The Crystal Structure of Phosphoglucose Isomerase/Autocrine Motility Factor/Neuroleukin Complexed with Its Carbohydrate Phosphate Inhibitors Suggests Its Substrate/Receptor Recognition*

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Phosphoglucose isomerase catalyzes the reversible isomerization of glucose 6-phosphate to fructose 6-phosphate. In addition, phosphoglucose isomerase has been shown to have functions equivalent to neuroleukin, autocrine motility factor, and maturation factor. Here we present the crystal structures of phosphoglucose isomerase complexed with 5-phospho-D-arabinonate and N-bromoacetylthanolamine phosphate at 2.5- and 2.3-Å resolution, respectively. The inhibitors bind to a region within the domains' interface and interact with a histidine residue (His506) from the other subunit. We also demonstrated that the inhibitors not only affect the enzymatic activity of phosphoglucose isomerase, but can also inhibit the autocrine motility factor-induced cell motility of CT-26 mouse colon tumor cells. These results indicate that the substrate and the receptor binding sites of phosphoglucose isomerase and autocrine motility factor are located within close proximity to each other. Based on these two complex structures, together with biological and biochemical results, we propose a possible isomerization mechanism for phosphoglucose isomerase.

Phosphoglucose isomerase (PGI)1 (EC 5.3.1.9), a glycolytic enzyme, is an essential enzyme in all tissues. It interconverts glucose 6-phosphate and fructose 6-phosphate, hence plays a central role in both the glycolysis and the gluconeogenesis pathways. PGI deficiency in humans is an autosomal recessive genetic disorder that has the typical manifestation of non-spherocytic hemolytic anemia of variable clinical expression (1, 2). The serum activity of human PGI serves as a tumor marker in cancer patients (3, 4) and elevation in PGI activity is closely correlated with metastasis (5, 6). In addition to its essential role in carbohydrate metabolism in the cytoplasm, PGI also functions as neuroleukin (NLK) (7–9), autocrine motility factor (AMF) (10, 11), and maturation factor (MF) (12).

NLK is a neurotrophic growth factor that promotes the survival of spinal and sensory neurons (13). Interestingly, PGI and NLK have previously been reported to have differential induction capabilities for certain neurons (9). AMF, a new class of cytokines, can stimulate cell migration in vitro and metastasis in vivo (10, 14–16). Amino acid sequencing and immunological cross-reactivity experiments suggested that mouse AMF is identical or closely related to PGI/NLK (10). MF is capable of mediating the differentiation of human myeloid leukemic HL-60 cells to terminal monocytic cells, and apparently a high degree of homology exists between the MF of myeloid leukemia cells and PGI or NLK (12).

It was previously suggested that PGI may recognize a sugar-containing molecule(s) at the cell surface (7) and that NLK binds to the cell surface in a carbohydrate-dependent manner utilizing a PGI-like structure. Watanabe (10, 16) also pointed out that AMF (PGI/NLK/MF) may contain a PGI-like structure and initiates signal transduction by interacting with the carbohydrate side chains of the extracellular domain of the AMF receptor (AMFR). Carbohydrate phosphates can act as AMF inhibitors probably by binding and blocking the receptor recognition site on AMF. These novel functions of PGI may depend directly on the catabolism of phosphosugar, making an understanding of the molecular catalytic mechanism of the enzyme potentially significant.

We previously reported the substrate-free structure of PGI/AMF/NLK and confirmed that PGI can function as AMF and NLK (17). Nonetheless, the nature of the substrate-active site or the receptor binding site of PGI/AMF/NLK remains poorly understood. In this report, we tested the inhibitory effect of two carbohydrates containing phosphate, 5-phospho-D-arabinonate (SPA), and N-bromoacetylthanolamine phosphate (BAP), on the PGI enzymatic activity and the AMF-induced cell motility. 5PA, the five-carbon homologue of 6-phosphogluconate, is the equivalent analogue of the cis-1,2-enediolate intermediate in the reaction of phosphoglucose isomerase (18), and BAP is characterized as an active-site directed inhibitor to mammalian phosphoglucose isomerase in an effort to identify active-site residues (19). We then presented the crystal structures of PGI from Bacillus stearothermophilus complexed with 5PA and BAP at 2.5- and 2.3-Å resolution, respectively.

