Molecular Docking Analysis of Sorbitol Dehydrogenase Using Ligandfit Algorithm

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Abstract

Diabetic retinopathy is a major cause of visual impairment worldwide. The polyol pathway is a two step metabolic process in which the enzyme aldose reductase reduces glucose to sorbitol using NADPH as a cofactor; Sorbitol is then metabolized to fructose by sorbitol dehydrogenase (SORD) that uses NAD^+ as cofactor. Human sorbitol dehydrogenase is a tetramer of identical, catalytically active sububits. Recent evidence suggests that the inhibition of both sorbitol and fructose is required to achieve beneficial effects in diabetic retinopathy. In our present study the molecular docking analysis of sorbitol dehydrogenase was carried out using Accelyr's Discovery studio software (uses LIGANDFIT algorithm). The binding affinity of several ligands to the target protein was calculated based on the dock scores obtained. It was found that out of 210 compounds 21 ligands docked to SORD whereas 17 ligands failed to dock and 59 ligands were found to be without hits. Among the 21 ligands, Dithiodialanine had greater binding affinity with the receptor thereby it can be recommended as a drug for the treatment of diabetic retinopathy.

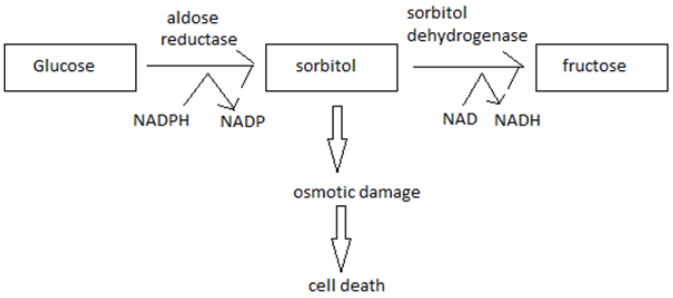
Keywords: Diabetic retinopathy, Sorbitol Dehydrogenase, Molecular Docking, LIGANDFIT.

1. Introduction

Diabetic retinopathy is one of the most important microvascular complications in both forms of diabetes mellitus. It has been considered as the major cause of visual impairment worldwide [1-4]. Many biochemical mechanisms have been proposed to explain the structural and functional abnormalities associated with diabetic retinopathy. These mainly include (i) enhanced glucose flux through the polyol pathway (ii) Increased intracellular formation of advanced glycation end products (AGE) (iii) Activation of protein kinase C (iv) stimulation of the hexosamine pathway [5].

The polyol pathway is a two step metabolic process in which the enzyme aldose reductase reduces glucose to sorbitol using NADPH as a cofactor; Sorbitol is then metabolized to fructose by sorbitol dehydrogenase (SORD) that uses NAD^+ as cofactor [Figure 1]. The fructose becomes phosphorylated to fructose 3 phosphate which is broken down to 3-deoxyglucosome; both components are glycosylating agents that enter in the formation of advanced glycation end products [6,7]. Since sorbitol is impermeable to cellular membranes it accumulates within the cell. This imbalance resulting in the acculmulation of sorbitol leads to several abnormalities in cellular metabolism and hence contributes to the development of diabetic microvascular complications [8, 9]. Polyol pathway is one of the important pathways that occurs because of hyperglycemic conditions and the products of this pathway alters the equilibrium of cofactors and hence generates cellular stress [10]. Inhibition of first enzyme (aldose reductase) of the polyol pathway prevents diabetic retinopathy in rodent models at a low success rate [11, 12].

Fig.1 *Mechanism of Polyol Pathway*



It is thought that this poor performance was due to the insufficient inhibition of the polyol pathway in human tissue [13]. Recent evidence suggests that the inhibition of both sorbitol and fructose is required to achieve beneficial effects in diabetic retinopathy [14].

Therefore in our study we have chosen the sorbitol dehydrogenase (SORD) as the target protein for analyzing the binding efficiency of various ligands to SORD. Some researchers have reported that SORD is one of the genetic factors associated with diabetic retinopathy [15].

2. Materials and methods

2.1 Ligand preparation

A total of 210 ligands were used for this study. The three dimensional structures of these ligands were downloaded in .sdf format from pubchem database. To these ligands the hydrogen bonds were added and energy minimization was carried out using CHARMm force field. Lipinski rule of 5 was calculated for the ligands used in this study.

