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Full Length Research Article

In silico study of the binding parameters of various antioxidants with human Paraoxonase 1

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Paraoxonases are a group of enzymes involved in the hydrolysis of organophosphates. Human Paraoxonase 1 is synthesized in liver and secreted into blood, where it is associated exclusively with High Density Lipoproteins and may protect against the development of atherosclerosis. Paraoxonase was identified as a genetic risk factor for cardiovascular disease. An enhancement of Paraoxonase 1 activity by well-known anti oxidant flavonols like Quercetin and its derivatives are therefore of interest. The aim of this study is to investigate the binding parameters of Quercetin and its derivatives such as Quercetin dihydrate, Quercetin pentaacetate and Quercetin -3-methylether with Paraoxonase. Human Paraoxonase 1 was modeled using Modeller and binding parameters were studied using Arguslab software. The interactions show the differences in their binding and will throw light on new ways of effecting High Density Lipoprotein content and action. The docking studies helped in understanding the binding parameters thereby giving a direction for future anti-atherosclerotic treatment options.

Key words: Paraoxonases, quercetin, anti-oxidant, high density lipoproteins (HDL), atherosclerosis.

INTRODUCTION

Paraoxonase

In serum, Paraoxonase (PON) is a high-density lipoprotein associated esterase that hydrolyses lipoperoxides and inhibits Low Density Lipoprotein oxidation. Paraoxonase 1 serves as a protective factor against oxidative modification of LDL, suggesting that it may play an important role in the prevention of atherosclerotic process. Paraoxonase, a member of the A-oxonzse family, breaks down acetylcholinesterase inhibitors before they bind to the cholinestarases, thus protecting from harm by low dose organophosphate pesticide exposure. Paraoxonase-1 possesses both arylesterase and organophosphatase activities.

The Paraoxonase gene family includes 3 genes, PON1, PON2, and PON3 aligned next to each other on chromosome 7. The human paraoxonase-1 (PON-1) is a serum high-density lipoprotein-associated phosphotriesterase secreted mainly by the liver. The enzyme encoded by this gene is an arylesterase that mainly hydrolyzes paroxon to produce p-nitrophenol. Paroxon is an organophosphorus anticholinesterase compound that is produced in vivo by oxidation of the insecticide parathion. Polymorphisms in this gene are a risk factor in coronary artery disease. Next to the PON1 gene is a gene that codes for 1 of the pyruvate dehydrogenase kinases and may explain the linkage of Paraoxonase (PON) genotypes with diabetic glycemic control in some studies.

The product of PON2 has not yet been identified in biological tissue, but the PON3 gene product has recently been identified as a lactonase located on rabbit high

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Abbreviations: PON, Paraoxonase; **OP,** organophosphates; **CHD,** coronary heart disease; **NCBI,** national center for biotechnology information; **BLAST,** basic local alignment search tool; **PDB,** protein data bank; **PFam,** protein family database; **SAVS,** structural analysis and validation server.

density lipoprotein.

In addition to its detoxification function, paraoxonase-1 is involved in the metabolism of endogenous substrates (Aviram et al., 1998). This enzyme metabolizes oxidized phospholipids in high- and low density lipoproteins, homocysteine thiolactoneactivating factor. It was shown that PON-1–deficient mice are more susceptible to atherosclerosis than wild-type littermates, and several clinical studies report that paraoxonase-1 plays a role in the physiological prevention of cardiovascular disease (Mackness et al., 2001).

Quercetin and its derivatives

Quercetin is a phytochemical found in the skins of apples and red onions. Quercetin - a powerful antioxidant is also natural anti-histamine (Boesch-saadatmandi et al., 2010). Research shows that Quercetin may help to prevent cancer, especially prostate cancer. Quercetin has demonstrated significant anti-inflammatory activity by inhibiting both manufacture and release of histamine and other allergic/inflammatory mediators. In addition, it exerts potent antioxidant activity and vitamin C-sparing action. Cultured skin and prostate cancer cells showed significant mortality when treated with a combination of Quercetin and ultrasound. Ultrasound also promotes topical absorption up to 1,000 times more, making the use of topical Quercetin and ultrasound an interesting proposition.