MATERIALS AND METHODS

Preparation of the Wild Type and N-Bromoacetylthanolamine Phosphate-Linked Phosphoglucose Isomerase—The isolation and purification of PGI have been previously reported (20). The complete inactivation of PGI was achieved by incubating the enzyme (0.6 mg/ml) at 30 °C overnight with 4 mM N-bromoacetylthanolamine phosphate (BrAcNH2OP) in a phosphate buffer (20 mM, pH 7.0). BrAcNH2OP was synthesized according to the protocol described by Hartman et al. (21).
Crystal Structure of PGI/AMF/NLK

TABLE I

Summary of diffraction and refinement data

|                  | PGI - BAP complex | PGI - 5PA complex |
|------------------|-------------------|-------------------|
| Data collection statistics |                  |                   |
| Resolution (Å)    | 2.3               | 2.5               |
| Number of reflections collected | 108,736 | 87,547 |
| Number of unique reflections | 30,710 | 21,464 |
| Redundancy of reflection | 3.57  | 4.08  |
| Data completeness (Å) (final shell) | 96.6 (98.0) | 99.4 |
| Rmerge (%) (final shell) | 7.5 (31.6) | 9.9 (61.5) |

Refinement statistics

|                  |                  |
|------------------|------------------|
| Resolution range (Å) | 8.0 – 2.3 |
| Number of used reflections (F > 2σ(F)) | 23,623 |
| Rmerge/Free | 17.2/24.2 |
| Protein atoms | 3,516 |
| Residues 200–205 (Å) | 0.46 |
| Solvent atoms | 134 |
| Overall isotropic B factor (Å²) | 19.1 |
| Side chain B factor (Å²) | 22.6 |
| Main chain B factor (Å²) | 32.4 |

Root mean square derivation from ideal geometry

| Bond lengths (Å) | 0.01 |
| Bond angles (degrees) | 1.2 |

Root mean square derivation superimposed with 2PGI (Ca)

|                  |                  |
|------------------|------------------|
| All (Å)          | 0.25 |
| Residues 140–145 (Å) | 0.26 |
| Residues 200–205 (Å) | 0.46 |

Results and Discussion

Cell Motility Assay—The cell motility assay of mouse CT-26 cells was measured by the method of Lin et al. (22) with minor modifications. Polyvinylpyrrolidone-free polycarbonate filters (Nuclepore, 8-µm pore size) were preincubated for 2 h before filling the bottom chamber with DMEM. Incubation was carried out at 37 °C for 16 h. The filters were removed and fixed in 4% paraformaldehyde for 15 min at room temperature. Cells on the upper filter surface were removed with a cotton swab. The filters were stained with hematoxycin for 40 min, and the cells on the lower filter surface were counted under a light microscope.

Enzymatic Assay—The phosphoglucoisomerase activity was determined at 30 °C by the coupled glucose-6-phosphate dehydrogenase method (23). The standard assay mixture (1 ml) contains 20 mM potassium phosphate buffer, pH 7.0, 10 units of glucose-6-phosphate dehydrogenase, 2 mM L-glutamate, 0.4 mM NAD+, 4 mM fructose-6-phosphate, and 0.5 µg of PGI or PGI covalently linked with BAP. To assess the inhibitory effect, 5-phosphoarabinonate was included in the assay mixture to a final concentration of 0.1 mM.

Crystallization and Data Collection—The PGI-BAP crystals were grown at room temperature by hanging-drop vapor diffusion of a 2-µl protein solution (15 mg/ml in 50 mM potassium phosphate buffer, pH 7.0) mixed with 2-µl reservoir solution containing 0.8 M potassium sodium tartrate in 0.1 M HEPES buffer (pH 7.5). The PGI-BAP crystals belong to space group I222 with cell dimensions of a = 74.94 Å, b = 93.64 Å, and c = 171.99 Å and a diﬀraction to a 2.3-Å resolution, with one molecule per asymmetric unit. For the PGI-5PA complex, crystals were also grown by the hanging-drop vapor diﬀusion method at room temperature from 2 µl of protein solution (15 mg/ml containing 0.05 mM 5PA in 50 mM phosphate, pH 7.0) mixed with 2 µl of reservoir solution containing 0.4 M ammonium dihydrogen phosphate. Crystals measuring 0.3 x 0.56 x 0.1 mm grew in about 4 days and had a diﬀraction resolution of 2.5 Å. The PGI-5PA crystal also belongs to space group I222 with cell dimensions of a = 74.80 Å, b = 94.71 Å, and c = 171.74 Å and contains one molecule per asymmetric unit.

