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Forum Review Article

Tocopherol-Binding Proteins: Their Function and Physiological Significance

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ABSTRACT

The present review is a continuation of earlier essays on the uptake mechanisms and the biological function of vitamin E. There are eight naturally occurring homologues of vitamin E, which differ in their structure and in biological activity *in vivo* and *in vitro*. Various studies have suggested that after normal gastrointestinal absorption of dietary vitamin E specific mechanisms favor the preferential accumulation of one of its homologues, α-tocopherol, in the human body. This process is thought to be mediated in part by the α-tocopherol transfer protein (α-TTP) in the liver cytoplasm. The mechanism and pathway by which α-TTP specifically incorporates α-tocopherol into plasma lipoproteins is not yet fully understood. Because α-tocopherol is widely distributed in tissues in various concentrations but α-TTP resides only in liver, its role as intracellular carrier of α-tocopherol seems unlikely. However, recent data indicate that a system of α-tocopherol-binding proteins is involved in these processes that favor the localization of α-tocopherol at the sites where it is required. The current status of the evidence for the regulation of α-tocopherol levels and their impact on cellular signaling is discussed. Antiox Redox Signal. 2, 397–404.

INTRODUCTION

VITAMIN E is one of the most researched compounds in medicine. It is an essential nutrient known to function as a chain-breaking antioxidant that prevents the propagation of free radical reactions in the human body (Burton *et al.*, 1983). It is essential, by definition, because the body cannot manufacture its own vitamin E and thus it must be provided by foods and supplements. The term vitamin E is actually a general name for the various chemical forms of this compound. In nature, molecules having vitamin E activity include two groups of closely related fat-soluble compounds, tocopherols and tocotrienols. The members of each group are designated as α, β, γ, or δ, depending on the numbers and position of the methyl groups attached to the chromanol ring structure (Kwiatkowska, 1988) (Fig. 1).

RRR-α-tocopherol and *RRR*-γ-tocopherol are the most common vitamin E homologs deriving mainly from diets rich in edible plant oils, vegetables, and fruits. Major sources for tocopherols are sunflower seeds, containing predominantly *RRR*-α-tocopherol, and oil from soybeans, which contain a mixture of γ-, δ-, and α-tocopherol (Crawley, 1993). Within the human body, all homologs of vitamin E encounter a rapid clearance from tissues and plasma with the exception of RRR-α-tocopherol (Traber and Kayden, 1989). Thus, the regulation of α-tocopherol levels in the plasma as well as the specific transport of α-tocopherol toward intracel-

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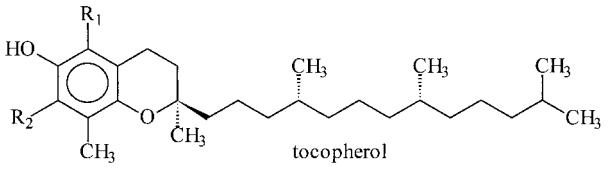

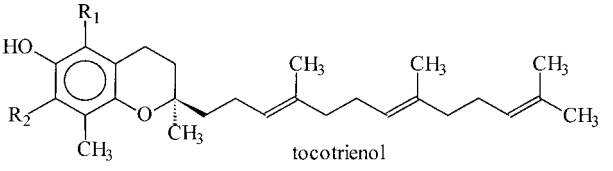

R_1	R_2	
CH_3	CH_3	α
CH_3	H	β
H	CH_3	γ
H	H	δ

FIG. 1. Naturally occurring components of vitamin E.

lular compartments may represent essential events in modulating the biological activity of this compound.

ABSORPTION, TRANSPORT, AND DISTRIBUTION OF VITAMIN E

Vitamin E requires, because of its hydrophobicity, special transport mechanisms in the aqueous environment of the plasma, body fluids, and cells (Buttriss and Diplock, 1988). In human vitamins, E is taken up in the proximal part of the intestine depending on the amount of food lipids, bile, and pancreatic esterases. Vitamin E is solubilized together with the fat-soluble components of the food. Lipolysis and emulsification of the formed lipid droplets leads then to the spontaneous formation of mixed micelles that are absorbed at the brush border membrane of the mucosa by passive diffusion (Gallo-Torres, 1970) (Fig. 2).

