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The Crystal Structure of Glucokinase from *Leishmania braziliensis*

Gregory S. Buechner^{§,a}, Matthew E. Millington^{§,a}, Kay Perry[‡], and Edward L. D'Antonio^{§,*}

[§]Department of Natural Sciences, University of South Carolina Beaufort, 1 University Boulevard, Bluffton, South Carolina 29909, USA

[‡]NE-CAT, Department of Chemistry and Chemical Biology, Cornell University, Building 436E, Argonne National Laboratory, 9700 S. Cass Avenue, Argonne, Illinois 60439, USA

Abstract

Glucokinase from pathogenic protozoa of the genus *Leishmania* is a potential drug target for the chemotherapeutic treatment against leishmaniasis because this enzyme is located at a nodal point between two critically important metabolic pathways, glycolysis and the pentose phosphate pathway (PPP). *L. braziliensis* glucokinase (*LbGlcK*) was evaluated for its structural characterization and enzymatic performance. The enzyme catalyzes the phosphorylation of D-glucose with co-substrate ATP to yield the products G6P and ADP. *LbGlcK* had K_M values determined as 6.61 ± 2.63 mM and 0.338 ± 0.080 mM for D-glucose and ATP, respectively. The 1.85 Å resolution X-ray crystal structure of the apo form of *LbGlcK* was determined and a homodimer was revealed where each subunit (both in open conformations) included the typical small and large domains. Structural comparisons were assessed in relationship to *Homo sapiens* hexokinase IV and *Trypanosoma cruzi* glucokinase. Comparisons revealed that all residues important for making hydrogen bonding interactions with D-glucose in the active site and catalysis were strictly conserved. *LbGlcK* was screened against four glucosamine analogue inhibitors and the stronger inhibitor of the series, HPOP-GlcN, had a K_i value of 56.9 ± 16.6 μM that exhibited competitive inhibition. For the purpose of future structure-based drug design experimentation, *L. braziliensis* glucokinase was observed to be very similar to *T. cruzi* glucokinase even though there was a 44% protein sequence identity between the two enzymes.

Keywords

Leishmaniasis; Glucokinase; Hexokinase; X-ray Crystallography; Neglected Tropical Diseases

*To whom correspondence should be addressed. E.L.D.: telephone: +1 843-208-8101; email: edantonio@uscb.edu.

^aAuthors contributed equally to this work.

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Accession Code

The atomic coordinates and structure factors for apo-*LbGlcK* were deposited in the Protein Data Bank (www.rcsb.org) having the accession code of 6EDI.

1. Introduction

Human pathogenic *Leishmania* protozoa comprise 20 different species that are distributed throughout the world and cause the disease leishmaniasis (1). Transmission primarily occurs through the bite of a female sand fly and clinical manifestations of the disease span from cutaneous lesions to visceral infections (2). The parasitic protozoa utilize D-glucose as a major preferential carbon source to support their cellular growth and survival (3). Amino acids and fatty acids can act as alternative carbon sources when D-glucose levels are limited (4); however, all cultured stages of *Leishmania* exhibit severe growth restraints under such conditions (3, 5). D-Glucose is also another important metabolic source for the generation of NADPH through the PPP, where NADPH has a role in serving as a reducing agent in maintaining redox balance and in producing precursors for DNA and RNA biosynthesis (6). Based on the utility of D-glucose for *Leishmania* proliferation, metabolic pathways that include this monosaccharide as a substrate can be the subject of inhibition for leishmaniasis drug discovery.