All data sets were taken at room temperature on a Rigaku R-AXIS II imaging plate system using double-mirror-focused CuKα x-ray radiation generated from a Rigaku RU-300 rotating anode operating at 50 kV and 80 mA. Data were indexed, integrated, and scaled using DENZO and SCALEPACK software packages (24) (Table I).

Structure Determination and Refinement—The AMoRe program package (25) was used in the rotation and translation search. The phase structure (17) was used as the search model (Protein Data Bank (PDB) accession code: 2PGI). The search model was placed in a P1 cell with a = b = c = 90 Å and α = β = γ = 90°. Data between 8.0 and 4.5 Å and a Patterson radius of 20 Å were used for all rotation and translation function calculations. The data sets from PGI-5PA and PGI-BAP both gave significant solutions. After rigid body refinement, the optimal solution was determined for PGI-BAP data at Euler angle α = 71.32°, β = 76.29°, and γ = 305.05°; x = 0.482, y = 0.139, and z = 0.150. When compare with the non-normalized structure factors between search model and target crystal in the definition region, the correlation coefficient and R-factor are 84.0 and 23.5%, respectively.

RESULTS AND DISCUSSION

Cell Motility and Enzyme Inactivation—Previously, we demonstrated by cell migratory stimulation assay that PGI from B. stearothermophilus exhibits AMF cell motility activity (17). To directly address the question whether the isomerase and the cell motility functions of the protein share the same substrate binding site, we tested the effect of two carbohydrates containing phosphates, 5PA and BAP, on these functions. The PGI activities were monitored by the rate of isomerizing fructose-6-phosphate (28). In the presence of 0.1 mM 5PA, the isomerase activity of the enzyme was decreased by 86%. PGI that has been conjugated with BAP was essentially inactive. A similar result with the BAP-linked PGI has been reported by Gibson et al. (19).

Results from the motility assay and the stimulatory effect on CT-26 cells in the absence (Fig. 2A) or presence (Fig. 2B) of PGI are presented. In the presence of 5PA (C) and BAP (D), the inhibitory effect on cell motility can be readily observed. The current data indicate that 5PA and BAP not only inhibit PGI enzymatic activity but also constrain the induced cell motility of PGI with no effect on basal migration. The results confirm that the substrate and the receptor binding site of PGI/AMF are overlapped.

General Structure—The orthorhombic crystals of PGI-BAP and PGI-5PA are isomorphous to the substrate-free PGI (17) crystals. The folding topology of these two inhibitor complexes is structurally identical to the substrate-free form as shown in Fig. 3a. Briefly, the structure is composed of two globular domains (designated as the large and small domain) and an “arm-like” C-terminal tail. Each domain has a β-sheet core surrounded by α-helices that link the β-strands. The active site of substrate/inhibitor binding is located within the two globular domains, the C-terminal tail, and the interface between the two subunits. The large domain also contains a protruding loop...
region opposite to the C-terminal tail. The structure can be represented by a prolate ellipsoid with an "arm-like" structural feature on each side. The dimensions of the monomer are about 71 x 75 x 33 Å.

Even though there is one subunit in the crystallographic asymmetric unit, the active form of the enzyme is a dimer. The monomer-monomer associate in an arm-to-arm hug fashion with intimate contacts and form a hydrophilic channel that coincides with the crystallographic 2-fold axis, which runs through the dimer as indicated by the arrow in Fig. 3b. The solvent-accessible surface (29) of the subunit is 19505 Å^2. The intermolecular contact area is 10,751 Å^2, with a summation of 3192 and 7559 Å^2 of the hydrophilic and hydrophobic surface areas, respectively. Dimer formation causes a burial of 55% of the monomer surface area.

Fig. 4 shows the structural comparisons of substrate-free PGI with those of the PGI-5PA and PGI-BAP complexes. Both inhibitors gave similar, but not identical, results. Their binding sites lie in the slight cleft located within the large domain, the small domain, and the C-terminal tail of the monomer as predicted (Sun et al., 1999). The cleft is close to the subunit interface, which is formed by the association of the two subunits. Least-squares superposition (Ca) of the PGI-BAP and PGI5PA complexes with the substrate-free structure gave root mean square deviations of 0.25 and 0.46 Å, respectively. This small difference indicates that the overall conformations of these three structures are very similar, except for local conformational changes in the loop and substrate binding regions. We will discuss some important local structural variations later to shed light on the action of the inhibitor binding.