Quercetin is a member of a group the flavonoids, which have a common flavone nucleus composed of two benzene rings linked through a heterocyclicpyrone ring. Epidemiological data suggest an inverse relation between flavonoid intake and the risk for cardiovascular disease [Aviram and Fuhrman 2001]. Although there is some evidence from cell culture studies that the dietary flavonoid Quercetin may induce the expression of PON1, systematic studies investigating the influence of a Quercetin supplementation on PON2 gene expression are missing.

The therapeutic effects of plasmid DNA containing the human PON1 gene (pcDNA/PON1) in hyperlipidemic model rats suggest the potential therapeutic effect of pcDNA/PON1 on hyperlipidemia (Fu and Shao, 2010). The role of PON1 in development of cardio-vascular disease has drawn considerable attention in recent years. Studies have shown decreased levels of High Density Lipoprotein and PON1 activity in CRF patients on hemodialysis and reported this to be a risk factor in the development of CVD. Prakash et al. (2010) enhancement or maintenance of the PON1 activity may prevent development of CVDs and its consequences in patients on hemodialysis.

PON1 and PON2 were associated with increased systemic oxidative stress and increased risk for CVD (Shih and Lusis, 2009) and atherogenesis (Thomas van Himbergen, 2006). They are basically Lactonases (Gupta et al., 2009). PON1 expression protected against *Pseudomonas aeruginosa* lethality in Drosophila, suggesting that PON1 can interfere with quorum sensing *in vivo*. PON2 attenuated macrophage triglyceride accumulation via inhibition of diacylglycerol acyltransferase 1. Over expression of PON2 protected against endoplasmic reticulum stress-induced apoptosis when the stress was induced by interference with protein modification but not when endoplasmic reticulum stress was induced by Ca^{2+} deregulation. Elucidation of the physiologic substrates of the PON proteins is of particular importance.

MATERIALS AND METHODS

The target protein was zeroed in as Paraoxonase 1 after screening the proteins involved in hyperlipidemia and atherosclerosis. The sequence was retrieved from the NCBI database whose SWISSPROT id is P27169. The protein sequences which have a similarity above 40% have been identified using the BLASTP tool. The protein 1V04 was chosen as the template as the similarity was 83%. The Template sequence and structure was retrieved from PDB database. Homology modeling was done using Modeller 9v5 and the 3-D structure of the target protein (Paraoxonase 1) was generated with the template structure (1V04) (Figure 1). Validation was done using the SAVS-PROCHECK. Q-site finder was used to identify the active site of the target protein (Paraoxonase 1) and the template protein.

Docking Studies: The 3-D structure of the ligands – Quercetin and its derivatives were downloaded from PUBCHEM. The Docking Analysis was done with the help of ArgusLab. The binding parameters of Paroxanase 1 with Quercetin and its derivatives are shown in Figures 4 to 7.

RESULTS

Blast results

The structure similarity for the sequence was carried out with the help of basic local alignment search tool. The similar structure for PON1_Human sequence was search against the PDB database.

The result indicates the degree of similarity between target and query sequences. Paraoxonase 1 from Homo sapiens had 83% similarity with 1V04 (Figure 2). Identities 83% indicate same amino acid, positives 91% indicates same group of amino acid and gaps 0%. As the criteria of the template selection is satisfied, 1V04 is used for homology modeling to model Paraoxonase1 (target).

Domain selection using PFAM

The domain region for Paraoxonase 1 was from 168 – 253. This family consists of arylesterases EC: 3.1.1.2. These enzymes hydrolyse organophosphorus esters such as paraoxon and are found in the liver and blood.

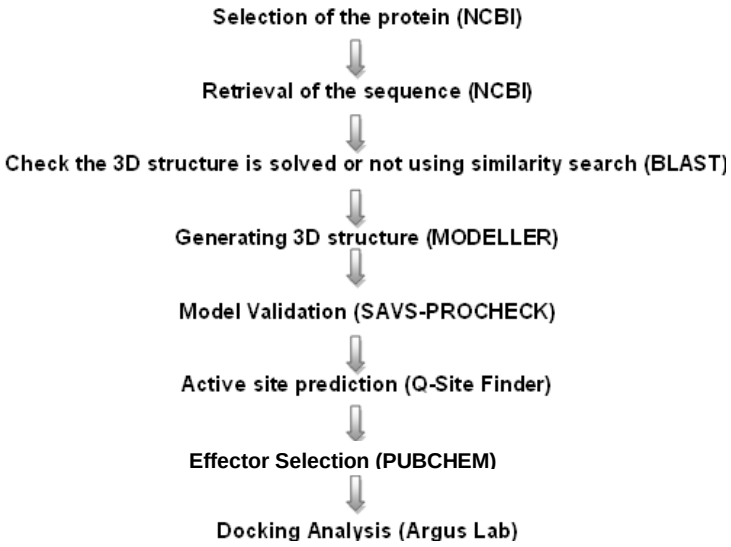

Figure 1. Methodology .