Enzymes of the glycolytic and PPP metabolisms have been studied as potential drug targets for the treatment of trypanosomatid diseases that include Chagas' disease, Human African Sleeping Sickness, and leishmaniasis (7–9). Up to the present time, there has been a lack of investigations on the *Leishmania* glucose kinases (i.e. hexokinase or glucokinase) as potential drug targets for the treatment of leishmaniasis. The glucose kinases are transferase enzymes responsible in the conversion of substrate D-glucose and co-substrate ATP to yield products G6P and ADP, where a Mg^{2+} ion is required for catalysis (10). These enzymes are principally distinguished by their amino acid 1° structure and molecular weight range, which was thoroughly described by Kawai and colleagues (11). *Leishmania* parasites have a hexokinase (12, 13) and a glucokinase (14) that are localized in the glycosome (15). This is different than human cells, which contain four isoenzymes of hexokinase, belonging to the hexokinase group, designated HsHxK I – IV. Furthermore, the inhibition of a glucose kinase from *Leishmania* may prove to be an important drug discovery strategy. The work herein focused on the X-ray crystal structure determination of glucokinase from the species *L. braziliensis* (LbGlcK) along with a kinetic characterization. Accordingly, active site structural comparisons to the previously solved structures of *T. cruzi* glucokinase (TcGlcK) (16) and HsHxKIV (17) will aid in the understanding of whether LbGlcK can be inhibited with monosaccharide-based competitive inhibitors in a selective manner.

2. Materials and methods

2.1. Materials.

Benzoyl glucosamine (BENZ-GlcN) and carboxybenzyl glucosamine (CBZ-GlcN) were purchased from Carbosynth. Hydroxyphenyloxopropyl glucosamine (HPOPGlcN) and dioxobenzylthiophenyl glucosamine (DBT-GlcN) were previously synthesized (17). β -Nicotinamide adenine dinucleotide phosphate (reduced form) tetrasodium salt (NADPH, 95%) was purchased from Enzo Life Sciences. Ethylenediaminetetraacetic acid tetrasodium salt hydrate (>99.0%), imidazole (99+%), bovine pancreas deoxyribonuclease I (DNase I), bovine pancreas ribonuclease A (RNase A), *Saccharomyces cerevisiae* glucose 6-phosphate dehydrogenase (G6PDH) (type XV), β -nicotinamide adenine dinucleotide

phosphate hydrate (NADP⁺, 95%), adenosine 5'-triphosphate disodium salt hydrate (ATP, 99%), terrific broth, modified (TB modified), and all buffer salts (98%) were purchased from Sigma. Cobaltnitilotriacetic acid (Co-NTA) resin, lysogeny broth (LB), lysozyme (type VI), kanamycin sulfate, protease inhibitor tablets (EDTA-free), and all other chemicals were purchased from Fisher Scientific.

2.2. Cloning.

Glucokinase from the *Leishmania braziliensis* (MHOM/BR/75/M2904) strain (GenBank accession number [XP_001568875](#)) was cloned into a kanamycin-resistant pET-28a(+) *Escherichia coli* expression vector at restriction sites 5' NcoI and 3' HindIII at Genewiz, Inc. (South Plainfield, NJ). Codons were optimized for protein expression and the plasmid was designated as the pET-*LbGlcK*-xtal construct. The pET-*LbGlcK*-xtal plasmid encodes the segment MGRGSHHHHHHGMA that precedes the start methionine and the 13-residue segment KSADQVVPTKSHL at the C-terminal region was not included.

2.3. Standard methods.

Standard methods are presented in the Supplementary Information (SI) section for the following: (a) the expression and purification of *LbGlcK*, (b) activity assays of *LbGlcK*, (c) a primary screening assay of *LbGlcK* vs. four glucosamine analogue compounds, (d) an *LbGlcK* – inhibitor (HPOP-GlcN) assay, and (e) crystallization and the X-ray crystal structure determination of apo-*LbGlcK*. X-ray diffraction data collection and refinement statistics for apo-*LbGlcK* are shown in Table 1. Enzyme kinetic parameters for *LbGlcK* were obtained by performing the coupled hexokinase – G6PDH assay that produces the chromophore NADPH (16). NADPH is useful as a compound to be detected for this assay because on its spectral properties [UV-visible absorbance (λ_{max} : 340 nm); fluorescence (λ_{ex} : 340 nm, λ_{em} : 485 nm)] (16, 22). All kinetic measurements, including the activity assays involving substrates D-glucose and ATP, the primary screening assay, and the enzyme – inhibitor assay, were each carried out in triplicate at room temperature and pH 7.6.

