REVIEW

Vitamin E transport, membrane incorporation and cell metabolism: Is α-tocopherol in lipid rafts an oar in the lifeboat?

Stéphanie Lemaire-Ewing[1,2], Catherine Desrumaux[1,2], Dominique Néel[1,2] and Laurent Lagrost[1,2]

[1] INSERM UMR866, Dijon, France
[2] Université de Bourgogne, Dijon, France

Vitamin E is composed of closely related compounds, including tocopherols and tocotrienols. Studies of the last decade provide strong support for a specific role of α-tocopherol in cell signalling and the regulation of gene expression. It produces significant effects on inflammation, cell proliferation and apoptosis that are not shared by other vitamin E isomers with similar antioxidant properties. The different behaviours of vitamin E isomers might relate, at least in part, to the specific effects they exert at the plasma membrane. α-Tocopherol is not randomly distributed throughout the phospholipid bilayer of biological membranes, and as compared with other isomers, it shows a propensity to associate with lipid rafts. Distinct aspects of vitamin E transport and metabolism is discussed with emphasis on the interaction between α-tocopherol and lipid rafts and the consequences of these interactions on cell metabolism.

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1 Structural heterogeneity and molecular properties of vitamin E: α-tocopherol above all

Vitamin E is composed of closely related compounds, each comprising a 6-chromanol ring and a polyisopentenyl side chain, which can be either saturated (tocopherols) or unsaturated with three double bonds at positions 3′,7′ and 11′ (tocotrienols). Tocopherols and tocotrienols have four isomers, each designated according to the position and number of the methyl groups on the phenol ring. Thus, the α-, β-, γ- and δ-vitamers contain three, two, two and one methyl groups, respectively [1]. Importantly, the α-tocopherol form, and especially its natural RRR-isomer, is the only one to be recognized with high selectivity by liver α-tocopherol transfer protein (α-TTP), which triggers its association with lipoprotein and the secretion of vitamin E into the bloodstream [2, 3]. As a consequence, α-tocopherol is the main vitamin E isomer found in plasma and tissues. Because all the tocopherol forms display similar antioxidative properties, it is tempting to speculate that the remarkable adaptation of the organism to select α-tocopherol may actually relate to some specific, non-antioxidative properties (including modulation of enzyme activity, regulation of gene expression and membrane stabilization [4]) that are not shared by other vitamin E forms [5]. Finally, recent studies came in support of tocopherol phosphate as a natural form of tocopherol with

Correspondence: Dr. Laurent Lagrost, Centre de Recherche INSERM UMR866, Faculté de Médecine, 7 boulevard Jeanne d'Arc, BP 87900, 21079 Dijon Cedex, France
E-mail: laurent.lagrost@u-bourgogne.fr
Fax: +33 3-80-39-34-47

Abbreviations: **α-TTP,** α-tocopherol transfer protein; **ABCA1,** ATP-binding cassette-A1; **apoB,** apolipoprotein-B; **CEHC,** carboxyethyl-hydroxychroman; **CETP,** cholesteryl ester transfer protein; **HDL,** high density lipoprotein; **LDL,** low density lipoprotein; **LPL,** lipoprotein lipase; **PKC,** protein kinase C; **PLTP,** phospholipid transfer protein; **PLTP-KO,** PLTP-knockout; **PP2A,** protein phosphatase 2A; **SHIP,** SH2-containing protein-tyrosine phosphatase-1; **SR-BI,** scavenger receptor-BI; **TAP,** tocopherol-associated protein; **VLDL,** very low density lipoprotein

putative greater biological effects than tocopherol [6–8]. However, whether tocopherol phosphate constitutes either a reserve form of α-tocopherol, a derivative with high transmembrane transport potential, or an activated form with specific regulatory properties remain to be clarified and will not be extensively discussed in the present review.

2 Absorption, intravascular transit and supply of vitamin E to tissues

The hydrophobicity of vitamin E is a major limitation to its transport and distribution in aqueous compartments. As for other hydrophobic/lipid compounds, including cholesterol, phospholipids and triglycerides, vitamin E isomers cannot distribute as free monomers in the bloodstream, but require association with lipoproteins. As a consequence tocopherol metabolism shares similar features with lipoprotein metabolism and cholesterol transport. The following paragraphs describe key steps of the lipoprotein-related pathways of tocopherol transport, with emphasis on the recently discovered impact of plasma phospholipid transfer protein (PLTP).

