Crystal Structures of an ATP-dependent Hexokinase with Broad Substrate Specificity from the Hyperthermophilic Archaeon *Sulfolobus tokodaii*  

Received for publication, November 17, 2006, and in revised form, December 27, 2006

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Hexokinase catalyzes the phosphorylation of glucose to glucose 6-phosphate by using ATP as a phosphoryl donor. Recently, we identified and characterized an ATP-dependent hexokinase (*SthHK*) from the hyperthermophilic archaeon *Sulfolobus tokodaii*, which can phosphorylate a broad range of sugar substrates, including glucose, mannose, glucosamine, and N-acetylglucosamine. Here we present the crystal structures of *SthHK* in four different forms: (i) apo-form, (ii) binary complex with glucose, (iii) binary complex with ADP, and (iv) quaternary complex with xylose, Mg$^{2+}$, and ADP. Forms i and iii are in the open state, and forms ii and iv are in the closed state, indicating that sugar binding induces a large conformational change, whereas ADP binding does not. The four different crystal structures of the same enzyme provide "snapshots" of the conformational changes during the catalytic cycle. *SthHK* exhibits a core fold characteristic of the hexokinase family, but the structures of several loop regions responsible for substrate binding are significantly different from those of other known hexokinase family members. Structural comparison of *SthHK* with human N-acetylglucosaminidase and other hexokinases provides an explanation for the ability of *SthHK* to phosphorylate both glucose and N-acetylglucosamine. A Mg$^{2+}$ ion and coordinating water molecules are well defined in the electron density of the quaternary complex structure. This structure represents the first direct visualization of the binding mode for magnesium to hexokinase and thus allows for a better understanding of the catalytic mechanism proposed for the entire hexokinase family. Phosphorylation of glucose to glucose 6-phosphate is important for both energy metabolism and biosynthesis in the cell. In eukaryotes, the reaction is catalyzed by hexokinases (EC 2.7.1.1), which can phosphorylate several hexoses, including mannose and fructose, in addition to glucose (1). In contrast, bacteria possess glucokinases (EC 2.7.1.2) that are specific for glucose. Bacterial glucokinases can be classified into two groups as follows: (i) glucokinases belonging to the repressors/open reading frames of unknown function/sugar kinases (ROK) family, which is characterized by two signature motifs (2), and (ii) glucokinases without the ROK motifs (3). On the other hand, most Archaea use two types of glucokinase as follows: (i) ADP-dependent glucokinases (4–9), or (ii) ATP-dependent glucokinases belonging to the ROK family (10, 11).

On the basis of amino acid sequence similarity, sugar kinases can be divided into three families as follows: (i) hexokinase family, (ii) galactokinase family, and (iii) ribokinase family (12). Hexokinases and glucokinases belong to the hexokinase family, with the exception of the archael ADP-dependent glucokinases (13–15), which are members of the ribokinase family. The crystal structures of several members of the hexokinase family have been reported, including human hexokinase (16–20), rat and *Schistosoma mansoni* hexokinase (21), yeast hexokinase (22–25), *Escherichia coli* glucokinase (26), *Arthrobacter* sp. strain KM glucomannokinase (27), and *E. coli* rhamnulose kinase (28). These molecules possess an identical core structure consisting of two domains with the ββββαβαβα fold and the active site located between the two domains. Two β-turns formed by the conserved DJXGTT and GTG motifs are involved in ATP binding. The hexokinase family is included in the ASKHA (acetate and sugar kinases/Hsp70/actin) superfamily (29, 30), the members of which have a common core fold and catalyze phosphoryl transfer or hydrolysis of ATP.