Inhibitor Binding Site of Transition State Analogue 5PA—5-Phosphoarabinonate (5PA) is a stable analogue of the cis-1,2-enediolate intermediate that is believed to occur transiently in the phosphoglucose isomerase reaction (18). 5PA is also one of the strongest known competitive inhibitors of PGI. Therefore, it possibly represents a transition state analogue and the 5PA-bound form of the protein may resemble a catalytic intermediate or a transition state.

**Motility Stimulation of CT-26 mouse colon cancer cell by PgiB**

| PgiB (fg/ml) | cell number | X-fold |
|-------------|-------------|--------|
| (A) 0       | 230         | 1      |
| (B) 1000    | 560         | 2.4    |
| (C) 1000    | 251         | 1.1    |
| (D) 1000    | 287         | 1.2    |
As shown in Fig. 5A, the specific binding of 5PA involves a network of polar interactions between 5PA and Ile\(^{80}\), Gly\(^{82}\), Gly\(^{201}\), Arg\(^{202}\), Glu\(^{285}\), His\(^{306}\), Gln\(^{413}\), and Lys\(^{420}\). The phosphate group on 5PA is essential for binding and is stabilized by interacting with residues Gly\(^{201}\), Arg\(^{202}\), Glu\(^{285}\), and Gln\(^{413}\). At the other end of 5PA, the O\(^{5}\) carboxylate oxygen of 5PA forms hydrogen bonds with the N\(^{31}\) atom of His\(^{306}\) and the O\(^{61}\) carboxylate oxygen of Glu\(^{285}\). The oxygens of the hydroxyl groups (O\(_2\) and O\(_4\)) on 5PA interact with His\(^{306}\), Lys\(^{420}\), Ile\(^{80}\), and Gly\(^{459}\) via hydrogen bonds. In addition, the backbone oxygen (O\(_3\)) is hydrogen-bonded with N\(^{42}\) of Glu\(^{281}\). It should be noted that one water molecule is H-bonded to the O\(_3\) oxygen atoms and that the interaction of 5PA and Thr\(^{143}\) is mediated via this water molecule.

In the PGI-5PA complex, a total of 16 hydrogen bonds, 7 from the large domain, 6 from the small domain, and 2 from the C-terminal region, are observed between the protein and the ligand. Residues participate in 5PA binding are shown in Table II and include charged, polar, and nonpolar side chains, as well as atoms from the peptide backbone. It is also interesting to point out that the His\(^{306}\) positioned in the binding site is contributed by the subunit of the dimer. This positioning may explain why the active form of the isomerase is a dimer.

**Inhibitor Binding Site of BAP**—Even though BAP may not represent a transition state analogue, it can modify PGI stoichiometrically, resulting in complete and rapid inactivation of the enzyme (19). In addition, data from mutagenesis (30) suggest that His\(^{306}\) is an active-site residue of PGI. The imidazole of His\(^{306}\) could be the nucleophile that attacks BrAcNHETOP. As shown in Fig. 1b, BAP formed a covalent bond with His\(^{306}\). Therefore, the complex structure of PGI-BAP can confirm that BAP is indeed an active site inhibitor and react with His\(^{306}\).

In contrast to the fairly deep binding location of 5PA (Fig. 5A), BAP is situated near the entrance of the binding pocket (Fig. 5B). The noteworthy difference between 5PA and BAP is the orientation of the phosphate moieties on these inhibitors. The phosphate group on 5PA is pointed toward the bottom of the pocket. Conversely, the phosphate moiety on BAP swings about 180° and orients in the opposite direction. As shown in Fig. 5B, the binding pocket of BAP is lined with Ser\(^{140}\), Thr\(^{142}\), Thr\(^{143}\), Glu\(^{145}\), His\(^{306}\), Glu\(^{417}\), and Lys\(^{420}\). The His\(^{306}\) residue was previously suggested to act as a general base to initiate the isomerization reaction (31). As expected, the PGI enzyme is alkylated by BAP specifically at position N\(^{41}\) of His\(^{306}\) contributed from the other subunit of the dimer. In addition to a water molecule, the phosphate moiety of BAP constitutes hydrogen bonds with the hydroxyl group of Ser\(^{140}\) and Thr\(^{143}\), whereas the side chain of Lys\(^{420}\) neutralizes the opposite charges and forms a salt bridge with the phosphate group.