2.1 Vitamin E absorption

Dietary vitamin E (mainly RRR-α-tocopherol and RRR-γ-tocopherol) is taken up in the proximal part of the intestine after solubilization by bile acids. Although intestinal uptake has been regarded for a long time as a passive process, it has recently been shown to be mediated, at least in part, by the scavenger receptor-BI (SR-BI), a transmembrane glycoprotein that mediates selective uptake of HDL-associated cholesteryl esters, especially in the liver and steroidogenic tissues [9]. After uptake, tocopherols are resecreted from the basal side of enterocytes as part of chylomicrons or nascent HDL [10]. The secretion of vitamin E in chylomicrons requires functional Microsomal Triglyceride Transfer Protein, while secretion in HDL involves the transmembrane transporter ATP-Binding Cassette-A1 (ABCA1) [11, 12] (Fig. 1).

2.2 Postprandial delivery of vitamin E to peripheral tissues

After secretion into the lymph duct by enterocytes, chylomicrons enter the bloodstream where they are subjected to hydrolysis by lipoprotein lipase (LPL). As a consequence, LPL plays a significant role in the cellular uptake of vitamin E from chylomicrons. It has been suggested that uptake relies on both the triglyceride hydrolysis activity and lipoprotein-cell bridging function of LPL [13]. Alternatively, the ABCA1-mediated association with HDL constitutes an independent pathway, which might become prominent when chylomicron assembly in the postprandial phase is defective, as in abetalipoproteinemia [11, 12, 14].

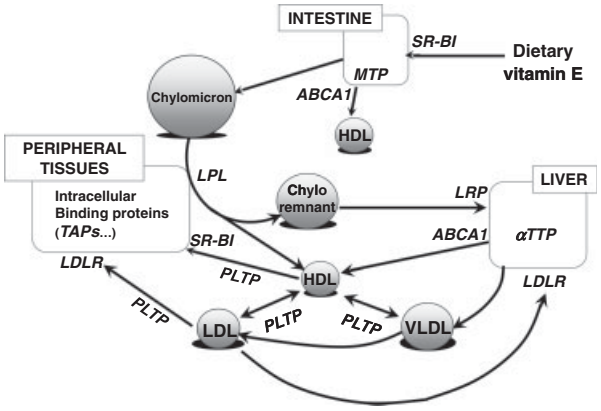

Figure 1. Absorption, intravascular transport, and tissue distribution of vitamin E. See main text for details. LDLR, LDL receptor; LRP, LDL receptor-related protein; MTP, microsomal triglyceride transfer protein.

2.3 Hepatic uptake and resecretion of α-tocopherol

After hydrolysis by LPL, chylomicron remnants containing vitamin E are captured by the liver *via* the interaction of apolipoprotein E with LDL receptor-related protein [15]. In hepatocytes, a cytosolic protein called α-tocopherol transfer protein (α-TTP) is responsible for the selective resecretion of this vitamin E isomer into the plasma compartment [16]. In humans, heritable mutations in the α-TTP gene cause ataxia with Vitamin E deficiency, an autosomal recessive disorder characterized by low plasma vitamin E levels and progressive neurodegeneration [17]. Although it has long been assumed that very low density lipoproteins (VLDLs) are the principal vehicle for α-tocopherol export from the liver during the interprandial phase, recent studies also pointed to the physiological role of ABCA1 and HDL in this process [18].

2.4 Intravascular transport of α-tocopherol

Under fasting conditions, low density lipoproteins and HDL become the major carriers of plasma α-tocopherol. Although spontaneous exchange/transfer of α-tocopherol occurs between circulating lipoproteins, it is rather slow and cannot account for the high exchange rates that have been reported *in vivo*. PLTP, *i.e.* a member of the lipid transfer/lipopolysaccharide binding protein family arose recently as a cogent candidate in governing vitamin E metabolism *in vivo* both at the intracellular and at the plasma levels [19–21]. First, PLTP may favour α-tocopherol export since it has been described as a major determinant of VLDL assembly and secretion in the liver [22]. Second, PLTP transfers