In the past, ATP-dependent glucose phosphorylating activity had been detected in cell extracts of the hyperthermophilic archaeon *Sulfolobus solfataricus* (31), but no homologs of known hexokinases and glucokinases have been found in the genomes of the *Sulfolobus* species (32–34). Recently, we purified the ATP-dependent glucose phosphorylating activity from cell extracts of *Sulfolobus tokodaii* and identified the gene responsible for the activity (35). Our kinetic analyses indicated that *S. tokodaii* hexokinase (SthHK) is a novel hexokinase that can phosphorylate not only glucose but also GlcNAc, glucosamine, and mannose. The enzyme differs from other known hexokinases and glucokinases in that its activity is strongly inhibited by ADP. SthHK shows ~25% amino acid sequence identity with mammalian GlcNAc kinases (supplemental Fig. S1). How
ever, StHK is distinct in its broad substrate specificity from the GlcNAc kinases (36, 37), which are specific for GlcNAc. Although mammalian GlcNAc kinases show no significant sequence similarity with hexokinases, they possess the two ATP-binding motifs (EGGGT and GTG) characteristic of the hexokinase family and are therefore classified into this family (38). Very recently, the crystal structures of human GlcNAc kinase in complex with GlcNAc and with glucose and ADP have been reported (39). The structures reveal that human GlcNAc kinase has a similar core fold to the hexokinase family. StHK also contains the DAGGT and GTG motifs at the amino-terminal and middle regions of the sequence, respectively (supplementary Fig. S1), indicating that StHK is a new member of the hexokinase family with unique substrate specificity.

Here we report the crystal structures of StHK in four different forms as follows: (i) the apo-form; (ii) complex with glucose; (iii) complex with ADP; and (iv) complex with xylose, Mg$^{2+}$, and ADP. These structures provide a molecular basis for the substrate specificity, conformational changes upon substrate binding, and catalytic mechanism of this enzyme.

**EXPERIMENTAL PROCEDURES**

Sample Preparation and Crystallization—StHK was expressed in *E. coli* and purified to homogeneity as described (35). The selenomethionine-substituted protein was expressed in *E. coli* B834 (DE3). The cells were grown at 37 °C for 20 hours in 1 liter of SeMet core medium (Wako) supplemented with 10 g of glucose, 0.25 g of MgSO$_4$$\cdot$7H$_2$O, 4 mg of FeSO$_4$$\cdot$7H$_2$O, 10 ml of vitamin growth supplement (Sigma), 0.1 g of ampicillin, and 25 mg of seleno-$\beta$-methionine (Wako). The protein was purified using the same protocol as for the native protein. The native and selenomethionine-substituted proteins were concentrated to 15 mg/ml in 10 mM Tris-HCl (pH 8.0) and used for crystallization.

Crystallization was performed at 25 °C using the hanging drop vapor diffusion method. Crystals of the apo-form were grown by mixing 2 μl of the protein solution and 1 μl of the reservoir solution consisting of 0.1 M HEPES-NaOH (pH 7.5) and 1.3 M Li$_2$SO$_4$. Crystals of the ADP complex were grown by mixing 2 μl of the protein solution containing 5 mM ADP and 10 mM MgCl$_2$, and 1 μl of the reservoir solution consisting of 0.1 M HEPES-NaOH (pH 7.7) and 1.3 M Li$_2$SO$_4$. Crystals of the glucose complex were grown by mixing 2 μl of the protein solution containing 10 mM glucose and 0.2 mM MgCl$_2$, and 1 μl of the reservoir solution consisting of 0.1 M Tris-HCl (pH 8.6) and 34% PEG3350. Crystals of the xylose-Mg$^{2+}$-ADP complex were grown by mixing 2 μl of the protein solution containing 10 mM xylose, 20 mM MgCl$_2$, and 10 mM ATP, and 1 μl of the reservoir solution consisting of 0.1 M Tris-HCl (pH 9.0) and 18% PEG3350. The selenomethionine-substituted protein was crystallized under conditions similar to those used for the native crystals of the glucose complex. All crystals grew to full size within 1 week.

Data Collection—X-ray diffraction data were collected using a charged-coupled device (CCD) camera at the BL-5 and BL-6A station at the Photon Factory, and the NW12 station at the Photon Factory AR, High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. Crystals of the apo-form were cryoprotected in the reservoir solution supplemented with 20% trehalose. Crystals of the ADP complex were cryoprotected in the reservoir solution supplemented with 5 mM ADP, 10 mM MgCl$_2$, and 20% trehalose. Crystals of the xylose-Mg$^{2+}$-ADP complex were cryoprotected in the reservoir solution supplemented with 10 mM xylose, 20 mM MgCl$_2$, 10 mM ATP, and 15% PEG400. All crystals were flash-frozen at 100 K in a stream of liquid nitrogen. Data were processed using the HKL2000 (40).

