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Docking studies on Aldose reductase for diabetic retinopathy

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Abstract: Aldose reducutase, is an enzyme that is normally present in many parts of the body and catalyzes one of the steps in the sorbitol (polyol) pathway that is responsible for fructose formation from glucose. The main mercial existed drugs, has potential side effects on individual patients. In this study, we have taken some of the naturally existing drugs such as flavonoids (Quercitin and Narningin) and marketly existing drugs Tolerostat and fiderostat were performed docking studies on Aldose reducutase enzyme with different docking algorithms like Cdocker, Ligand fit and Libdock. Docking studies were confirmed that present natural drugs have higher binding affinity and least energy, which internally helps more precisely binding with the target.
Key words: Aldose reductase, Diabetis retinopathy, Drug derivatives, flavonoids, docking.

Introduction

Aldose reductase activity increases as the glucose concentration rises in diabetes in those tissues that are not insulin sensitive, which include the lenses, peripheral nerves and glomerulus. Sorbitol does not diffuse through cell membranes easily and therefore accumulates, causing osmotic damage which leads to retinopathy and neuropathy. In diabetic retinopathy new blood vessels form at the back of the eye, they can bleed (hemorrhage) and blur vision. It will leave just a few specks of blood or spots. So these spots are often followed within a few days or weeks by a much greater leakage of blood, which blurs vision.

Diabetic cataract formation follows an increase in sugars in the lens. The excess sugar within the lens is reduced by aldose reductase to its alcohol, but the lens capsule is relatively impermeable to sugar alcohols. Because of the excess sugar alcohol (polyol), the lens imbibes water, causing osmotic imbalance. Eventually, increased sodium and decreased potassium levels and decreased glutathione levels lead to cataract formation. Topical administration of aldose reductase inhibitors have been shown to prevent the cataract in rats.

Diabetic retinopathy is a microvascular complication of diabetes mellitus and is a significantcause of new-onset blindness. Diabetic macular changes in the form of yellowish spots and fullor partial thickness extravasations through the retina were observed for the first time by Eduard Jäger. In 1855, he published "Beiträge zur Pathologie des Auges" where he included his fundus paintings. Jaeger's findings were controversial until 1872, when Edward Nettleship published his seminal paper on "Oedema or cystic disease of the retina", providing the first histopathological proof of "cystoid degeneration of the macula" in patients with diabetes. In1876, Wilhelm Manz described the proliferative changes occurring in diabetic retinopathy and the importance of tractional retinal detachments and vitreous haemorrhages. However, it was not until 1943 that the work of Arthur James Ballantyne provided evidence that diabetic retinopathy represents a unique form of vascular disease. A number of multi-centred clinical trials during the last ten years have contributed substantially to the understanding of the natural history of diabetic retinopathy and have established the value of intensive glycaemic control in reducing both the risk of onset and the progression of diabetic retinopathy[1].

Diabetic retinopathy (DR) is a common diabetes complication that affects the small blood vessels of the retina. It remains one of the leading causes of vision loss despite the availability of effective treatment. In the early stages, known as non-proliferative DR, the blood vessels of the retina swell and leak fluid. This stage is not usually associated with visual impairment and there are no symptoms. As the disease progresses (known as proliferative DR) abnormal blood vessels grow on the surface of the retina and, without treatment, these can bleed causing cloudy vision or blindness[2]. Abnormal fibrous tissue may also develop, leading to retinal detachment and severe vision loss. Blurred central vision may occur when the macula swells from leaking fluid (called macular oedema) or due to macular ischaemia from poor perfusion consequent upon perifoveal capillary loss. It is estimated that about 133,900 Australians aged 55 or more had diabetic retinopathy in 2004, which represents 2.8% of that population (AIHW 2005). The prevalence of DR was greater in the older age groups. Published results from the Diabetes, Obesity and Lifestyle Study reported that the prevalence of DR was similar in men and women. Also, any form of DR occurred in 22% of those with known type 2 diabetes and 6% in those who had not previously been diagnosed[3].