α-tocopherol between isolated lipoproteins in the bloodstream. The pathophysiological relevance of these observations has been demonstrated in the PLTP-knockout (PLTP-KO) mouse model, in mice injected with an adenovirus-associated virus encoding human PLTP [21, 23, 24], as well as in diabetic patients and control subjects [25]. Since PLTP has the ability to interact with and transfer a number of amphipathic compounds with a broad diversity of molecular structures (including phospholipids, tocopherols, lipopolysaccharides, unesterified cholesterol and diacylglycerides [26]), it is conceivable that it might also bind a large variety of tocopherol derivatives such as tocopheryl phosphate. This latter view will deserve direct attention. Cholesteryl ester transfer protein (CETP), another member of the lipid transfer/lipopolysaccharide binding protein gene family mediates the exchange of neutral lipids. Like PLTP, CETP is also thought to play a role in vitamin E transport and metabolism since a CETP inhibitor was found to decrease the transfer of vitamin E from triglyceride-rich lipoproteins to HDL *in vitro* [27, 28]. However, and unlike the vitamin E transfer activity of PLTP, no evidence for the physiological relevance of the vitamin E transfer activity of CETP has been reported so far.

2.5 Interprandial delivery of α-tocopherol to peripheral tissues

The distribution and metabolism of α-tocopherol is closely related to that of other lipids that are transported by plasma lipoproteins. Although both LPL and LDL receptors were proposed earlier as key players in vitamin E supply to tissues, the SR-BI, tocopherol-associated proteins (TAP) and PLTP emerged only recently as additional determinants of vitamin E distribution *in vivo*.

2.5.1 LPL

Although the LPL-mediated pathway may mostly apply to the cellular uptake of tocopherol from chylomicrons during the postprandial phase, it was also found to drive cell delivery from VLDL, LDL and HDL *in vitro* [29], suggesting that it may also operate in the fasting state. *In vivo* studies have shown the impact of LPL on vitamin E uptake in the mammary gland [30], skeletal muscle [31] and brain [32].

2.5.2 LDL receptor

LDL is a major carrier of vitamin E in plasma, and earlier studies revealed the role of the LDL receptor in delivering vitamin E to cultured cells through LDL endocytosis [33]. However, subsequent studies conducted in animal models as well as in human populations led to rejection of the physiological involvement of the LDL receptor, which was eventually declared not essential to maintain normal tissue levels of vitamin E *in vivo* [34–36].

2.5.3 SR-BI

Earlier observations made in HepG2 cells, type II pneumocytes and primary porcine brain capillary endothelial cells identified HDL as the preferential source of α-tocopherol for these cell types and demonstrated that α-tocopherol is incorporated through selective lipid uptake [37–39]. Later studies conducted with transfected Chinese hamster ovary cells showed the function of the SR-BI in selective α-tocopherol uptake [40]. The relevance of SR-BI as a cell surface receptor for α-tocopherol uptake was established *in vivo*, since SR-BI knockout mice show increased plasma and HDL α-tocopherol levels, decreased biliary excretion of α-tocopherol and reduced α-tocopherol contents in several peripheral organs [36]. Thus, the SR-BI-deficient phenotype is characterized by a marked, 70% decrease in brain α-tocopherol content, suggesting a role for SR-BI in selective α-tocopherol uptake across the blood–brain barrier [36]. Although no significant neurological impairment was noted in SR-BI knockout mice, a detailed evaluation of learning and memory as well as susceptibility to neurodegenerative disorders would be warranted. Another feature of SR-BI deficiency in mice is a reduction of the α-tocopherol content in lungs. Although the relevance of SR-BI-mediated α-tocopherol supply to the lungs remains to be established, its implication in the uptake of the antitumoral agent α-tocopheryl succinate and protection against lung cancer *in vivo* has been demonstrated [41]. SR-BI deficiency in mice is accompanied by decreased α-tocopherol content in the ovary and testis. Since vitamin E was first described as an essential micronutrient for fertility in rats, the impact of SR-BI on fertility was investigated. SR-BI deficiency was accompanied by female infertility, which was associated with decreased *in vitro* viability and development of embryos [42]. Finally, recent studies have suggested a role for SR-BI in α-tocopherol uptake at the inner blood–retinal barrier and in maintaining α-tocopherol in the neural retina [43].