Together with triglycerides, phospholipids, cholesterol, and apolipoproteins, vitamin E is then reassembled to chylomycrons in the Golgi apparatus of the mucosa cells (Bjorneboe *et al.*, 1990). The chylomycrons are stored as secretory granula and eventually excreted by exocytosis to the lymphatic compartment, from where they reach the blood stream via the ductus thoracicus (Bjornson *et al.*, 1976). The rather high clearance rate (24–48 hr) of a bolus of vitamin E from the plasma and the concomitant rapid uptake by the liver parenchyma indicate that the intravascular degradation of the chylomycrons to remnants by the endothelial lipoprotein lipase (LPL) is a prerequisite for the hepatic uptake of vitamin E (Mathias *et al.*, 1981; Handelman *et al.*, 1985). Most probably, the exchange between apolipoproteins of the cylomycrons (type AI, AII, and B_{48}) and high-density lipoprotein (HDL) (types C and E) triggers the formation of the remnants and in this way favors their rapid uptake via hepatic receptors for apolipoprotein E (apo-E) and (apo-B) (Fig. 3).

LIVER α-TOCOPHEROL TRANSFER PROTEIN

In contrast to the unspecific uptake pathway of vitamin E from ingested food to the liver parenchyma, the transfer of α-tocopherol from the hepatic cells into plasma is mediated by a specific protein, the α-tocopherol transfer protein (α-TTP) (Arita *et al.*, 1995). This protein specifically selects α-tocopherol from all incoming tocopherols and promotes its transfer into lipoproteins (Hosomi *et al.*, 1997). It has

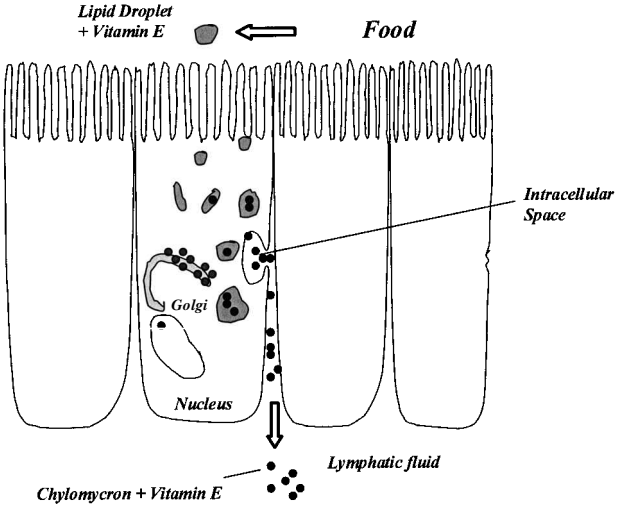

FIG. 2. Duodenal absorption of vitamin E from the food and its transport into the intestinal lymphatic fluid by exocytosis of the chylomycrons. (Adapted from Löffler and Petrides, 1997).

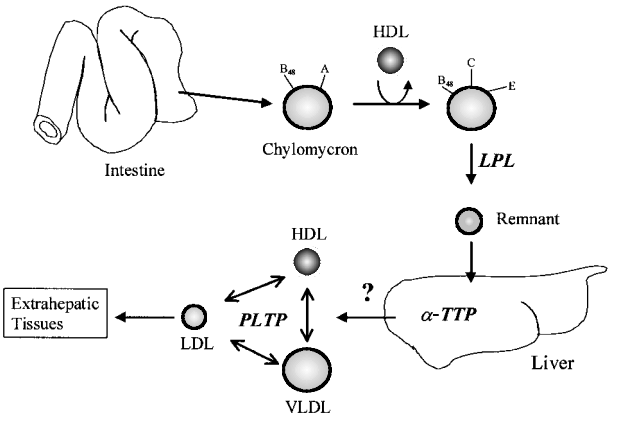

FIG. 3. Unselective hepatic uptake of vitamin E via remnants and subsequent selective transfer of α-tocopherol to extrahepatic tissues.

