In silico studies on lycopene metabolism, pharmacokinetics, transport and redox potential

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Abstract

The increasing importance of lycopene as a nutraceutical led us to investigate its fate in human system. In addition to being a potent antioxidant, lycopene has chemopreventive activity, reducing the risk of several types of cancers and heart ailments. Present work is an effort to study in silico the mechanism of interaction of lycopene with lipoxigenase, an enzyme responsible for its pharmacokinetics and permeability glycoprotein involved in its efflux and transport and thio redoxin, an indicator of its redox status inside cells. It can be inferred from these results that proper formulations of lycopene or its covalent linking with appropriate ligands can make it a potent anti-inflammatory/anti-tumor/anti-cancer prodrug in future.

Keywords: lycopene, metabolism, transport, redox, efflux, pharmacokinetics, pharmacodynamics

Introduction

Lycopene is a member of carotenoid family, the naturally occurring pigments produced by yeasts and all photosynthetic organisms. Lycopene is synthesized in vitro by green plants, bacteria and some fungi. It is an antioxidant that once absorbed by the body, helps to repair damaged cells. Lycopene interacts with ROS (reactive oxygen species) in the body and has been shown to inhibit DNA oxidation which sometimes leads to cancer. Antioxidants are the internal bodyguards that protect the cells from the degenerative influence of free radicals. These free radicals or reactive oxygen species cause cancer, blockages in the arteries, joint deterioration, nervous system degradation and ageing.

Lycopene is known to reduce the risk of muscular degenerative disease, serum lipid oxidation and cancers of the lung, bladder, cervix and skin. It is one of the three most concentrated carotenoids in blood serum and is especially abundant in the prostate gland. Dietary consumption of lycopene (mostly from tomato products) has been associated with a lowered risk of prostate cancer. It reduces LDL (low-density lipoprotein) oxidation and helps to reduce cholesterol levels in the blood.

It has been reported that men with higher levels of lycopene in their blood have statistically significant lower rates of occurrence of cancer than men with lower lycopene levels in blood. Studies have also shown that men who eat more lycopene-rich foods are less prone to heart diseases.

Significant amount of lycopene is found in the fruits including watermelon, pink grapefruit, apricot, strawberries, papaya, and tomatoes. The tomatoes and tomato-based products account for approximately 85% of dietary lycopene. The bright red, just ripe tomatoes have more lycopene than green or yellow unripe ones.
The efficiency of absorption of lycopene from tomatoes is much higher in processed tomato products. More lycopene is absorbed from cooked or processed tomatoes like sauce, pizza than from raw ones. Absorption of lycopene increases by adding a few drops of oil in processed tomatoes. The yield of lycopene extracted from tomatoes is dependent on temperature.

The recent reports regarding the medicinal properties of lycopene against several types of malignant cancers (e.g. cervical and prostate), diabetes and heart diseases has made it an exceedingly important nutraceutical requiring large scale commercial production. This has necessitated computational research to be directed towards increase of its absorption, transport and excretion of lycopene. Its different natural resources such as plants and microbes are required to be genetically manipulated so that the yield of lycopene is enhanced, thus resulting in the reduction of cost of its different drug formulations in the market. For study of the mechanism of metabolism, absorption, distribution and excretion of lycopene the authors have now studied in silico binding modes of lycopene with lipoxygenase, HSA, P-glycoprotein and thioredoxin.

**Material and Methods**

**Protein Data Bank (PDB)**: It is a collection of 3-D structural data of proteins. These data are typically obtained by X-ray crystallography or NMR spectroscopy.

**ACD/Chemsketch**: It is 2-D structure of lycopene obtained drawing on ACD/chemsketch software.

**Chimera**: It is 3-D structure of lycopene (Fig. 1a & b) obtained from chimera. This software generates three dimensional atomic coordinates from the 2-D structure of a molecule. It is a phyton based software.

**Autodock**: It is a automated docking tool. It is used to find out the binding modes of receptor and protein. It predicts the orientation and actual position of ligand at the predefined active site.

**Proteins used for docking**

All the selected protein structures were downloaded from PDB.

**Lipoxygenase**: Lipoxygenase is considered as one of the metabolizing enzymes of lycopene. Lipoxygenase is not a single enzyme but it is a family of iron-containing enzymes. These are known to catalyse the dioxygenation of polyunsaturated fatty acids in lipids. Lipoxygenases are found in plants, animals and fungi but abundant in plants. The crystal structures of soybean and rabbit lipoxygenases are known. The protein consists of two domains (i) N-terminal PLAT domain which is smaller and (ii) C-terminal catalytic domain which is larger. It contains the active site of the enzyme. Its structure was downloaded from PDB having id 1jng.