3. Results and discussion

We report for His₆-*LbGlcK* a K_M (D-glucose) of 6.61 ± 2.63 mM, a k_{cat} (D-glucose) of $3.59 \times 10^3 \pm 0.72 \times 10^3$ min⁻¹, a k_{cat}/K_M (D-glucose) of $5.71 \times 10^2 \pm 1.08 \times 10^2$ mM⁻¹ min⁻¹, and a K_M (ATP) of 0.338 ± 0.080 mM (see Figure S1 in SI for Michaelis-Menten plots). The *LbGlcK* K_M (D-glucose) is close to the previously reported *L. major* glucokinase K_M (D-glucose) of 3.30 mM and the K_M values with respect to ATP are essentially identical (e.g. K_M (ATP) of 0.35 mM for *L. major* glucokinase) (14). Table S1 in SI shows a comparison between kinetic parameters of recombinant glucokinases from selected trypanosomatid species. Briefly, the K_M values (with respect to D-glucose) for the listed *Leishmania* spp. glucokinases are slightly higher when compared to *T. cruzi* glucokinase (K_M of 1.00 mM) and the K_M with respect to ATP is principally the same for all of these glucokinases. The cDNA construct used in this study produced a truncation mutant of *LbGlcK* that excluded a 13-residue segment of the C-terminal region (residues: KSADQVVPTKSHL). We decided to use such a construct design because the analysis of the *LbGlcK* amino acid full-length sequence using the program *DISOPRED3* (20) suggested that this segment, in particular,

was disordered and could thereby hinder crystallization. The truncation created for full-length *LbGlcK* compared similarly to the truncation that was previously employed for that of full-length *TcGlcK* (a 9-residue C-terminal amino acid exclusion) (16). In that, full-length *TcGlcK* also revealed the same disordered region at the C-terminus. Moreover, although we did not assay the full-length *LbGlcK* enzyme, we did not expect that the removal of its C-terminal segment would make any difference in enzymatic assay properties. This can be explained by the comparison to the results observed for *TcGlcK*, in which the full-length protein (14) and its truncation mutant (exclusion of a 9-residue C-terminal segment: VGKKQKAQL) (16) were previously assayed. The assays in the two studies revealed the same K_M value for the substrate D-glucose, which was $K_M = 1$ mM (see Table S1 in the Supplementary Section). In terms of the possibility of changes in structural folding due to the *LbGlcK* truncation, we did not expect such an issue since disorder defined by Ward and colleagues (20) is a protein segment that is flexible and dynamic that usually extends partially or fully into bulk solvent.

LbGlcK crystallized as a ligandless dimer in the asymmetric unit of space group $P2_1$ (Figure 1a) with two identical subunits stabilized by a large contact surface area of $2,295 \text{ \AA}^2$ (13% of the total surface area) as determined by the Protein Interfaces, Surfaces, and Assemblies (PISA) server (18). Each monomer has a large $\alpha + \beta$ domain (residues 157 – 383) and a small α/β domain (residues 1 – 142 and 384 – 398) (Figure 1b). Each subunit of *LbGlcK* was observed in an open conformation between large and small domains and this comparison was made by having a superposition against the *TcGlcK* – BENZ-GlcN complex (PDB entry 5BRD) (Figure S2, SI), which has a low bending region angle of 4.9° (16). Specifically, the large domains of *LbGlcK* and *TcGlcK* were superimposed onto each other while the small domains oriented differently. The small domain of *LbGlcK* (open form) had a bending region angle that was greater than 20° from the small domain of *TcGlcK* (closed form), as determined by the program *DynDom* (21). When the *LbGlcK* closed form crystal structure becomes available, a better analysis of the bending region angle can be established between the two domains. For a trypanosomatid glucokinase, this structure reveals the first open conformation structure reported. The overall fold of the *LbGlcK* dimer is almost similar when compared to that of the *TcGlcK*-BENZ-GlcN complex (PDB entry 5BRD) (16) except with changes noted from the bending angle between large and small domains. When superimposed, this resulted in a median root-mean-square deviation (r.m.s.d.) of $4.10 \text{ \AA}$ for 733 C α atoms or an r.m.s.d. of $1.673 \text{ \AA}$ for 522 C α atoms (after 5 cycles of atom rejections). The differences in fold are attributed to the partially open conformation (*TcGlcK*) and the full open conformation (*LbGlcK*) between crystal structures. A superposition of the crystal structures reveals conserved features within the active site (Figure S3, SI). R.m.s.d. values were determined through the MacPyMOL software (19).

