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Structural Insight into Mechanism and Diverse Substrate Selection Strategy of L-Ribulokinase

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Abstract

The *araBAD* operon encodes three different enzymes required for catabolism of L-arabinose, which is one of the most abundant monosaccharides in nature. L-ribulokinase, encoded by the *araB* gene, catalyses conversion of L-ribulose to L-ribulose-5-phosphate, the second step in the catabolic pathway. Unlike other kinases, ribulokinase exhibits diversity in substrate selectivity and catalyses phosphorylation of all four 2-ketopentose sugars with comparable k_{cat} values. To understand ribulokinase recognition and phosphorylation of a diverse set of substrates, we have determined the X-ray structure of ribulokinase from *Bacillus halodurans* bound to L-ribulose and investigated its substrate and ATP co-factor binding properties. The polypeptide chain is folded into two domains, one small and the other large, with a deep cleft in between. By analogy with related sugar kinases, we identified ⁴⁴⁷GGLPQK⁴⁵² as the ATP binding motif within the smaller domain. L-ribulose binds in the cleft between the two domains *via* hydrogen bonds with the sidechains of highly conserved Trp126, Lys208, Asp274, and Glu329 and the main chain nitrogen of Ala96. The interaction of L-ribulokinase with L-ribulose reveals versatile structural features that help explain recognition of various 2-ketopentose substrates and competitive inhibition by L-erythrulose. Comparison of our structure to that of the structures of other sugar kinases, revealed conformational variations that suggest domain-domain closure movements are responsible for establishing the observed active site environment.

Keywords

Crystal structure; ribulokinase; ribulose; *araBAD*; *araB*; arabinose-catabolism

Introduction

L-ribulokinase (RK; EC 2.7.1.16) is one of three major enzymes of arabinose catabolic pathway¹. In nature, L-arabinose is among the most abundant monosaccharides. In *Escherichia coli* (*E. coli*), catabolism of L-arabinose involves expression of six distinct genes, including *araA*, B, C, D, E, and F². Three such genes (*araB*, *araA*, and *araD*) constitute the *araBAD* operon^{2,3}. L-ribulokinase (*araB*)^{4–6}, L-arabinose isomerase (*araA*)^{5,7,8}, and L-ribulose 5-phosphate 4-epimerase (*araD*)⁹ are the first three enzymes of L-arabinose catabolism. They break down the monosaccharide sugar L-arabinose into D-xylulose-5-phosphate, which is then metabolized *via* the pentose phosphate pathway¹ (Fig. 1). RK, believed to be biological dimer^{4,10}, catalyses phosphorylation of L(or D)-ribulose producing L(or D)-ribulose 5 phosphate⁴.

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Previously published biochemical studies of *E. coli* RK have revealed its substrate preferences and identified L-erythrulose as a competitive inhibitor¹¹. Unlike some of other sugar kinases, RK phosphorylates all four 2-ketopentoses (L or D-ribulose and L or D-xylulose) with similar k_{cat} values¹¹. Despite RK's central role in arabinose catabolism, its three-dimensional structure, mechanism of action, and the bases of this broad substrate selectivity have not been elucidated. In order to fill this void ribulokinase from *Bacillus halodurans* was chosen as a target for structure determination by the New York Structure GenomiX Research consortium.

In this paper, we present the first crystal structure of L-ribulokinase from *Bacillus halodurans* bound to one of its substrates (L-ribulose) at 2.3Å resolution. Within the structure, we identified ATP binding and ATPase signature motifs. Our structural analysis also explains the diverse substrate selectivity of this enzyme and inhibition by L-erythrulose, and sheds some light on its mechanism of action.

Materials and Methods

Gene cloning, Protein expression and Purification

The gene for L-ribulokinase (encoding residues 3–563) from *Bacillus halodurans* (Uniprot: Q9KBQ3) was amplified using the following PCR primers: forward-ACGAAATACACAATTGGGGTTGACT and reverse-CCGTAAACAAAGAAGACGGACGATGC and cloned in pSGX3 (BC) vector. A sequence verified clone was then transformed into BL21(DE3)-codon+RIL cells for the expression. Protein expression and purification followed the standard NYSGXRC protocol (www.pepcDB.org).