2.2 Protein selection

The PDB structure of sorbitol dehydrogenase chosen for our study was downloaded from RCSB protein databank (PDB id: 1PL6). SORD is a tetramer [16-18] (dimer of identical dimmers (dimer AD and dimer BC) [19]) with each subunit consisting of 356 aminoacids (38kda) and one catalytic zinc atom [20,21]. The PDB structure chosen has a good resolution of 2.00A^{o} and R factor 0.183.

2.3 Protein preparation

The active site of the protein molecule was analyzed and it is defined in a cavity size of 3475 point units which were followed by site sphere definition. In our study site 1 was chosen as the binding site. The water molecules were removed from the target protein. Like the preparation of ligands for the target protein also the energy minimization was carried out using CHARMm force field which is explained by the equation

E = Eb + Eq + Ef + Ew + Evdw + Eel + Ehb + Ecr + Ecj

where E = total energy; Eb = Bond potential energy ; Eq & Ef = bond angle potential energy ; Ew = torsion energy ; Evdw = vanderwaals interaction energy; Eel = electrostatic potential energy ; Ehb = hydrogen bond energy ; Ecr = energy constraints ; Ecj = energy function.

2.4 Docking study

The docking method used for our study is LIGANDFIT (Accelyr's Discovery Studio 2.0). LIGANDFIT is based on a cavity detection algorithm which allow us to virtually screen a database of compounds and predict the strongest binders based on various scoring function. For our study the molecular docking analysis of sorbitol dehydrogenase with ligands was carried out using Dreiding parameter. In this energy grid parameter the Gasteiger charging method is employed to calculate the partial charges of target protein and ligand. The energy grid extension was set to 5.0A° and the conformation search number of Monte carlo trial was set to '0'. Also the number of poses for ligands in receptor cavity was set to 10 [22]. Apart from these, other input parameters for docking were set as default options. Then the ligands were docked into the active site of the protein using Libdock procedure. The final energy refinement of the ligand pose (or) pose optimization in LIGANDFIT occurs by Broyden-Flecher Gold Farbshanno (BFGS) method. The docked poses were minimized using CHARMm force field and evaluated with a set of scoring functions.

3. Result and Discussion

Human sorbitol dehydrogenase is a tetramer of identical, catalytically active sububits. SORD 'A' chain was analyzed for all the possible binding sites. Ten active sites were found from the receptor according to the volume of their binding cavity. Among the ten active sites site 1 was chosen for our study as it is evident that in the apo and NAD+ complex catalytic zinc of SORD is coordinated by Histidine, Cystine, Glutamic acid and a water molecule which is found in site 1 of SORD. As a result, docking was performed for all 210 ligands in site 1 of SORD. Best poses of ligands were analyzed for different energy parameters using scoring functions.

3.1 Scoring functions

In LIGANDFIT, the scoring function is used to predict the binding affinity of one ligand to the receptor molecule. The determination of the ligand binding affinity was calculated using Ligscore 1, Ligscore 2, PLP1, PLP2, JainScores and Dockscores.

3.2 Dock score

Docking was separated from general ligand scoring. Internal scoring function is very popular which is used by Ligandfit algorithm. Dock score, to select and return a maximum of 10 dissimilar poses for each compound. Dock score is a simple force field based scoring function [23]. It is the sum of the ligand/ protein interaction energy and the internal energy of the ligand which is used in

grading the energy [24]. Though 10 poses were returned by Ligandfit, the highest dock score pose was used for post docking scoring. Scoring was performed employing a set of scoring functions as implemented in Discovery studio software.

Out of 210 compounds, 21 ligands docked to site 1 of SORD whereas 17 ligands failed to dock and 59 ligands were found to be without hits. The number of conformation poses, dpmi value, dock score, Ligscore1, Ligscore 2, PLP1, PLP2, Jain scores for all the docked 21 ligands are given in. The highest dock score 30.528 on molecular interaction with SORD was found with the drug molecule "DITHIODIALANINE" (Table 1).