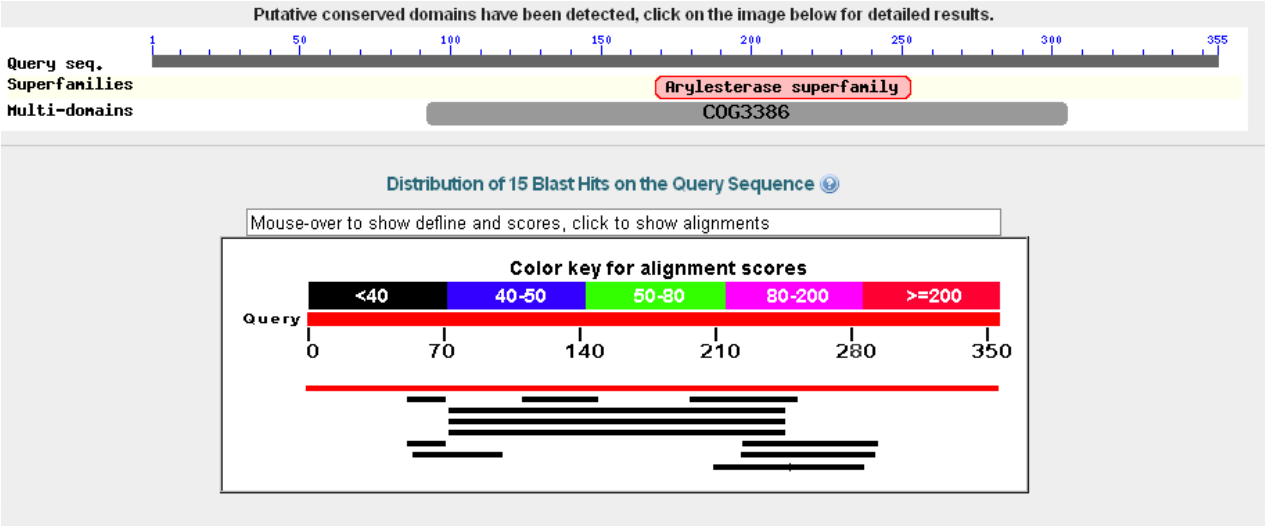

Figure 2. Graphical representation of sequence alignment from BLAST.

They confer resistance to organophosphate toxicity. Human arylesterase (PON1) is associated with HDL and may protect against LDL oxidation.

The structure model obtained was evaluated further using SAVS server. The RMSD value of 0.23**Å** revealed deviation between target and template structure.

SAVS server result

SAVS server analyzed the target (PON1) protein structure and showed the validity. PON1 had 88.3% residues in core region, 10.4% residues in allowed region, 1.3% residues in generously allowed region and 0.0% in disallowed region (Figure 3).

Protein ligand binding and the orientation within the targeted binding site by a suitable ligand is chosen and docked with the help of ArgusLab.

The docking result of Paraoxonase 1 with Quercetin is as follows

Best Ligand Pose: energy = -8.34 kcal/mol

ArgusLab predicted 117 poses with minimum energies. It had 69 final unique configurations. The pose 1 has the least energy of -8.34 kcal/mol. The best pose is selected