2.5.4 TAP

In humans, three TAPs (hTAP1, hTAP2 and hTAP3 also called SEC14L2, SEC14L3 and SEC14L4 as referred to the yeast SEC14p secretory proteins) have been described. TAP-1 was first identified in the cytosol of bovine liver [44]. This was followed by the cloning of TPA-2 and TAP-3 [45]. Unlike α-TTP, TAPs can bind the four tocopherol analogues as well as tocotrienols and a number of other ligands such as squalene, phosphatidylinositol and phosphatidylcholine [46]. Even though their role in binding to and transporting vitamin E is still unclear, TAP1 and

TAP2 might be involved in the intracellular transport of α-tocopherol to mitochondria [46]. This observation suggests that hTAPs could play a role in vitamin E transport to subcellular compartments.

2.5.5 PLTP

While *in vitro* studies indicated that PLTP can transfer α-tocopherol between lipoproteins and mediates its incorporation in cultured endothelial cells, complementary *ex vivo* studies on rabbit aortic segments indicated that the impairment of the endothelium-dependent arterial relaxation induced by oxidized LDL can be counteracted by pretreament with purified PLTP and α-tocopherol–albumin complexes. These observations suggest that PLTP, by supplying endothelial cells with α-tocopherol, may preserve the normal relaxing function of vascular endothelial cells [20].

The *in vivo* relevance of the impact of PLTP on α-tocopherol delivery to peripheral organs was investigated using the PLTP-KO mouse model. In these mice, significant alterations of the α-tocopherol content were observed in several tissues, which led to the unravelling of novel biological functions and pathophysiological implications of PLTP. Forthcoming evaluations of PLTP inhibition as a new cardioprotective strategy should ideally incorporate the possible consequences on vitamin E distribution and availability.

Although the accumulation of α-tocopherol and increased antioxidant protection of apolipoprotein-B (apoB)-containing lipoproteins [21] are plausible anti-atherogenic mechanisms associated with PLTP deficiency, the substantial reduction in the hepatic secretion of apoB (the major protein of atherogenic lipoproteins) appeared as another important feature of the PLTP-deficient state. Similarly, it was reported that animals overexpressing PLTP exhibit hepatic VLDL overproduction and increased susceptibility to atherosclerosis [47]. Recently, associations of plasma PLTP activity with elevated apoB levels and increased cardiovascular risk have been found in humans as well [48, 49]. Interestingly, PLTP-deficient livers were found to exhibit a relative deficiency of vitamin E and an excess of intracellular lipid peroxides, which led to excessive ROS-dependent degradation of newly synthesized apoB [23]. Thus, the α-tocopherol transfer activity of PLTP provides a likely explanation for its modulating role on apoB-lipoprotein production by the liver and its impact on atherogenesis.

Macrophages are the hallmark of atherosclerotic lesions. Interestingly, bone-marrow transplantation studies in mice indicated that, in contrast to systemic PLTP, which increases atherosclerosis susceptibility [22, 50], macrophage-derived PLTP could be atheroprotective when produced locally in the absence of major change in circulating level of the protein [51]. When the biochemical characteristics of peritoneal macrophages isolated from PLTP-KO mice were examined, they were shown to have increased basal cholesterol content and to accumulate more cholesterol in the presence of LDL than did their wild-type counterparts [52]. Moreover, cholesterol parameters in the macrophages of PLTP-deficient mice were normalized by dietary α-tocopherol supplementation. These data led to the conclusion that, unlike systemic PLTP, macrophage-derived PLTP has antiatherogenic properties that are related at least in part to its ability to reduce cholesterol accumulation in macrophages through changes in the α-tocopherol content and oxidative status of the cells [52].

The brain is the major reservoir of vitamin E in the body and shows a peculiar sensitivity to vitamin E depletion and oxidative injury. PLTP is expressed in the brain [53], and PLTP deficiency results in significant depletion of brain α-tocopherol in both homozygous and heterozygous PLTP knocked-out mice. α-Tocopherol depletion in PLTP-deficient homozygotes was associated with the elevation of lipofuscin, cholesterol oxides and cellular peroxides in the brain. Moreover, complete PLTP deficiency in homozygotes was accompanied by increased anxiety in the absence of locomotor deterioration. Thus, the vitamin E transfer activity of PLTP appeared to be a key process in preventing oxidative damage in the brain, and PLTP-deficient mice could be a new model to study the contribution of oxidative brain injury in the etiology of neurodegenerative diseases [54].