Table.1 *The dock scores of ligand DITHIODIALANINE with SORD*

	Conformation pose	dpmi	ligscore1	ligscore 2	PLP1	PLP2	JAIN Scores	PMF	Dock score
Dithiodialanine	1	1.88	4.04	5.07	62.46	62.24	-0.6	115	30.528
	2	1.94	4.04	5.07	62.34	62.27	-0.53	112.7	30.524
	3	1.7	4.05	5.09	63.32	61.63	-0.65	111.5	30.456
	4	1.7	4.14	5.09	64.17	63	-0.64	109.8	30.423
	5	1.79	4.12	5.12	62.79	63	-0.61	114.7	29.519
	6	1.66	3.06	4.6	55.25	51.51	-1.31	87.72	29.087
	7	1.93	3.08	4.59	55.02	51.46	-1.32	86.6	29.083
	8	1.66	3.08	4.6	55.25	51.5	-1.32	86.94	29.077
	9	1.98	3.72	4.9	60.46	61.95	-0.35	113.3	28.909
	10	1.86	3.89	4.97	61.51	62.7	-0.14	114.8	28.842

The two dimensional structure of Dithiodialanine is shown in Figure 2. The binding of ligand Dithiodialanine to SORD target protein is shown in Figure 3. The ligand interacts with all amino acids present in SORD A chain, among which GLU 155 and THR 121 is hydrogen bonded with the ligand Dithiodialanine at a distance of 2.011 A° and 2.299 A° respectively [Figure 4]. The attributes are:

DITHIODIALANINE H25: A:GLU 155 OE2
A:THR 121 HG1: DITHIODIALANINE O4

Fig.2 *2D structure of Dithiodialanine.*

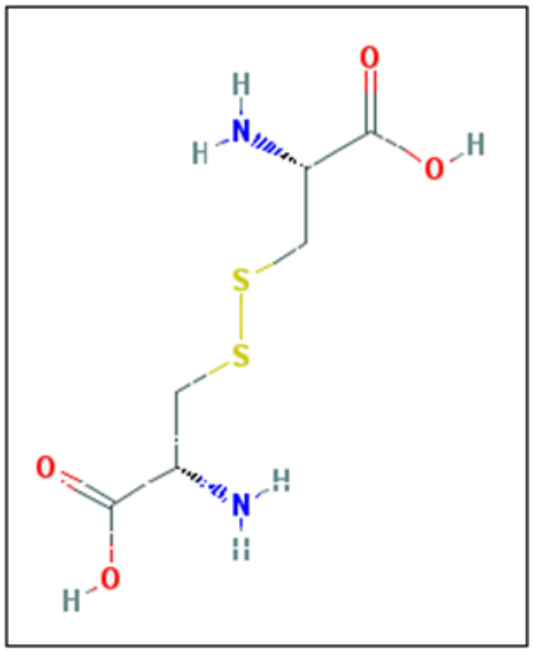


Fig.3 *Ligand DITHIODIALANINE bound to SORD target protein (stick representation).*

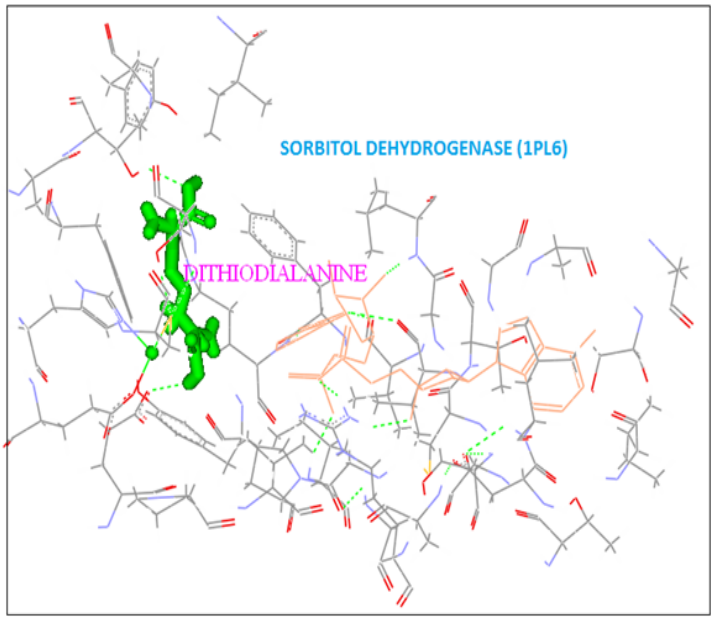


Fig.4 *Amino acids GLU 155 and THR 121 of SORD protein interacting with ligand Dithiodialanine at a distance of 2.011 A° and 2. 299 A° respectively.*