Crystallization and Data collection

Diffraction quality crystals of seleno-methionine (Se-Met) protein were obtained in the monoclinic space group $P2_1$ at room temperature *via* vapor diffusion against a reservoir solution containing 30% PEG4000, 0.2M Sodium acetate, 0.1M Tris, pH 8.5 (1µl reservoir solution plus 1µl protein at 20 mg/ml). Crystals were frozen by direct immersion in liquid nitrogen using the mother liquor with glycerol [final concentration=15% (v/v)] as a cryo-protectant. X-ray diffraction data from 2 crystals were collected under standard cryogenic conditions at Beamlines X12C and X25, National Synchrotron Light Source, Brookhaven National Laboratory. Data were processed, merged, and scaled using HKL2000¹². Assuming four molecules/asymmetric unit, Matthews' coefficient was determined to be 2.31 Å³/Da. Data collection and refinement statistics are provided in Table I.

Structure Determination by Se-SAD and Refinement

Single wavelength anomalous diffraction (SAD) data recorded at beamline X12C with a Se-Met crystal yielded the locations of 50 of a possible 64 selenium atoms (16 Se-Met/protomer) with occupancies estimated >0.4 using ShelxD¹³. Phases were improved using SHARP¹⁴ and SOLOMAN¹⁵, and by NCS averaging followed by density modification using CCP4¹⁶ at 2.7 Å resolution. Phases were ultimately extended to 2.3Å resolution using data collected at beamline X25. The polypeptide chain model was automatically built in part into the electron density map using Arp/wArp, followed by manual building with COOT¹⁷ and O¹⁸. The structure of the best resolved protomer was used to generate the remaining three molecules comprising the asymmetric unit. CNS¹⁹ and reftmac5¹⁶ were used to refining the atomic model to convergence (Table I).

Results and Discussion

Overall Structure

The X-ray structure of full length RK includes four copies of the polypeptide chain (molecules A, B, C, and D) arranged into two dimers, each with a buried surface area of $\sim 4690 \text{ \AA}^2$ (12% of the total surface area). Subunits A and B form one dimer, and C and D form the other. Missing portions of the polypeptide chains include residues 1–4 and 554–563 of protomers A and B, and residues 1–4 and 559–563 of protomers C and D. The overall structure of each protomer is very similar to the other three, with root-mean-square deviations (r.m.s.d.s) of 0.4–0.7 $\text{\AA}$ for α -carbon atomic pairs (Fig. 2).

RK belongs to the carbohydrate kinase FGGY superfamily²⁰. A VAST²¹ search of the Protein Data Bank (www.pdb.org) with our structure of RK revealed two xylulose kinases (XKs) as its nearest structural neighbors (from *E. coli*, ecXK, PDB Code: 2ITM, r.m.s.d.=3.0 $\text{\AA}$ for 466 α -carbon atomic pairs with 20% sequence identity and from *Lactobacillus acidophilus*, laXK, PDB Code: 3LL3, r.m.s.d.=2.5 $\text{\AA}$ for 482 α -carbon atomic pairs with 17% sequence identity). A BLAST²² amino acid sequence search revealed similarity to both the glycerol kinases (GKs) and XKs (sequence identities ranging between 24 and 28%).

Each polypeptide chain is folded into two main domains (Fig. 2; domain I: residues 5–273; domain II: 274–563). Although no substrate was added during or prior to crystallization, a continuous interpretable electron density was observed in protomer A within the inter domain cleft in SigmaA weighted $|F_o| - |F_c|$ difference Fourier synthesis and could be modeled unambiguously as L-ribulose. Despite their similarity to protomer A, protomers B, C, and D lack discernable ligand(s) in their inter domain clefts. In crystal structures, the absence of ligands in some protomers within the asymmetric unit is not uncommon (PDB ids: 1Z2L and 2PUZ).

Substrate Binding

L- and D-Ribulose are known substrates for RK¹¹, which phosphorylates both compounds at the C5 position. In our X-ray structure, L-ribulose binds in the inter domain cleft and interacts primarily with the larger domain II (Fig. 3). Four of the five oxygen atoms of L-ribulose (O1, O2, O3, and O4) make hydrogen bonds with conserved residues in domain II (Table II), including Glu329 (OE2), Lys208 (NZ), Ala96 (N), and Asp274 (OD1 and OD2). An aliphatic chain portion of the substrate interacts with π -electron cloud of Trp126 of domain I (Fig. 3).

