REVIEW

α-Tocopheryl phosphate – An active lipid mediator?

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Received: August 17, 2009
Revised: September 17, 2009
Accepted: September 24, 2009

The vitamin E (α-tocopherol, αT) derivative, α-tocopheryl phosphate (αTP), is detectable in small amounts in plasma, tissues, and cultured cells. Studies done *in vitro* and *in vivo* suggest that αT can become phosphorylated and αTP dephosphorylated, suggesting the existence of enzyme(s) with αT kinase or αTP phosphatase activity, respectively. As a supplement in animal studies, αTP can reach plasma concentrations similar to αT and only a part is dephosphorylated; thus, αTP may act both as pro-vitamin E, but also as phosphorylated form of vitamin E with possibly novel regulatory activities. Many effects of αTP have been described: in the test tube αTP modulates the activity of several enzymes; in cell culture αTP affects proliferation, apoptosis, signal transduction, and gene expression; in animal studies αTP prevents atherosclerosis, ischemia/reperfusion injury, and induces hippocampal long-term potentiation. At the molecular level, αTP may act as a cofactor for enzymes, as an active lipid mediator similar to other phosphorylated lipids, or indirectly by altering membrane characteristics such as lipid rafts, fluidity, and curvature. In this review, the molecular and cellular activities of αTP are examined and the possible functions of αTP as a natural compound, cofactor and active lipid mediator involved in signal transduction and gene expression discussed.

Keywords:
EPC-K1 / Kinase / Phospholipids / Tocopherol / Tocopheryl phosphate

1 Introduction

Vitamin E was discovered almost 100 years ago by Evans and Bishop as an essential nutrient for reproduction in rats [1]. Since then, the biological activity of vitamin E has been mainly ascribed to its ability to chemically act as a free radical chain breaking molecule in the lipid phase (lipoprotein and membranes) and to exert its action in concert with vitamin C (L-ascorbic acid) by protecting the organism against the attack of those radicals [2–4]. The supplementation of the diet with high levels of vitamin E is thus mainly aimed at reducing the propagation of reactive oxygen and nitrogen species (ROS and RNS) associated with several diseases. During the last 20 years, however, several alternative roles for vitamin E have been proposed that are independent of its radical chain breaking function (reviewed in [5, 6]). Vitamin E has been shown to influence cellular behavior by modulating the activity of several enzymes involved in signal transduction, ultimately leading to changes in gene expression [7–10].

Eight major natural analogues of vitamin E (α-, β-, γ-, δ-tocopherol/tocotrienols), which occur as *RRR*- side-chain stereoisomers, have been described. Although the overall antioxidant activity of these molecules is more or less similar, clear individual physicochemical and biological effects can be distinguished at a molecular level. In humans and higher animals, only α-tocopherol (αT) is enriched in plasma 10- to 100-fold, from about 0.3 to 2.5 μM (as measured for the non-αT analogues) to an average of 23.2 μM [11]. This enrichment is the consequence of selective retention of *RRR*-αT by the liver α-tocopherol

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Abbreviations: αT, α-tocopherol; **αTA,** α-tocopheryl acetate; **αTP,** α-tocopheryl phosphate; **αTS,** α-tocopheryl succinate; **α-TTP,** α-tocopherol transfer protein; **hTAP,** human TAP; **PI3K,** phosphatidylinositol-3-kinase; **RNS,** reactive nitrogen species; **ROS,** reactive oxygen species; **SMC,** smooth muscle cell; **TAP,** tocopherol associated protein; **VEGF,** vascular endothelial growth factor

transfer protein (α-TTP), or *vice versa*, of the enhanced metabolic degradation of the other tocopherols/tocotrienols (β-, γ-, and δ-) by cytochrome P450 enzymes and their subsequent elimination (reviewed in [12]). As a consequence of enrichment, only the αT analogue is defined as the vitamin E analogue with essential function, regardless of basically equal antioxidant activities of the eight analogues.

The ability to act as antioxidants renders the natural vitamin E analogues unstable; thus, several stabilized vitamin E derivatives (*e.g.* α-tocopheryl acetate (αTA), α-tocopheryl succinate (αTS), α-tocopheryl phosphate (αTP) and others) have been synthesized for usage in dietary supplements, cosmetics and even as anti-cancer agents (reviewed in [11]). In addition to stabilization, the solubility, transport, metabolism, and cellular activities of these derivatives are also different. Most tocopherol derivatives modified at the 6-hydroxyl group of the chromanol ring are not susceptible to oxidation and cannot act as antioxidants. These derivatives are usually prepared using αT, since this is the analogue that is selectively enriched by the liver α-TTP. Some of these stabilized esters of αT can be considered to be pro-vitamins, since they are readily converted to the natural parent forms by intestinal or epidermal esterases and thus ultimately perform the same function in the body as the natural αT. Once in the gut, the esters of αT are split to their unesterified forms under the action of pancreatic and intestinal esterases and only the non-esterized tocopherols are efficiently taken up and appear in plasma within hours [13–17]. αTP appears to be an exception, not being easily hydrolyzed and being able to be absorbed to a large extent as such.