Active site residues of the *TcGlcK*-BENZ-GlcN complex (PDB entry 5BRD) (16) that are indeed responsible in making hydrogen bonding interactions with the glucose moiety of the competitive inhibitor were evaluated with respect to the conserved residues of *LbGlcK* through a superposition of their crystallographic coordinates (Figure S3, SI). *TcGlcK* was selected as the comparison model because it has the highest protein sequence identity to *LbGlcK* across all deposited PDB entries. A structure-based sequence alignment between

the two enzymes revealed which active site residues were conserved and indicated a 44% sequence identity (Figure S4, SI). Since *LbGlcK* is in the apo form (ligandless) in the structure presented in this work, we assumed that its following residues make hydrogen bonding interactions with Dglucose during catalysis: N142, D143, E262, and E220 (note: residues N130, D131, E236, and E207 of *TcGlcK* correspond respectively as the conserved residues). Furthermore, all residues of the active site and the outer part of the active site were presented in Figure S3, SI. Similar to the *TcGlcK* active site structural environment, *LbGlcK* also includes a hydrophobic pocket at the outer part region. Hydrophobic residues F368 and M364 of *LbGlcK* are in the same superimposed positions as *TcGlcK* residues F337 and M334, respectively. Interestingly, as some of the glucosamine analogue inhibitors from our previous work were observed to make π -stacking and van der Waals interactions in the hydrophobic pocket, which afforded enhanced competitive inhibitor binding strength (16), we predict that residue F368 in *LbGlcK* will participate in the same kind of role as F337 of *TcGlcK*.

Structural and enzyme – inhibitor kinetics comparisons were made between *LbGlcK*, *TcGlcK*, and *HsHxKIV* to assess whether glucosamine analogues, that have a linker extended from the C2 position of the glucose moiety (refer to the SI section for example chemical structures of such compounds), could indeed bind in the active site cavity of *LbGlcK*. Pertaining to structural assessments, they included the analysis of residues within the active site that are subject to cause deterrence for the binding of a glucosamine analogue and the analysis of two protein loops by the outer part of the active site for the possibility of deterrence of inhibitor access into the active site.

The manner in which various residue side chains are oriented in the active site of *HsHxKIV* was a driving factor for why glucosamine analogues could not bind, as we previously proposed (16). These compounds would have difficulty managing access into the active site based on residue P153 in addition to side chains that could potentially cause steric hindrance (i.e. T168, N166, Q286, and K56). Our previous work considered the possibility of HPOP-GlcN as a potential inhibitor to bind in the active site of *HsHxKIV*, through a molecular modeling analysis. This assumed that the glucose moiety of HPOP-GlcN would maintain the key binding interactions at the glucose binding site (16). We reasoned that HPOP-GlcN (and similar analogues) may not have the ability to bind favorably in *HsHxKIV*, because in the closed conformation a narrow channel (bordered by residues P153 and T168) that the HPOP-GlcN linker group would need to extend through, would certainly have unavoidable close contact interactions (e.g. the rigid side chain of P153, in particular). The other *HsHxKIV* residues in the vicinity, such as K56, N166, T168, and Q286 have flexible side chains and could possibly reposition, but overall, the active site appeared too congested for HPOP-GlcN. On the other hand, *TcGlcK* was observed to have a much wider channel (bordered by residues P94 and N105) and was shown to accommodate HPOP-GlcN in the active site, as evidenced from a *TcGlcK* – HPOP-GlcN complex from crystallography (see PDB entry 5BRF (16)). P94 (*TcGlcK*) was the conserved residue to P153 (*HsHxKIV*) and P94 (*TcGlcK*) was observed to be positioned in a different manner that could avoid the close contact of the HPOP-GlcN linker moiety. Moreover, enzyme – inhibitor K_i values determined for *TcGlcK* and *HsHxKIV* revealed a value of 186 for a *TcGlcK* selectivity ratio (16). In glucokinase from *L. braziliensis*, residue P106 (*LbGlcK*) is the conserved residue of