To understand better the broad substrate selectivity of RK, the active sites of RK, ecGK (Glycerol kinase from *E. coli*, PDB ID: 1GLE, r.m.s.d.=4.4 $\text{\AA}$ for 465 α -carbon atomic pairs with 20% identity), and ecXK and laXK were compared in detail. Not surprisingly, the substrate binding sites of RK and the XKs are similar in size, whereas the substrate binding site of GK is smaller. Comparisons of representative enzyme:substrate co-crystal structures of XKs and GK revealed conservation of Asp274^{23–26} (RK numbering), which engages in analogous interactions with substrate oxygen atoms. In ecGK²⁴, glycerol O2 interacts with the OD1 of conserved Asp246 and the main chain N of Arg83. O3 of glycerol makes a hydrogen bond with OD2 of Asp246, like O4 of L-ribulose with Asp274. At the other terminus of the substrate, O1 of glycerol forms hydrogen bonds with sidechains of Glu84 and Arg83, not unlike the interactions of RK Lys208 and Glu329 to O2 and O1 of L-ribulose, respectively.

Previously reported structures of XKs from different organisms bound to D-xylulose reveal heterogeneity in substrate binding. In the structure of laXK (PDB ID: 3LL3) bound to both ATP and D-xylulose, the substrate O5 interacts with N of Met78 and OD2 of Asp237, and

O2 hydrogen bonds to NE2 of His79. On the other hand, in the structure of ecXK²³ bound to D-xylulose, the substrate O3 interacts with N of Met77 and OD1 of Asp233, whereas O1 and O2 make hydrogen bonds with His 78 (NE2) and Asn234 (ND2), respectively.

The most significant difference in substrate binding between RK and the XKs involves the location of C5 (where catalytic addition of phosphate occurs). Unlike D-xylulose binding to laXK, wherein a direct interaction is observed (closest approach 2.76 Å, OD2 of Asp237), L-ribulose O5 does not interact directly with Asp274 (Asp237 in laXK) of RK in our structure. We, therefore, suggest that RK-catalyzed phosphate addition to L-ribulose requires movement of enzyme and/or substrate with respect to the C5-O5 portion upon Mg⁺⁺-ATP binding, to bring the C5-bound hydroxyl group near the catalytic Asp274 sidechain.

ATP Binding Site

The sequence and structural similarity of RK and laXK allowed us to identify a putative ATP binding motif (⁴⁴⁷GGLPQK⁴⁵²) and binding site in RK (Fig. 4). Having made multiple unsuccessful attempts to soak RK crystals with ATP and co-crystallize the protein with ATP (with and without L-ribulose), we were forced to resort to modeling ATP into our structure of RK using the ternary complex structure of laXK as a guide (Fig. 4). Although this exercise provides some insight into RK's mode of ATP binding, the conformations of RK and laXK are not entirely analogous. Even though, the active site cleft of our structure of RK appears more open than that of laXK, O5 of L-ribulose is ~9 Å away from the γ- and β-phosphates of ATP in the model depicted in Figure 4. In the experimental structure of the laXK ternary complex, O5 of D-xylulose is ~10.7–12.2 Å from the γ- and β-phosphates of ATP. Above observation suggests the possible conformational changes in two domains to bring substrate and ATP more closely to achieve the catalysis.

Putative Conformational Changes Required for Catalysis

A Hingeplot²⁷ analysis of our RK structure identified a potential hinge region that appears capable of supporting the conformational rearrangement posited above. Residues 286, 472, and 508 divide residues 5–79, 83–286, and 473–508 (rigid portion 1) from residues 287–472 and 509–553 (rigid portion 2). Rotation of rigid portions 1 and 2 with respect to one another could open and close the inter domain cleft where L-ribulose and ATP bind. A second potential hinge region involving residues 38, 63, 95, 186, 204, 241, 331, 425, and 526 also emerged from our analysis. Given the distance of the substrate from ATP (~9 Å) in the model depicted in Figure 4, both hinges may be required to bring the substrate close enough to the enzyme co-factor. Analogous structural differences involving ~12–37 Å domain movements^{23–26,28,29} have been observed crystallographically for both GK and XK^{23–26,28,29}.