been suggested by several groups that α-tocopherol taken up by the liver is resecreted into the plasma in very low density lipoprotein (VLDL) (Peake *et al.*, 1972; Bjornson *et al.*, 1976). Nevertheless, it could be shown recently that α-TTP present in the liver cytosol functions to stimulate secretion of cellular α-tocopherol into the extracellular medium and that the reaction uses a novel non-Golgi-mediated pathway. It was concluded that this novel pathway may be linked to cellular cholesterol metabolism and/or transport (Arita *et al.*, 1997; Fragoso and Brown, 1998). α-TTP has been shown to possess both stereospecificity as well as regiospecificity toward the most abundant isomer of vitamin E, *RRR*-α-tocopherol. As consequence of the selectivity of α-TTP, major parts of the natural homologs and nonnatural isomers of α-tocopherol are excluded from the plasma and secreted with the bile (Traber and Kayden, 1989). Genetic defects in the hepatic α-TTP have been reported that have led to the discovery of a rare genetic disease, resulting in vitamin E deficiency (ataxia with isolated vitamin E deficiency, AVED) (Ouahchi *et al.*, 1995). AVED patients have very low plasma vitamin E concentrations and suffer from progressive peripheral neuropathy and ataxia (Amiel *et al.*, 1995). The highly tissue-specific pathology associated with AVED would suggest a tissue target of action rather than a general antioxidant function for vitamin E. Several studies have shown that α-TTP is expressed only in liver in significant amounts. Thus the incorporation of extracellular α-tocopherol into extrahepatic tissues would relate to a series of unknown transport processes (Fig. 3).

PHOSPHOLIPID TRANSFER PROTEIN AND LIPOPROTEINS

Because of its hydrophobicity, α-tocopherol is mainly transported in association with lipoproteins in the plasma compartment (Kayden and Traber, 1993). All plasma lipoproteins can constitute α-tocopherol vehicles, and the contribution of distinct lipoprotein fractions to α-tocopherol transport actually depends on their relative proportions in one given plasma sample (Desrumaux *et al.*, 1999). The plasma phospholipid transfer protein (PLTP), which is known to catalyze the exchange of phospholipids and other amphipatic compounds between lipid structures, has been reported to facilitate several fold the exchange of α-tocopherol between lipoproteins (Kostner *et al.*, 1995). The binding of tocopherol-containing lipoproteins to cells and the subsequent preferential uptake of LDL into these cells has been well documented. Using LDL and HDL labeled with [^{3}H]-α-tocopherol characteristics for receptor binding and cell uptake could be established (Gurusinghe *et al.*, 1988). It was shown that binding and uptake appear to be specific to LDL receptors whereas HDL, which also binds to cells, shows no evidence of internalization (Fig. 3).

Although a basal α-tocopherol uptake by muscle cells from both HDL and LDL has been observed, the receptor-mediated uptake appears to be about one order more effective (Cohn and Kuhn, 1989). These findings are supported by the fact that the highest α-tocopherol content of all subcellular compartments is found in lysosomes (14.6 mmol/mol lipid) (Buttriss and Diplock, 1988).

TOCOPHEROL-ASSOCIATED PROTEIN

Recently, a cytosolic tocopherol binding protein with broad tissue distribution has been discovered in our group (Stocker *et al.*, 1999). This

46-kD protein has been purified from bovine liver by conventional chromatographic methods using [^{3}H]-α-tocopherol. The corresponding human 46-kD protein has been identified, but its function is still unknown and therefore it has been given the name tocopherol-associated protein (TAP). So far, human TAP has been shown to be ubiquitous, but more highly expressed in adult liver, prostate, and brain tissue. Sequence homology of TAP ascribes it to a family of hydrophobic ligand binding proteins including α-TTP (Gu *et al.,* 1992). Another member of this family is phosphatidylinositol-transfer protein (SEC14). This protein catalyzes the transfer of phospholipids between membrane bilayers and plays an essential role in yeast Golgi function (Bankaitis *et al.,* 1990). A structural analysis of SEC14 obtained by X-ray diffraction provides insights in its function and new information concerning the architecture of the entire family of evolutionary conserved proteins (Sha *et al.,* 1998) (Fig. 4).

The structural homology of TAP with phosphatidylinositol-transfer protein (SEC14) and its broad tissue distribution make TAP a probable candidate responsible for the regulation of tissue α-tocopherol levels. Moreover, regulatory functions of this protein cannot be excluded at the present time and are the object of present studies.