The impact of PLTP on fertility was investigated using the PLTP-KO mouse model. This study showed that PLTP deficiency is associated with lower fertility in male mice, but not in female mice [55]. Spermatozoa motility was significantly decreased in PLTP-KO male mice, and PLTP-KO spermatozoa were less likely to induce oocyte fertilization. Abnormalities were shown to be due at least in part to alterations in vitamin E transfer, since vitamin E was reported to be markedly reduced in the spermatozoa and epididymis of PLTP-KO mice [55]. Thus, compared with other previously identified vitamin E transport factors, PLTP arises as a unique vitamin E transporter that acts primarily at the post-testicular stage of epididymal spermatozoa maturation. The expected benefit of vitamin E supplementation in the treatment of human male infertility might well be dependent on both the epididymal expression of PLTP and the initial vitamin E content of spermatozoa in treated patients, a hypothesis that deserves further attention.

Although a number of tissues, including liver, brain, testis, macrophages and the vascular wall, were shown to contain fewer vitamin E molecules in the PLTP-deficient state, circulating erythrocytes were found to behave rather like circulating apoB-containing lipoproteins, accumulating vitamin E in the PLTP-deficient state [56]. Interestingly, vitamin E enrichment of PLTP-KO erythrocytes occurred in the absence of significant alterations in the overall cellular content in phospholipids, *i.e.* other amphipathic compounds known to be transferred by PLTP. Thus, it was proved for the first time that complete as well as partial PLTP deficiency in mice is a relevant, yet unrecognized means to produce significant alteration in the vitamin E content of

circulating erythrocytes. Moreover, a direct relationship between vitamin E content and phosphatidylserine exposure in erythrocytes was shown in PLTP-deficient mice, and the high-vitamin E/low-phosphatidylserine exposure phenotype of circulating $PLTP^{-/-}$ erythrocytes decreased whole blood coagulation [56].

2.6 α-Tocopherol excretion

The metabolism and excretion of vitamin E are tightly regulated, and in contrast with other lipophilic vitamins, no toxic accumulation occurs even when high-dose supplements are ingested. About one-third of dietary α-tocopherol is not absorbed and is excreted in feces. α-Tocopherol is excreted in bile and urine. α-Tocopherol can be excreted in the bile in its natural form, a process that involves ABC-transporters located in the canalicular membranes of hepatocytes [57]. It has also been suggested that SR-BI plays a key role in controlling biliary α-tocopherol levels [36]. α-Tocopherol is mainly excreted as a carboxyethyl-hydroxychroman (CEHC) metabolite. The transformation of α-tocopherol into α-CEHC is achieved *via* a metabolic cascade involving the cytochrome P450 system of xenobiotic metabolizing enzymes [58]. A number of *in vivo* observations have indicated that this system is auto-regulated, as α-tocopherol is able to modulate its own metabolism and thus prevent toxic accumulation [59]. α-CEHC is excreted in bile and urine either as a sulfate ester or as a glucuronide conjugation product.

3 Does α-tocopherol act as an antioxidant?

In plants, where tocopherols are synthesized, they seem to play a mainly protective role against unsaturated fatty acid oxidation [60]. In animals, it has been suggested that vitamin E and in particular its major isomer α-tocopherol, can exhibit both antioxidant and non-antioxidant properties. Traber and Atkinson recently suggested that all of the biological effects of tocopherols are in fact related to their antioxidant properties [2]. For instance, they asserted that α-tocopherol-induced inhibition of protein kinase (PKC) is due to an antioxidant phenomenon in the plasma membrane, arguing that low concentrations of peroxide added to cell cultures induced a reversible oxidation/activation of PKC [61]. On the other hand, for Azzi, the differences in the molecular functions of the different tocopherols are related to the non-antioxidant properties of α-tocopherol [5]. Other biological properties of vitamin E, such as the stimulation of diacylglycerol kinase [62], the inhibition [63] or activation [64] of Akt-PKB or the activation of protein tyrosine phosphatase [65] are also thought to be independent of the antioxidant properties of α-tocopherol. The molecular mechanism that explains the effect of α-tocopherol on the control of enzymatic activities remains a matter of controversy. α-Tocopherol is not able to protect against oxidant-induced damage *in vivo* [66], and Brigelius-Flohé suggested that the rather weak antioxidant properties of vitamin E could actually involve the detection of oxidative membrane disturbances, which would trigger cellular responses by modulating lipid mediator production [4].