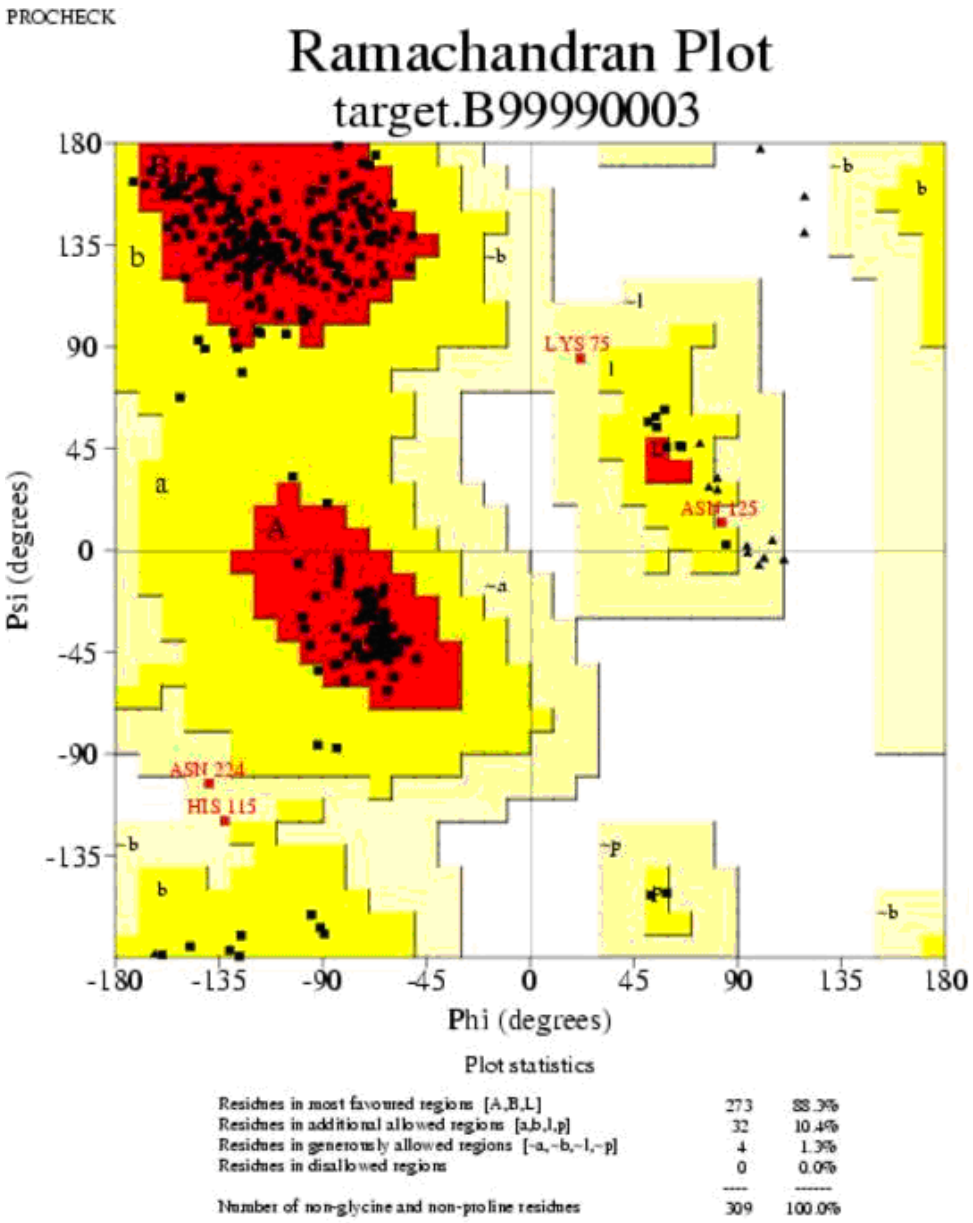

Figure 3. Protein structure validation: Ramachandran Plot.

for viewing the hydrogen bond interactions. The docking result shows six hydrogen bond interactions with the Paraoxonase 1 protein (Figure 4).

Hydrogen bond information dialog box shows the hydrogen bond interaction with the distance in angstrom.

The docking result of Paraoxonase 1 with Quercetin dihydrate is as follows:

Best Ligand Pose: energy = -8.55557 kcal/mol

ArgusLab predicted 120 poses with minimum energies. It had 69 final unique configurations. The first pose has the least energy of -8.555 kcal/mol. The best pose is selected for viewing the hydrogen bond interactions. The docking result shows seven hydrogen bond interactions with the Paraoxonase 1 protein (Figure 5).

The docking result of Paraoxonase 1 with Quercetin pentaacetate is as follows:

Best Ligand Pose: energy = -7.90468 kcal/mol

Paraoxonase 1 with Quercetin pentaacetate – 8 hydrogen bonds. ArgusLab predicted 115 poses with minimum energies. It had 72 final unique configurations.

