

## Towards the interaction mechanism of tocopherols and tocotrienols (vitamin E) with selected metabolizing enzymes

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### Abstract:

Vitamin E is a mixture of eight compounds  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ - tocopherols and  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ - tocotrienols. Their individual role in cellular transport as antioxidants and in metabolic pathways has been highlighted in the present work. All the eight compounds have been docked with the respective metabolizing enzymes ( $\alpha$ -tocopherol transfer protein (ATTP),  $\alpha$ -tocopherol associated protein (TAP), P-glycoprotein (P-gly) and human serum albumin (HSA)) to understand molecular interactions for pharmacokinetics. These have been structurally aligned against the four human phospholipids in order to reveal their individual role in chylomicron formation and hence the mechanism of cellular transport. The study of their binding with their metabolizing enzymes provides insight to the comparative antioxidant activity of each of these isomers.

**Keywords:** docking data; vitamin E, enzymes, mechanism; anti-oxidant

### Background:

It has now been realized that Vitamin E, traditionally known as  $\alpha$ - tocopherol, is a mixture of eight different compounds, four tocopherols and four tocotrienols, each one being designated as  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  forms. The two groups differ in the hydrophobic tridecyl side chain which is saturated (phytyl) in tocopherols and unsaturated having three double bonds (geranyl) in tocotrienols. Detailed reports are available on  $\alpha$ -tocopherol. However, during the last few years it has been found that all the eight forms are biologically active and perform specific functions. Clinical research has shown that mixture of tocotrienols and tocopherols offer synergistic protective action against heart ailments and cancer that is not exclusively offered by  $\alpha$ -tocopherol. The other advantage of mixed tocopherols and tocotrienols is their role in slowing down aging. Diseases like diabetes 1 and 2, autoimmune diseases, bacterial and viral infections, Alzheimer disease, fungal (*Candida*) infections are prevented by these compounds. It helps in the maintenance of bones, muscles, eyes (vision), memory, sleep, lungs, infertility, skin and wrinkles. [1]

The main function of  $\alpha$ -Tocopherol is to terminate a chain reaction of lipid peroxidation and thus protecting the cell membranes and LDL from oxidative disintegration. It has been reported in literature that the cell signaling activity of the enzyme protein kinase C is considerably reduced due to the presence of  $\alpha$ -tocopherol [2]. This also affects the activity of both inflammatory and immune cells [3].  $\alpha$ -Tocopherol also dilates blood vessels and interferes with aggregation of platelets. Recent reports have suggested better activity of  $\gamma$ -tocopherol Vis-a-Vis  $\alpha$ -tocopherol.  $\gamma$ -tocopherol can trap NO and other nitrogen free radicals more efficiently than  $\alpha$ -tocopherol [4].  $\gamma$ -Tocopherol is known to have a balanced effect on the transport of Na<sup>+</sup> (sodium ions) thus affecting beneficially the uretic activity [5].  $\delta$ -Tocopherol and its metabolites have been found to

be the most effective inhibitors for the proliferation of prostate cancer cells [6].

$\gamma$ -Tocopherol has unique properties unlike alpha-tocopherol including the ability to neutralize certain free radicals and suppress expression of a gene (ras-p21) that is known to cause cancer [7]. Stone and colleagues have reported that  $\gamma$ -tocopherol can hinder the growth of colon cancer [8]. An interesting observation has been reported elsewhere [9], that if  $\alpha$  and  $\gamma$ -tocopherols are taken up together into the cells, the  $\gamma$ -tocopherol increases the level of  $\alpha$ -tocopherol. It has been reported that excess of  $\alpha$ -tocopherol displaces the more important gamma component in the body. The cellular uptake of tocotrienols through lipid bilayer is more due to the presence of double bonds in their side chains, enhancing their bioavailability and hydrophobicity. This makes them potentially more useful for cosmetic products. It is evident from the fact that amongst the two  $\alpha$ -components, the  $\alpha$ -tocotrienol has been found to be 40 to 60 times more potent than  $\alpha$ -tocopherol in preventing lipid peroxidation and 6.5 times better at defending cytochrome P-450 against oxidative damage. The efficiency of  $\alpha$ -tocotrienol in scavenging peroxy radicals in liposomes is 1.5 fold more than  $\alpha$ -tocopherol.

