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Full length Research Paper

Protein ligand interaction analysis an insilico potential drug target identification in diabetes mellitus and nephropathy

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Diabetes mellitus is a multisystem disorder leading to hyperglycemia and other metabolic abnormalities leading to complications in many organs of the human body including the kidney. Oxidative stress induced by hyperglycemia can produce dysfunction of pancreatic beta-cells, as well as lead to various other forms of tissue damage in patients with diabetes mellitus. Therefore, it seems reasonable that antioxidants can play a role in the management of diabetic nephropathy. Brain-derived neurotrophic factor (BDNF), which plays a role in human glucose metabolism, has been implicated in the pathogenesis of Alzheimer's disease and depression, which co-exist with type II diabetes. Ras homolog gene is one of the genes associated with Diabetic nephropathy. Three Dimensional structures of the proteins RHOD, BDNF were taken from the PDB databank and the ligands, Astaxanthin, Beta carotene were downloaded from Ligand database. Protein Ligand Docking was done for the target proteins and antioxidant ligands. BDNF and Astaxanthin-Docking Energy range: Emin = -225.39, Emax = -74.12, BDNF and β-carotene- Docking Energy range: Emin = -220.68, Emax = -69.21, RHOD and Astaxanthin Docking Energy range: Emin = -247.72, Emax = -88.39, Rhod and β-carotene Docking Energy range: Emin = -232.07, Emax = -86.55. In docking the lowest minimum energy has the highest affinity. It is concluded that astaxanthin docking score when compared with β-carotene is lowest so, it has the highest affinity with the target proteins. In conclusion, Astaxanthin has powerful antioxidant properties, with the potential to be used in reducing glucose toxicity.

Key words: Diabetes mellitus, antioxidants, docking, Astaxanthin.

INTRODUCTION

In the area of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second (Lengauer et al., 1996).

Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed toward improving the methods.

Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex calculates protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. It is still the only docking and superposition program to use spherical polar Fourier correlations to accelerate the calculations, and it is one of the few docking programs which has built-in graphics to view the results (Ritchie et al., 2003).

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Oxidative stress is thought to contribute to the development of a wide range of diseases including the pathologies caused by diabetes (Davi et al., 2005). Oxidative stress and oxidative damage to tissues are common end points of chronic diseases, such as atherosclerosis, diabetes, and rheumatoid arthritis. Increased oxidative stress has a primary role in the pathogenesis of diabetic complications and it is a secondary indicator of end-stage tissue damage in diabetes. The increase in glycoxidation and lipoxidation products in plasma and tissue proteins suggests that oxidative stress is increased in diabetes. Elevated levels of oxidizable substrates may also explain the increase in glycoxidation and lipoxidation products in tissue proteins, without the necessity of invoking an increase in oxidative stress.

Diabetes mellitus is characterized by oxidative stress, which in turn determines endothelial dysfunction. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.

In chemical terms, oxidative stress is a large rise in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione (Schafer et al., 2001). The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis (Lennon et al., 1991).

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German et al., 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Knight et al., 1998).

Astaxanthin's potent antioxidant activity may be beneficial in cardiovascular, immune, anti-inflammatory, and neurodegenerative diseases. Astaxanthin is a carotenoid that is found in marine animals and vegetables. Several previous studies have demonstrated that AST exhibits a wide variety of biological activities including antioxidant, antitumor, and anti-*Helicobacter pylori* effects (Ohgami et al., 2003).

Astaxanthin has 100-500 times the antioxidant capacity of Vitamin E and 10 times the antioxidant capacity of beta-carotene. Many laboratory studies also indicate astaxanthin is a stronger antioxidant than lutein, lycopene and tocotrienols.

Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic beta-cells and various forms of tissue damage in patients with diabetes mellitus. Astaxanthin, a carotenoid of marine microalgae, is reported as a strong anti-oxidant inhibiting lipid peroxidation and scavenging reactive oxygen species. Astaxanthin can exert beneficial effects in diabetes, with preservation of beta-cell function. This finding suggests that anti-oxidants may be potentially useful for reducing glucose toxicity (Uchiyama et al., 2002).

β-carotene is an organic compound a terpenoid, a red-orange pigment abundant in plants and fruits. As a carotene with β-rings at both ends, it is the most common form of carotene. It is a precursor (inactive form) of vitamin A being highly conjugated, it is deeply colored, and as a hydrocarbon lacking functional groups, it is very lipophilic (Susan 1998).

The active site of an enzyme contains the catalytic and binding sites. The structure and chemical properties of the active site allow the recognition and binding of the substrate.

Background

Diabetes mellitus is an increasingly common metabolic disorder that affects both large vessels (macrovessels) and small vessels (microvessels) of the body (Sridhar and Rao, 2003). Microvascular involvement of the kidney results in diabetic nephropathy (Satyavani et al., 2010). Both genetic and metabolic causes including oxidative stress are implicated in the pathogenesis of the disease and its complications. Increased levels of oxidative stress indicators occur in individuals with diabetic complications, implying antioxidants can be useful in their prevention and management.