In order to get a sense of the conformational changes that occur on substrate binding and substrate- ATP/ADP binding, we superimposed structures of ecGK, laXK, and ecXK on RK (Fig. 5). Proteins ecXK, ecGK, laXK have 20, 20 and 17% sequence identity with RK for 466, 465 and 482 Cα atoms, respectively. The domain II of ecGK (PDB ID: 1GLE; 4–244), laXK (PDB ID: 3LL3, 1–236), and ecXK (PDB ID: 2ITM, 1–232) were superposed on RK (PDB ID: 3QDK, 5–273) (Fig. 5). The two domains make varying angles in ecGK, laXK, ecXK and RK of 105.4°, 119.15°, 128.57°, and 126.74°, respectively.

As expected, structures of enzymes bearing only bound substrate were more open (ecXK: 2ITM and RK: 3QDK) than substrate-ADP/ATP bound ternary complexes (ecGK: 1GLE and laXK: 3LL3) (Fig. 5). In the absence of substrate, these structurally-related kinases appear to adopt a more open conformation that narrows somewhat upon substrate binding.

Further narrowing occurs when ATP binds to achieve a closed, active, conformation²³. This assertion is supported by our finding that L-ribulose interacts only weakly with the smaller domain I of RK in the absence of bound ATP. We propose that augmented interactions of substrate with domains I will eventually lead to conformational change from open to close.

Structural Basis of Substrate Selectivity of RK

RK can recognize both L- and D-configurations of ribulose and catalyze phosphate addition to C5 with comparable k_{cat} values. The measured K_m ¹¹ values for binding of these two substrates document a preference for L-configuration versus D¹¹. Detailed analysis of the RK substrate binding site, depicted in Figures 3 and 6, does provide a structural basis for rationalizing the observed differences. Interactions of O4 with Asp274 OD1 and OD2 and, at the same time maintaining other close hydrogen bond interactions of O1, O2, O3 with other residues Lys208, Glu329, and Ala96 are very critical for optimal positioning of L-ribulose (Fig. 3, 6 (a)). The same will be compromised due to steric hindrances at C5-C4-C3 in D-configuration, which in turn leads to K_m variations (Fig. 6 (a)). However, we do not exclude the possibility of sidechain conformational changes in residues directly involved in substrate binding to facilitate binding of D-ribulose in a competent manner (Fig. 6(b)). Besides this, in pentose sugars the presence of C=O bond at C2 position or D-configuration if its CHOH, is preferred¹¹. C=O at C2 or D-configuration at C2 poses O2 to make its important interaction with Lys208, which will vanish due to L-configuration at C2. Also C1-C2-C3 is planar due to C=O, which has larger bond angle (120°) as compared to CHOH (109°), at C2 position. This in turn helps substrate to interact with protein in more extended way (Fig. 6(a) and (b)). Overall, the steric hindrances, bond angles and required length (5 Carbon) of ketopentoses decide the binding and catalysis (Fig. 6). Above argument also confirms why RK phosphorylates only at C5 and not at C1 in either configuration of ketopentoses. Therefore, we assume that binding of the sugar in competent way, as per the aligning residues of the active site of RK is key point to the substrate selection and phosphorylation.

Although the substrate binding sites of the XKs and RK are not identical, both enzymes do catalyze phosphate addition to the substrates of the other. Results of modeling of Land D-xylulose in the RK active site (data not shown) suggest that both of these other 2-ketopentoses can form interactions fairly similar to those seen for L-ribulose. Similar results were obtained with the obverse exercise using the structure laXK and and L- and D-ribulose (data not shown).

L-Erythrulose Inhibition

L-Erythrulose is a competitive inhibitor of RK¹¹. We analyzed the structural basis of inhibition by L-erythrulose by modeling it into the RK L-ribulose binding site. The two compounds are so similar that inhibitor binding could exploit three of the hydrogen bonds observed between the enzyme and L-ribulose, including O1-OE2 of Glu239, O2-NZ of Lys208, and O3-N of Ala96. A modest change in the position of the sidechain of Asp274 would suffice to support hydrogen bonding between O4 and OE1 and/or OE2. This proposed mode of binding for the isostructural tetrose explains competitive inhibition. Presumably, this mode of binding also explains why L-erythrulose is not a substrate for RK. By mimicking the binding mode of L-ribulose, L-erythrulose is simply too short in length to engage both the sidechain of Asp274 and the γ - and β -phosphate groups of ATP, even in the closed active enzyme conformation.