PHYSIOLOGICAL SIGNIFICANCE OF VITAMIN E REGULATION

The mechanisms by which tissue specific levels and turnover rates of *RRR*-α-tocopherol are regulated still remain obscure. Nevertheless, the role of *RRR*-α-tocopherol in intracellular signaling has been intensively studied on different cellular levels in the last decade (Azzi *et al.,* 1998). RRR-α-tocopherol was identified as a cell-cycle specific negative regulator of cell proliferation in our group (Azzi *et al.,* 1997). Numerous experiments consistently show that *RRR*-α-tocopherol, but not *RRR*-β-tocopherol, inhibits proliferation of vascular smooth muscle cells from rats and humans at physiological concentrations, although both homologs are taken up at the same rate. The lack of inhibition by β-tocopherol cannot be explained by physicochemical arguments, since the relative

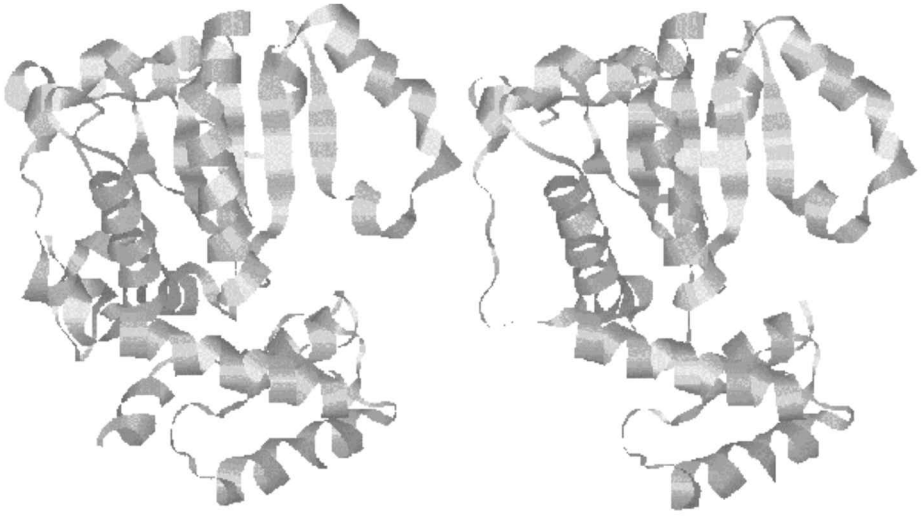

FIG. 4. (Left) Crystal structure of yeast phosphatidylinositol-transfer protein (SEC14), residues 1–246. (Right) Computational model (Swissmodel) of the three-dimensional structure of human TAP, residues 1–246.

free radical quenching efficiencies of both homologs are very similar (Kaiser *et al.*, 1990). The preferential uptake of α-tocopherol in the human body is accompanied by specific inhibition of protein kinase C (PKC), a key enzyme in the proliferative pathway of a number of cells (Boscoboinik *et al.*, 1991). PKC inhibition may be linked to the activity of protein-phosphatase 2A (PP2A). This phosphatase is stimulated by *RRR*-α-tocopherol in a dose-dependent way and may lead to the dephosphorylation and inactivation of PKC-α (Boscoboinik *et al.*, 1991). The data reported can be rationalized by a model in which α-tocopherol affects molecules, at the level of signal transduction, ending with the inhibition of cell proliferation. Platelet aggregation, monocyte adhesion, oxygen radical release from macrophages and neutrophils, and mesangial cell growth inhibition are examples of some of the consequences of PKC inhibition in different cells. Genes that directly or indirectly are under the control of this cascade are the gene of collagenase (MMP1), the gene of α-tropomyosin (Aratri *et al.*, 1999), and the gene for one of the scavenger receptors (CD36) (Ricciarelli *et al.*, 1999). Thus, it is reasonable to postulate a regulation system that controls vitamin E at cellular levels as well as a direct molecular interaction of α-tocopherol in analogy with the mechanisms involved in retinoid function (Morriss-Kay and Ward, 1999).

VITAMIN E REQUIREMENTS

Recent studies on the uptake, metabolism, and degradation of vitamin E have shown that synthetic all-rac-α-tocopherol, as well as the natural homologs of α-tocopherol, are preferentially degraded in humans (Traber *et al.*, 1998; Swanson *et al.*, 1999). Despite this known human preference for RRR-α-tocopherol, synthetic α-tocopherol (all-rac-α-tocopherol) is widely used in many types of medical preparations and food additives. A number of articles support the notion that an assessment of vitamin E requirements must be based on protective levels, preventing undesirable chronic lipid peroxidation, and not on suppressing signs of deficiency, which are infrequently seen in humans. Overt vitamin E deficiency occurs only rarely in humans and is caused by a genetic defect of the α-TTP (AVED) (Cavalier *et al.*, 1998) or as the result of various malabsorption syndromes (Traber and Sies, 1996). Severe vitamin E deficiency has also been reported in cases of Retinitis pigmentosa (Yokota *et al.*, 1996).