The phosphorylated form of αT, αTP (Fig. 1), was synthesized and tested in several experimental systems since the early 1940s [18]. After developing a novel isolation and detection method, αTP was shown only recently to occur naturally in foods and in animals as well as in human tissues [19, 20]. The natural presence of αTP in the human body prompts a number of questions: is αTP a means of transport or storage of αT, is it a non-functional metabolite, or is it an active form of αT such as a cofactor or ''second messenger'' capable of exerting regulatory effects at a cellular level [21]? Here, the molecular and cellular activities of αTP are reviewed and the possible functions of αTP as a natural compound and active lipid mediator involved in signal transduction and gene expression are discussed.

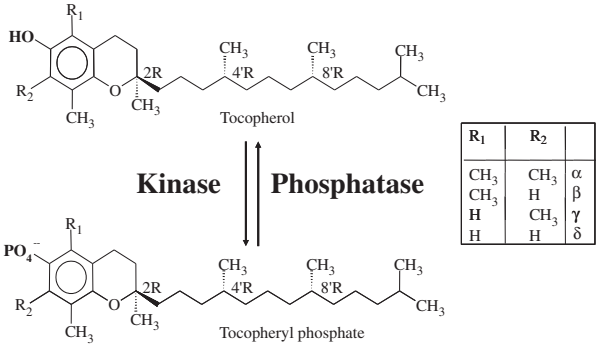

Figure 1. Chemical structures of tocopherol and tocopheryl phosphate and their interconversion by kinases and phosphatases.

2 Occurrence of αTP

αTP has recently been identified as a natural analogue occurring in low amounts in foods and in animals as well as in human tissues [19, 20]. The phosphorylated forms of β-, γ-, and δ-tocopherols/tocotrienols have so far not yet been detected because they are either not formed or are rapidly hydrolyzed and their endogenous level is too low to detect. The amounts of αTP in foods are variable and generally lower than the free αT [19]. In animal tissues (including humans), the amount of αTP (0.1 μg/g) is about 100 times lower than that of αT (10 μg/g) [20]. Supplementation of the diet of rats with αTP results in an increased appearance of αTP and αT in liver and adipose tissue [19], and no significant toxicity is observed in several animal models [22, 23]. αTP levels in plasma of un-supplemented rats, minipigs, and humans (Zingg *et al.*, unpublished) are similar as measured in un-supplemented rabbits, having a basal plasma αTP level of 0.19 μM that increased to 29.4 μM after supplementation with 1.33 g/kg of body weight of αTP for 4 wk [24]. An increase of plasma levels after αTP supplementation suggests mechanisms of transport of the intact form, but it is unknown whether the same route is taken as demonstrated for αT or αTA and where and to what degree αTP is hydrolyzed [25, 26].

3 Interconversion of αT and αTP by kinases and phosphatases

For the phosphorylation reaction an αT kinase, and for the de-phosphorylation reaction an αTP phosphatase or esterase can be postulated; both activities have been detected in cells in culture or in tissues [20, 21, 27, 28]. Studies using an *in situ* αT kinase activity assay with HMC-1 human mast cells, primary human coronary artery smooth muscle cells (SMC), and 3T3-L1 mouse adipocytes suggest that αT can be phosphorylated in small amounts in cell culture [20, 21]. αT can also become phosphorylated *in vivo*, as demonstrated by feeding rats with radioactive [^{14}C]-αT as precursor and isolating the labeled αTP from the liver [20]. Other studies suggest that αTP can be de-phosphorylated at a low rate in cell lines (THP-1 monocytes and human brain microvascular endotheliocytes) [21, 29, 30], in microsomal and mitochondrial suspensions [31], as well as *in vivo* in mouse keratinocytes and in rabbits [24, 28].

4 Lipid transport proteins with possible role in αT and αTP function

Since αT is a hydrophobic and αTP an amphipathic molecule, they are located mainly in membranes, and transporters and specific lipid transfer proteins may be required to make them more accessible to modifying (*e.g.* kinases and phosphatases) and degrading enzymes, or to present them to specific receptors, membrane transporters, transcription factors, membrane domains and organelles (reviewed in [25, 32]). So far most experiments with tocopherol binding proteins have only tested tocopherols and tocotrienols and much work has to be done to elucidate whether they play a similar role for αTP, a molecule that occurs in plasma and tissues in much lower amounts.

For the transport of αT across the plasma membrane, the ATP binding cassette transporter A1 [33–35] and the multidrug resistance protein P-glycoprotein [36, 37] have been identified. For the import of αT, the LDL receptor [38] and the scavenger receptor SR-BI has been described [39], whereas glybenclamide sensitive organic anion transporters have been suggested as αTP transmembrane carriers [29]. Uptake and hydrolysis of αTP in isolated hepatocytes was increased when extracellular [Ca^{2+}] was depleted, suggesting that the cellular [Ca^{2+}] content modulates the transport efficiency and metabolism of αTP [40].

