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In silico studies on the substrate specificity of an L-arabinose isomerase from *Bacillus licheniformis*

Ponnandy Prabhu [a], Marimuthu Jeya [b], Jung-Kul Lee [b,c,*]

[a] Department of Bioscience and Biotechnology, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, Republic of Korea
[b] Department of Chemical Engineering, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, Republic of Korea
[c] Institute of SK-KU Biomaterials, Konkuk University, 1 Hwayang-Dong, Gwangjin-Gu, Seoul 143-701, Republic of Korea

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ABSTRACT

L-Arabinose isomerase (BLAI) from *Bacillus licheniformis* was found to be active only with L-arabinose, unlike other L-arabinose isomerases (L-AIs) active with a variety of aldoses. Therefore, the differences in molecular interactions and substrate orientation in the active site of L-AIs have been examined and the residue at position 346 is proposed to be responsible for the unique substrate specificity of BLAI.

L-Arabinose is commonly found in nature as a component of biopolymers such as hemicellulose and pectin.[1] The enzyme L-arabinose isomerase (L-AI) permits the conversion of L-arabinose to L-ribulose, an intermediate in the pathway for L-arabinose utilization in many bacteria.[2] L-Ribose, an important precursor in the synthesis of anti-viral drugs is not available in nature at abundant levels.[3] One of the alternative possibilities to synthesize L-ribose is based on the enzymatic isomerization of L-ribulose.[4]

L-AIs have been reported from various sources of microorganisms.[5] All of these L-AIs show the ability to isomerize many aldo-hexoses (D-galactose or D-allose or D-mannose) or aldo-pentoses (D-ribose or D-xylose) in addition to their natural substrate, L-arabinose. Thus, broad substrate specificity is common among the bacterial L-AIs. Among those reported, the L-AI (BSTAI) from *Bacillus stearothermophilus* efficiently isomerizes both L-arabinose and D-galactose. However, *Bacillus licheniformis* L-AI (BLAI) differs from previously reported L-AIs in that it displays distinct substrate specificity, acting only on L-arabinose. Thus, study of substrate binding to the active site of BLAI would be informative in understanding the molecular mechanisms and interactions of this enzyme reaction.

The *araA* gene from *B. licheniformis* was cloned and heterologously expressed in *Escherichia coli* BL21(DE3) as described previously.[6] Substrate specificity of BLAI towards various aldoses was investigated at 250 mM. BLAI had a high preference only for L-arabinose, which is atypical of L-AIs. Other aldoses including D-galactose were ineffective as substrate (1% or less, compared with L-arabinose). The non-linear regression fitting of the Michaelis–Menten equation for the conversion of L-arabinose under standard assay conditions showed that k_{cat} = 12,455 min^{-1} and k_{cat}/K_m = 34 min^{-1} mM^{-1} for L-arabinose (Table 1). However, BSTAI had high activity with both L-arabinose (k_{cat}/K_m = 71.4 min^{-1} mM^{-1}) and D-galactose (k_{cat}/K_m = 8.4 min^{-1} mM^{-1}).[7]

Homology models of BLAI (Q65J10) and BSTAI (Q9S467) were constructed based on the X-ray crystal structure of *E. coli* L-AI (PDB entry 2ajtA).[8] The sequence identity between BLAI and BSTAI was 56%, while the sequence identities of BLAI and BSTAI towards the template (2ajtA) were 50% and 60%, respectively (Supplementary data, Fig. S1). The Ramachandran plots for local backbone conformation of each residue in the final models were produced by RAMPAGE.[9] In the BLAI model (Supplementary data, Fig. S2), 97.8% of residues were located within the allowed region, constituting 91.8% of the residues in the favorable region and 6% of the residues in the allowed region. Only 2.2% of the residues were located in the outlier regions of the Ramachandran plot. In BSTAI model (Supplementary data, Fig. S2), 92.2%, 6.2%, and 1.6% of residues were in the favorable, the allowed, and the outlier regions, respectively. The 1D–3D compatibility score (Profile-3D score) of BLAI and BSTAI were 175 and 213, respectively, compared with the expected maximum expected scores of 212 and 223. The root mean square deviation (RMSD) between the models and template was calculated by superimposing the BLAI and BSTAI models onto

* Corresponding author. Tel.: +82 2 450 3505.
E-mail address: jkrhee@konkuk.ac.kr (J.-K. Lee).