**HSA**: 3-D structure of human serum is downloaded from PDB, ID is 1e7h. This is the protein of human blood plasma. The liver produces this important protein. Concentration of albumin in blood is about half of the blood serum protein. HSA is predominantly characterized by its incredible ability to tie an extensive variety of hydrophobic small molecule, ligands including fatty acids, bilirubin, thyroxine, bile acids and steroids; it acts as a transporter and solubilizer for these compounds.

**P-glycoprotein**: For docking with p-glycoprotein PDB ID 2ghi was downloaded. P-glycoprotein are members of family of efflux transporters found in the organs as kidneys, brain, gonads, gut, biliary system etc. P-gp is also known as ATP-binding cassette sub-

![Fig 1 (a and b)- 3-D model and chemical structure of lycopene](image)
family B member 1, ABCB1, MDR1, and PGY\textsuperscript{13,14}. In humans two genes encoding the p-glycoprotein \textit{mdr-1} and \textit{mdr-2}. The \textit{mdr-1} gene is responsible for drug resistance and transport and \textit{mdr-2} has role in the secretion of phosphatidylcholine into bile\textsuperscript{15}.

\textit{Thioredoxin}: For docking with thioredoxin pdb id 1vdc was downloaded. Thioredoxin is a 12-kD oxidoreductase enzyme containing a di-thiol-disulfide active site. Thioredoxin (Trx) is a small redox protein present everywhere. It has been found in both prokaryotes and eukaryotes and the sequence around the redox-active disulfide bond seems to be highly conserved. It is one of the antioxidant proteins. The thioredoxin system keeps a reducing environment in cells. Thioredoxin functions depend on the protein's redox state, as determined by two conserved cysteines. It can eliminate hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) as from a cell and also makes active other antioxidant molecules, such as glutathione peroxidase and thioredoxin peroxidase\textsuperscript{16}. Two main forms of Thioredoxin have been identified; Thioredoxin 1 (Trx1)\textsuperscript{17} and Thioredoxin 2 (Trx2)\textsuperscript{18}. Trx 1 present in cytosol is 104 amino acids long whereas Trx2 located in mitochondria is 166 amino acids long with a calculated molecular mass of 18.2 kDa.

All the selected proteins were downloaded from PDB and the bound ligands were removed using Chimera software, for active site or binding site prediction. Casp server was used. The 2-D structures of ligands were designed by using Chemskech software and conversion to 3-D and energy minimization was done by Chimera software. The Lamarckian Genetic Algorithm (LGA) in Autodock3 was used to explore the energy landscape. A grid box of maximum limit 126x126x126 points with a grid spacing of 0.375 Å was used in the calculations. The maximum number of energy evaluation was 2,500,000 and the maximum number of generations was 2700000. The number of cycles was set to 100. So a total of 100 docking configurations were determined in each docking calculation. The "preferable" docking configuration, which was selected based on the lowest empirical binding free energy and the most frequent cluster, was chosen as the "active" binding conformation\textsuperscript{19}.

\section*{Results}

\textit{Binding mode of lycopene with relevant enzymes with lipoxygenase}

This enzyme consists of two domains; a smaller N-terminal PLAT domain and a larger C-terminal catalytic domain containing the active site/cavity.

Volume and area of the selected cavity(ID 145) were simulated by the CASTp programme where lycopene binds. Volume of A chain cavity is 660.2 Å\textsuperscript{3} and area is 569.8 Å\textsuperscript{2}. Active site having 67 (Leu277 to Iso 857) amino acid residues was exposed to the solvent.

Lycopene docked at the active site with lowest binding energy (-9.17kcal/mol) and docking energy-(15.52kcal/mol). Docked conformation of lycopene with lipoxygenase is shown in Fig 2. Lycopene lacks the hydrogen bond interaction with this enzyme but electrostatic interactions are seen with interacting amino acids like Pro 435, Leu 389, Asp 431, Trp 593 as shown in Fig 3.

\textit{Binding mode of lycopene with Human serum albumin}

Volume and area of predicted cavity(ID 53) was 88.7m\textsuperscript{3} and 90.2m\textsuperscript{3} respectively. Active site has residues from lys 413 to lys 538.

Lycopene binds at the active site with 8.81kcal/mol binding energy and 12.88 kcal\textperthousand mol docking energy. (Fig 4) Lycopene had no H bond interaction with HSA. Fig 5a and Fig 5b show the electrostatic interaction and hydrophobic region with interacting amino acids.