The study addressed the role for aldose reductase (AR) in retinal oxidative stress and vascular endothelial growth factor (VEGF) over expression in early diabetes, and high glucose induced oxidative stress in retinal endothelial cells. In vivo experiments were performed on control rats and diabetic rats treated with or without low or high dose of the AR inhibitor (ARI) fidarestat. (In vitro studies were performed on bovine retinal endothelial cells (BREC) cultured in either 5 or 30 mol/l glucose with or without 1 mol/l fidarestat. Intracellular reactive oxygen species were assessed using the 5-(and-6)-chloromethyl-2, 7 dichlorodihydrofluorescein diacetate (H2DCFDA) probe and flow cytometry. Both low and high doses of fidarestat (i.e., the doses that partially and completely inhibited sorbitol pathway hyperactivity) arrested diabetes induced retinal lipid peroxidation. This was achieved due to upregulation of the key antioxidative defense enzyme activities rather than changes in reduced glutathione, oxidized glutathione, ascorbate and dehydroascorbate concentrations, and the glutathione and ascorbate redox states. Diabetes-associated 2.1-fold VEGF protein over expression (enzyme-linked immunosorbent assay; ELISA) was dose-dependently prevented by fidarestat, whereas total VEGF mRNA and VEGF-164 mRNA (RTPCR) abundance were not affected by either diabetes or the ARI. In BREC, fidarestat corrected hyperglycemia induced increase in H2DCFDA fluorescence but not oxidative stress caused by three different pro-oxidants in normoglycemic conditions. In conclusion, increased AR activity contributes to retinal oxidative stress and VEGF protein over expression in early diabetes. The findings justify the rationale for evaluation of fidarestat on diabetic retinopathy[4].

Aldose reductase (ALR2) is thought to be involved in the pathogenesis of various diseases associated with diabetes mellitus, such as cataract, retinopathy, neuropathy, and nephropathy. However, its physiological functions are not well understood. We developed mice deficient in this enzyme and found that they had no apparent developmental or reproductive abnormality except that they drank and urinated significantly more than their wild-type littermates. These ALR2-deficient mice exhibited a partially defective urine-concentrating ability, having a phenotype resembling that of nephrogenic diabetes insipidus[5].

Recent data obtained from the Diabetes Control and Complications Trial clearly indicate that intensive insulin treatment effectively delays the onset and slows the progression of longterm diabetic complications in patients with insulin-dependent diabetes mellitus (IDDM) b (Diabetes Control and Complications Trial Research Group, 1993). Nevertheless, even with the best clinical management available at present, it is practically impossible to maintain normoglycemia at all times throughout the life of diabetic individuals. Accordingly, chemical agents that effectively halt the hyperglycemic injury in diabetic patients would be of great clinical importance[6].

The present study is designed to detect potential variants in the putative promoter and encoding regions and to determine their potential association with early diabetic retinopathy in adolescents with type 1 diabetes. A novel polymorphism in the promoter region of the aldose reductase gene was identified, which was located at C (–106) T. The frequencies of homozygote CC and C alleles were significantly higher in those with retinopathy compared with those without retinopathy (P =0.0035 and 0.005, respectively). We also confirmed that the Z-2 allele (2) was strongly associated with retinopathy among patients ($P < 0.0005$). The two polymorphisms are in strong linkage disequilibrium (x2 test, $P = 0.001$). The C allele and the Z-2 allele in the promoter region of the aldose reductase gene increase the risk of diabetic retinopathy in type 1diabetic adolescents[7-11].

The main aim of the study is to find the natural existing drug which is the alternative for commercial existed drugs, has potential side effects on individual patients The main objective of our studies is to find the natural existing drug which is the alternative for commercial existed drugs, has potential side effects on individual patients. We have taken some marketly existing drugs (fiderostat and Tolerostat) and natural compounds flavonoids,quercitin, and performed docking studies on Aldose reducutase enzyme with different docking algorithms like Cdocker, Ligand fit and Libdock. Our docking studies were confirmed that present natural drugs have higher binding energy which internally helps more precisely binding with the target.

