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REVIEW

α-TOCOPHEROL TRANSFER PROTEIN: MOLECULAR ASPECTS OF SPECIFIC RECOGNITION OF α-TOCOPHEROL

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Vitamin E is an essential nutrient for reproduction with very complex biological activities. Apart from the classical anti-oxidant functions, in the past decades scientists discovered that vitamin E is involved in the modulation of gene expression and cell signal transduction pathways through proteins that bind tocopherol with high specificity. One of these proteins is α-tocopherol transfer protein (α-TTP) whose function is to select α-tocopherol from other phenolic diet compounds and to regulate α-tocopherol concentration in plasma. α-TTP has the ability to retain in the human body the most biologically by active form of vitamin E. The central role of α-TTP in the metabolism of α-tocopherol is illustrated by the fact that mutations in the *ttpA* gene result in a progressive neurodegenerative spinocerebellar ataxia (ataxia with vitamin E deficiency, AVED) and retinitis pigmentosa.

INTRODUCTION

Vitamin E designates a group of chemically-related, neutral plant lipids with isoprenoid structure. They were first described in 1922 as essential micronutrients for normal fertility in rats. There are eight chemically distinct forms of vitamin E (4 tocopherols and 4 tocotrienols), amongst which *RRR*-α-tocopherol (α-T) is considered the most biologically active form (Fig.1).

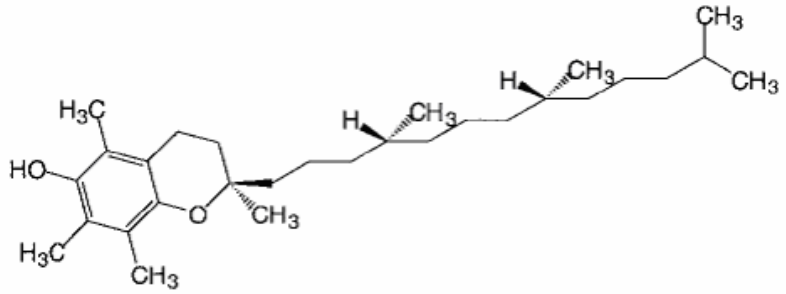

Fig. 1 – Structure of RRR-α-tocopherol showing the configuration of stereocenters (C_2, $C_{4'}$, C_8).

Vitamin E has many functions that can be divided into anti-oxidant (free radical chain-breaking antioxidant) and non-antioxidant (mediated through protein kinase C and tocopherol-associated proteins and tocopherol-binding proteins).[1,2] Vitamin E alone and in conjunction with other compounds (vitamin C, polyphenols, selenium) is one of the components of the first line of defense against lipid peroxidation. For many years α-tocopherol has been considered to play a central role in the prevention of pathological states induced by oxidative stress (atherosclerosis, Alzheimer's disease and cancer).[3] Non-antioxidant functions

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include modulation of cellular signaling pathways, transcriptional regulation, modulation of immune function and induction of apoptosis.[4,5] α-Tocopherol transfer protein (α-TTP) is one of the most important proteins implicated in the metabolism of α-tocopherol due to its ability of specific recognition, selection and retention in the body of α-tocopherol. This paper will focus on the molecular aspects of the specific recognition and selection of α-tocopherol by α-TTP.

INTRACELLULAR TRAFFICKING OF VITAMIN E

Vitamin E is absorbed by entherocites from the lumen of the small intestine, along with other hydrophobic dietary compounds. Inside the entherocytes tocopherols are assembled together with triacilglycerols (TAG), cholesterol, phopsholipids, carotenoids and apolipoproteins (apoB 48) into chylomicrons.[6] In the circulation, the endothelial-bound enzyme, lipoprotein lipase (LPL), which hydrolyzes TAG, catabolizes chylomicrons. During chylomicron lipolysis, a part of vitamin E is distributed to the tissues. After partial delipidation by LPL and acquisition of apo E, remnant chylomicrons are endocytosed to a great extent by the liver parenchymal cells.[7] Endocytosed plasma lipoproteins are transported to late endosomes/lysosomes, where the lipoproteins are hydrolyzed and α-tocopherol associated with them is released.