For the intra- and extracellular transport of αT, several proteins such as the microsomal triglyceride transfer protein [41], afamin [42], phospholipid transfer protein [43, 44], the Niemann-pick C1-like protein [45], the α-TTP [46],] and three tocopherol associated proteins (TAP1, TAP2, and TAP3 or SEC14L2, SEC14L3, and SEC14L4, respectively) [47–51] have been identified, but so far only TAP1 which is also known as supernatant protein factor has been demonstrated to bind αTP (reviewed in [25, 52]).

The three TAP proteins are highly homologous and related to the *Saccharomyces cerevisiae* SEC14p protein, which is the prototype of a large eukaryotic family of proteins containing a SEC14-lipid binding domain (reviewed in [53–55]). The relatively large binding pocket of TAPs can accommodate several different ligands that within cells may form a group of lipids competing for the same binding site. Several hydrophobic ligands are bound by the TAP proteins *in vitro*, such as α-, β-, γ-, δ-tocopherols and tocotrienols, α-tocopheryl quinone, phosphatidylcholine, phosphatidylserine, and squalene, as well as αTS, phosphatidylinositol and phosphatidylinositol-3,4,5-phosphate [47–50, 56–62]. Using an isoelectric point mobility shift assay [59], αTP was able to compete *in vitro* with phosphatidylinositol for binding to recombinant human TAP1 (hTAP1) in a similar manner as αT, suggesting that αT and αTP may influence cellular events *via* competition for the same binding site. Since hTAP1 is the only protein so far tested for αTP binding, it is not yet clear whether hTAP2 or hTAP3 can do the same.

Although the *in vivo* physiological ligand(s) has not yet been defined, a role of the three hTAP proteins in lipid transport, metabolism, and trafficking is likely [63]. A tocopherol transport function of these proteins is supported by the finding that the cellular uptake of αT and αTS is increased by hTAP1 over-expression [49, 50], that the *in vitro* αT transport to mitochondria is augmented [51], and that mitochondria-mediated apoptosis is induced by αTS in hTAP1-overexpressing mesothelioma cells, most likely resulting from increased transport to Bcl-xL/Bcl-2 or mitochondrial succinate oxidase [49, 50, 64–66]. Since the TAP proteins are predominantly expressed in epithelial duct cells of several glands, a role in uptake and secretion of ligands into or out of the extracellular space appears possible [51].

Intracellular transport of lipids and tocopherols by TAP proteins may also facilitate their presentation to specific enzymes involved in signal transduction or metabolic conversion. In line with this, αT stimulates *in vitro* phosphatidylinositol-3-kinase (PI3K) gamma activity in the presence of recombinant hTAP1, probably by forcing the release of phosphatidylinositol and/or facilitating its presentation to the enzyme [48, 49, 60, 62]. In mice and humans cells, TAP1 interacts directly with PI3K and modulates its activity *in vitro* and *in vivo* [48, 49]. Moreover, TAP proteins also stimulate squalene epoxidase, possibly by facilitating squalene transport and correct presentation to the enzyme, which is important for the biosynthesis of cholesterol [58, 67]. As shown *in vivo* with TAP1/supernatant protein factor-knockout mice, TAP1 plays a role in facilitating cholesterol synthesis during fasting by compensating for decreased squalene epoxidase and HMG-CoA reductase activity [68].

5 Antioxidant activity of αT and αTP

Soon after its discovery, αT was recognized as an antioxidant molecule [69], acting chemically as a scavenger of ROS and RNS in the lipid phase, but alternative mechanisms of action, such as a cofactor or precursor of a cofactor, have also been suggested [70, 71]. In recent years, αT has been shown to modulate the activity of several enzymes involved in signal transduction and gene expression (reviewed in [5, 10]). At concentrations normally found in plasma and tissues, αT may act as a lipid mediator and modulate enzymes and transcription factors without necessarily affecting markers of oxidative stress [72, 73]. Interestingly, the four tocopherols and tocotrienols (α-, β-, γ-, δ-) often affected signal transduction and gene expression differently, despite having essentially equal antioxidant activity, therefore suggesting non-antioxidant cellular effects (reviewed in [5, 6, 74]). Although some of the cellular effects seen with the tocopherols are the result of scavenging free radicals, it also appears possible that at least for αT, some of the observed effects may have occurred after the phosphorylation of low amounts of αT to αTP. In fact, the negative

charge of αTP renders it more similar to phosphorylated messenger lipids such as phosphatidylinositol-phosphates, possibly modulating specific and non-specific protein-membrane interactions (reviewed in [75]).