Astaxanthin, a carotenoid of marine microalgae, is reported as a strong anti-oxidant inhibiting lipid peroxidation and scavenging reactive oxygen species. Astaxanthin is a fat-soluble, oxygenated pigment called a xanthophyll and a member of the carotenoid family. It has a unique molecular structure that gives it powerful antioxidant function. It is synthesized by plants and algae, and distributed in marine seafood. Astaxanthin can exert beneficial effects in diabetes, with preservation of beta-cell function.

MATERIALS AND METHODS

Hex calculates protein-ligand docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. Hex is still the only docking and superpostion

Table 1. Docking result energy values in tabular format.

S/No.	Protein	Ligand	Energy range: Emin	Energy range: Emax
1	BDNF	AXT	-225.39	-74.12
2	BDNF	BCR	-220.68	-69.21
3	RHOD	AXT	-247.72	-88.39
4	RHOD	BCR	-232.07	-86.55

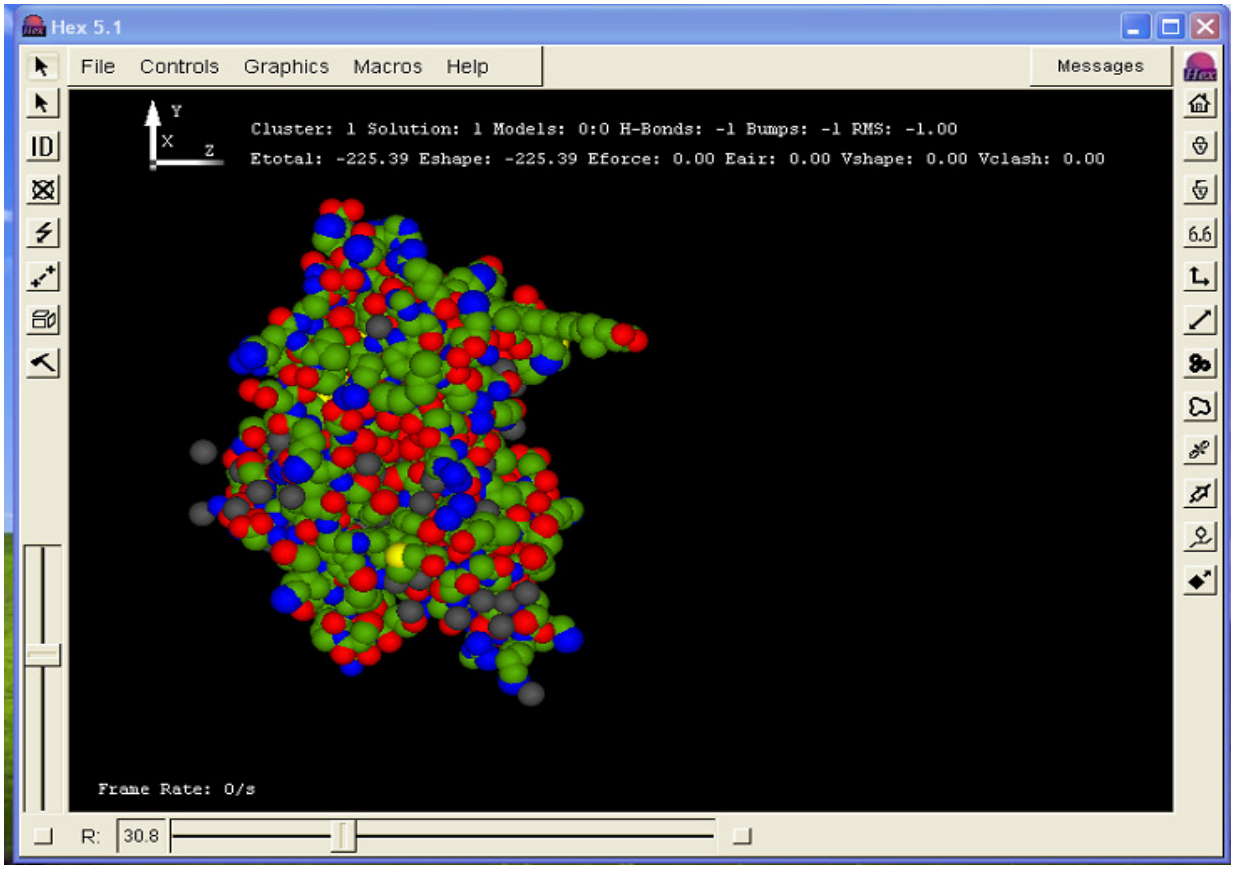

Figure 1. Hex docking result BDNF and astaxanthin.

program to use spherical polar Fourier (SPF) correlations to accelerate the calculations, and its still one of the few docking programs which has built-in graphics to view the results.