It is found that  $\delta$ -tocotrienol is uniformly distributed in cell membranes due to its hydrophobic nature which is due to its unsaturated side chain.  $\delta$ - tocotrienol has been shown to possess anti-thrombic activity and decreases platelet aggregation.  $\delta$ -tocotrienol is the most effective form of vitamin E family in reducing the risk of developing atherosclerotic plaque. One of the most striking discoveries in tocotrienol research is their ability to clear atherosclerotic blockages (stenosis) in the carotid artery, potentially reducing the risk of stroke. These are more potent in quenching and scavenging the free radicals. Due

to their molecular structure they have greater recycling activity and more effective collusion with free radicals [10].

One of the metabolite of  $\gamma$ -tocotrienol is LLU-alpha which reduces the high blood pressure and congestive heart failure [11]. Tocotrienols inhibit cancer cell proliferation through apoptosis. Studies on estrogen responsive and estrogen non-responsive human breast cancer cells have shown that tocotrienols are effective in preventing the proliferation of both [12]. Tocotrienols inhibit NF  $\kappa$ B signaling pathway leading to suppression of antiapoptotic gene products and starts apoptosis. Tocotrienols are known to cross the blood-brain barrier, and are potent protectors of neuron cells that may get destroyed through stroke and other neurodegenerative diseases.

The reports discussed thus far about the enhanced activity of other members of the vitamin E family besides the mostly studied  $\alpha$ -tocopherol, motivated us to study the structure-activity relationship of the whole group. It is known that  $\alpha$ -tocopherol is taken up together with dietary lipids and bile in the proximal part of the intestine. The tocopherols get assembled together with triglycerides, cholesterol, phospholipids, and apolipoproteins into chylomicrons. The exact mechanism of this process of chylomicron formation is not very well known. In this paper we have made an effort to structurally align all the four mammalian phospholipids viz. phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, lysophosphatidyl inositol, present in the human liver with tocopherols and tocotrienols to establish their mechanism of action.

#### Methodology:

##### ACD/CHEMSKETCH:

2-D structures of all the eight compounds of vitamin E were developed using ACD/chemsketch software (to draw molecules, reactions and schematic diagrams and calculate their properties).

##### CHIMERA:

3-D structures of all the eight isomers of vitamin E were obtained from chimera (python based software generates three dimensional atomic coordinates from 2-D structure of a molecule).

##### AUTODOCK:

Docking was performed using AUTODOCK version 3.0 software package running on PC Intel-based Pentium 4, running Linux operating system.

#### Protein receptors for docking:

The following four target proteins were selected for the study,

##### Alpha-tocopherol transfer protein (ATTP)

Alpha-tocopherol ( $\alpha$ -T) transfer protein (ATTP) is a member of the family of lipid-binding proteins containing two CRAL-TRIO domains, pfam03765 (residues 11–83) and pfam00650 (residues 89–275). The corresponding structure with PDB ID 1R51 is downloaded from PDB.

##### Alpha-tocopherol associated protein (TAP)

It is tocopherol binding protein with a molecular mass of 46 kDa present in the cytosol of bovine liver. This is referred as alpha tocopherol-associated protein (TAP). The corresponding structure with PDB ID 1OLM is downloaded from PDB.

##### P-glycoprotein (P-gp)

3-D structure of p-glycoprotein was downloaded from PDB (PDB ID 2GHI). P-glycoprotein is found in the gut, gonads, kidneys, biliary system, brain and other organs. It belongs to the family of efflux transporter. P-gp is also called ABCB1, ATP-binding cassette sub-family B member 1, MDR1, and PGY1. It is a 170 kDa encoded by the MDR (multidrug resistance) gene(s) that are highly conserved across species.

##### Human serum albumin (HSA)

3-D structure of human serum was downloaded from PDB (PDB ID: 1E7H). The protein is a helical monomer of 66 kDa containing three homologous domains (I-III) each of which is composed of A and B sub-domains.

#### Structural Alignment

We used the VMD software for structural alignment of the phospholipid molecules present in human liver with all the eight forms of tocopherols and tocotrienols. The lipid structures were first downloaded from lipid database, and thereafter the 2D structures were drawn using the ChemsKetch software. Root mean square deviation was then calculated using appropriate tools in ChemsKetch.