4 Cellular properties of α-tocopherol: The lipid raft hypothesis

4.1 Lipid raft association of α-tocopherol

As a hydrophobic vitamin, α-tocopherol is present in cell membranes, where it has been shown to spontaneously associate with polyunsaturated fatty acids present in phosphatidylcholine [67]. The incorporation of α-tocopherol in model membranes has a stabilizing effect by decreasing fluidity. This stabilizing effect may be due to the formation of complexes with membrane lipid components (such as free fatty acids or lysophospholipids), which have a destabilizing effect [4]. Interestingly, the stabilizing property might be a specific characteristic of α-tocopherol since it has been reported that only α-tocopherol, but not β-, γ- or δ-tocopherol, is able to decrease the fluidity and thus to stabilize intestinal brush-border membranes [68]. In fact, α-tocopherol does not appear to randomly distribute throughout the phospholipid bilayer of biological membranes, but it rather forms complexes with specific membrane constituents and could segregate in specific membrane domains such as lipid rafts [4, 69]. The latter point was brought to the fore only recently, and the isolation and characterization of membrane microdomains demonstrated that, as compared with other vitamin E isomers, only α-tocopherol can incrementally associate with lipid raft structures [70]. Lipid rafts are cholesterol and sphingolipid-enriched microdomains that serve as a platform for signalling complexes. It should be emphasized that most of the enzymes known to be regulated by α-tocopherol are associated with lipid rafts and can modulate raft-embedded signal transduction pathways, possibly by modifying protein–lipid and protein–protein interactions [4, 70].

4.2 Effect of α-tocopherol on cell signalling

Over the last decade, α-tocopherol has been reported to regulate cell signalling, and in this context, the pathways involved were found to vary according to cell type. In vascular smooth muscle cells, PKC inhibition appears to be related to an α-tocopherol-induced inhibition of phosphorylation and thus translocation to the plasma membrane [71, 72]. Although the exact mechanism has not yet been elucidated, it has been shown that α-tocopherol-induced dephosphorylation of active PKC occurs *via* the activation of protein phosphatase 2A (PP2A) [73]. Since PKC and PP2A have been shown to physically interact in human and mouse mast cells [69], this interaction is likely to take place at the plasma membrane and

especially in lipids rafts where a pool of PP2A has been reported [74]. With regard to Akt/PKB, it has been shown to reside in lipid rafts when activated [75], so it is quite conceivable that the regulation of Akt/PKB activity by α-tocopherol may also be the consequence of affecting its translocation to lipid rafts. In the same way, it has been demonstrated that diacylglycerol kinase is activated by α-tocopherol-induced translocation of the enzyme to the plasma membrane [76]. Finally, according to Cuschieri *et al.* [77], α-tocopherol succinate could also act through the inhibition of the endotoxin-mediated transport of phosphatases, such as SH2-containing protein-tyrosine phosphatase-1 (SHIP), to lipid rafts, which would lead to the increased activation of Akt and attenuated activation of the MAPK pathway.

4.3 α-Tocopherol and cell proliferation

Tasinato *et al.* [78] reported a significant inhibition of vascular smooth muscle cell proliferation by α-tocopherol. This effect was claimed to be mainly independent of the antioxidant property of α-tocopherol since its effect was abrogated by β-tocopherol. In this case, inhibition of PKC activity might be the molecular mechanism involved [78]. Alternatively, the antiproliferative effect of α-tocopherol on HMC1 mast cells has been shown to involve the inhibition of the Akt-PKB pathway [63].

4.4 α-Tocopherol and inflammation

As far as inflammation is concerned, it has been reported that α-tocopherol exerts pro- or anti-inflammatory effects depending on cell type and experimental models. However, it is worthy of note that in many cases the direct involvement of lipid raft domains was suspected. Vitamin E was shown to reverse the age-associated decline in effective immune synapse formation of $CD4^+$ T cells. This occurred by enhancing the redistribution and the phosphorylation of the adapter linker for the activation of T cells to the immune synapse [79], which is known to be embedded in lipid rafts [80]. It has been suggested that the mechanism involved in the vitamin E-induced increase in linker for the activation of T cells phosphorylation is related to the redistribution of lipid raft-associated signalling proteins, such as the protein tyrosine phosphatase SHIP-1 [81]. Finally, and according to Cuschieri *et al.* [77], the tocopherol succinate-mediated inhibition of the transport of SHIP to lipid rafts, would result in attenuation of the liberation of inflammatory cytokines.