Structure Determination and Refinement—Initially, the structure of the glucose complex was determined by multi-wavelength anomalous diffraction using a selenomethionine-substituted crystal. All eight selenium atoms were located, and initial phases were calculated using SOLVE/RESOLVE (41), followed by automated model building using ARP/wARP (42). The resultant model was refined against the native data set of the glucose complex. Manual model building and refinement were performed using XetalView (43) and CNS (44), respectively. The structure of the glucose complex was refined at 1.65 Å to an R factor = 18.8 (R$_{free}$ = 21.8). The crystal belongs to space group C222$_1$ with one molecule in the asymmetric unit. The final model contains residues 1–299, 1 glucose molecule, and 375 water molecules.

The structure of the apo-form was determined at 1.9 Å resolution by molecular replacement using MOLREP (45) in CCP4 (46) with the small and large domains of the glucose complex structure as search models, and refined to an R factor = 17.5 (R$_{free}$ = 21.0). The crystal belongs to space group P2$_1$ with two molecules in the asymmetric unit. The final model contains molecules A (residues 1–298) and B (residues 1–297) as well as three sulfate ions, one HEPES molecule, and 573 water molecules. The two sulfate ions are bound at the dimer interface, whereas the other sulfate ion and the HEPES molecule are located far from the active site allowing them to participate in crystal packing interactions. As the two molecules are essentially identical (r.m.s.d. for Cα atoms = 0.44 Å), molecule A is described as the apo structure.

The structure of the ADP complex was determined at 2.0 Å resolution by molecular replacement and refined to an R factor = 18.3 (R$_{free}$ = 22.6). The crystal belongs to space group P2$_1$ with two molecules in the asymmetric unit. The final model contains molecules A (residues 1–298) and B (residues 1–297) as well as two ADP molecules, three sulfate ions, one HEPES molecule, and 482 water molecules. As the two molecules are essentially identical (r.m.s.d. for Cα atoms = 0.38 Å), molecule A is described as the ADP complex structure.

The structure of the xylose-Mg$^{2+}$-ADP complex was determined at 2.0 Å resolution by molecular replacement and refined to an R factor = 21.3 (R$_{free}$ = 25.5). The crystal belongs to space group C2 with two molecules in the asymmetric unit. The final model contains molecule A (residues 1–297) and B (residues 1–296) in addition to one xylose molecule, one Mg$^{2+}$ ion, two ADP molecules, and 261 water molecules. Two ADP molecules are included in the model, because no electron density was observed for the γ-phosphate of ATP, probably because of hydrolysis of ATP during crystallization, although the protein was crystallized in the presence of ATP. Molecule A adopts a closed conformation with bound xylose, Mg$^{2+}$, and ADP, whereas molecule B adopts an open conformation with
bound ADP and is essentially identical to the ADP complex structure (r.m.s.d. for C-α atoms = 0.66 Å). Molecule A is described as the xylose-Mg\(^{2+}\)·ADP complex structure.

Data collection and refinement statistics are shown in Table 1. Figures were prepared using PyMol (47).

### RESULTS AND DISCUSSION

**Apo-form**—The structure of StHK (299 amino acid residues) in the apo-form was determined at 1.9 Å resolution. The structure consists of two domains, each of which has the βββααβαβα fold characteristic of the ASKHA superfamily (Fig. 1A). The small domain (residues 1–107 and 274–299) is composed of a five-stranded mixed β-sheet (β3, β2, β1, β4, and β5), with strand β2 antiparallel to the rest, flanked on one side by a pair of α-helices (α2 and α3) and on the other by helix α11. The large domain (residues 108–273) is composed of a five-stranded mixed β-sheet (β8, β7, β6, β9, and β10), with strand β7 antiparallel to the rest, six α-helices (α5 to α10), and a 3\(^\text{10}\)-helix. The β-sheet is flanked on one side by helix α4 and on the other side by a pair of α-helices (α9 and α10). The two domains are connected by helix α4. An intramolecular disulfide bond is formed by the conserved cysteine residues Cys-21 and Cys-291.