αTP has *per se* no antioxidant activity since it is phosphorylated at the chromanol -OH group, which in αT is essential for the scavenging of free radicals. Despite that, it has been suggested to reduce oxidative stress by preventing the propagation of free radicals in membranes from one polyunsaturated fatty acid to another or possibly by interfering with their enzymatic generation by specific interaction with enzyme(s) and/or receptor(s) [30, 76]. However, the amounts of αTP used in the above-mentioned experiments were orders of a magnitude higher than those possibly present in cells and therefore of no physiological relevance. An ''indirect'' antioxidant function of αTP *via* induction or regulation of enzymes involved in scavenging ROS and RNS was so far not observed; in fact, in contrast to αT [77], αTP was ineffective in elevating the intracellular level of the redox-active antioxidant glutathione (GSH). The observed inhibition by αTP of glutathione S-transferase omega 1 *in vitro* possibly plays a role in modulating the anti-inflammatory effects of αT [78].

6 Biological activities of αTP

Early after the first synthesis of αTP, much work was done on evaluating its cellular effects *in vitro* and in animal models; however, since the biochemical knowledge and methodology was less advanced at that time and often was based on usage of crude homogenates and high concentrations of αTP, the specificity and relevance of these effects is difficult to evaluate and may need to be reassessed with today's techniques. When tested *in vitro*, αTP modulated many enzymes (Table 1); nevertheless, since αTP was later demonstrated to form an insoluble salt with Ca^{2+} [79], some of these effects were suggested to be the results of Ca^{2+} removal [79–81]. Animal studies with rabbits showed that parenteral administration of αTP had a higher preventive activity against muscular dystrophy induced by vitamin E deficiency than αT [82, 83]. In rabbits, αTP inhibited the augmented activity of succinoxidase as well as the higher rate of oxygen consumption observed in vitamin E-deficient muscles, whereas αT had no effect [84, 85]. Interestingly, in contrast to αTP, αTA was not effective when administered parenterally, but was equally effective when administered orally [82]. In the search for possible mechanisms for this phenomenon, αTP was proposed to inhibit the enzyme diphosphopyridine nucleotidase (DPN), leading to inhibition of succinoxidase by the promotion of oxalacetate formation by malic dehydrogenase [81]. However, later studies indicated that the observed effects of αTP on the succinoxidase system may be due to its properties as a surface-active anion similar to SDS, preventing the interaction of succinic dehydrogenase with cytochrome c [86].

Table 1. Enzymes modulated by α-tocopheryl phosphate *in vitro*

Enzymes	Inhibition (I) or activation (A)	References or references therein
Acid and alkaline phosphatase	I	[116]
Adenosinetriphosphatase	I	[167]
Amylase	I	[18]
cAMP and cGMP phosphodiesterase II (PDE II)	A	[88, 168]
Catalase	A	[169, 170]
Cytochrome c reductase	A	[80]
Diphosphopyridine nucleotidase (DPNase)	I	[80, 81]
Fructosidase	I	[18]
Glutathione *S*-transferase omega	I	[78]
Hyaluronidase	I	[80]
Lactic dehydrogenase	I	[171]
Leucoprotease	I	[101]
Lipoxidase	I	[80]
Liver acid phosphatase	I	[80]
Liver esterase	I	[80]
Malic oxidase	I	[169, 170]
Papain	I	[101]
Phenylalanine hydroxylase	I	[18]
Phenylalanine hydroxylase	A	[89, 172]
Plasma protease	I	[101]
Succinic oxidase	I	[80, 84]
Transaminase	I	[116]
Trypsin	I	[80, 101]

Much later, diphosphopyridine nucleotide- and succinate-cytochrome c reductase was found to be activated by αT and αTP after inhibition by digitonin [87]. In addition to the enzymes described early on (Table 1), αTP inhibits glutathione S-transferase omega [78], cAMP and cGMP phosphodiesterase [88], and mitochondrial succinate oxidase of complex II [86]; further, it stimulates rat liver phenylalanine hydroxylase, which converts L-phenylalanine to L-tyrosine [89]. A possible role of αTP as intermediate in oxidative phosphorylation was not supported experimentally [90].

In cell culture, αTPm (a mixture αTP, di-αTP, and αT), and also the pure αTP, are more potent than αT in reducing the proliferation of human THP-1 monocytes and rat aortic SMCs, as well as in normalizing CD36 mRNA and protein expression [29, 91, 92]. αTP inhibits cellular proteasome activity in THP-1 monocytes [93], and stimulates telomerase activity with consequent prevention of telomere shortening [30]; however, the *in vivo* relevance and the molecular mechanisms involved are not known but could contribute to the anti-aging effects seen with vitamin E [94, 95]. Contrary to αT, αTP is cytotoxic to THP-1 monocytes and murine MG-63 melanoma cells [92, 96], but cytotoxicity and apoptosis is only observed at high concentrations ($>50\,\mu M$), possibly reflecting an activity seen with synthetic vitamin E derivatives, such as αTS or 2,5,7,8-tetramethyl-2R-(4R,8R,1

2-trimethyltridecyl) chroman-6-yloxy acetic acid (reviewed in [11]).