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Table 1
Kinetic properties of L-AIs from different organisms

Organism	k_{cat} (min^{-1})		k_{cat}/K_m (min^{-1} mM^{-1})		ΔG (kcal mol^{-1})	
	L-Arabinose	D-Galactose	L-Arabinose	D-Galactose	Docked with L-arabinose	Docked with D-galactose
Alicyclobacillus acidocaldarius[10]	1989	420	41.5	3.2	—	—
Geobacillus stearothermophilus T6[11]	2047	516	32.5	4	—	—
Thermoanaerobacter mathranii[12]	NR	NR	NR	NR	—	—
Thermotoga neapolitana[13]	6740	810	58	3.2	—	—
Thermotoga maritima[5]	2340	504	75	8.4	—	—
Lactobacillus plantarum[14]	671	111.5	15.5	1.6	—	—
Bacillus stearothermophilus[7]	2035	484	71.4	8.4	−41.9	−37.5
Bacillus licheniformis	12,455	ND	34	ND	−28.1	−5.6

the template crystal structure, for the reliability of the models. RMSD values of BLAI and BSTAI were found to be 0.51 Å and 0.40 Å, respectively, based on Cα atoms. This result indicates strong confidence in the generated models.

The catalytic site residues of 2ajtA (E306, E333, H350, and H450) are highly conserved in the same positions of both modeled structures; E306, E331, H348, and H447 in BLAI and BSTAI (Fig. 1). In the crystal structure of *E. coli* fucose isomerase (ECFI)[15] and ECAI,[8] C1 and C2 of the substrate have been reported to transfer protons via an enediol intermediate. During the aldose–ketose inter-conversion two hydrogen atoms are transferred via an ene–diol intermediate. It is highly evident, based on the active site residue of crystal structure of 2ajtA, that there are two glutamates (Fig. 1) functioning as catalytic residues in the BLAI and BSTAI models. They are located in the active site near the C1–C2 region of the substrate. The proton transfer is facilitated by two bases Glu-306 and Glu-331, corresponding to the mechanism suggested in ECAI and ECFI.

Superimposition of BLAI and BSTAI onto 2ajtA allowed determination of the catalytic sites. Figure 2 illustrates the docking model of different substrates into the active site pocket of BLAI and BSTAI. The best conformation of substrates inside the active site region of BLAI and BSTAI were retrieved for post-docking analysis. The substrate docking poses were screened based on the total docking energy. The selected docking pose was used to analyze the orientation of a substrate molecule in the active site region and the interactions of substrate molecule with the active site residues. When L-arabinose was docked into the active site pocket of BLAI or BSTAI, a hydrogen bond of 2.07 Å (BLAI) or 3.66 Å (BSTAI) between hydrogen in the C2 hydroxyl group of L-arabinose and oxygen of

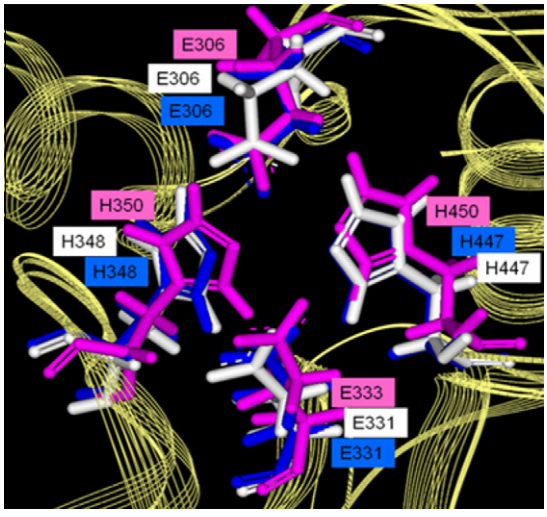

Figure 1. Superimposition of catalytic residues from *E. coli* L-AI (PDB, 2ajtA; in pink) onto *Bacillus licheniformis* L-AI (BLAI) in blue and *Bacillus stearothermophilus* L-AI (BSTAI) in white.