A structural similarity search using Dali (48) indicated that StHK shows relatively high degrees of similarity to the hexokinase family members, including *E. coli* glucokinase (26) (Protein Data Bank code 1Q18; Z score = 15.9, and root mean square deviation (r.m.s.d.) for 237 C-α atoms = 3.6 Å), *Arthrobacter* sp. strain KM glucomannokinase (27) (Protein Data Bank code 1WOQ; Z score = 15.0, and r.m.s.d. for 205 C-α atoms = 3.2 Å), the carboxyl-terminal catalytic domain (residues 525–917) of human hexokinase I (18) (Protein Data Bank code 1QHA; Z score = 15.9, and r.m.s.d. for 241 C-α atoms = 3.4 Å), and yeast hexokinase PII (23) (Protein Data Bank code 2YHX; Z score = 13.3, and r.m.s.d. for 228 C-α atoms = 3.7 Å). StHK also shows modest structural similar-
Crystal Structures of S. tokodaii Hexokinase

**Glucose Complex**—StHK was crystallized in the presence of glucose, and the glucose complex structure was determined at 1.65 Å resolution. Clear electron density for glucose is observed at the pocket formed by four loops, the β3-α1 and β4-α3 loops of the small domain and the β5-α4 and β8-α5 loops of the large domain (Fig. 2A). As observed in *E. coli* glucokinase (26), glucose is bound in the β-anomeric configuration. The hydroxyl groups of glucose form direct hydrogen bonds with Gly-69, Asp-71, His-94, Asp-95, Gly-135, and Asp-140 and water-mediated hydrogen bonds with Gly-11, Asn-35, and Gly-117. In addition, the 2-hydroxyl group is hydrogen-bonded with Tyr-189 of the adjacent subunit. The extensive hydrogen bonding interactions between the enzyme and glucose can explain the high affinity of the enzyme for glucose (*K_m* = 0.050 mM) (35). These residues involved in glucose binding are completely conserved in the homologs from *Sulfolobus* species (supplemental Fig. S1).

**ADP Complex**—The enzyme was crystallized in the presence of Mg²⁺ and ADP, and the structure was determined at 2.0 Å resolution. As no electron density was observed for a Mg²⁺ ion in this form, we call it the “ADP complex” structure. Clear electron density for ADP is observed in the cleft of the large domain formed by helices α5, α8, and 3_10 and a turn between β6 and β7 (Fig. 2B). The ribose moiety adopts the C2'-endo pucker, and the adenine ring is in the anti-conformation. The adenine ring is sandwiched by the side chains of Lys-203 and Arg-251, with the N-6 atom interacting with the carboxyl group of Asp-206 through a hydrogen bond. The 2'-hydroxyl group of the ribose forms a hydrogen bond with the backbone carbonyl of Ala-199. The 3'-hydroxyl group of the ribose forms a water-mediated hydrogen bond with the backbone carbonyl of Asp-196. In addition, the ribose ring makes van der Waals interactions with the side chains of Ala-144, Ala-202, and Met-249.

StHK exists as a dimer in solution (35). Consistent with this observation, the two molecules related by the crystallographic 2-fold symmetry form a dimer in the glucose complex structure, whereas two molecules in the asymmetric unit form a similar dimer in the other crystal forms (Fig. 1B). The two subunits are tightly connected by hydrogen bonds, hydrophobic interactions, salt bridges, and a disulfide bond. Two α-helices (α5 and α7) and two loop regions (β4-α3 and β8-α5) mainly contribute to dimerization. Trp-146, Arg-149, Lys-150, and Arg-153 on helix α5 form a hydrogen bonding network through water molecules with the counterparts of the adjacent subunit. Asp-141 on the β8-α5 loop forms salt bridges with Arg-153 and Lys-157 on helix α5 of the adjacent subunit. Cys-193 forms an intermolecular disulfide bond with the counterpart of the adjacent subunit. In the apo-form and the ADP complex, two sulfate ions derived from the crystallization buffer are bound at the dimer interface instead of water molecules to allow interactions with the protein.