Apparently very little vitamin E is required by healthy adults (10–40 mg/day) to prevent nutritional deficiency (Weber *et al.*, 1997). However, possible effects of inadequate vitamin E intake may develop over a long time, typically decades, and have been linked to degenerative diseases such as atherosclerosis (Davey *et al.*, 1998) and with prostate cancer (Heinonen *et al.*, 1998). The Cambridge Heart Antioxidant Study (CHAOS) (Stephens *et al.*, 1996) reported in over 2,000 patients with angiographycally proven coronary atherosclerosis that vitamin E supplementation (400–800 IU/day) significantly reduced the incidence of cardiovascular death.

A decrease in lipid peroxidation of low-density lipoproteins (LDL), due to the antioxidant action of α-tocopherol, has been assumed to be the major mechanism leading to such a result. The discovery that LDL oxidation is a prerequisite for foam cell formation has led to the "oxidative modification hypothesis" of atherosclerosis (Witztum and Steinberg, 1991). According to this hypothesis, LDL traverses the subendothelial arterial space where it is subjected to oxidation. *In vitro* evidence indicates that once oxidized, LDL becomes a ligand for scavenger receptors, leading to foam cell formation. Oxidized LDL is also chemotactic for cultured monocytes (Quinn *et al.*, 1987) and stimulates cellular production of chemokines (Cushing *et al.*, 1990), potentially leading to inflammatory cell recruitment into the arterial wall. Thus, LDL oxidation triggers a number of events that can promote establishment and progression of atherosclerosis. Studies on cholesterol-induced atherosclerosis in rabbit have shown no protection by probucol but a strong protection by α-tocopherol, indicating a specific role for vitamin E (Ozer *et al.*, 1998). Recent studies in Watanabe heritable hyperlipidemic rabbits have shown that inhibition of aortic lipid peroxidation might not correlate with an antiatherogenic effect in this animal model (Wit-

ting *et al.,* 1999). These findings are in line with our observation that the major role of α-tocopherol is not in its action as antioxidant by preventing the oxidation of LDL, but by down-regulating the scavenger receptor, leading to a diminution of the uptake of oxidized LDL (Ricciarelli *et al.,* 2000). Nevertheless, the mechanisms by which vitamin E is transported and regulated within cells and how it is involved in cellular signaling still remain obscure. Furthermore, the possibility that α-tocopherol acts similarly to retinol derivatives is being considered. Recently, the major urinary metabolite (α-CEHC) of α-tocopherol has been discovered (Schonfeld *et al.,* 1993). It appears in human urine after vitamin E supplementation and is formed directly from α-tocopherol without previous oxidative splitting of the chromane ring. The correlation of tocopherol intake and urinary excretion of α-CEHC was examined in human volunteers supplemented with *RRR*-α-tocopherol in the range from 0 to 800 mg/day. The analysis revealed that α-CEHC is only excreted above a daily intake of 150 mg of α-tocopherol. This amount was interpreted as an indicator of plasma saturation by vitamin E (∼80 μM) and may be considered as marker of optimum vitamin E intake (Schultz *et al.,* 1995). If prevention of oxidative damage and promotion of an optimal health status are taken as end point, current estimates suggest that roughly an amount 10 times higher than that recommended to prevent symptoms of vitamin E deficiency is needed (Lemoyne *et al.,* 1987). In the light of our current knowledge, it might be reasonable to conclude that supplementation with *RRR*-α-tocopherol is preferable for human supplementation and prevention of disease.

ABBREVIATIONS

α-CEHC, α-Carboxyethylhydroxychroman; α-TTP, α-tocopherol transfer protein; phosphatidylinositol-transfer protein (SEC14); apo-E, Apolipoprotein E; AVED, ataxia with isolated vitamin E deficiency; CD36, platelet glycoprotein IV/thrombospondin receptor/class B scavenger receptor; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPL, endothelial lipoprotein lipase; MMP1, matrix metalloproteinase I; PKC protein kinase C; PLTP, plasma phospholipid transfer protein; PP2A, protein phosphatase 2A; SEC, secretory protein gene products; TAP, α-tocopherol associated protein; VLDL, very-low-density lipoprotein.

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