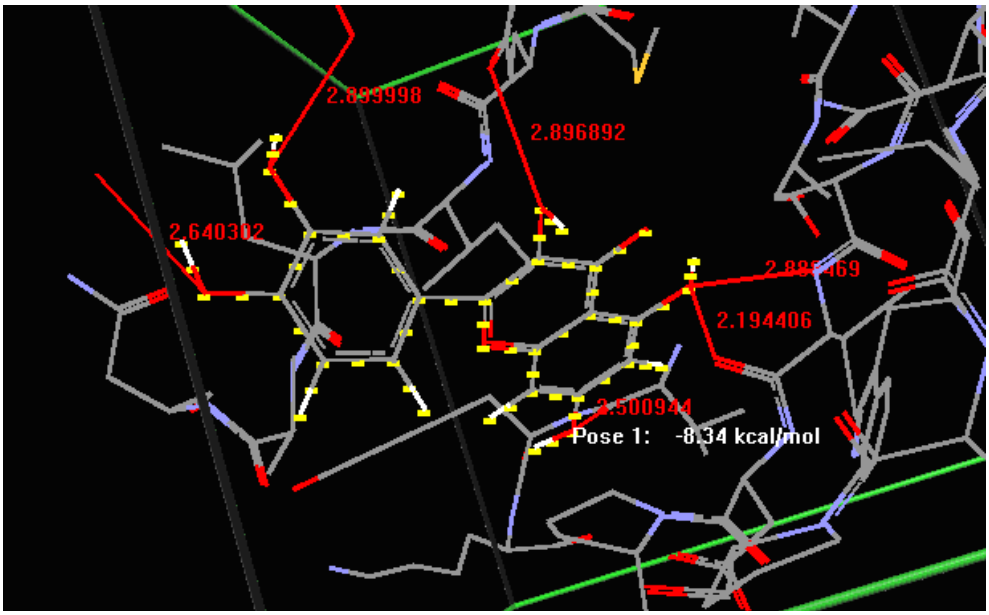

Figure 4. Docking result of Paraoxonase 1 with Quercetin.
Pose 115 fitness=-1.90066; pose 116 fitness=0.926483; pose 117 fitness=26.4906.
Refining candidate poses
Clustering the final poses:69 final unique configurations; number of local searches that succeeded in locating new minima=3; re-clustering the final poses: 69final unique configurations.
Best Ligand Pose: energy=-8.33652 kcal/mol.

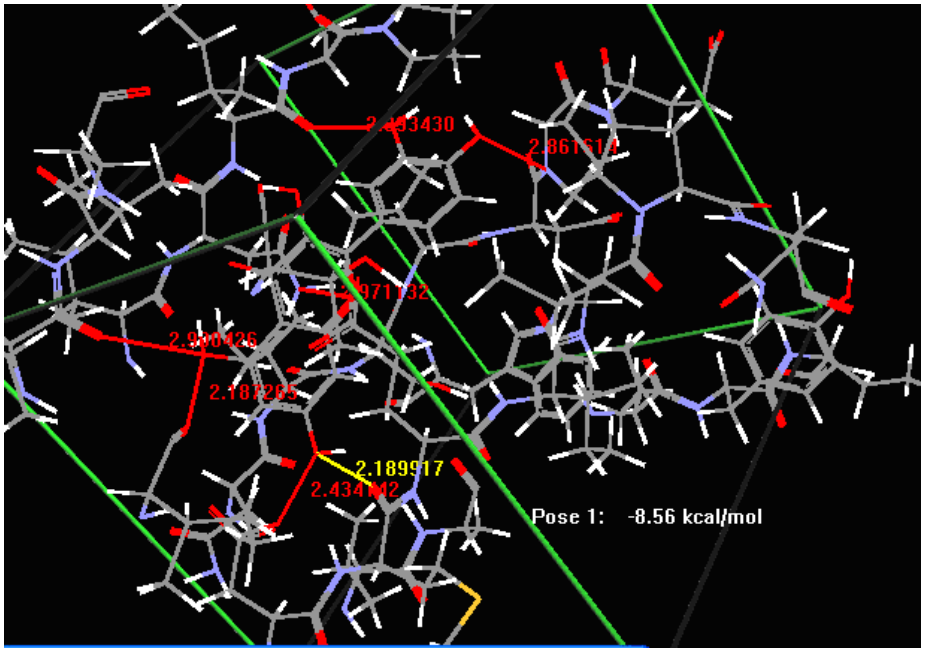

Figure 5. Docking result of Paraoxonase 1 with Quercetin dehydrates.