Materials and Methods

Data Base:

1. Swiss-Prot:

Swiss-Prot is a manually curated biological database of protein sequences.

2. Protein Data Bank:

The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids.

3. PubChem:

PubChem is a database of chemical molecules.

4. DrugBank:

The Drug Bank database available at the University of Alberta is a bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, pathway) information.

Tools:

1. SOPMA:

SOPMA is a secondary structure prediction method. SOPMA (Self-Optimized Prediction Method with Alignment) is an improvement of SOPM method.

2. **Protoparam**:

Protparam is used to compute molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half life, instability index, aliphatic index and grand average of hydropathicity.The protein can either be specified as s Swiss Prot/Tr EMBL accession number or ID or in form of raw sequence. It is used to analyse the given protein sequence and to retrieve the physical and chemical parameters for a particular protein sequence.

3 Blast:

A heuristic approach that approximates the Smith-Waterman algorithm. **.** BLAST is actually a family of programs (all included in the blastall executable). The following are some of the programs Blast BLAST searches for high scoring sequence alignments between the query sequence and sequences in the database using

Web Based Servers:

Rapper (Ramachandran plot analysis):

RAPPER is an ab initio conformational search algorithm for restraint-based protein modelling. It has been used for all-atom loop modelling whole protein modelling under limited restraints comparative modelling

ab initio structure prediction, structure validation and experimental structure determination with X-ray and nuclear magnetic resonance spectroscopy

Softwares used

Bioedit, Chemsketch, Accelerys, Gold (Genetic optimization for Ligand docking), Admet:

Molecular Modelling:

Molecular modeling is a collective term that refers to theoretical methods and computational techniques to model or mimic the behavior of molecules. The techniques are used in the fields of computational chemistry, computational biology and materials science for studying molecular systems ranging from small chemical systems to large biological molecules and material assemblies. The simplest calculations can be performed by hand, but inevitably computers are required to perform molecular modeling of any reasonably sized system. The common feature of molecular modeling techniques is the atomistic level description of the molecular systems; the lowest level of information is individual atoms (or a small group of atoms). This is in contrast to quantum chemistry (also known as electronic structure calculations) where electrons are considered explicitly. The benefit of molecular modeling is that it reduces the complexity of the system, allowing many more particles (atoms) to be considered during simulations.

Molecular mechanics is one aspect of molecular modeling, as it is refers to the use of classical mechanics/Newtonian mechanics to describe the physical basis behind the models. Molecular models typically describe atoms (nucleus and electrons collectively) as point charges with an associated mass. The interactions between neighbouring atoms are described by spring-like interactions (representing chemical bonds) and van der Waals forces. The Lennard-Jones potential is commonly used to describe van der Waals forces. The electrostatic interactions are computed based on Coulomb's law. Atoms are assigned coordinates in Cartesian space or in internal coordinates, and can also be assigned velocities in dynamical simulations. The atomic velocities are related to the temperature of the system, a macroscopic quantity. The collective mathematical expression is known as a potential function and is related to the system internal energy (U), a thermodynamic quantity equal to the sum of potential and kinetic energies. Methods which minimize the potential energy are known as energy minimization techniques (e.g., steepest descent and conjugate gradient), while methods that model the behaviour of the system with propagation of time are known as molecular dynamics.