**FIGURE 1. Overall structures of StHK.** A, stereo view of the monomer structure in the apo-form. The small and large domains are colored cyan and pink, respectively. The β1-β2 and β6-β7 turns are colored dark blue. The disulfide bond between Cys-21 and Cys-291 is shown in stick representation. B, stereo view of the dimer structure in complex with glucose. The bound glucose is shown in stick representation with carbon atoms in yellow. The region involved in dimer formation is colored green. The adjacent subunit is colored gray.
water molecules coordinating the Mg^{2+} ion, are well defined in the electron density map (Fig. 2C). As with other kinases, Mg^{2+} is required for the enzyme activity of StHK (35). In the complex structure, the Mg^{2+} ion is octahedrally coordinated by a β-phosphoryl oxygen of ADP and five water molecules. Asp-8, Lys-15, and Asp-95 form hydrogen bonds with the water molecules coordinating the Mg^{2+} ion. One of the five water molecules coordinating the Mg^{2+} ion does not interact with the protein but forms a hydrogen bond with the α-phosphate of ADP.

As no crystal structure bound to Mg^{2+} has been reported for the hexokinase family, this is the first report of visualization of the binding mode between the enzyme and Mg^{2+} in this family. The binding mode observed here is consistent with that proposed previously for other family members. In human hexokinase I, electron paramagnetic resonance (50) and modeling (17) suggested that Mg^{2+} interacts with Asp-532, Arg-539, and Asp-657, which are equivalent to Asp-8, Lys-15, and Asp-95 of StHK, through water molecules. Furthermore, the importance of these residues for activity has been confirmed by mutagenesis (51–53).

In the complex structure, the hydroxyl groups of xylose are recognized by the enzyme in a manner similar to that in the glucose complex, except for the absence of the hydrogen bond between the enzyme and the 6-hydroxyl group of glucose. The binding mode between the enzyme and the AMP portion of ADP is also similar to that in the ADP complex. In contrast, the binding mode between the enzyme and the β-phosphate in the xylose-Mg^{2+}-ADP complex is remarkably different from that in the ADP complex. The β-phosphate interacts with the backbone amides of Gly-11, Thr-12, and Lys-13 in the β1-β2 turn in addition to the backbone amide and the hydroxyl group of Thr-116 in the β6-β7 turn. The additional interactions result from the closed conformation of the xylose-Mg^{2+}-ADP complex.
Crystal Structures of S. tokodaii Hexokinase

FIGURE 3. Stereo view of the superposition of the apo-form (gray), the glucose complex (green), and the xylose-Mg$^{2+}$-ADP complex (magenta) based on the large domains. Ligands are shown in stick representation. The Mg$^{2+}$ ion is shown as a magenta sphere. Water molecules involved in the hydrogen bonding interactions between the small and large domains of the glucose complex are shown as green spheres. The β1-β2 turn is indicated by blue arrows. The ADP complex structure is not shown because it is essentially identical to the apo structure.

The two β-turns involved in ATP binding are a hallmark of the ASKHA superfamily (29, 30).

Conformational Change upon Substrate Binding—Comparison of the crystal structures of STHK in four different states revealed the conformational changes in the enzyme upon binding of substrates during catalysis (Fig. 3). Superposition of the apo and the glucose complex structures indicated a large conformational change from the open to closed state, accompanied by ~25° rotation of the small domain relative to the large domain (r.m.s.d. for C-α atoms = 3.0 Å) (Fig. 3). The conformational change upon glucose binding is supported by our observation that the apo crystals dissolved immediately when soaked in a glucose-containing solution. In the glucose complex structure, the water-mediated hydrogen bonding network between helices α1 and α8 and between helix α1 and the β8-α5 loop, in addition to the interactions through bound glucose, contributes to stabilization of the closed state (Fig. 3). Notably, the side chains of His-94 and Tyr-189 rotate to form hydrogen bonds with the hydroxyl groups of glucose. The conformational change upon sugar binding, exemplified by yeast hexokinase (54), is a common feature of the hexokinase family. Previous studies have shown that two domains generally rotate by ~10° around the hinge region upon sugar binding in the hexokinase family (28). In contrast, the crystal structures of human GlcNAc kinase have demonstrated a 26° rotation of the small domain relative to the large domain upon GlcNAc binding (39), which is comparable with that observed in STHK. These observations suggest that the sugar kinases phosphorylating GlcNAc generally undergo a larger conformational change than the other members of the hexokinase family.