αTP also protects neurons against oxidative damage by attenuating Ca^{2+} influx and *via* a genomic action [97, 98]. In immature cerebellar granule cells subjected to ischemia/reoxygenation, αTP increased the production of ROS and increased intracellular [Ca^{2+}], lipid peroxidation and cell death, and the effect on intracellular [Ca^{2+}] was age-dependent [99]. In cultured rat hepatocytes, αTP protects against ethyl methanesulfonate-induced cell death and lipid peroxidation, and protects against lethal doses of gamma irradiation, possibly by increasing the tissue αT levels (reviewed in [31]). αTP inhibited gap junctional intercellular communication in rat liver epithelial cells (IAR203) with no effect on connexin 43 phosphorylation, toxicity, and cell proliferation [100].

In vivo, several functions and activities have been suggested for αTP but a common molecular target has not yet been identified. Intraperitoneal injection of αTP to rats increased their thrombin clotting time; *in vitro* plasma coagulation time was also prolonged, most likely as a result of the anti-proteolytic activity of αTP [101]. Such an anti-thrombotic activity of vitamin E is consistent with an increased tendency of thrombosis in vitamin E deficient vessels (reviewed in [102]).

Other studies assessed the effects of αTP on the metabolism of carbohydrates after injection into rat skeletal muscle and reported a suppression of glycogenolysis in skeletal muscle [103] by modulating the phosphoglucomutase system [104, 105] (reviewed in [106]), or investigated the preventive action of αTP against muscular dystrophy induced in rabbits by vitamin E deficiency [82, 83].

More recently, an induction of hippocampal long term potentiation by αTP was seen in tissue slices isolated from guinea pig hippocampal CA1 pyramidal neurons, possibly indicating some effects on memory and learning [107]. A protective function of αTP against ultraviolet-induced damage was observed in mouse skin [28], and as well as against methamphetamine- and morphine-induced toxicity [108]. αTP also prolonged the therapeutic action of barbiturates, prevented the agitation phase [109], and exerted a pronounced prolonging effect on thiopental-induced depression in mice and rats, but not in rabbits and dogs [110].

Atherosclerosis progression and CD36 over-expression in hypercholesterolemic rabbits were better prevented by dietary supplementation with αTP than with αTA [24]. A protective effect against myocardial ischemia/reperfusion injury was observed in rats after αTP supplementation for 30 days [111]. In contrast to that, potentiation of cell death and lipid peroxidation by αTP treatment was observed after ischemia/reperfusion injury in immature cerebellar granule cells, possibly as a result of increasing the level of intracellular free [Ca^{2+}] in an age-dependent manner [99].

Differences seen in the biological effects between αTP and αT may be the consequence of their action on different molecular targets, as well as their different chemical ability to scavenge free radicals. Alternatively, it appears possible that the cellular uptake of αTP by OAT transporters [29] and its intracellular hydrolysis by esterases may lead to higher levels of intracellular αT at specific sites than after treatment directly with αT [20, 21, 27, 28]. However, at least in THP-1 cells, only 10% of the added αTP was hydrolyzed and even an αT concentration tenfold higher than that produced by αTP hydrolysis was not effective in inhibiting cell proliferation [29]. A direct interaction of αTP with specific proteins and cellular structures, as described with αTS binding to Bcl-xL/Bcl-2 or mitochondrial succinate oxidase [49, 50, 64–66], may also occur. It must be emphasized that the levels of αTP used in most *in vitro* experiments are much higher than the levels found in plasma and tissues [24]; hence, the cellular response may not necessarily reflect physiological effects. However, due to the presence of $CaCl_2$ and $MgSO_4$ in the cell culture media, which are capable of precipitating αTP, its cellular concentration may substantially differ from the added one and the amount of αTP transferred inside the cells should be measured. In the *in vivo* situation, high local concentrations of αTP with cellular importance may be achieved by local production of αTP inside the cells by an αT kinase or αTP phosphatase possibly induced by cellular triggers only in specific cells or circumstances, or by cellular enrichment and compartmentalization of αTP at specific subcellular sites.

7 Is αTP an active lipid mediator with essential vitamin E function?

The fact that only low amounts of αT become phosphorylated and de-phosphorylated in cell culture and animal tissues [20, 21, 28] suggests that the interconversion could serve some cellular signaling functions. In cells, αTP may be a cofactor for enzymes, a ligand of a receptor or transcription factor, or it may act as a ''second messenger'' or active lipid mediator capable of exerting regulatory effects at a cellular level [21]. Although the antioxidant effects of αT have been clearly demonstrated *in vitro*, in the *in vivo* situation, a decrease of markers of lipid and protein oxidation are not consistently observed after αT supplementation [112] and may require levels much higher than needed for essential vitamin E functions (>11.6 αM in plasma [113]). Moreover, only rarely oxidized tocopherol metabolites are detected reflecting action as a chemical antioxidant, and vitamin E deficient α-TTP knockout mice show only a modest increase of the levels of free radicals in some tissues [72, 73, 114, 115]. In fact, prolonged αT deficiency paradoxically even decreases oxidative stress in the brain [72, 73, 114, 115].