The first pose has the least energy of -7.90468 kcal/mol. The best pose is selected for viewing the hydrogen bond interactions. The docking result shows eight hydrogen bond interactions with the Paraoxonase 1 protein (Figure

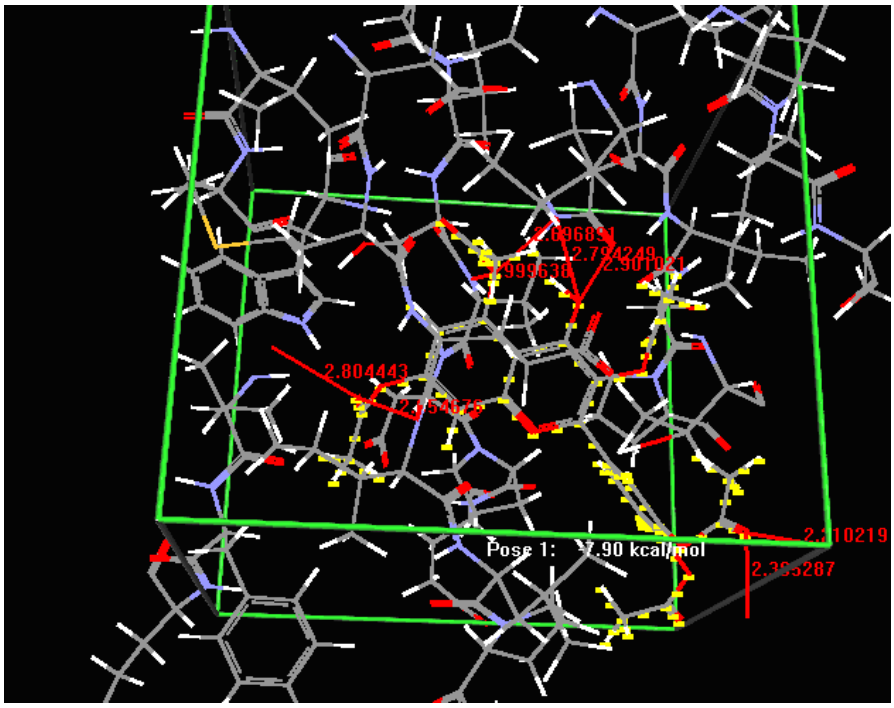

Figure 6. Docking result of Paraoxonase 1 with Quercetin pentaacetate. Best Ligand pose: energy = -7.90468 kcal/mol

6).

The docking result of Paraoxonase 1 with Quercetin -3-methylether is as follows:

Best Ligand Pose: energy = -7.5832 kcal/mol

Paraoxonase 1 with Quercetin -3- methylether – 5 hydrogen bonds. ArgusLab predicted 109 poses with minimum energies. It had 72 final unique configurations. The first pose has the least energy of -7.5832 kcal/mol. The best pose is selected for viewing the hydrogen bond interactions. The docking result shows five hydrogen bond interactions with the Paraoxonase 1 protein (Figure 7).

DISCUSSION

Of the 69 unique configurations with Quercetin the one with the least energy of -8.34 kcal/mol was selected for viewing the hydrogen bond interactions. The six hydrogen bonds had lengths ranging from 2.1 to 2.89 Angstrom, the lesser the distance, the stronger the interaction. In this context the aliphatic and hydrophobic amino acid Valine (VAL273) of Paraoxonase 1 has a length of 2.1944 Angstrom thus showing the strongest interaction with the Quercetin.

The docking result of Paraoxonase 1 with Quercetin dihydrate showed seven hydrogen bond interactions with distances ranging from 2.1 to 2.97 Angstrom. It is interesting to note that Methionine (MET55) and Isoleucine (ILE117) had lesser distances of 2.189 and 2.187 Angstrom. Therefore Paraoxonase 1 with Quercetin derivative has two strong interactions. That could be involved in binding. The residues of the predicted active site are different in the modified ligand, which can be taken up for further study.