The xylose-Mg$^{2+}$-ADP complex structure can be superimposed with the glucose complex structure with r.m.s.d. for C-α atoms of 0.79 Å (Fig. 3). However, local structural differences are observed between them. In the xylose-Mg$^{2+}$-ADP complex, the β1-β2 turn moves slightly away from the β6-β7 turn to accommodate the phosphoryl groups of ADP. The C-α atom of Thr-12 in the β1-β2 turn shifts by 1.7 Å from its position in the glucose complex. In addition, the side chain of Lys-15, which forms a salt bridge with Glu-278 in the glucose complex, is redirected toward ADP to interact with the α-phosphoryl group. The glucose complex crystals cracked and dissolved when soaked in an ADP-containing solution, supporting the notion that additional conformational changes would occur upon ADP binding from the closed conformation stabilized by glucose binding.

The apo and ADP complex structures are essentially identical (r.m.s.d. for C-α atoms = 0.17 Å), indicating that ADP can bind to the apoenzyme without causing conformational changes in the enzyme. This was supported by our observation that the apo crystals were stable when soaked in an ADP-containing solution and its remarkable inhibition by ADP, in marked contrast to other members of the hexokinase family. No crystal structure bound to only ADP has been reported among other members of the hexokinase family, although a number of crystal structures of these enzymes bound only to glucose or to both glucose and ADP have been solved. For example, it has been reported that the co-crystal structure of E. coli glucokinase with ADP could not be obtained in the presence or absence of glucose (26), suggesting that the enzyme in the open conformation may have low affinity for ADP. In the E. coli enzyme, conformational changes from the open to closed state have been suggested to be a prerequisite for ATP binding, and the activity of the enzyme has not been reported to be inhibited by ADP.

Substrate Specificity—In the ASKHA superfamily, substrate specificity of each member is thought to be mediated by diverged insertions into the conserved core fold (30). The crystal structures of STHK represent the first example of a sugar kinase that can phosphorylate both glucose and GlcNAc efficiently, and thus provide new insights into substrate recognition.

Comparison of STHK with human GlcNAc kinase explains why STHK can phosphorylate not only glucose but also GlcNAc. As shown in Fig. 4A, in the structure of human GlcNAc kinase in complex with GlcNAc, the carbonyl oxygen of the N-acetyl moiety of GlcNAc forms a hydrogen bond with N-δ of Asn-36, and its methyl group is recognized by a hydrophobic pocket formed by Trp-38 and Trp-146, and Leu-201 and Tyr-205 from the adjacent subunit (39). Asn-35, His-37, Arg-134, Val-185, and Tyr-189 of STHK correspond to Asn-36, Trp-38, Trp-146, Leu-201, and Tyr-205 of human GlcNAc kinase, respectively, suggesting that in STHK these residues are involved in binding the N-acetyl moiety of GlcNAc. However, modeling of GlcNAc into the active site of the glucose complex of STHK, simply based on the position of glucose, indicates that there would be...
Steric hindrance between the N-acetyl moiety and the side chains of Asp-71 and Tyr-189. The side chain of Tyr-189 may rotate to form a hydrophobic interaction with the GlcNAc methyl group. Superposition of the glucose complex of StHK and the GlcNAc complex of human GlcNAc kinase, based on the large domains, indicates that StHK adopts a more closed conformation relative to human GlcNAc kinase (Fig. 4B). This suggests that StHK may adopt a more open conformation when it accommodates GlcNAc. StHK was also crystallized in the presence of GlcNAc using a crystallization buffer similar to that used for the glucose complex, but the crystals did not diffract to high resolution. This result supports the assumption that the GlcNAc complex of StHK would adopt a similar but not identical conformation to that of the glucose complex.