**Inhibitor-induced Local Conformational Changes**—Achari et al. (32, 33) have proposed an occurrence of local conformational changes upon inhibitor binding. We compared the structures of the inhibitor-bound complexes with that of the uncomplexed PGI (17) and observed significant conformational changes in the active site of either PGI-5PA or PGI-BAP. The comparison of the PGI structures with and without 5PA bound is shown in Fig. 5A. The most significant difference appears at the region...
between residues Gly\textsuperscript{200} and Val\textsuperscript{205}. This variation may be due partially to the enhanced interaction between Arg\textsuperscript{202} and the phosphate group on 5PA. The quanidino group of Arg\textsuperscript{202} in the PGI-5PA complex structure moved 3–4 Å toward the inhibitor from its original position in the unbound protein.

Because the phosphate moiety of BAP points to the entrance of the binding pocket, a local conformational change upon BAP binding is found at the region between residues Lys\textsuperscript{139} and Thr\textsuperscript{144} as shown in Fig. 5A. Ser\textsuperscript{140} is the key residue responsible for this structural variation. Upon inhibitor binding, the side-chain hydroxyl group of Ser\textsuperscript{140} moves into proximity with BAP and forms two hydrogen bonds with an oxygen atom of the phosphate group and the backbone oxygen O\textsubscript{4}.

Proposed Catalytic Mechanism of Phosphoglucose Isomerase—Previous results suggest that the availability of the active site on PGI is required for stimulating the migration of tumor cells. Therefore, the understanding of the molecular catalytic mechanism of the enzyme should be physiologically significant.

It has long been proposed that the catalytic mechanism of phosphoglucose isomerase should include the following steps: 1) binding of the cyclic form of the substrate to the enzyme, 2) ring opening of the substrate, 3) base-catalyzed isomerization via a cis-enediol intermediate, 4) ring closure of the product, and 5) release of product (31, 34–35). Isotope effect studies suggest that the isomerization step is rate-limiting (34). The transition state should have a structure similar to cis-enediolate, because both 5PA (18) and 5-phosphoarabinohydroxamate (36) behave as transition state inhibitors. The isomerization activity is pH-dependent and follows a bell-shaped curve and, together with the temperature dependence of the pK\textsubscript{a} values, suggests that histidine and lysine residues participate in the catalysis (31). Mutagenic studies indicate that one of the conserved lysine (Lys\textsuperscript{420} in PGI) and arginine (Arg\textsuperscript{202} in PGI) residues play indispensable roles in catalysis (30). Affinity labeling of the conserved histidine (His\textsuperscript{306} in PGI) by BrAcNHEtOP, an active site-directed inhibitor of phosphoglucose isomerase, suggests that the conserved histidine functions as a general base in the isomerization step (30).

Considering all of the above data from the literature in combination with the active-site structure of phosphoglucose isomerase unveiled in the current study (Fig. 5), we propose the following molecular catalytic mechanism for PGI: (i) The reaction is initiated by binding the substrate to the enzyme. A network of hydrogen bonds between the substrate and the active site amino acids stabilizes this binding. It is worth noting that the amino acids comprising the active site, especially...
those directly contacting the substrate, are conserved throughout the PGI family (17). (ii) Lys420 is involved in the opening of the phosphoglucopyranose ring by functioning as a general base. The side-chain amino group of Lys420 is within 3.0 Å of the C1 and C2 oxygen atoms of the transition state isomerization presumably has a structure similar to the cis-enediol intermediate and inaugurates the isomerization step. By reversing their roles in the subsequent step, the carboxylate of Glu285 could be lowered and the acidic role of Glu285 in isomerization suggested for the active-site Lys in the present study. A conserved Glu (Glu285 in this study) is thought to be the residue that donates a proton to the substrate in the initial isomerization step (Fig. 6). The proposed role of Glu285 is supported by the following reasons: 1) the closeness of the O1 carboxylate oxygen of 5PA and the Oβ1 carboxylate oxygen of Glu285; 2) the indispensable role of Glu285 in catalysis suggested by affinity labeling by 1,2-anhydrohexitol 6-phosphate (38). No glutamate was assigned in the proposed mechanism of rPGI. In our catalytic model, all three residues (His306, Glu285, and Lys420) play significant roles in the isomerization step; mutation of any one of them would drastically impair the catalytic ability of PGI. The function of Arg272 in rPGI (corresponding to Arg202 in this study) was proposed to make the overall electrostatic potential of Arg272 in rPGI. In our catalytic model, all three residues (His306, Glu285, and Lys420) play significant roles in the isomerization step; mutation of any one of them would drastically impair the catalytic ability of PGI. The function of Arg272 in rPGI (corresponding to Arg202 in this study) was proposed to make the overall electrostatic potential of Arg272 in rPGI. In our catalytic model, all three residues (His306, Glu285, and Lys420) play significant roles in the isomerization step; mutation of any one of them would drastically impair the catalytic ability of PGI. The function of Arg272 in rPGI (corresponding to Arg202 in this study) was proposed to make the overall electrostatic potential of Arg272 in rPGI. In our catalytic model, all three residues (His306, Glu285, and Lys420) play significant roles in the isomerization step; mutation of any one of them would drastically impair the catalytic ability of PGI. The function of Arg272 in rPGI (corresponding to Arg202 in this study) was proposed to make the overall electrostatic potential of Arg272 in rPGI. In our catalytic model, all three residues (His306, Glu285, and Lys420) play significant roles in the isomerization step; mutation of any one of them would drastically impair the catalytic ability of PGI. The function of Arg272 in rPGI (corresponding to Arg202 in this study) was proposed to make the overall electrostatic potential of Arg272 in rPGI. In our catalytic model, all three residues (His306, Glu285, and Lys420) play significant roles in the isomerization step; mutation of any one of them would drastically impair the catalytic ability of PGI.