E306 was found (Fig. 2A and D). These interactions are evident in both models, suggesting that L-arabinose isomerization is efficiently catalyzed by both BLAI and BSTAI. Comparative analysis of the molecular interactions of D-galactose in the active site region of BLAI (Fig. 2B) and BSTAI (Fig. 2E) was also carried out. Clear interactions between the substrate (D-galactose) and the active site residues of BSTAI were indicated; where possible hydrogen bonds of 1.96, 2.0, and 1.99 Å were seen between hydrogen in the C1, C3, and C4 hydroxyl groups of D-galactose and the oxygen molecules of E306, E331, and E331, respectively (Fig. 2E). These hydrogen bonds would help to maintain the proper orientation of the C2 of the substrate with respect to E306 to allow efficient isomerization.

In contrast, in BLAI, no hydrogen bonding between C1 and C2 existed that could facilitate proper orientation of D-galactose (Fig. 2B) and D-ribose (Fig. 2C) inside the active site. Thus, the base-mediated proton transfer is prohibited, in agreement with the poor activity of BLAI for D-galactose and D-ribose. Ligand binding energy of BLAI and BSTAI was calculated. It was found to be −28.1 kcal/mol for BLAI with L-arabinose and was −41.9 kcal/mol for BSTAI. While D-galactose binding of BSTAI was stronger with the binding energy of −37.5 kcal/mol, rather BLAI was exhibiting a low D-galactose binding energy of −5.6 kcal/mol where no proper interaction was witnessed.

Upon multiple sequence alignment, glycine (G) was found at position 346 in other L-AIs except for BLAI having glutamine (Q) at the same position (Supplementary data, Fig. S1). Structural location of this residue in BLAI and BSTAI was assessed using the models. Position 346 is located exactly beneath the catalytic residue E331. Presence of glutamine in BLAI led to decrease in the size of substrate binding pocket which is evident from the distance between each catalytic amino acid and the metal ion (Supplementary data, Fig. S3). BSTAI has the smallest amino acid (G) at position 346, thus giving a bigger substrate binding pocket; which is helpful in accommodating bigger substrate like D-galactose. Substrate binding pockets of BLAI and BSTAI were further analyzed with L-arabinose and D-galactose as substrate (Fig. 3). It shows that BSTAI has much deeper and wider pocket than BLAI, which allowed both substrate L-arabinose (Fig. 3C) and D-galactose (Fig. 3D) into it, rather BLAI had narrow binding pocket which could facilitate L-arabinose interaction only (Fig. 3A), but not D-galactose (Fig. 3B). Orientation of L-arabinose in the binding pocket of both BLAI and BSTAI was found to be similar, but the orientation of D-galactose was different in both cases, where wider binding pocket of BSTAI facilitated proper orientation and thus binding with catalytic residues. It could not be seen with BLAI due to narrow binding pocket and thus no hydrogen bonding with catalytic amino acids.

In addition, docking of substrate D-galactose into the active site of BLAI required significant conformational alterations of the side chains of Arg-96, Leu-202, and Phe-264. Therefore, the residue Q346 might be involved in the narrow substrate specificity of BLAI. This proposed amino acid residue could be the potential target for further detailed mutational analysis and biochemical study to