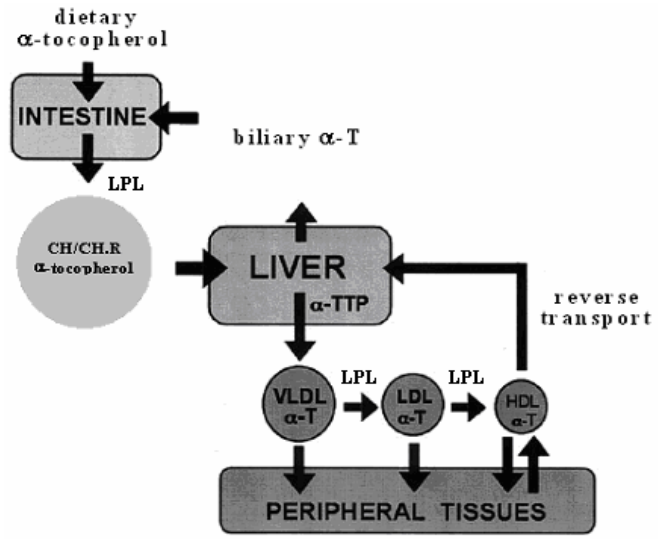

Fig. 2 – Overview of α-tocopherol (α-T) metabolism showing the central role of hepatic α-TTP. α-T is absorbed in the intestine and incorporated into chylomicrons, which are converted to remnant particles by LPL. Remnant particles are then transported to the liver where α-T is selectively incorporated into VLDL by the α-TTP. Between VLDL, LDL and HDL is a rapid spontaneous exchange of α-T. HDL can remove excess α-T from cells. α-T is mainly excreted in the bile and a small fraction is eliminated in the urine (CH = chylomicrons; CH.R = remnant chylomicrons; LPL = lipoprotein lipase) [after, Mardones, 2004].[8]

The liver plays the central role in the regulation of vitamin E. The cytochrome P_{450} isoforms CYP3A4, CYP3A5, CYP4F2 catabolize all the other forms of vitamin E except α-T, into water-soluble compounds that are excreted in the urine. The remaining α-T is then incorporated with other lipids into nascent very low-density lipoproteins (VLDL) and secreted from the liver into the bloodstream. The liver is able to make the discrimination between different tocopherol stereoisomers with the help of **α-tocopherol binding protein**. α-T is not assembled into nascent VLDL in the liver cells, but rather becomes associated with VLDL after its secretion, possibly in the sinusoidal space.[9] Brefeldin A, which inhibits VLDL secretion by disrupting the Golgi apparatus, had no effect on α-T secretion. In circulation VLDL particles are catabolized by LPL to form low-density lipoproteins (LDL) particles, which also deliver α-T to the peripheral tissues (Fig. 2).

High-density lipoprotein (HDL) particles are responsible for the reverse transport of α-tocopherol from peripheral tissues back to the liver but also for the delivery of α-T between different tissues. About 30-50% of the circulating α-T is transported in association with HDL particles. The uptake of HDL-associated α-T occurs *via* a selective pathway implicating a scavenger receptor class B type I (SR-BI).[10]

Oram and colleagues incriminate for the first time a link between ABCA1 (*A*TP *b*inding *c*assette transporter *A1*) and α-T secretion from cells.[11] Recent data suggest that α-TTP transfers α-T to ABCA1 and then to the apo A1, the apolipoprotein of HDL, but the exact mechanism remains still elusive.[12]

The catabolism of tocopherols takes place in the liver and requires cytochrome P_{450} isoforms CYP3A4, CYP3A5 and CYP4F2.[13] The first step is a peroxisomal ω-hydroxylation of the side chain by the action of some CYP-dependent hydroxylases. The initial ω-hydroxylation is followed by oxidation of the hydroxyl group to the carboxyl group and β-oxidation to final carboxyethyl hydroxychromans (CEHCs).[14]

α-TOCOPHEROL TRANSFER PROTEIN

α-TTP was identified in 1975 by Catignani and collab., as a cytosolic protein with a molecular weight of 32 kDa (278 aminoacids). Both Southern-blot hybridization and fluorescence *in situ* hybridization revealed a single α-TTP gene corresponding to the 8q13.1-13.3 region of chromosome 8.[7]

α-TTP is a member of the SEC14 family of proteins that bind specific lipid ligands: α-tocopherol to α-TTP,[15] phosphatidyl inositol to Sec14 (*sec*retory pathway, from *Saccharomices cerevisiae*),[16] retinol and retinal to 11-*c*is-retinol/11-*c*is-retin*al b*inding *p*rotein (CRALBP) [17] and squalene to the supernatant factor protein.[15]

Subcellular distribution of α-TTP is static and is not affected by the presence of vitamin E. α-TTP exhibits partial co-localization with EEA1 (*e*arly *e*ndosomal *a*utoantigen *1*), a marker for early endosome and significant co-localization with LAMP-1 (*l*ysosomal-*a*ssociated *m*embrane *p*rotein *1),* a highly glycosilated protein from the lysosomal membrane with an uncertain function.[12]