The crystal structure of rabbit skeletal muscle PGI (rPGI) with a competitive inhibitor d-glucose 6-phosphate was published by Jeffery et al. (37) during the preparation of the present manuscript. The topology of rPGI is very similar to that of PGI from B. stearothermophilus, but the orientation of the bound d-glucose 6-phosphate is different from that of 5PA in the present study. Consequently, the catalytic mechanism proposed by Jeffery et al. (37) has features that differ from that proposed in this study. In rPGI, a His306-Glu216 dyad and Lys518 (corresponding to His306, Glu145, and Lys420 of the B. stearothermophilus PGI) were proposed as the general base-acid pair to initiate the isomerization step. The function of the

### Table II

| Protein residue | Distance |
|-----------------|----------|
| BAP             |          |
| O2              | N’ (Lys420) 3.0 |
| O4              | N’ (Lys420) 3.2 |
| O1P             | O’ (Ser440) 3.5 |
| O2P             | O’ (Ser440) 3.3 |
| O3P             | O’ (Thr343) 3.5 |
| O3              | O’ (Thr9) 3.0 |
| O3P             | O’ (Glu417) 3.5 |
| O3P             | N’ (Lys420) 2.8 |
| O3P             | Water 2.2 |
| 5PA             |          |
| O1A             | O31 (Glu285) 3.5 |
| O1              | N2 (His306) 3.1 |
| O2              | N’ (Lys420) 2.8 |
| O2              | N’ (His306) 3.5 |
| O3              | Water 2.7 |
| O4              | O (His96) 2.9 |
| O4              | N (Gly262) 2.8 |
| O5              | N2 (Glu285) 3.5 |
| O1P             | N (Glu285) 3.4 |
| O1P             | N (Arg202) 2.5 |
| O1P             | O2 (Glu285) 3.2 |
| O2P             | N2 (Arg202) 3.5 |
| O2P             | O3 (Glu285) 3.0 |
| O2P             | O2 (Glu285) 2.8 |
| O3P             | N2 (Glu285) 3.1 |
| O3P             | N (Gly262) 3.2 |

* a His306 belongs to the other subunit in a dimer.

**FIG. 6.** A schematic drawing of the proposed mechanism for the conversion of glucose 6-phosphate to fructose 6-phosphate as catalyzed by phosphoglucone isomerase. The mechanism includes the ring opening (a and b), isomerization (b through e), and ring closure steps (e and f). The putative residues involved in isomerization are shown. TS stands for the cis-enediol intermediate transition state.
that Arg202 also plays a key role for the recognition of phosphosugar during isomerization. It should be noted that our crystal structure does not rule out the possibility of Lys420 being the role of a general acid during isomerization as suggested by the previous rPGI study. The actual function of each active-site residue may be defined in more precise fashion in the future after more biochemical data are available.

In summary, we have elucidated the crystal structures of PGI complexed with the transition state analogue 5-phospho-D-arabinonate (5PA) and N-bromoacetylethanolamine phosphate (BAP) at 2.5- and 2.3-Å resolution, respectively. We also demonstrate that the inhibitors, 5PA and BAP, not only inhibit the enzymatic activity of PGI but also inhibit the AMF-induced cell motility of CT-26 mouse colon tumor cells. Thereby, the present study provides the first view that the locations of the substrate binding site for phosphoglucose isomerase and the receptor binding site for autocrine motility factor are overlapped. We also propose a possible isomerization mechanism for PGI.

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