4.5 α-Tocopherol and cholesterol oxide-mediated apoptosis

Cholesterol oxides, in particular 7-ketocholesterol and 7-β-hydroxycholesterol, are potent inducers of apoptosis. α-Tocopherol was found to prevent cholesterol oxide-mediated mitochondrial dysfunction and apoptosis in various cell types of the vascular wall [64, 70, 82, 83]. In the A7R5 aortic smooth muscle cell line, protection against 7-ketocholesterol-induced apoptosis was found to be restricted to α-tocopherol; γ-tocopherol had no effect [70]. This protective effect was related to the impairment of oxysterol incorporation into lipid rafts when α-tocopherol was added before oxysterol; again, pre-treatment with γ-tocopherol had no effect [70]. The proapoptotic signalling pathway triggered by 7-ketocholesterol involves the inactivation of the kinase Akt-PKB [64, 70] leading to activation of the pro-apoptotic protein Bad by dephosphorylation [84, 85]. Bad has three sites for phosphorylation, including Ser75, Ser118 and Ser99, with only Ser99 being phosphorylated by Akt/PKB [86]. Earlier studies demonstrated that single phosphorylation on Ser99 is actually sufficient for its sequestration by the 14-3-3 protein in the cytosol, thus inhibiting its interaction with the mitochondria and leading to its inactivation [86]. When α-tocopherol is added before the 7-ketocholesterol, sustained activation of Akt/PKB is observed as is Bad phosphorylation and thus cell survival (Fig. 2). These observations constitute the first direct evidence that inhibition of apoptosis by α-tocopherol relates to its selective

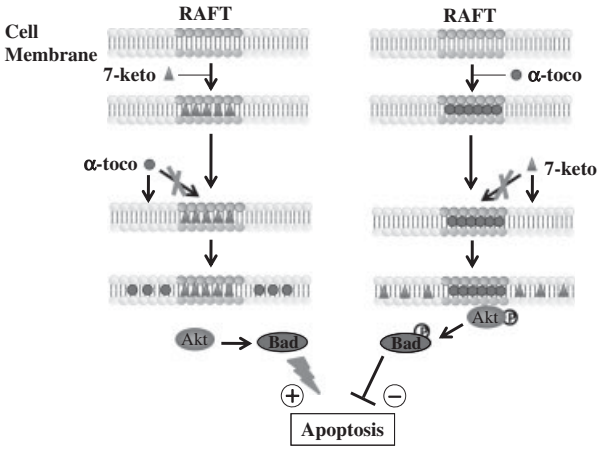

Figure 2. Mechanism of inhibition of 7-ketocholesterol-induced apoptosis by α-tocopherol. Left panel. Step 1: when 7-ketocholesterol (7-keto) is added to A7R5 cells, it incorporates preferentially into lipid raft domains of the plasma membrane. Step 2: when α-tocopherol (α-toco) is added after 7-ketocholesterol, it is unable to incorporate into lipid rafts and thus associates with non-raft membrane. Step 3: 7-ketocholesterol induces Akt and Bad dephosphorylation leading to apoptosis. Right panel. Step 1: when α-tocopherol is added to A7R5 cells, it shows a propensity to associate with lipid rafts. Step 2: when 7-ketocholesterol is added after α-tocopherol, it loses its ability to incorporate into lipid rafts and then associates with non-raft domains of the plasma membrane. Step 3: Akt is recruited and activated in the α-tocopherol-containing lipid rafts, leading to phosphorylation and inactivation of Bad, and finally to inhibition of 7-ketocholesterol-induced apoptosis.

incorporation into lipid rafts, and is associated with changes in raft-associated signalling pathways.

5 Concluding remarks

Although α-tocopherol is unable to protect the entire membrane from oxidative damage, it might modulate locally the synthesis of oxidized lipids, and as a consequence, raft-embedded signalling cascades [4, 87]. Alternatively, α-tocopherol might still act at specific sites through putative non-antioxidant functions. By facing the deleterious effect of cholesterol oxides, α-tocopherol in lipid rafts can prevent cell death, and as such may be considered as the oar in the lifeboat. A key issue now is then to work out what is the molecular basis of the selective association of α-tocopherol with lipid rafts. Are there specific receptor(s) in lipid rafts that allow α-tocopherol to act as a ligand or is the random incorporation into rafts sufficient to disturb signal transduction within these microdomains?

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