$$E = E_{bonds} + E_{angle} + E_{dihedral} + E_{non-bonded}$$

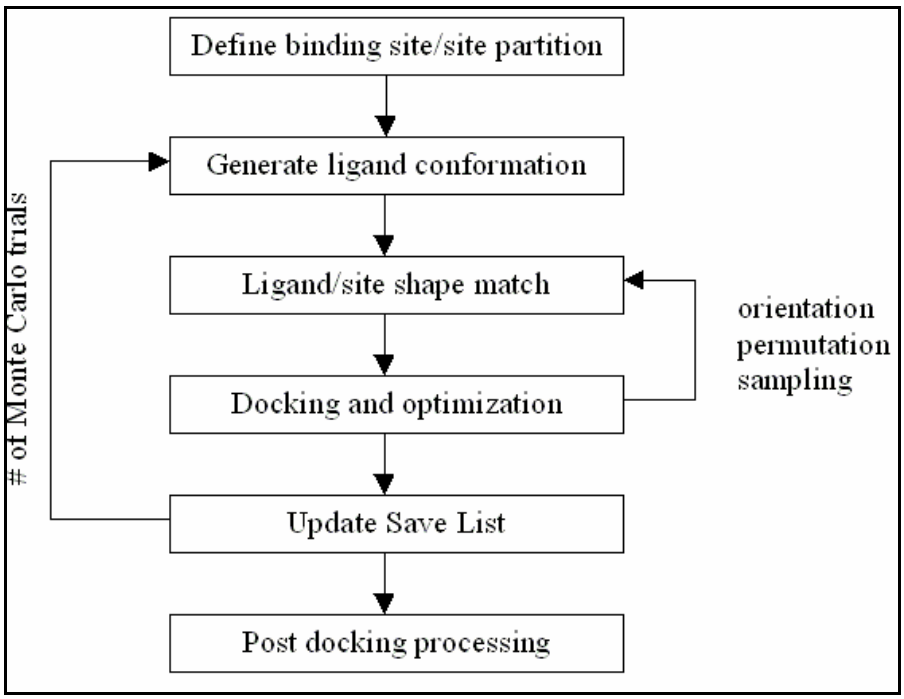
$$E_{non-bonded} = E_{electrostatic} + E_{vanderWaals}$$

This function, referred to as a potential function, computes the molecular potential energy as a sum of energy terms that describe the deviation of bond lengths, bond angles and torsion angles away from equilibrium values, plus terms for non-bonded pairs of atoms describing van der Waals and electrostatic interactions. The set of parameters consisting of equilibrium bond lengths, bond angles, partial charge values, force constants and van der Waals parameters are collectively known as a force field. Different implementations of molecular mechanics use slightly different mathematical expressions, and therefore, different constants for the potential function. The common force fields in use today have been developed by using high level quantum calculations and/or fitting to experimental data. The technique known as energy minimization is used to find positions of zero gradient for all atoms, in other words, a local energy minimum. Lower energy states are more stable and are commonly investigated because of their role in chemical and biological processes. A molecular dynamics simulation, on the other hand, computes the behavior of a system as a function of time. It involves solving Newton's laws of motion, principally the second law, F = ma. Integration of Newton's laws of motion, using different integration algorithms, leads to atomic trajectories in space and time. The force on an atom is defined as the negative gradient of the potential energy function. The energy minimization technique is useful for obtaining a static picture for comparing between states of similar systems, while molecular dynamics provides information about the dynamic processes with the intrinsic inclusion of temperature effects.

Molecules can be modeled either in vacuum or in the presence of a solvent such as water. Simulations of systems in vacuum are referred to as gas-phase simulations, while those that include the presence of solvent molecules are referred to as explicit solvent simulations. In another type of simulation, the effect of solvent is estimated using an empirical mathematical expression; these are known as implicit solvation simulations.

Molecular modeling methods are now routinely used to investigate the structure, dynamics and thermodynamics of inorganic, biological, and polymeric systems. The types of biological activity that have been investigated using molecular modeling include protein folding, enzyme catalysis, protein stability, conformational changes associated with biomolecular function, and molecular recognition of proteins, DNA, and membrane complexes.