The structure of human GlcNAc kinase in complex with GlcNAc revealed that the hydroxyl groups of GlcNAc form hydrogen bonds mainly with the large domain but only one direct hydrogen bond with the small domain, and the interactions between the N-acetyl moiety of GlcNAc and the side chains of Asn-36 and Trp-38 located on the small domain of the enzyme are a prerequisite for domain closure (39). In addition, the structure of human GlcNAc kinase in complex with glucose and ADP indicated that because glucose binding cannot stabilize the closed conformation of the enzyme, human GlcNAc kinase exhibits 20,000-fold lower catalytic efficiency for glucose than for GlcNAc (39). In contrast, in the glucose complex of StHK, there are seven direct hydrogen bonding interactions between bound glucose and the small domain of the enzyme. Notably, Asp-71 and His-94 of the small domain form multiple hydrogen bonds with the hydroxyl groups of glucose. Therefore, the extensive hydrogen bonds between glucose and the small domain of StHK to stabilize the closed conformation are likely responsible for the ability of the enzyme to phosphorylate glucose as well as GlcNAc. On the other hand, other hexokinase members, including human hexokinase I and bacterial glucokinases, appear to be unable to accommodate GlcNAc because they are largely different from StHK and human GlcNAc kinase in loop regions involved in sugar bind-

**FIGURE 4.** A, stereo view of the superposition of the active sites of StHK in complex with glucose (green) and human GlcNAc kinase in complex with GlcNAc (dark blue) based on the large domains. Hydrogen bonds between ligands and the small domains are shown as dashed lines. The residues from the adjacent subunits are indicated by primes. B, stereo view of the superposition of the overall structures of StHK in complex with glucose (green) and human GlcNAc kinase in complex with GlcNAc (dark blue) based on the large domains. C, stereo view of the superposition of the active sites of StHK in complex with glucose (green) and human hexokinase in complex with glucose and ADP (Protein Data Bank code 1DGK, magenta) based on the positions of glucose. The bound ADP molecule is not shown for clarity.
Crystal Structures of S. tokodaii Hexokinase

The catalytic mechanism where an aspartate residue abstracts the proton from the 6-hydroxyl group of glucose at the initial stage of the reaction is commonly proposed for the hexokinase family. However, an aspartate is not likely able to abstract the proton from the 6-hydroxyl group of glucose because the \( pK_a \) of an aspartate is low. It may be more likely that the phosphoryl transfer would proceed through the metaphosphate-like transition state and an aspartate residue would accept the substrate proton from ADP and form a direct bond to the \( \gamma \)-phosphate of ATP, which is consistent with the previously proposed models based on the crystal structures of human hexokinase I (17) and human GlcNAc kinase (39). The other \( \gamma \)-phosphoryl oxygen is positioned in close proximity to the water molecules coordinating the \( \text{Mg}^{2+} \) ion (Wat\(_A\)), suggesting that during catalysis this \( \gamma \)-phosphoryl oxygen might coordinate the ion in place of Wat\(_B\) with the \( \beta \)- and \( \gamma \)-phosphates bridged by the ion. The \( \text{Mg}^{2+} \) ion together with Lys-15 would play a crucial role in stabilizing the developing negative charge on the phosphoryl oxygens during the transition state to promote catalysis.

**Biological Implications**—Here we describe the structural basis of the specificity of StHK for both glucose and GlcNAc. It has been reported that several enzymes involved in sugar metabolism in *Sulfolobus* also exhibit broad substrate specificity. In *Sulfolobus*, glucose is metabolized to pyruvate via the nonphosphorylative Entner-Doudoroff pathway (31). In the pathway, glucose dehydrogenase catalyzes the oxidation of glucose to gluconate, and then gluconate dehydratase catalyzes the dehydration of gluconate to KDG. Subsequently, KDG aldolase catalyzes the cleavage of KDG to glyceraldehyde and pyruvate. These three enzymes are involved in the metabolism of not only glucose but also galactose (57–59). Crystal structures of glucose dehydrogenase (60) and KDG aldolase (61) have been solved, and the structural bases for their substrate promiscuity have been determined. In addition, the nucleotidyltransferase from *S. tokodaii* has been reported to exhibit both glucose-1-phosphate thymidyltransferase and GlcNAc-1-phosphate uridylyltransferase activities (62). No genes encoding sugar kinases responsible for phosphorylation of mannose, glucosamine, and GlcNAc have been found in the genomes of *Sulfolobus* species, suggesting that StHK may also be involved in phosphorylation of these sugars in vivo. Promiscuous enzymes may be generally used in some metabolic pathways in addition to central metabolism in *Sulfolobus*, which is in contrast with the situation in...
most other organisms where separate enzymes with higher specificity are usually used.

Acknowledgment—We thank the staff at the Photon Factory for the x-ray data collection.

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