Molecular aspects of specific recognition of α-tocopherol by α-TTP

α-TTP contains two CRAL-TRIO domains: *pfam03765* (residues 11-83) and *pfam00650* (residues 89-275). These domains where described for the first time in the retinol/retinal-binding protein (CRALBP) and Trio protein.[18]

The crystallographic studies showed that the two domains of α-TTP are N-terminal and, respectively C-terminal, the latter having a *βαβαβαβ* folding unit. α-T is bound in a hydrophobic pocket of α-TTP where several amino acids make van der Waals contacts with this compound. In this pocket there are also four water molecules: two are hydrogen-bonded to the hydroxyl group of the chromanol ring, one is hydrogen-bonded to the oxygen atoms of V182 and L189, and the fourth water molecule is hydrogen-bonded to the hydroxyl group of S140.[18]

The pyran ring of chromanol is in half-chair conformation, having the methyl group from C_2 stereocenter into an indent of the hydrophobic cavity formed by residues P133, V182 and I179 from α-TTP protein.[19]

One of the factors, which affect the affinity of α-TTP for the four tocopherols, is the degree of methylation of the chromanol ring (α >> β > γ > δ). This is due to the change of the number of the van der Waals contacts between aminoacid side chains and α-TTP (Fig. 3). The extreme lower affinity of α-TTP for tocotrienols is explained by the presence of the three double bonds, with rigid configurations, which enable the unsaturated tail to accommodate in the hydrophobic pocket of the protein. α-TTP selectively binds to *RRR*-α-T (100%) relative to *RRR*-β-T (38%), *RRR*-γ-T (9%) and *RRR*-δ-T (2%).[19]

The specificity of α-TTP for α-tocopherol is due to: (1) the three methyl groups from the chromanol ring, the methyl group from C_5 being critical for the discrimination between α-,β- and γ-Ts; (2) the hydroxyl group on the chromanol ring; (3) the structure and spatial configuration of the phytyl chain.

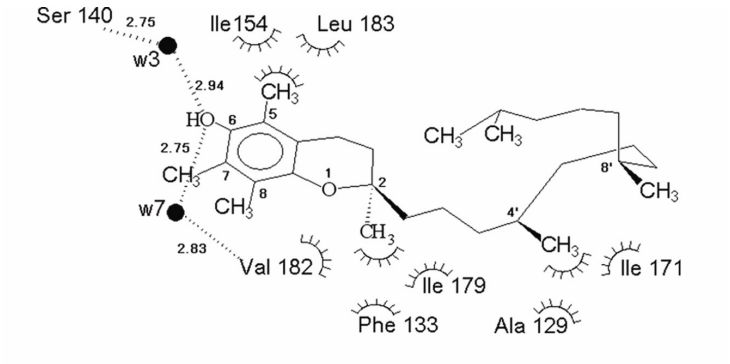

Fig. 3 – The conformation of α-tocopherol adopted in the hydrophobic pocket of α-TTP (dark balls are 2 of the four water molecules found in the hydrophobic pocket; indication is given of the van der Waals contacts between the methyl groups of α-tocopherol and the side chain of aminoacids from the pocket) [courtesy of A. Stocker].

Mechanism of function of α-TTP

Crystallographic studies have revealed that α-TTP can adopt two conformations. In the α-T charged form a mobile helical lid seals the hydrophobic binding pocket of the protein. In the presence of detergents, an open conformation is observed, which probably represents the membrane-bound form of α-TTP.[20] The helical lid has two faces, a polar one, oriented towards the solvent, and a hydrophobic one oriented towards the hydrophobic pocket. In the closed conformation the hydrophobic area of the lid (P203, V206, P207, I210, L214) is in direct contact with the phytyl side-chain of *RRR*-α-T. The opening of the lid shifts these residues towards the exterior making possible the contact with lipids or hydrophobic surfaces and the exchange of α-T. In the closed conformation the lid exposes the polar face and the charged protein can leave the membrane.[19]

Tissue distribution of α-TTP

α-TTP is expressed at high levels in the liver and at low levels in other tissues such as brain,[21] slpeen, lung, kidney and placenta. In the liver α-TTP is expressed by parenchymal hepatocytes, but not by other types of cells.