Flowchart with the major steps of the Ligand Fit algorithm:



Graphical representation of amino acid composition is presented below:

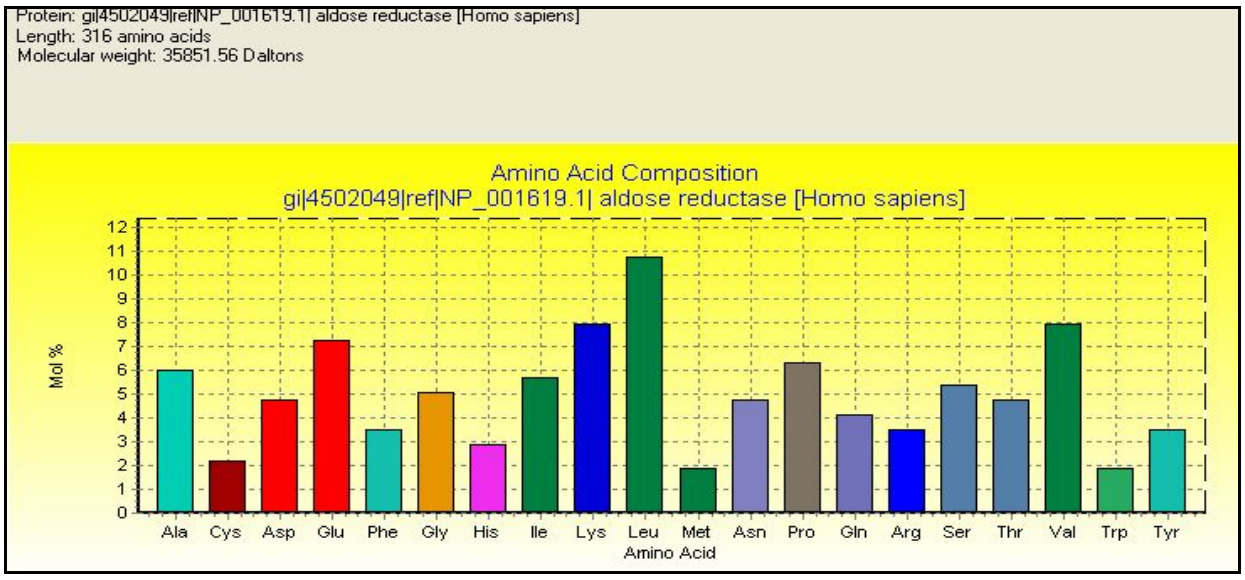
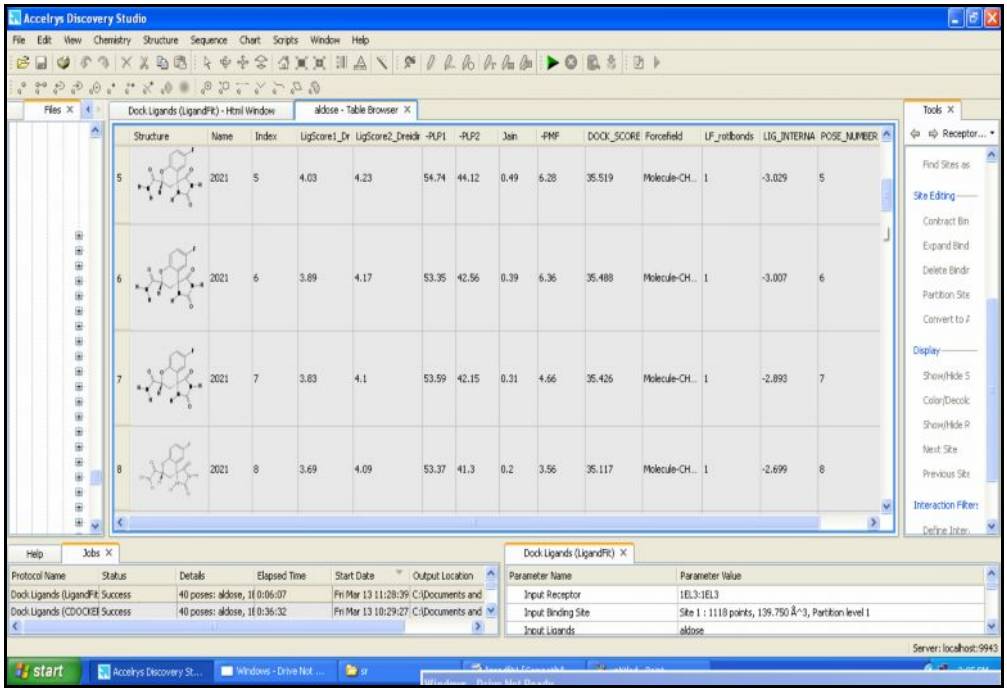