#### Molecular docking:

##### ATTP:

The PDB ID used for ATTP is 1R51. The crystal structure of human alpha-tocopherol transfer protein bound to its ligand  $\alpha$ -tocopherol is used. The bound tocopherol was removed and the active site of the ligand was analyzed with the help of CASTP server. Volume of the active site is 1030  $\text{\AA}^3$  and surface area is 727  $\text{\AA}^2$ . This active site is made of residues TYR100 to ILE222. This is tested for all the eight isomers.

##### TAP:

The PDB ID used for TAP is 1OLM. The structure is protein complex with  $\alpha$ -tocopherolquinone. The active site has volume 10262  $\text{\AA}^3$  and surface area 6402.2  $\text{\AA}^2$ .

##### HSA:

The PDB ID used for HSA is 1E7H. HSA forms complex with palmitic acid. The volume of the active site (LYS413-LYS538) 88.7  $\text{\AA}^3$  and the surface area is 90.2  $\text{\AA}^2$ .

##### P-gp:

The PDB ID used for P-gp is 2GHI. P-gp has four chains with ATP binding sites. The volume of the active site (LYS37-ILE231) is 10.1  $\text{\AA}^3$  and the surface area is 18.9  $\text{\AA}^2$ .

#### Docking procedure:

The active sites are located in each of the target proteins before being loaded to AUTODOCK. Polar hydrogens were then added using the protonate utility available in AUTODOCK. The ligand is made flexible by providing rotation to bonds. AUTOGRID (using Kollman united atom charges and salvation parameters) is then used to map grid to active sites of target protein. The grid dimensions are [1.344, 63.053, 14.596] for TTP, [-20.33, 19.575, 87.289] for TAP, [-3.188, 1.003, 26.114] for HSA and [45.57, 14.8, -17.9] for P-gp. The genetic search algorithm with 100 iterative runs was selected for dockings.

### Discussion:

We aligned all the structures of eight tocopherols and tocotrienols with four human phospholipids to elucidate the exact binding mode in the formation of chylomicrons before reaching the intestine [13]. Literature reports suggest that  $\alpha$ -tocopherol along with lipids is packed into chylomicrons and transported to the liver [14]. They appearance in plasma is only after passing through the liver. Most of the ingested  $\beta$ -,  $\gamma$ -, and  $\delta$ - tocopherols are secreted into bile are not taken up and hence excreted in the feces. This is true for all the other compounds used in this study. However, results show that  $\alpha$ -tocopherol and  $\alpha$ -tocotrienol are more structurally similar to phosphatidyl inositol.  $\beta$ -tocopherol is similar to phosphatidyl choline and beta-tocotrienol is aligned with phosphatidyl ethanolamine.  $\delta$  and  $\gamma$  forms are more similar to lysophosphatidyl ethanolamine. Nonetheless, all the four phospholipids are associated with the transportation process. The binding mode of these eight components of vitamin E with different proteins in metabolism, transport and absorption is of interest. The binding energy of these compounds with four metabolizing enzymes is given in Table 1.

Alpha-tocopherol transfer protein ( $\alpha$ -TTP) present in liver is selectively involved in retention of  $\alpha$ - tocopherol from dietary vitamin E (a mixture of  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  tocopherols and corresponding tocotrienols). The components of vitamin E are taken up along with emulsified lipid molecules, extracted from food, in equal amounts in an unspecific manner. These are transported from intestine packed in chylomicrons and subsequently found as remnants in the liver. The cytosolic protein  $\alpha$ -TTP is then released into circulation from the liver. This enzyme is responsible for the stereo-selective transfer of  $\alpha$ - tocopherol to VLDL. *In vitro* and *in vivo* assays have shown that  $\alpha$ - TTP preferentially binds to the  $\alpha$ -tocopherol. However, our docked complex shows that  $\alpha$ -tocotrienol shows the highest binding affinity among all the eight isomers tested for the protein. The other three tocotrienols ( $\beta$ ,  $\gamma$ ,  $\delta$ ) have appreciably high binding energy as compared to the corresponding tocopherols. Data also show that the four tocopherols have almost equivalent binding energy. We then selected the specific active sites for docking of all the eight isomers. The docked conformation of  $\alpha$ -tocotrienol with  $\alpha$ - TTP has two hydrogen bonded interaction with  $\alpha$ -TTP (Figure 1). The interaction is with ser140 of intensity -0.28 and with ser136 of intensity -1.26.