\textit{Binding mode of lycopene with p-glycoprotein (p-gp)}

For \textit{in silico} study the authors have selected the ATP binding site for docking in order to analyze their efflux potential. The volume of the active site is 10.1m\textsuperscript{3} and area 18.9m\textsuperscript{2} This site has residue from LYS 37 to ILE231.

Lycopene binds at the active site with -7.49kcal\textperthousand mol binding energy and-13.31 Kcal\textperthousand mol docking energy. Fig. 5(a) and 5(b) shows the bound conformation of lycopene with P-gp. and electrostatic interaction among the interacting amino acid residues Lys 130, Asp 136, Gin 133, Arg 163 Gly 159, Ser 167 and Gln 162 of P-gp respectively.

\textit{Binding mode of lycopene Thioredoxin}

Thioredoxin was targeted due to its antioxidant property. The selected active site(ID 34) has volume of chain A cavity as 2447.74 Å\textsuperscript{3} whereas area as 1775.10 Å\textsuperscript{2}, it has 229 amino acid residues. Lycopene binds at this active site with -7.8 kcal\textperthousand mol binding energy and -8.52 kcal\textperthousand mol docking energy. The bound conformation of lycopene and thioredoxin is shown in Fig. 6(a) and the electrostatic interactions are shown in Fig. 6(b).
Fig. 2 – Bound conformation of lycopene with lipooxygenase

Fig. 3 – Electrostatic interaction of lycopene with lipooxygenase

Fig. 4(a) – Bound conformation of lycopene with HSA
Fig. 4b– Electrostatic interaction of lycopene with interacting amino acids of HSA

Fig. 5a– Round conformation of lycopene with P-glycoprotein

Fig. 5b– Electrostatic interaction of lycopene with interacting amino acids of P-glycoprotein (p-gp)
Fig. 6a – Bound conformation of lycopene with interacting amino acid of Thioredoxin

Fig. 6b – Electrostatic interaction of lycopene with interacting amino acid of thioredoxin

Table 1 – Comparative docking result of lycopene

<table>
<thead>
<tr>
<th>Protein</th>
<th>Docking energy (Kcal/mol)</th>
<th>Binding energy (Kcal/mol)</th>
<th>Interacting residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-glycoprotein</td>
<td>-13.31</td>
<td>-7.49</td>
<td>Lys 130, Asp136, Gln 133, Arg 163, Gly 159, Ser 167, Gln 162</td>
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<tr>
<td>Human serum albumin</td>
<td>-12.8</td>
<td>-8.81</td>
<td>Phe 551, Lys 526, Leu 529, Val 493, Gln 492</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>-8.52</td>
<td>-7.8</td>
<td>Pro 15, Ser 13, Glu 11, Leu 43, Ala 295, Arg 293</td>
</tr>
<tr>
<td>Lipoxygenase</td>
<td>-15.52</td>
<td>-9.17</td>
<td>Arg 439, Pro 438, Leu 389, Asp 431, Trp 593, Gln 598</td>
</tr>
</tbody>
</table>
Discussion

The docking of lycopene with lipoxygenase, the enzyme responsible for its metabolism, shows that it’s interaction is weak and exists only through electrostatic forces. The inhibition of the enzyme can delay it’s metabolism, thereby enhancing its bioavailability. The interaction with HSA is also through weak electrostatic forces and hydrophobic interactions, which can also be enhanced through proper modification, tagging or using appropriate formulation of lycopene molecule. The interaction of lycopene with p-glycoprotein in indicative of it’s efflux problem. The binding of lycopene at the ATP binding site of P-glycoprotein shows that it can inhibit the efflux of many drugs which depends on the activation of substrate bind ATPase. This is a significant finding, since the bioavailability of many antibiotics and other drugs can be enhanced, if given together with lycopene. Thioredoxin has a significantly strong binding with lycopene, which is suggestive of the active participation of lycopene in redox control of the cell. Therefore it can be concluded that lycopene can prove to be an excellent antioxidants and also as a synergist along with the marketed drugs.

Conclusion

The fate of lycopene once it enters the human body has been studied in the present work by studying it’s interaction with four important enzymes, that is, HSA, P-glycoprotein, Thioredoxin and Lipoxygenase responsible for its absorption, efflux, redox control and metabolism. It can be inferred that proper formulations of lycopene or its coherent linking with appropriate ligands can make it a potent anti-inflammatory/anti-tumor/anti-cancer prodrug in future.

References