In light of these findings, the primary function of αT as an essential vitamin has been suggested not to be the result of its antioxidant, but rather of its non-antioxidant action as a molecule able to modulate cellular events [5, 6, 70, 80,

116–119]. In some experimental settings, it appears that the primary and essential cellular function of vitamin E as a specific cofactor may have been masked by general and pleiotropic antioxidant effects, and depending on the assay used both effects may be observed at the same time after vitamin E supplementation. A possible cofactor function of vitamin E has been previously hypothesized by postulating that α-tocopheryl quinone acts as an essential cofactor for the mitochondrial, long chain fatty acid desaturase [120, 121] or by proposing a condensation product with inositol (tocopherol-inositol ether) as the active form [71]. Whereas such cofactor functions of vitamin E remain speculative they cannot be excluded at the present time. It appears possible that αT is activated by phosphorylation to αTP and becomes an active cofactor [10] for specific enzymatic reactions similar to other phosphorylated vitamins (*e.g.* thiamine/B1 and pyridoxine/B6), which both undergo phosphorylation and dephosphorylation during their transport and act as active cofactors in only their phosphorylated form (*e.g.* as thiamine mono-, di-, and tri-phosphate and pyridoxal phosphate/pyridoxamine phosphate, respectively) [10]. In this context it is interesting to note that thiamine di-phosphate in addition to its action as cofactor of several enzymes, can directly bind and regulate mRNAs encoding enzymes involved in its biosynthesis, offering a further possibility for a regulatory action of αTP [122].

αTP (and possibly also αT) may bind to membrane receptors and activate or inhibit signal transduction *via* G-proteins or receptor tyrosine kinases similar to other phosphorylated lipids, such as ceramide-1-phosphate [123], sphingosine-1-phosphate [123],] or others (Table 2). Alternatively, it is also plausible that αTP acts as an intracellular signaling molecule mediating some of the effects seen with αT on gene expression and cellular signaling (Fig. 2) (reviewed in [10]). At the molecular level, αTP may act as a ''second messenger'' or membrane address similar to the phosphorylated forms of phosphatidylinositol [124] and other lipids, by attracting or preventing the access of structural proteins or enzymes such as kinases and phosphatases to the plasma membrane leading to their activation/inactivation. In this model, the negative charge of αTP would render it more similar to phosphorylated lipids such as phosphatidylinositol phosphates and thus increase its ability to modulate specific protein-membrane interactions (reviewed in [75]). Some evidence for such a mechanism of action was described in HMC-1 mast cells, in which αT inhibited Akt translocation to the plasma membrane [125], an event that may also be modulated by αTP. Such an action could also be the consequence of affecting general membrane properties, like membrane curvature, fluidity [126], or the composition of lipid rafts [127].

Table 2. Examples of phosphorylated lipids able to modulate signal transduction and gene expression

Lipid	Lipid-monophosphate	Lipid-di- or tri-phosphate	Reference and references therein
Ceramide	Ceramide-1-phosphate		[173]
Diacylglycerol	Phosphatidate		[174]
Dihydro-Sphingosine	Dihydro-Sphingosine-1-phosphate		[173]
Farnesol	Farnesyl-monophosphate	Farnesyl-diphosphate	[175–178]
FTY720	FTY720-phosphate		[173, 179]
Geranyl	Geranyl-monophosphate	Geranyl-diphosphate	[178]
Geranyl-geranol	Geranylgeranyl-monophosphate	Geranyl-geranyl-diphosphate	[180]
Isopentenyl	Isoprene-monophosphate		[178]
Lipid A /LPS	Monophosphoryl Lipid A (MPL-A)		[181]
Mevalonate	Mevalonate-5-phosphate	Mevalonate-5-diphosphate	[182]
Monoacylglycerol	Lysophosphatitic acid		[177]
Phosphatidylinositol	Phosphatidylinositol-3-phosphate Phosphatidylinositol-4-phosphate Phosphatidylinositol-5-phosphate	Phosphatidylinositol-3,4-diphosphate Phosphatidylinositol-3,5-diphosphate Phosphatidylinositol-4,5-diphosphate Phosphatidylinositol-3,4,5-triphosphate	[183]
Phyto-Sphingosine	Phyto-Sphingosine-1-phosphate		[173]
Sphingosine	Sphingosine-1-phosphate		[123, 173]
Squalene	Presqualene-monophosphate	Presqualene-diphosphate	[184, 185]
Tocopherol	Tocopheryl phosphate		[21]
Ubiquinone	Ubiquinone-phosphate		[186]