Tocopherol-associated protein (TAP) is also been documented extensively as a metabolizing enzyme. It is

present in brain, liver and prostate gland. It increases the uptake and absorption of Vitamin E and hence facilitates the anti proliferation effect in prostate cancer cells. TAP also functions like a tumor suppressor gene to control cancer cell viability through a non-vitamin E functional mechanism. Therefore, TAP is a new prophetic marker for prostate cancer progression. TAP is a vitamin-binding protein and it is involved in transport of Vitamin components specifically tocopherols. Docking data suggests that  $\gamma$ -tocotrienol binds better than others. Three tocotrienols except  $\delta$ -tocotrienol have comparable binding energies higher affinity than tocopherols. They are easily transported between the membranes due to unsaturated side chains. Thus, trienols are potential tumor suppressors for prostate cancer. Docking data shows that gamma tocotrienol have four hydrogen bond interactions with active site residues of TAP (Figure 2). The interactions are with Ile80 of intensity -0.899, Leu84 of intensity -0.4962, Leu84 of intensity -0.30 and Asn259 of intensity -2.203.

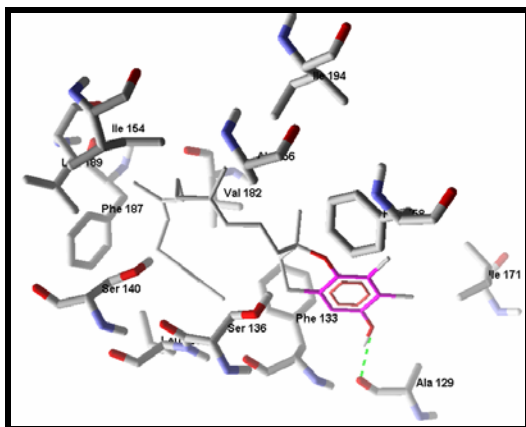
HSA is the target protein for circulation of tocopherols and tocotrienols through blood. This protein has an ability to bind variety of hydrophobic small molecules. These include fatty acids, bilirubin, thyroxin, bile acids, steroids and a group of other bio active molecules. HSA can solubilize the ligands to facilitate transport and buffering effect in free concentration. It is documented that HSA binds a wide variety of drugs in two primary sites which overlap with the binding locations of endogenous ligands [15]. We then selected a site specific for palmitic acid. Data suggest that  $\gamma$ - tocotrienol and  $\delta$ -tocotrienol have highest binding energy with HSA as compared to other members of the family. Overall the binding of tocotrienols with HSA is comparatively higher than the four tocopherols. The docked conformation of  $\gamma$ - tocotrienol with the highest binding energy -11.34 and docking energy is -14.24 kcal/mol. Figure 3 shows the electrostatic interactions of  $\gamma$ - tocotrienol with the aminoacid of HAS. This shows the better absorption and transport potential of tocotrienols.

P-glycoproteins are involved in active transport of various ligands across cell membranes through ATP mediated processes. These are either localized in the plasma membrane or intracellular and are responsible for efflux of various foreign substances including drugs. This protects the body against toxic xenobiotics and drugs. It prevents their accretion in sensitive organs like brain, placenta and gonads by removing these compounds into bile, urine, and the intestinal lumen. The role played by P-gp efflux transporters in assaying the overall bioavailability of drugs is emphasized in recent years. The involvement of P-gp in drug metabolism has immense pharmacokinetic importance. The role of efflux transporters in determining the permeability and overall bioavailability of drugs has gained considerable attention. P-gp efflux pump is localized in a wide range of tissues, including enterocytes of the GI tract. The interaction of P-gp with vitamin E components highlights their bioavailability and pharmacokinetics for distribution in organs/tissues. The docked conformation of  $\alpha$ -tocotrienol with P-gp shows a single hydrogen bond interaction (Figure 4). The

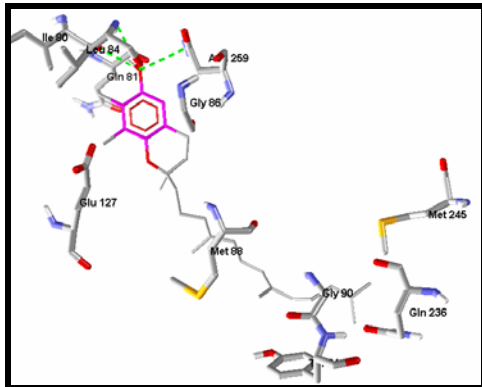
interaction is with Gln100 of interaction energy -0.781 kcal/mol, binding energy -9.06 kcal/mol and docking energy -11.91 kcal/mol.

