Docking Study of Human Galactokinase Inhibitors

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Abstract

Galactosemia is a potentially lethal disorder caused by the deficiency of the enzyme galactose-1-phosphate uridylytransferase (GALT) within the Leloir pathway. Galactokinase (GALK) is the enzyme in Leloir pathway which converts α-D galactose to galactose 1-phosphate. The elevated level of galactose-1-phosphate, the product of GALK plays a major role in Galactosemia. Therefore the inhibition of GALK is a novel therapy for this disorder. Hence in the present study, we performed molecular docking of twenty inhibitors with different activity against galactokinase into the active site of galactokinase enzyme. The binding mode of these inhibitors was obtained using Surflex dock program interfaced in Sybyl-X2.0. The residues such as SER141, TYR109, ARG105, ARG228, TYR106, GLY346, GLY136, ASP86, ASP186 and SER142 found to interact with inhibitors.

Keywords: Galactokinase, Inhibition, Active Site, Enzyme

1. Introduction

In Leloir pathway, the conversion of β-D galactose to glucose-1-phosphate is facilitated by the action of four enzymes[1,2]. Galactokinase (GALK) is an enzyme which catalyzes the second step in the Leloir pathway which phosphorylates the α-D galactose to galactose 1-phosphate at the expense of one molecule of ATP[3]. A series of additional steps converts this galactose 1-phosphate to another simple sugar called glucose which is the main source of energy for most of the cells. Galactokinase belongs to the class of ATP-dependent enzymes known as GHMP superfamily and this enzyme is composed of two domains called N and C-terminal domain separated by a large cleft[4,5]. The deficiency of an enzyme galactose-1-phosphate uridylytransferase (GALT) within the Leloir pathway leads to rare inherited metabolic disorder called Galactosemia[6]. GALT is responsible for the conversion of galactose-1-phosphate and UDP-glucose to glucose-1-phosphate and UDP-galactose. GALK is an upstream of GALT in Leloir pathway, and the elevated level of galactose-1-phosphate, the product of GALK plays a major role in Galactosemia[7]. Therefore inhibitor molecule of human GALK would act to prevent the accumulation of gal-1-p and offer a novel entry therapy for this disorder.

In the present study, the identification of potent inhibitor molecule and its ability to bind within the galactokinase enzyme was identified by in silico approach, molecular docking. The molecules were docked into its binding site and its score and binding mode was obtained using Surflex dock module of SYBYL. The role of active site residues in human galactokinase have become understood recently. It was reported that aspartic and arginine residues in galactokinase are highly conserved play a vital role in galactokinase function[2,5]. The inhibitor molecules used possess favorable H-bond interaction with the SER141, TYR109, ARG105 and ARG228 and moderate interaction with TYR106, GLY346, GLY136, ASP86, ASP186 and SER142.

2. Materials and Methods

2.1. Ligand Preparation

The dataset comprising of twenty galactokinase inhibitors and its biological activity were taken from the literature[8]. The Ligand molecules were sketched using sketch molecule function in SYBYL[9]. The energy minimization of the molecules was performed using Tripos

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Table 1. Structures and biological activities (pIC$_{50}$) of galactokinase inhibitor

| Compound | Structure | Compound | Structure |
|----------|-----------|----------|-----------|
| 1        | ![Structure 1](image1.png) | 11       | ![Structure 11](image11.png) |
| 2        | ![Structure 2](image2.png) | 12       | ![Structure 12](image12.png) |
| 3        | ![Structure 3](image3.png) | 13       | ![Structure 13](image13.png) |
| 4        | ![Structure 4](image4.png) | 14       | ![Structure 14](image14.png) |
| 5        | ![Structure 5](image5.png) | 15       | ![Structure 15](image15.png) |
| 6        | ![Structure 6](image6.png) | 16       | ![Structure 16](image16.png) |
| 7        | ![Structure 7](image7.png) | 17       | ![Structure 17](image17.png) |
| 8        | ![Structure 8](image8.png) | 18       | ![Structure 18](image18.png) |

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force field and atomic charges were assigned using Gasteiger Huckel method. The structure and of all molecules are tabulated in Table 1.

2.2. Protein Preparation

The protein structure for docking was prepared using protein preparation tool in biopolymer module of SYBYL. The crystal structure of human galactokinase (1WUU) in complex with galactose and phosphoaminophosphonic acid - adenylate ester (ANP) was downloaded from PDB. The chain A of galactokinase was taken and the ligand molecules, metal ions and water molecules were removed from the structure. The hydrogen molecules and Gasteiger Huckel charge was added to the protein structure during preparation. The energy minimization was performed for 100 steps utilizing Tripos force field, Gasteiger Huckel charge and Powell method.