RK Mechanism of Action

The evolutionary relationship between the RKs and both the GKs and the XKs suggests that these three enzyme classes share a similar mechanism of action^{23–26,28–30}. The three

residues in ecGK and ecXK thought to be directly involved in ATP hydrolysis are conserved in RK (Asp11, Thr14, and Asp274; RK numbering; Table III). Structural overlays confirm that residues Asp11 and Asp274 in RK occur in the same relative locations as Asp6 and Asp233 in ecXK²³ and Asp10 and Asp245 in ecGK^{25,26}. Given the similarity of amino acid sequences, overall protein folds, and substrate and cofactor binding sites among the GKs, XKs, and RK and the results of site-directed mutagenesis of the two aspartic acid residues in both XK and GK^{23,25,26}, we can propose confidently that the mechanisms by which these three carbohydrate kinases work are fundamentally similar. In all three cases, binding of both substrate and Mg⁺⁺-ATP (either sequentially or together) are required to bring the two domains of the enzyme together. Closure of the inter domain cleft would bring the γ - and β -phosphate groups of ATP, the catalytic aspartic acid sidechain (Asp274 in RK, Asp233 in ecXK, and Asp245 in ecGK), and the terminal carbon atom together. Once the required configuration is realized, activation of the terminal carbon atom (C5 in RK, C5 in ecXK, and C3 in ecGK) and nucleophilic attack can occur, leading to phosphoryl transfer. Elucidation of the precise roles of other RK active site residues in stabilizing the transition state will require further study.

Structural Data Deposition—Atomic coordinates and structure factors have been deposited to the Protein Data Bank (www.pdb.org; PDB Code: 3QDK).

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Abbreviations

AMPPNP	5'-adenylyl- β , γ -imidodiphosphate
ATP	Adenosine 5' – triphosphate
ecXK	<i>E. coli</i> xylulose kinase
ecGK	<i>E. coli</i> glycerol kinase
r.m.s.d	root-mean-square deviation
laXK	<i>L. acidophilus</i> xylulose kinase
SAD	Single wavelength Anomalous Diffraction
RK	ribulokinase

References

- Englesberg E, Anderson RL, Weinberg R, Lee N, Hoffee P, Huttenhauer G, Boyer H. L-Arabinose-sensitive, L-ribulose 5-phosphate 4-epimerase-deficient mutants of *Escherichia coli*. *J Bacteriol.* 1962; 84:137–146. [PubMed: 13890280]
- Englesberg E, Wilcox G. Regulation: positive control. *Annu Rev Genet.* 1974; 8:219–242. [PubMed: 4374117]
- Schleif R, Lis JT. The regulatory region of the L-arabinose operon: a physical, genetic and physiological study. *J Mol Biol.* 1975; 95(3):417–431. [PubMed: 168391]