Hex can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes (Ritchie et al., 2003). It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result (Ritchie et al., 2000). Hex 5.1 is downloaded on to the system and the molecular Interphase tool is opened.

The PDB (Protein Data Bank) is the single world wide archive of Structural data of Biological macromolecules, established in Brookhaven National Laboratories (BNL) in 1971. It contains Structural information of the macromolecules determined by X-ray crystallographic, NMR methods. The three dimensional structure of the protein BDNF: Brain derieved neurofactor (1BND) and RHOD: Ras homolog gene, memberD (1JIL), are downloaded from the protein databank website and the Antioxidant ligands Astaxanthin, β-carotene from Ligand Database opened onto the HEX molecular Interface tool. The proteins Brain derieved neurofactor (BDNF) and RHOD: Ras homolog gene member (1JIL), were docked with Astaxanthin and β-carotene. The HEX molecular Interface tool Figure displayed in the results.

RESULTS

Docking result of BDNF and AXT

The Protein- Ligand interaction plays a significant role in structural based drug designing.The proteins were docked with Astaxanthin, B- carotenene and the energy values obtained are shown in Table 1, Figures 1 and 2.

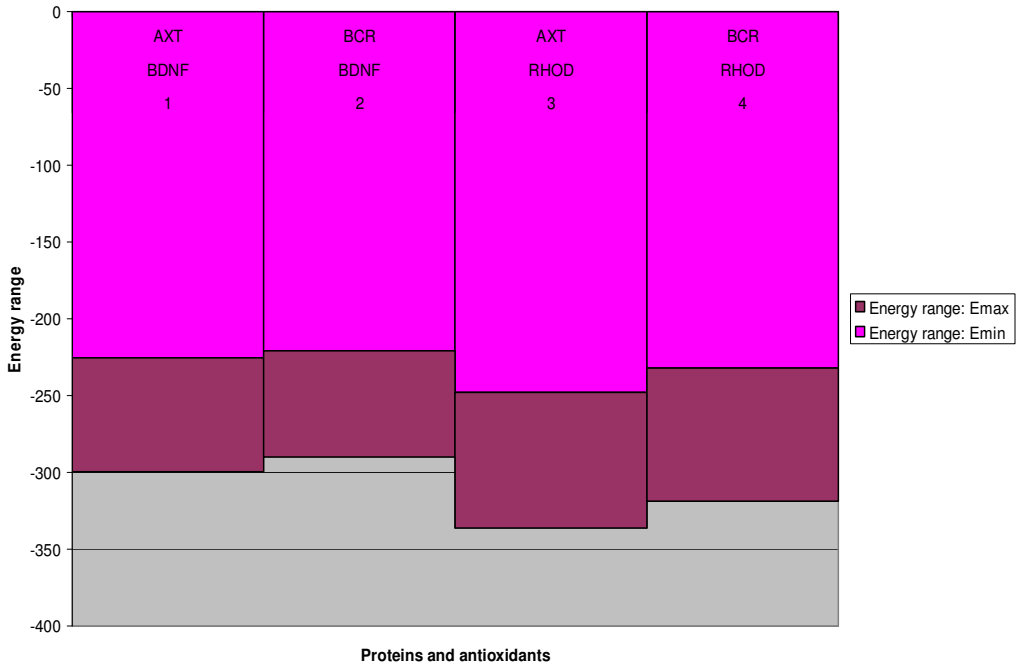

Figure 2. Docking result energy values in graphical format.

Energy range: Emin = -225.39, Emax = -74.12

The docking results are ordered by energy values and the lowest energy docking solution is the seed member for drug design. Lowest energy orientation is the prediction for target (Ritchie, 2002). Using the HEX 5.1 docking software, depending on the energy values I conclude that astaxanthin is the best and the powerful antioxidant to treat Diabetic nephropathy.

DISCUSSION

In this study we have focused on BDNF: Brain-derived neurotrophic factor and RHOD: Ras homolog gene family; proteins from the list of biomarkers of diabetic nephropathy are selected and docked with the antioxidant ligands Astaxanthin and β-carotene. It is observed that the docking energy for Astaxanthin is low when compared with β-carotene. In conclusion, Astaxanthin has powerful antioxidant properties, with the potential to be used in reducing glucose toxicity.

Future works

In future research works, successful re-analysis of the samples to determine appropriate genes, it would be very helpful to assay more individuals with known material backgrounds. All the 46 genes identified as the biomarkers for Diabetic nephropathy are further studied in detail of each protein and checked for any drug molecule or docked with the same antioxidants and results are compared. ADME/T (Absorption, Distribution, Metabolism, Excretion / Toxicity) properties of these compounds can be calculated using the commercial ADME/T tools available thus reducing the time and cost in drug discovery process. These results are studied further in animal models and their response to the drug is monitored.

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