We selected the ATP binding site for docking of all the eight components of vitamin E in order to analyze their efflux potential. When binding intensity is high efflux is low resulting in more bioavailability. In this study, data shows that  $\alpha$ - and  $\delta$ -tocotrienols show highest binding affinity at the ATP site. Therefore, it can stop the efflux of

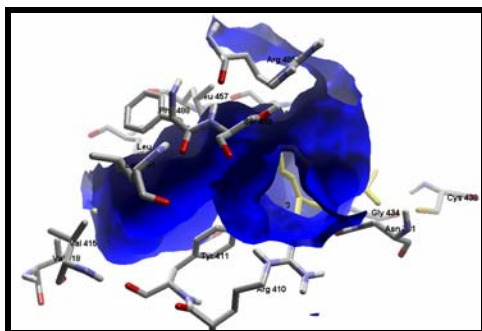
all other components more efficiently and thus, enhancing their bioavailability. The other two tocotrienols ( $\beta$  and  $\gamma$ ) have comparable activity. Tocotrienols are potent candidate as antioxidant and anticancer agent and thus it is important to increase their bioavailability. The bioavailability of all the components of vitamin E is increased when taken with compounds (eg. piperine) that bind strongly at the ATP site of P-glycoprotein which enhances the bioavailability of many antioxidants like curcumin and vitamin C.



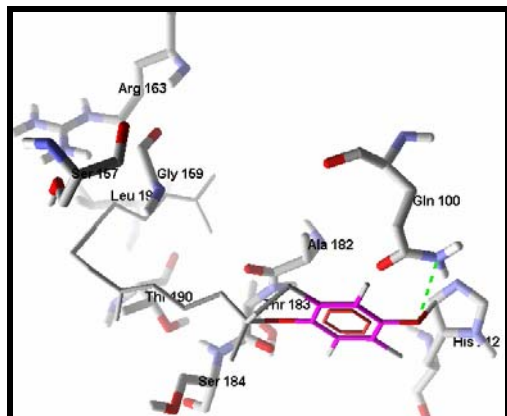
**Figure 1:** The docked conformation of  $\alpha$ -tocotrienol with  $\alpha$ -TTP has two hydrogen bonded interaction with  $\alpha$ -TTP.



**Figure 2:** Docking data shows that gamma tocotrienol have four hydrogen bond interactions with active site residues of TAP



**Figure 3:** The electrostatic interactions of  $\gamma$ -tocotrienol with the aminoacid of HSA.



**Figure 4:** The docked conformation of  $\alpha$ -tocotrienol with P-gp shows a single hydrogen bonded interaction

### Conclusion:

A study to understand the mechanism of cellular uptake of phospho-lipids through the formation of chylomicron with lipids is of interest. The eight components of vitamin E show similar mechanism of cellular uptake (chylomicron formation with lipids). The docked complexes of ATTP, TAP, HSA and P-gp with tocotrienols and tocopherols provide insight to their binding mode during metabolism, absorption, transport and efflux. Data shows that the tocotrienols with unsaturated side chain show better activity than tocopherols due to high binding with the corresponding metabolizing enzymes. This suggests tocotrienols as potential antioxidants and tumor suppressors than tocopherols. Nonetheless, it should be noted these docked data should be verified using appropriate experiments.

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### Supplementary material

**Table1: Binding energies for the docked conformations of eight tocopherols and trienols with four enzymes**

Ligand/Protein	A TTP	p-glycoprotein	HSA	TAP
$\alpha$ -tocopherol	-10.61	-7.71	-6.37	-9.76
$\alpha$ -tocotrienol	-12.1	-9.08	-7.60	-10.82
$\beta$ -tocopherol	-9.74	-6.89	-6.08	-8.83
$\beta$ -tocotrienol	-11.46	-8.52	-7.42	-10.73
$\delta$ -tocopherol	-10.39	-7.72	-6.60	-9.13
$\delta$ -tocotrienol	-11.68	-9.03	-7.74	-10.85
$\gamma$ -tocopherol	-10.86	-7.72	-6.50	-8.71
$\gamma$ -tocotrienol	-11.34	-8.57	-7.80	-11.28

ATTP = alpha-tocopherol transfer protein; HSA = Human serum albumin; TAP = alpha-tocopherol associated protein