4. Lee N, Bendet I. Crystalline L-ribulokinase from *Escherichia coli*. *J Biol Chem*. 1967; 242(9):2043–2050. [PubMed: 5336963]
5. Lee N, Englesberg E. Dual effects of structural genes in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 1962; 48:335–348. [PubMed: 14463540]
6. Lee N, Patrick JW, Barnes NB. Subunit structure of L-ribulokinase from *Escherichia coli*. *J Biol Chem*. 1970; 245(6):1357–1361. [PubMed: 4910050]
7. Patrick JW, Lee N. Subunit structure of L-arabinose isomerase from *Escherichia coli*. *J Biol Chem*. 1969; 244(16):4277–4283. [PubMed: 4896751]
8. Patrick JW, Lee N. Purification and properties of an L-arabinose isomerase from *Escherichia coli*. *J Biol Chem*. 1968; 243(16):4312–4318. [PubMed: 4878429]
9. Lee N, Patrick JW, Masson M. Crystalline L-ribulose 5-phosphate 4-epimerase from *Escherichia coli*. *J Biol Chem*. 1968; 243(18):4700–4705. [PubMed: 4879898]
10. Lee N, Gielow W, Martin R, Hamilton E, Fowler A. The organization of the *araBAD* operon of *Escherichia coli*. *Gene*. 1986; 47(2–3):231–244. [PubMed: 3549454]
11. Lee LV, Gerrata B, Cleland WW. Substrate specificity and kinetic mechanism of *Escherichia coli* ribulokinase. *Arch Biochem Biophys*. 2001; 396(2):219–224. [PubMed: 11747300]
12. Otwinowski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol*. 1997; 276:307–326.
13. Schneider TR, Sheldrick GM. Substructure solution with SHELXD. *Acta Cryst*. 2002; D58:1772–1779.
14. Fortelle EDL, Bricogne G. Maximum-Likelihood Heavy-Atom Parameter Refinement for Multiple Isomorphous Replacement and Multi-wavelength Anomalous Diffraction Methods. *Methods in Enzymology Macromolecular Crystallography Part A*. 1997; 276:472–494.
15. Abrahams JP, Leslie AG. Methods used in the structure determination of bovine mitochondrial F1 ATPase. *Acta Crystallogr D Biol Crystallogr*. 1996; 52(Pt 1):30–42. [PubMed: 15299723]
16. Potterton E, Briggs P, Turkenburg M, Dodson E. A graphical user interface to the CCP4 program suite. *Acta Crystallogr D Biol Crystallogr*. 2003; 59(Pt 7):1131–1137. [PubMed: 12832755]
17. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr*. 2004; 60(Pt 12 Pt 1):2126–2132. [PubMed: 15572765]
18. Jones TA, Zou J-Y, Cowan SW, Kjeldgaard M. Improved methods in building protein models in electron density map and the location of errors in these models. *Acta Crystallogr*. 1991; A47:110 – 119.
19. Brunger AT, Adams PD, Clore GM, Delano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Somonsom T, Warren GL. Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr*. 1998; D54:905–921.
20. SWISSPROT. Swiss Institute of Bioinformatics. Updated 2009. 24 May 2010. <[Http://Expasy.Org/Sprot/](http://Expasy.Org/Sprot/)>
21. Madej T, Gibrat JF, Bryant SH. Threading a database of protein cores. *Proteins*. 1995; 23(3):356–369. [PubMed: 8710828]
22. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. 1990; 215(3):403–410. [PubMed: 2231712]
23. Di Luccio E, Petschacher B, Voegtli J, Chou HT, Stahlberg H, Nidetzky B, Wilson DK. Structural and kinetic studies of induced fit in xylulose kinase from *Escherichia coli*. *J Mol Biol*. 2007; 365(3):783–798. [PubMed: 17123542]
24. Feese M, Pettigrew DW, Meadow ND, Roseman S, Remington SJ. Cation-promoted association of a regulatory and target protein is controlled by protein phosphorylation. *Proc Natl Acad Sci U S A*. 1994; 91(9):3544–3548. [PubMed: 8170944]
25. Feese MD, Comolli L, Meadow ND, Roseman S, Remington SJ. Structural studies of the *Escherichia coli* signal transducing protein IIAGlc: implications for target recognition. *Biochemistry*. 1997; 36(51):16087–16096. [PubMed: 9405042]

26. Feese MD, Faber HR, Bystrom CE, Pettigrew DW, Remington SJ. Glycerol kinase from *Escherichia coli* and an Ala65-->Thr mutant: the crystal structures reveal conformational changes with implications for allosteric regulation. *Structure*. 1998; 6(11):1407–1418. [PubMed: 9817843]
27. Emekli U, Schneidman-Duhovny D, Wolfson HJ, Nussinov R, Haliloglu T. HingeProt: automated prediction of hinges in protein structures. *Proteins*. 2008; 70(4):1219–1227. [PubMed: 17847101]
28. Bystrom CE, Pettigrew DW, Branchaud BP, O'Brien P, Remington SJ. Crystal structures of *Escherichia coli* glycerol kinase variant S58-->W in complex with nonhydrolyzable ATP analogues reveal a putative active conformation of the enzyme as a result of domain motion. *Biochemistry*. 1999; 38(12):3508–3518. [PubMed: 10090737]
29. Yeh JI, Charrier V, Paulo J, Hou L, Darbon E, Claiborne A, Hol WG, Deutscher J. Structures of enterococcal glycerol kinase in the absence and presence of glycerol: correlation of conformation to substrate binding and a mechanism of activation by phosphorylation. *Biochemistry*. 2004; 43(2):362–373. [PubMed: 14717590]
30. Ormo M, Bystrom CE, Remington SJ. Crystal structure of a complex of *Escherichia coli* glycerol kinase and an allosteric effector fructose 1,6-bisphosphate. *Biochemistry*. 1998; 37(47):16565–16572. [PubMed: 9843423]
31. DeLano, WL. Pymol. San Carlos, CA, USA: 2002.