Figure 1: The above graph is showing amino acid composition of Query sequence, The amino acid LEU is present highest percentage compared to other residues.

Visulization of ligand fit energy values is shown below:



Interaction of ligand molecule with active site is shown below:

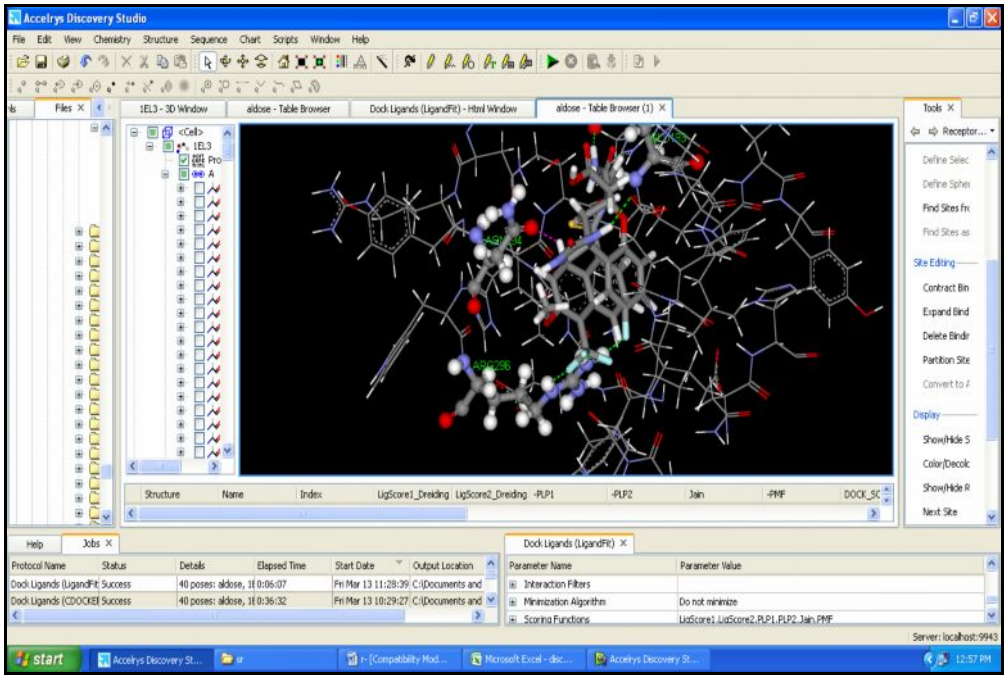


Figure 2: Bondend interaction of ligand molecule with protein the best ligand we found

Summary and Conclusion

The literatures studies were confirms that Aldose reductase is crucial enzyme which responsible for diabetic retinopathy. Structure –based drug design tools is an effective route of drug discovery, based on available structure or fragment which can be targeted by various drug molecules. The docking approach was performed to get the best drug, alternative for the existing drugs which have potential adverse effect after long time usage. Flexible and rigid docking studies were carried out on aldose reducutase enzyme by using with marketly exisited drugs and (fiderostat and Tolerostat) and Natural inhibitory compounds of aldose reducutase (Flavonoids) to find which drug molecule has highest binding energy and high affinity to bind the target protein

.For this we have done docking studies by using various algorithams like Ligand fit ,Cdocking and Libdock .finally we found the best pose DB03467 Quercetin has highest binding free energy and high affinity to binding the target protein.

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