2.3. Molecular Docking

Molecular docking was performed utilizing Surflex dock module of SYBYL. 20 galactokinase inhibitors taken from Liu et al were docked into the binding site of human galactokinase (1WUU). The docking algorithm in surflex dock uses an idealized active site called protomol. The protomol is the representation of intended binding site to which the ligand molecules were docked. Two parameters, such as threshold and bloat, determine the extent of a protomol. The protomol was generated based on ligand inside the active site. Surflex dock uses an empirical scoring function to score the docked ligand conformation which takes into account several terms, including hydrophobic, polar, repulsive, entropic and solvation. To evaluate the docking results, the docking scores are expressed in terms of $-\log_{10}K_d$ units, where $K_d$ represents a dissociation constant of a ligand.

3. Results and Discussion

3.1. Validation of Surflex Dock

To validate the Surflex dock software, we have performed re-docking on the crystal structure of human galactokinase (1WUU) utilizing the co-crystallized ligand molecule phosphoaminophosphonic acid - adenylate ester (ANP) into the binding pocket of galactokinase. The Surflex score of 9.25 was obtained for ANP molecule and it forms H-bond with ARG37, SER79, GLY136, GLY138, SER140, SER141, SER142, ARG228 and GLY346. It was reported that if the RMSD of the best conformation is <2.0 Å from the bound ligand in the experimental crystal structure then the used scoring function is successful. Therefore, the docked mode of ANP was compared to the crystal structure of bound ligand-protein complex. The RMSD of the docked pose of ANP with the co-crystal ANP was found to be 1.452 Å which authenticates the accuracy of the software. The superimposition of docked pose of ANP with the co-crystal ANP is shown in Fig. 1.

3.2. Molecular Docking

Molecular docking was performed for 20 galactokinase inhibitors and 20 different conformations was generated for each molecule and the best conformation was

| Compound | Structure | Compound | Structure |
|----------|-----------|----------|-----------|
| 9        | ![Structure 9](image) | 19       | ![Structure 19](image) |
| 10       | ![Structure 10](image) | 20       | ![Structure 20](image) |
chosen based on surflex score and better interaction with active site residues. The score and H-bond interactions for all the molecules are tabulated in Table 2. We found that all the inhibitor molecules have favorable interaction with SER141, TYR109, ARG105 and ARG228 residues. In addition to these residues TYR106, GLY346, GLY136, ASP86, ASP186 and SER142 also had moderate interaction with the inhibitor molecules. The residues such as SER141, ARG228, GLY136, GLY346 and SER142 are found in interaction between galactokinase and ANP ligand. Hence these inhibitors are found to have similar binding site as ANP

| Compound | Surflex Score | Total no. of H-bonds | Residues involved in forming H-bond |
|----------|---------------|----------------------|-----------------------------------|
| 1        | 5.34          | 4                    | SER141, TYR109, ARG105            |
| 2        | 5.70          | 3                    | SER141, ARG105                    |
| 3        | 3.30          | 3                    | TYR109, ARG228, ARG105            |
| 4        | 5.39          | 2                    | SER141, ARG105                    |
| 5        | 4.51          | 5                    | ARG105, TYR109, SER141            |
| 6        | 4.04          | 2                    | SER141, SER142                    |
| 7        | 3.53          | 3                    | ARG105, TYR109, SER142            |
| 8        | 5.69          | 1                    | TYR109                            |
| 9        | 6.26          | 4                    | TYR109, SER141, ARG105            |
| 10       | 3.00          | 4                    | GLY346, ARG228, TYR109,           |
| 11       | 4.15          | 3                    | GLU174, TYR2236, ASP186           |
| 12       | 5.66          | 4                    | TYR109, SER141, ARG105            |
| 13       | 3.74          | 4                    | ARG228, TYR109, GLY346            |
| 14       | 4.98          | 4                    | ARG228, SER141, GLY346, GLY136    |
| 15       | 5.34          | 8                    | ARG228, ARG105, TYR109,           |
| 16       | 6.01          | 6                    | ARG141, ARG228, ARG105, TRP106    |
| 17       | 5.96          | 3                    | ARG105, ARG228, SER141            |
| 18       | 6.00          | 3                    | ARG105, SER141, ARG228            |
| 19       | 4.34          | 3                    | ARG105, ASP83                     |
| 20       | 5.52          | 5                    | ARG105, TYR109, SER141            |
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4. Conclusion

The inhibition of human galactokinase would prevent the accumulation of galactose-1-phosphate and offer a novel entry therapy for Galactosmia. Hence, in this study the docked pose of galactokinase inhibitors was obtained through molecular docking approach. These inhibitors bind well within the binding site of galactokinase and shows strong H-bond interaction with the active site residues such as SER141, TYR109, ARG105, ARG228, TYR106, GLY346, GLY136, ASP86, ASP186 and SER142.

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