both P153 (*HsHxKIV*) (Figure 2) and P94 (*TcGlcK*) (Figure S4, SI). Although our *LbGlcK* structure is observed in the open conformation, P106 (*LbGlcK*) is located on the small domain, and the P106 side chain is therefore shifted away from the active site. We cannot accurately predict if P106 would deter these kinds of glucosamine analogues to bind. Interestingly however, it does not appear that the P106 side chain would interfere with a bound HPOP-GlcN inhibitor in the active site and this is based off of a superposition from the A-chains of *LbGlcK* and the *TcGlcK* – HPOP-GlcN complex.

With active site comparisons between *LbGlcK* and *TcGlcK* appearing to be rather close from a structural superposition (Figure S3, SI), it appears that glucosamine analogues have the ability to bind in the active site of *LbGlcK*. In order to test this hypothesis, we screened *LbGlcK* against four glucosamine analogue compounds that were previously determined as strong competitive inhibitors of *TcGlcK* (16). These compounds were BENZ-GlcN, CBZ-GlcN, HPOP-GlcN, and DBT-GlcN. The primary screening involved having percent *LbGlcK* inhibition to be greater than 34.2% (a calculated cutoff percentage) as the concentration of compounds was set to 20 μ M, in order to be considered as candidate hits. From the series tested, none of the compounds were candidate hits. HPOP-GlcN was the stronger *LbGlcK* inhibitor of the series, in which it inhibited *LbGlcK* by $26.9 \pm 1.2\%$. Inhibition by the other compounds was weaker, such as BENZ-GlcN with $14.2 \pm 3.9\%$ inhibition, DBT-GlcN with $13.7 \pm 3.0\%$ inhibition, and CBZ-GlcN with $10.6 \pm 3.1\%$ inhibition. The cutoff percentage of 34.2% was a calculated value that takes into consideration the positive controls when analyzed by the fluorescence microplate reader, and also, the variability that occurs from the fluorescence readout. A candidate hit was defined as an activity measurement being three standard deviations away from the mean of the positive controls and additional information on the calculation is provided in the SI section. In various target-based high-throughput screening campaign studies (22–24), the progression cascades (screening compounds to confirmed hits) reveal that compounds not passing requirements in the primary screen step will not proceed into *in vitro* parasite biological assays. As such, the four weak glucosamine analogue inhibitors of *LbGlcK* were excluded from any *anti-Leishmania in vitro* parasite testing. Since HPOP-GlcN had a higher inhibitory strength, its mode of inhibition and inhibitory constant (K_i) were sought after. The K_i for HPOP-GlcN *vs.* *LbGlcK* was determined to be $56.9 \pm 16.6 \mu$ M and the mode of inhibition was competitive (see Figure S5 for the Lineweaver-Burk plot). The K_i was observed at a much higher value when compared to *TcGlcK*/HPOP-GlcN ($K_i = 1.3 \pm 0.6 \mu$ M) (16), in which HPOP-GlcN exhibited a 43.8-fold weaker inhibition to *LbGlcK*. For selectivity *vs.* *HsHxKIV* ($K_i = 242 \pm 0.6 \mu$ M) (16), there was a 4.25 selectivity index that indicated HPOP-GlcN was not very selective towards *LbGlcK*. In terms of the observed competitive inhibition, HPOP-GlcN is binding in the active site of *LbGlcK* and this corroborates well for a glucosamine analogue inhibitor having the expectation to bind in the *LbGlcK* active site based on superposition structural data, as revealed in Figure S3.