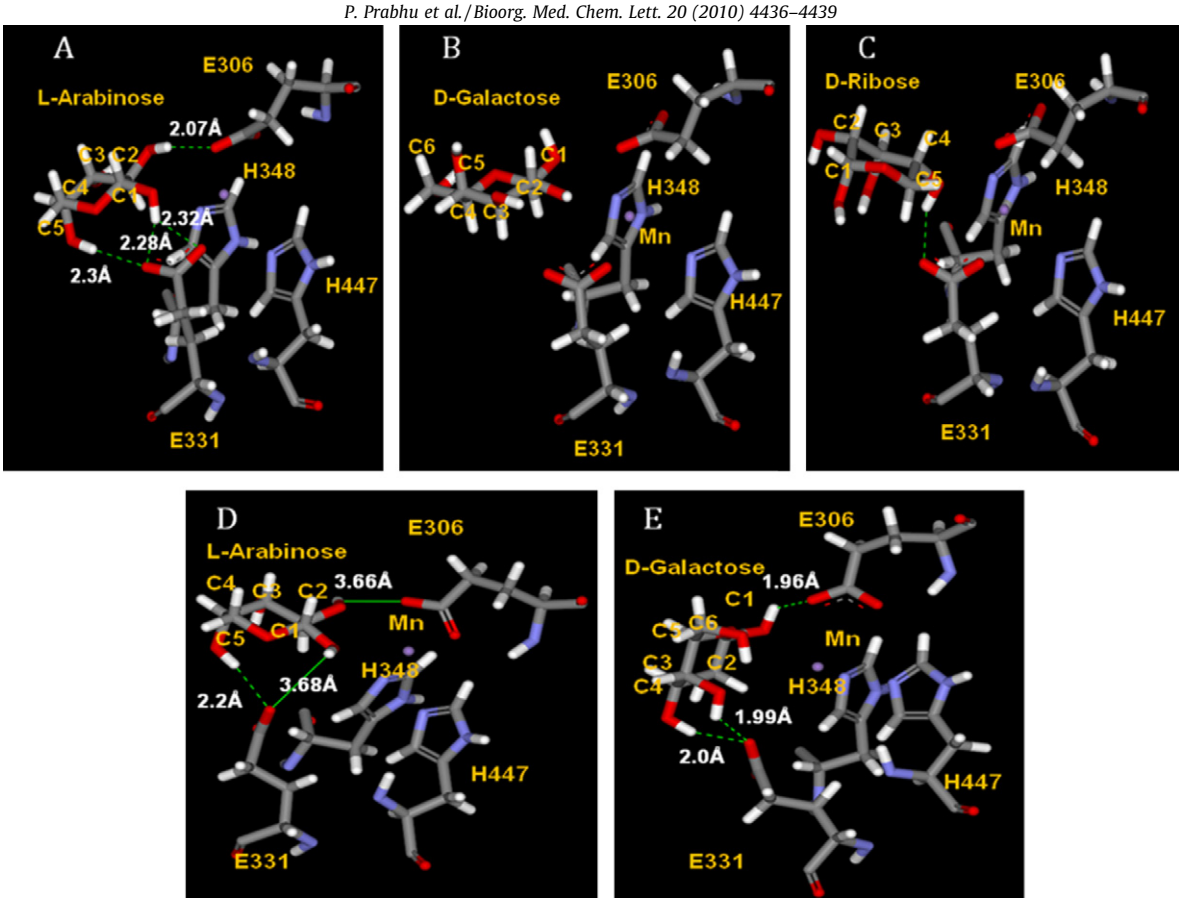

Figure 2. Docking of different substrates into active site of BLAI and BSTAI. (A) BLAI with L-arabinose; (B) BLAI with D-galactose; (C) BLAI with D-ribose; (D) BSTAI with L-arabinose and (E) BSTAI with D-galactose.