Figure 1.

The biochemical pathway of arabinose utilization¹. The *araA*, *B* and *D* are the genes from *araBAD* operon that code for isomerase, kinase and epimerase proteins, respectively.

Figure 2. Ribbon representation of *Bacillus halodurans* L-ribulokinase. L-ribulose is shown in middle of the cavity in blue colored ball and sticks model. The small and large domains are labeled in black as I and II. All figures were made using PYMOL³¹ software.

Figure 3. Stick figure stereo drawing of L-ribulose (C: yellow; O: red) and interacting RK residues (C: green, O: red, N: blue). Electron density corresponding to the ligand from a sigmaa weighted $2|Fo| - |Fc|$ map contoured at 1σ is shown as a blue mesh. Intermolecular interactions are shown as dashed red lines with distances (Å).

Figure 4.

Ribbon stereo-drawing of the RK active site with ball-and stick representations of bound L-ribulose (C: light blue, O: red) and the modeled binding pose of ATP (C: cyan, N: dark blue, O: red, P: orange). Active site residues are shown as atomic stick figures (C: yellow, N: dark blue, O: red).

Figure 5.
 α -Carbon stereo drawing representation of side-view of RK (3QDK; blue), ecXK (2ITM; orange), ecGK (1GLE; magenta), and laXK (3LL3; green) with their respective domain II components superimposed in COOT¹⁷.

Figure 6.

(a) Modeling of D- and L- forms of ribulose in $2F_o - F_c$ sigmaa weighted omit map. L-ribulose in yellow color (C1–C5 positions labeled 1–5 in black color) fits better as compared to D-ribulose in magenta, at the existing electron density. The distances of modeled D-ribulose O1, O2 and O4 are greater than L-ribulose with residues Asp274, Lys208 and Glu329, shown in magenta and orange colored dashes and label, respectively, and (b) L-ribulose model in 2 different conformations. The conformation in yellow fits relatively better than orange in the electron density. However, L- ribulose conformation in orange color model is generated by rotation between C3–C4 axes to get C5 close to Asp274 (distances shown in orange dash and label). In this conformation, O4 goes relatively out of the density and also it does not suffices the electron density pointed as arrow ($\rightarrow$) sign. However, this putative conformation of L-ribulose at C4–C5 seems to be more competent and presumably generated during transition state.

Table 1

Data collection and refinement statistics for L-ribulokinase.

Data collection	
Space group	P2 ₁
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	56.58, 88.84, 230.52
$\alpha\beta\gamma$ (°)	90, 92.75, 90
Resolution (Å)	2.31 (2.35–2.31) ^a
<i>R</i> _{sym} or <i>R</i> _{merge}	0.06 (0.16)
<i>I</i> / σ <i>I</i>	13.4 (~5)
Completeness (%)	98.1 (87.8)
Redundancy	3.6 (2.7)
No. of molecule/asymmetric unit	4
Refinement	
Resolution (Å)	50–2.31
No. reflections	97059
<i>R</i> _{work} / <i>R</i> _{free}	0.22/0.27
No. of atoms	
Protein	16759
Water	395
Heterogens	19
<i>B</i> -factors (Average) (Å ²)	36
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.50
Ramachandran plot analysis	
Most favored region (additionally allowed) (%)	88.9 (10.3)
Generously allowed region (disallowed) (%)	0.4 (0.4)

^aValues for the highest-resolution shell are provided in parentheses.

Table 2

L-ribulose interactions with RK active site residues.

L-ribulose	[O1]	2.9Å	RK -A: GLU 329 [OE2]
	[O2]	3.0Å	A: LYS 208 [NZ]
	[O3]	2.4Å	A: ALA 96 [N]
	[O4]	3.4Å	A: ASP 274 [OD1]
	[O4]	2.7Å	A: ASP 274 [OD2]
[aliphatic portion:]		stacking	A: Trp 126 [sidechain]

Table 3

Sequence alignment of the ecGK, ecXK, and RK ATP binding motifs with highlighted catalytic residues (green).

GLPK_ECOLI	8	VALDQGT	15	243	IAGDQQAA	250
XYLB_ECOLI	3	IGIDLGTS	10	230	GGGDAAG	237
ARAB_BACHD	7	IGVDYGT	14	271	GNVDAHAA	278
		*	**		*	*