Quercetin pentaacetate shows eight hydrogen bond interactions with distance ranging from 2.2 to 2.99 Angstrom. The distance of Threonine (THR121) with Quercetin pentaacetate was found to be 2.21 Angstrom. The active site also has Threonine which mimics hydrophobicity. Of the various amino acids that have hydrogen bonding Threonine is common in the base Quercetin and the pentaacetate derivative. It should be noted that the pentaacetate and the dihydrate derivatives have Asparagine in the active site and its mode of action could be similar

The methyl ether of Quercetin showed five hydrogen bond interactions with distance ranging from 2.2 to 2.69 Angstrom with Isoleucine (ILE117) having a distance of 2.245 Angstrom suggesting stronger interaction. Only one residue Isoleucine has a strong hydrogen bonding capacity in this derivative.

The present study infers that Paraoxonase 1 with Quercetin derivatives like Quercetin dihydrate, Quercetin pentaacetate has more number of stronger interactions when compared to Quercetin itself and the methyl ether

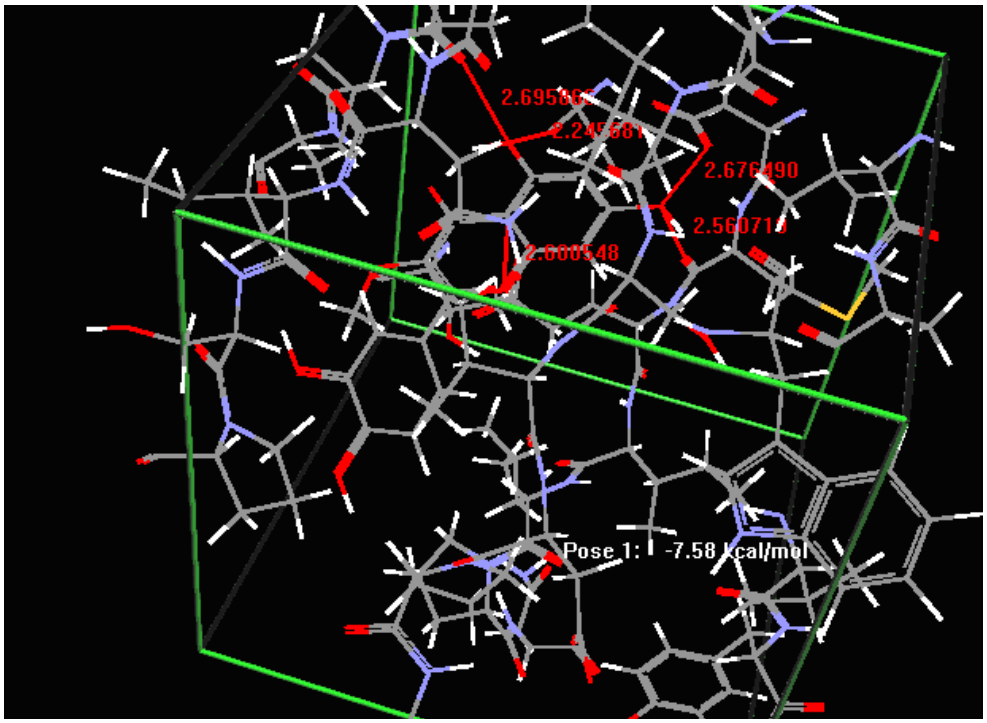

Figure 7. Docking result of Paraoxonase 1 with Quercetin -3-methylether. Clustering the final poses: 72final unique configurations.Number of local searches that succeeded in locating new minima = 2.
Re-clustering the final poses: 72 final unique configurations. Best Ligand Pose: energy = -7.5832 kcal/mol.

derivative has less binding. These results can be used to further research on the effect of various functional groups on the Quercetin molecule to increase the impact on Paroxonase, thereby leading to effective treatment of Hyperlipidemia especially to increase the high density lipoprotein which is the need of the hour.

Conclusion

The presence of Paraoxonase 1 in High Density Lipoprotein may thus be a major contributor to the anti-atherogenicity of this lipoprotein. In the presence of Quercetin HDL, up-regulation is increased thereby giving indications of alternative therapy modules. The various derivatives such as Quercetin dihydrate, Quercetin pentaacetate showed more number of stronger interactions when compared to Quercetin itself, but the methyl ether derivative has less binding. These results can be used for further research to identify derivatives for maximum impact on Paroxonase. The study demonstrates that a Quercetin supplementation may up-regulate Paraoxonase 1 activity giving us a valuable tool to treat or manage Hyperlipidemia.

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