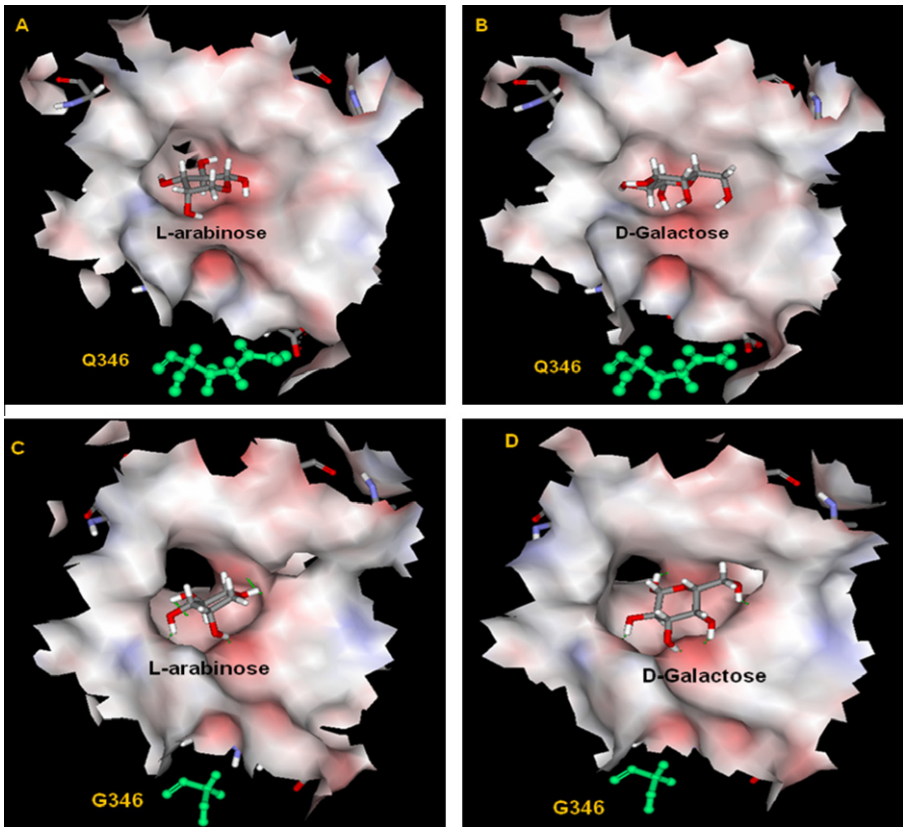

Figure 3. Molecular surfaces in the substrate binding pockets of BLAI and BSTAI. (A) BLAI with L-arabinose; (B) BLAI with D-galactose; (C) BSTAI with L-arabinose and (D) BSTAI with D-galactose. BSTAI has much deeper, wider pocket than BLAI, which facilitate binding of both substrates D-galactose and L-arabinose, rather BLAI had narrow binding pocket which could facilitate L-arabinose only.

modulate the substrate specificity of L-AIs. Additionally, it is evident from the substrate specificity constant (k_{cat}/K_m) that BLAI is specific only for L-arabinose (Table 1). It has negligible activity with D-galactose. Despite the similarity in the active site residues of BLAI and BSTAI, orientation of substrate inside the catalytic pocket emphasizes the differences in substrate specificity between BLAI and BSTAI. The present work constitutes the first step in a structure-based L-AI docking and post-docking analysis, designed to address substrate specificity issues.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.055.

References and notes

1. Hayn, M.; Klinger, R.W.S.; Steinmüller, H.; Sinner, M.; Esterbauer, H. *Bioconversion of Forest and Agricultural Plant Residues*, 1993, p 33.
2. Patrick, J.; Lee, N. *Methods Enzymol.* **1975**, *41*, 453.
3. Akagi, M.; Omae, D.; Tamura, Y.; Ueda, T.; Kumashiro, T.; Urata, H. *Chem. Pharm. Bull. (Tokyo)* **2002**, *50*, 866.
4. Ahmed, Z. *Electron. J. Biotechnol.* **2001**, 4.
5. Lee, D. W.; Jang, H. J.; Choe, E. A.; Kim, B. C.; Lee, S. J.; Kim, S. B.; Hong, Y. H.; Pyun, Y. R. *Appl. Environ. Microbiol.* **2004**, *70*, 1397.
6. Prabhu, P.; Tiwari, M. K.; Jeya, M.; Gunasekaran, P.; Kim, I. W.; Lee, J. K. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 283.
7. Rhimi, M.; Bejar, S. *Biochim. Biophys. Acta* **2006**, *1760*, 191.
8. Manjasetty, B. A.; Chance, M. R. *J. Mol. Biol.* **2006**, *360*, 297.
9. Lovell, S. C.; Davis, I. W.; Arendall, W. B., 3rd; de Bakker, P. I.; Word, J. M.; Prisant, M. G.; Richardson, J. S.; Richardson, D. C. *Proteins* **2003**, *50*, 437.
10. Lee, S. J.; Lee, D. W.; Choe, E. A.; Hong, Y. H.; Kim, S. B.; Kim, B. C.; Pyun, Y. R. *Appl. Environ. Microbiol.* **2005**, *71*, 7888.
11. Lee, D. W.; Choe, E. A.; Kim, S. B.; Eom, S. H.; Hong, Y. H.; Lee, S. J.; Lee, H. S.; Lee, D. Y.; Pyun, Y. R. *Arch. Biochem. Biophys.* **2005**, *434*, 333.
12. Jorgensen, F.; Hansen, O. C.; Stougaard, P. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 816.
13. Kim, B. C.; Lee, Y. H.; Lee, H. S.; Lee, D. W.; Choe, E. A.; Pyun, Y. R. *FEMS Microbiol. Lett.* **2002**, *212*, 121.
14. Chouayekh, H.; Bejar, W.; Rhimi, M.; Jelleli, K.; Mseddi, M.; Bejar, S. *FEMS Microbiol. Lett.* **2007**, *277*, 260.
15. Seemann, J. E.; Schulz, G. E. *J. Mol. Biol.* **1997**, *273*, 256.