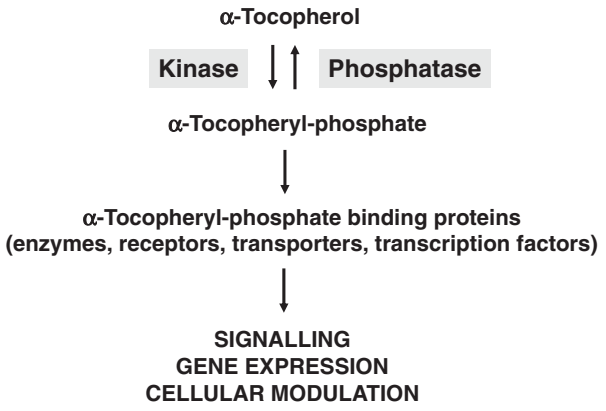

Figure 2. Model for a biological activity of α-tocopheryl phosphate in cells. Phosphorylation of α-tocopherol by a kinase yields small amounts of α-tocopheryl phosphate with a signaling function that is stopped by the activity of a phosphatase. αTP as an active lipid mediator or cofactor may bind and modulate enzymes, receptors, transporters, or transcription factors and thus change cellular behaviour by influencing signal transduction and gene expression.

Using gene expression microarrays, more genes were regulated by αTP than by αT, and most of the αTP-regulated genes were up-regulated, suggesting that αTP may act as an activating lipid mediator (Zingg *et al.*, unpublished). Preliminary results carried out *in vitro* suggest activation of the PI3K/Akt signaling pathway in THP-1 monocytes by αTP, ultimately leading to induction of vascular endothelial growth factor (VEGF) expression (Zingg *et al.*, unpublished). A similar stimulation of the PI3K/Akt pathways has been observed with αTS in lipopolysaccharide-stimulated THP-1 cells [128], and possibly contributes to the cardioprotective effect of αTP observed in dogs and rats after ischemia/reperfusion injury [111, 129]. Other experiments showed inhibition of Akt phosphorylation by αTS in NIH3T3 cells by down-regulating the oncogenic Ras signaling pathway [130], or by activating protein phosphatase 2A in prostate cancer cells leading to down-regulation of the androgen receptor [131]. In 3T3-L1 preadipocytes, α-and γ-tocotrienols inhibit Akt phosphorylation and adipocyte differentiation [132]. In a rat ischemia/reperfusion model, αTP normalizes reduced phosphorylation of Akt, p44/42 mitogen activated kinase β (MAPK), p38 MAPK β, and NF-κB binding, but decreased phosphorylation of Src and MAPKα and increased survival by decreasing apoptosis [111]. In response to αT, both induction [127, 133, 134] and inhibition [125, 135, 136] of the PI3K/Akt pathway have been observed, and the cellular response may depend on the degree of conversion of αT to αTP in a given tissue and cell type.

In vivo, the modulation of Akt and subsequently VEGF by αT and αTP may be relevant for cell survival, wound repair, and tissue homeostasis, and provide neuro-, myo-, and cardio-protection after exposure to various stressors including ischemia/reperfusion (Zingg *et al.*, unpublished). In this context, it is interesting to speculate that the observed induction of Hif1α, VEGF, and HO-1 by αT after focal brain ischemia could have been the consequence of Akt activation after conversion to αTP [137]. An antagonistic effect of αT and αTP on Akt phosphorylation may reflect an activity described for many natural compounds with possible relevance to maintain cellular homeostasis [138]. Moreover, it can be speculated that the main neurological symptoms of severe vitamin E deficiency result in part from the lack of conversion of αT into the more potent αTP, which may activate PI3K/Akt essential for the survival of specific neurons or muscle cells particularly in response to ischemia/reperfusion injury [137, 139]. It can be assumed that by interconverting inhibitory αT and activating αTP, the PI3K/Akt pathway is controlled by the kinases and phosphatases involved and not by the fluctuating dietary intake of αT or αTP. Interestingly, the levels of αTP may change in an age-dependent manner, since the rate of αTP to αT conversion in human brain microvascular endotheliocytes was reduced during aging [30].

8 Synthetic tocopherol derivatives with increased pharmacological activity

Synthetic derivatives of αT and αTP with increased potencies could be used for pharmacological purposes. Such derivatives could act on the same targets as αT or αTP but with higher or lower potency, or they may recognize related or completely novel molecular targets (reviewed in [11]). Similar to αT and αTP, some non-natural tocopherol derivates such as 2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxy acetic acid or αTS modulate signal transduction and gene expression in human MDA-MB-435 breast or PC3 prostate cancer cells as monitored by gene expression microarrays [140, 141]. As shown mainly for αTS, at the molecular level, synthetic derivatives may exert their effect by directly interacting with specific proteins and cellular structures or by generally influencing organelles and membrane properties [49, 50, 64, 66, 128, 131]. Similar to results with αT, experiments with a synthetic phosphatidylinositol ether lipid analogue have shown that Akt activation by membrane translocation can be specifically modulated, and it is possible that αT and αTP or other natural or synthetic vitamin E analogues can act in a similar manner [125, 142, 143]. αT and αTP could also be envisioned as anchors for other synthetic inhibitors, as recently shown for the sterol-linked α-secretase (BACE) transition state inhibitor showing increased activity due to its higher local concentration in lipid rafts [144]. The phosphorylated derivative of the antidiabetic molecule troglitazone, ''phosphoglitazone,'' which contains the phosphorylated chromanol moiety of αTP and a 2,4-thiazolidinedione nucleus, acts as a PPARγ agonist and inhibits vascular SMCs proliferation and proteoglycan synthesis with a potency similar to troglitazone but possibly with less toxicity [145].