LbGlcK and the *HsHxKIV*-D-glucose complex (PDB entry 3IDH) (17) were superimposed from individual subunits. The active site regions revealed the key glucose binding residues to be present for *LbGlcK*; also, the superposition showed that *HsHxKIV* had residues that were absent in *LbGlcK*, such as K169 and T168 (Figure 1c). A structure-based sequence alignment was also established between the same crystal structures (Figure 2). The overall

sequence identity based on that alignment was 15%, which is quite low but desirable from a drug discovery perspective, as an overall goal is to find inhibitor selectivity toward the parasite homologue. A noteworthy structural difference is with two distinct loops at the outer part of the active site. In *HsHxKIV*, there exists two large loop regions [(L165 – G178); (S281 – Q287)] that interact with each other through van der Waals contact. Such loops were evaluated from our previous work and hypothesized to cause steric hindrance to glucosamine analogue inhibitors in accessing the active site. The S281 – Q287 loop is oriented adjacent to the glucose binding site and N283, Q286, and Q287 have potential to block this region due to the proximity of their side chains (16). In *TcGlcK*, these two loop regions [(G102 – I110); (K230 – N234)] also exist but they are substantially shorter in residue count. As a result, they do not interact with each other and they also do not preclude inhibitor access to the active site. *LbGlcK* also has these two loop regions [G114 – I122); (A248 – I260)]. The first loop region contains 9 residues and is very similar to the loop segment found in *TcGlcK*; however, the second loop region is a 13-residue loop and is longer than both the loop segments of *TcGlcK* and *HsHxKIV*. Through our crystallography, we observed a 4-residue segment [K247 – A250] in both the A- and B-chains that had disorder and could not be modeled in. As this segment is part of the 13-residue loop, although being a longer loop, it appears that it still would not cause deterrence to inhibitor access to the active site even if the 4-residue segment could be modeled in, from a visual observation.

4. Conclusions

With glucose kinases being critical enzymes for glycolysis and the PPP due to the requirement of D-glucose as a major carbon source for *Leishmania* spp., the crystal structure of *LbGlcK* will provide important guidance regarding the design of new inhibitors. Competitive inhibitor designs for *LbGlcK* can follow a structure-activity relationship method because HPOP-GlcN was shown to have competitive inhibition, as such, variations in the structure of HPOP-GlcN will provide a starting point for new inhibitor designs. Thus, different variants of glucosamine analogue inhibitors are expected to strongly inhibit *LbGlcK*. Such inhibitors may also be biologically active against *Leishmania* parasites. Future studies in this regard will be reported in the course of time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BENZ-GlcN	benzoyl glucosamine
CBZ-GlcN	carboxybenzyl glucosamine
DBT-GlcN	dioxobenzylthiophenyl glucosamine
G6PDH	glucose-6-phosphate dehydrogenase
HPOP-GlcN	hydroxyphenyloxopropyl glucosamine
LbGlcK	<i>Leishmania braziliensis</i> glucokinase
PDB	Protein Data Bank
PPP	pentose phosphate pathway
r.m.s.d.	root-meansquare deviation
TcGlcK	<i>Trypanosoma cruzi</i> glucokinase
TcHxK	<i>Trypanosoma cruzi</i> hexokinase

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Highlights

- The X-ray crystal structure of *L. braziliensis* glucokinase was solved at 1.85 Å resolution.
- *L. braziliensis* glucokinase crystallized in the apo form and open conformation.
- Designs of competitive inhibitors for parasite homologue selective inhibition over the four human hexokinases are highly achievable.

Figure 1.