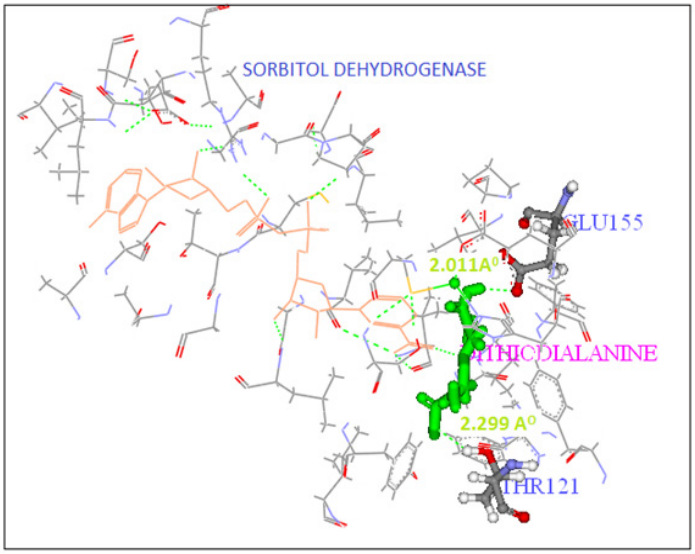
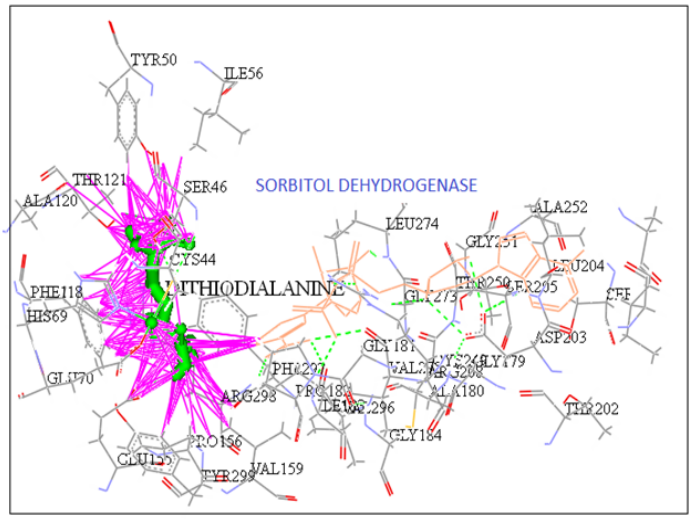


Fig.5 *Non bonded interaction (contacts) between ligand Dithiodialanine and amino acids in SORD*



LIGAND DITHIODIALANINE HAS NON BONDED INTERACTION (CONTACTS) WITH HIS69, PHE 118, ARG 298, GLU155, PRO 156, VAL 159, SER 46, TYR 50, ILE 56, THR 121, CYS 44, TYR 299, (CONTACTS SHOWN IN PINK DOTTED LINES)

Also apart from hydrogen bond interaction Dithiodialanine shows non bonded interaction (contacts) with amino acids HIS69, PHE 118, ARG 298, GLU155, PRO 156, VAL 159, SER 46, TYR 50, ILE 56, THR 121, CYS 44, TYR 299 [Figure 5].

All compounds docked with all 10 conformation poses except 'Azathioprine' drug molecule which docked with only pose 1 and pose 2. Also Azathioprine molecule showed the lowest dock score of 1.16. Similarly 17 molecules failed to dock to target protein as all of their 170 poses were rejected by dockscore filtering. For the remaining 59 compounds the ligands internal energy was above the threshold (1e+004). Therefore these compounds did not give hits.

As a result, 1138 poses (113 ligands) were rejected by dockscore filtering and 202 poses (21 ligands) were docked to site 1 of SORD A chain [Table 2]. Therefore it is concluded that the inhibition of sorbitol dehydrogenase is beneficial in delaying the onset of diabetic retinopathy.

Table.2 *The number of failed and docked ligands*

Total Ligands	210 (2100 poses)
Total Docked Ligands	21 (202 poses)
Failed Ligands	17 (170 poses)
Ligands without hits	59 (590 poses)
Total ligands rejected	113 (1138 poses)

We also extended our study by inhibiting the aldose reductase, the first rate limiting enzyme of polyol pathway with all 210 compounds used in this study using LIGANADFIT algorithm and found that this same drug molecule "DITHIODIALANINE" is found to inhibit the aldose reductase enzyme with a high dock score of 31.524 (Unpublished data). Therefore the drug molecule Dithiodialanine may be recommended to act upon the enzymes of the polyol pathway and hence can be used in the treatment of diabetic retinopathy.

4. References

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