A molecule related to αTP is EPC-K1 (L-ascorbic acid 2-[3,4-dihydro-2,5,7,8- tetramethyl-2-(4,8,12-trimethytridecyl) -2H-1-benzopyran-6-yl-hydrogen-phosphate] potassium salt), a composite molecule between vitamin E (αT) and vitamin C (L-ascorbate), linked by a phosphodiester bond. EPC-K1 acts chemically *via* its enolic hydroxyl group as a potent scavenger for both hydrophilic and hydrophobic radicals, including hydroxyl radicals, superoxide, peroxynitrites, as well as alkyl and lipid radicals [146]. EPC-K1 decreased oxidative DNA damage (8-hydroxy-2′-deoxyguanosine formation) in rat brain neuronal cells after cerebral artery occlusion [147]. In addition to that, EPC-K1 is chelating Cu^{2+} and Fe^{2+}, thus reducing free radicals generation *via* the Fenton reaction [148]. Chelation of Ca^{2+} has been linked with a dentin dissolving activity of high concentrations of EPC-K1 [149].

Similar to αTP, EPC-K1 protects against ischemia/reperfusion injury and lipid peroxidation in several experimental models (reviewed in [150]) and reduces NO-induced neurotoxicity by preventing apoptosis and mitochondrial dysfunction in cerebellar granule cells [151]. Furthermore, EPC-K1 modulates NF-κB and the glucocorticoid receptor *via* redox regulation [152, 153], inhibits phospholipase A2 activity, and stimulates endothelial nitric oxide production leading to endothelium-dependent relaxation [154]. EPC-K1 prevents 6-hydroxydopamine-induced dopamine depletion in mouse striatum by increasing the activity of superoxide dismutase and catalase [155]. EPC-K1 has not been tested as a preventive agent against atherosclerosis, although combinatorial treatment with vitamin E and C was more potent in several studies [156–159]. However, EPC-K1 showed some cardioprotective effects by affecting neutrophil function in myocardial infarction-induced rats by reducing superoxide generation and acid phosphatase activity [160] and by reducing reperfusion injury after heart transplantation in a canine model [161]. Scavenging of ROS by EPC-K1 increases endothelin-1-stimulated contractions in aortic rings in obese mice, whereas lean mice were not affected [162].

In vitro stability studies suggest that some αTP is produced as a hydrolytic decomposition product of EPC-K1 suggesting that some of the effects seen with EPC-K1 may occur after a cleavage into αTP and L-ascorbic acid. To date, it is unknown to what degree EPC-K1 is cleaved by enzymes in cells or *in vivo* [163]; however, in view of what we have discussed regarding overlapping effects seen with both αTP and EPC-K1, it is intriguing to speculate that EPC-K1 represents a synthetic pro-form of the phosphorylated vitamin E, αTP.

9 Concluding remarks

αTP has only recently been identified as a naturally occurring form of αT, and therefore the biological function of endogenous αTP remains to be elucidated. The low amount of αTP present in plasma and tissues makes it unlikely that it is a storage form. Alternatively, αTP may be an intra- or extracellular transport form of αT, a metabolite, a cofactor for enzymes, a ligand of a receptor or transcription factor, or a ''second messenger'' in the membrane capable of exerting regulatory effects [21]. The evidence presented in this review indicates that αTP can act as an active lipid mediator by modulating signal transduction and gene expression, but the exact molecular mechanism of action and physiological relevance are not yet resolved. Further confirmation of a signaling function of αTP requires the cloning of an αT kinase as well as an αTP phosphatase, and the discovery of a unique *in vivo* biological function. These kinases/phosphatases may become activated by specific triggers and/or in a cell type and tissue specific manner and thus determine the cellular level of αTP/αT [20, 21, 27, 28].

Whether αT or αTP acts *via* a membrane receptor, as a membrane address for enzymes regulated by membrane translocation, or as a ligand to specific receptors or transcription factors remains to be further explored. The regulatory influence of αT and αTP on PI3K/Akt activity could represent an important signal for neurons, and play an essential role in preventing the predominant neurological symptoms of severe vitamin E deficiency and of other neurodegenerative disorders as well as the cellular damage particularly after ischemia/reperfusion injury (reviewed in [164, 165]). Assuming a cellular or even essential importance of αTP in the human body, it appears possible that treatment directly with αTP or a synthetic precursor such as EPC-K1 may be more potent than with αT in preventing these diseases [166]. Further research is required to establish the biological function of αTP and the possible role of the three hTAP proteins in its transport and effects on signal transduction and gene expression.

This work is supported by grant USDA contract ♯58-1950-7-707. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the US Department of Agriculture. The authors thank Stephanie Marco for correcting the manuscript.

The authors have declared no conflict of interest.

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