α-TTP also plays a major role in regulating the cerebral status of vitamin E. In a knockout mouse model for *ttpA* gene, ataxia and retinal degeneration were observed after 1 year of age, symptoms very similar to those seen in the human AVED disease. In the brain, α-TTP is expressed mainly in the Purkinje cell layer of the cerebellar cortex.

Using monoclonal antibody α-TTP was localized in the syncytio-trophoblast and invasive trophoblast of the term human placenta, suggesting that α-T accumulation in the amniotic fluid involves the presence of α-TTP.[22] In α-TTP knockout mice pregnancy develops normally until day 9.5 *post coitum*, but between days 11.5 and 14.5 *post coitum*, the fetuses showed marked impairment of brain development with neural tube defects.[23]

Until recently, it was not clear when or where vitamin E is required during pregnancy. Jishage and colleagues have demonstrated that vitamin E is indispensable for the proliferation and/or function of the placenta but not necessarily for the development of the embryo itself.[24] Recent experimental evidence has indicated that vitamin E could protect against ovarian metaplasia by neutralizing the oxidative stress associated with ovulation and by enhancing the repair capacity of the surface epithelium, respectively.[25] There are some epidemiological trials suggesting an inverse relationship between consumption of vitamin E and risk of ovarian carcinoma.[26]

The level of α-T in the diet modulates the expression of α-TTP gene. Refeeding tocopherol to vitamin E-depleted rats increased the expression of α-TTP mRNA about 7-fold.[27] Also, an increase in the α/δ ratio in individual lipoprotein fractions is evidence of an increased α-TTP action concomitantly with the increased α-TTP mRNA expression.

Pathological states implicating α-TTP

In humans, mutations in the *ttpA* gene are associated with low plasma tocopherol levels and with a progressive peripheral neuropathy, which finally results in ataxia (AVED), very similar with Friedreich's ataxia.[7] Similarly, $TTP^{-/-}$ mice display low vitamin E levels, are infertile and exhibit an AVED-like pathology.[28] When patients with AVED received a high dose of α-tocopherol, the progression of the disease was reduced.[6] Mapping the known mutations leading to AVED onto the crystal structure of α-TTP shows that no mutation occurs directly in the hydrophobic pocket of the protein. This observation indicates that these mutations seem to influence the function of α-TTP by impairing the stability of the protein rather than by affecting ligand transfer.[20] However only one mutation involving the hydrophobic pocket of α-TTP (L183P) was reported. The side chain of L183 in combination with those of V191 and I194 forms an indent for the methyl group of C_5 on the chromanol ring of α-T.[18]

Substitutions of the amino acids that are not conserved in other members of the Sec14 family (CRALBP, SEC14) (semi-conservative missens mutations: R192H, A120T, H101Q) cause mild forms of AVED. Patients with these mutations may be able to incorporate the natural *RRR*-α-T into plasma lipoproteins, but to a lesser extent than normal subjects. In contrast, the substitutions of amino acids that are highly conserved in other members of the Sec14 family (non-conservative missens mutations: R59W, E141K, R221W) are associated with severe forms of AVED, suggesting an important role for the amino acids R59, E141 and R221.[7]

The mutation H101Q in the *ttpA* gene is responsible for Friedreich-like ataxia with retinitis pigmentosa.[29]

CONCLUSIONS

This review summarizes recent findings concerning the molecular mechanism underlying the ability of α-TTP to specifically recognize, select and retain α-tocopherol in the body. Vitamin E is one example of how human organism develops molecular mechanisms for retaining the most biologically active form of a compound. The central role of α-TTP in the trafficking of α-tocopherol is illustrated by the fact that mutations in the α-TTP gene cause severe deficiency in the nervous system development and function, and in the materno-fetal unit during pregnancy. Also, this is a very good example of how biomolecules can make very sensitive discriminations at the molecular level through stereochemistry of their ligands.

A better understanding of gene products and cellular pathways participating in α-tocopherol transfer and metabolism *in vivo* may offer novel targets for pharmacological interventions in many pathological conditions (cancer, neurodegenerative states, cardiovascular diseases).

ABREVIATIONS: α-T, α-tocopherol; α-TTP, α-tocopherol transfer protein; TAG, triacylglycerol; LPL, lipoprotein lipase; CYP, cytochrome P_{450}; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SR-BI, scavenger receptor class B type I; ABCA1, ATP-binding cassette A1; CEHC, carboxyethyl hydroxychroman; CRALBP, 11-*cis*-retinol/11-*cis*-retinal binding protein; EEA1, early endosomal autoantigen 1; LAMP-1, lysosomal-associated membrane protein 1; AVED, ataxia with vitamin E deficiency.

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