Quaternary, tertiary, and active site representations of apo-*LbGlcK*. (a) The 4° structure of *LbGlcK* is shown as a dimer with N- and C-termini indicated for each subunit and is color-coded as follows: blue for the small domain, green for the large domain, and grey for the linking segments between domains. The same colors in a shaded appearance represent the other subunit. (b) The 3° structure of *LbGlcK* is presented as a monomer revealing secondary structure elements (in sequential order) of α -helices, 3_{10} -helices, and β -strands as symbols of α , η , and β , respectively. (c) Active site view for the superposition of unliganded *LbGlcK* (chain A) and the *HsHxKIV*-D-glucose complex (PDB entry 3IDH) (17). Atoms are color-coded as follows, *LbGlcK* structure: yellow for carbon, blue for nitrogen, red for oxygen, and black labels; *HsHxKIV*-D-glucose complex: light blue (protein) and grey (D-glucose) for carbon, blue for nitrogen, red for oxygen, hydrogen bond interactions are represented as green dashed lines, and red labels.

Figure 2.

Structure-based sequence alignment of *LbGlcK* and *HsHxKIV* (PDB entry 3IDH) (17). The structural alignment was determined and prepared by (i) the web-based protein structure comparison service Fold at the European Bioinformatics Institute (*PDBeFold*), (ii) the program *STRIDE*, and (iii) the program *ESPrpt* (version 3.0); references for these programs are provided in the figure legend of Figure S4, SI. The overall sequence identity is 15% based on this alignment. The symbols α , η , and β indicate the secondary structural elements α -helices, 3_{10} helices, and β -strands, respectively. Residues having exact identity are boxed and indicated by white letters on red background; residues with near identity are boxed and indicated by red letters on white background. Active site residues are indicated by blue triangles.

Table 1.

Data Collection and Refinement Statistics.

Crystallographic details of apo-LbGlcK.	
	apo- <i>LbGlcK</i>
<i>Data Collection</i>	
resolution limits (Å) ^a	50.0 – 1.85 (1.88 – 1.85)
total/unique reflections measured	201229/58585
space group symmetry	P 1 21 1
unit cell dimensions	
a, b, c (Å)	68.5, 63.3, 85.6
α, β, γ (deg)	90.0, 107.8, 90.0
$R_{\text{merge}}^{a,b}$	0.098 (1.174)
$I/\sigma(I)^a$	10.3 (1.1)
completeness (%) ^a	97.4 (88.5)
CC _{1/2} ^a	0.994 (0.438)
<i>Refinement</i>	
reflections used in refinement/test set	58205/2955
R_{work}^c	0.2124
R_{free}^d	0.2618
protein chains ^{e,f}	2
protein residues ^{e,f}	788
protein atoms ^{e,f}	6126
solvent molecules ^{e,f}	176
<i>Root Mean Square Deviation^f</i>	
bonds(Å)	0.007
angles (deg)	1.18
<i>Wilson B-factor^f (Å²)</i>	31
<i>Average B-factor^f (Å²)</i>	
main chain	35
solvent	35
<i>Ramachandran Plot^g (%)</i>	

allowed	89.8
additionally allowed	9.0
generously allowed	0.7
disallowed	0.4
PDB accession code	6EDI

^aValues in parenthesis are for the highest resolution shell.

^b $R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$, where I is the observed intensity, $\langle I \rangle$ is the average intensity calculated from replicate data.

^c $R_{\text{work}} = \sum ||F_{\text{O}}| - |F_{\text{C}}|| / \sum |F_{\text{O}}|$ for reflections contained in the working set.

^d $R_{\text{free}} = \sum ||F_{\text{O}}| - |F_{\text{C}}|| / \sum |F_{\text{O}}|$ for 5% of reflections contained in the test set held aside during refinement. $|F_{\text{O}}|$ and $|F_{\text{C}}|$ are the observed and calculated structure factor amplitudes, respectively.

^ePer asymmetric unit cell.

^fCalculated using *PHENIX* (refer to references in SI).

^gCalculated using *PROCHECK* (refer to reference in SI).