the negative charge of the phosphate group on the reverse side of the active-site cleft may also inhibit the enzymatic activity by 1) electrostatic repulsion between the negative charges of the phosphate groups of Ser185 and the substrate molecule and/or 2) affecting the pH, values of the catalytic residues in the active site. It is also possible that the negative charge modifications induced by phosphorylation are below the resolution of the crystallographic method used. The point mutation at Ser185 may induce a subtle structural change that could not be detected by homology modeling techniques or a change of electrical charges at the active site. Read et al. (29) examined the crystal structures of 28 PGI mutants associated with hemolytic anemia and classified them into three categories: those that alter the PGI structure, those that disrupt dimerization, and those that affect active sites. Phosphorylated PGI may thus be classified into the last category.

The results from this study suggest that phosphorylation of PGI may influence glucose metabolism. It is well known that malignant tumors show an enhancement of glycolysis (46). Transfection with src and ras oncogenes results in an increase in glucose transport and augmented transporter protein and mRNA levels (47). Hexokinase, the first enzyme of the glycolytic pathway, is increased in a rapidly growing hepatoma (48). PGI deficiency and/or reduced activity due to sequence mutations causes hemolytic anemia, and PGI deficiency affects the glucose usage and energy metabolism in a mouse model (49).

In conclusion, PGI belongs to the family of “moonlighting proteins” that exhibit multiple cellular and extracellular functions (50). The data presented here show for the first time that phosphorylation of PGI regulates its enzymatic activity and suggest that this post-translational modification contributes to its multiple functions. It remains to be resolved whether the observed decreased enzymatic activity of phosphorylated PGI results from changes in enzyme-substrate binding ($K_m$) or reaction rate ($k_{cat}$), and the mutant PGI proteins generated here should be useful in future analyses of how changes in glucose metabolism affect the pathology of the cell.

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Phosphorylation of PGI/AMF Regulates Its Enzymatic Activity

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