

Hypothesis

On the Existence of Cellular Tocopheryl Phosphate, its Synthesis, Degradation and Cellular Roles: A Hypothesis

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Abstract

The finding that α -tocopheryl phosphate is present in cells in small amounts, that it can be synthesized and hydrolyzed supports the hypothesis that α -tocopheryl phosphate might be a signaling molecule. The possible pathways needed for the synthesis, hydrolysis and signaling are considered in this hypothesis as well the possible extension of this reaction to additional molecules such as tocopherols and tocotrienols. A possible mechanism of action of other tocopherol esters (succinate and maleate) is also hypothesized.

IUBMB *Life*, 57: 23–25, 2005

Keywords α -tocopherol; vitamin E; tocopherol esters; α -tocopheryl phosphate; cell signaling.

α -Tocopherol is a free radical scavenger (1) and, at the same time, a potential source of free radicals (2). At a cellular level, it has other functions not related with its redox chemistry (3), however, the exact molecular mechanism of α -tocopherol action in cells is not completely understood (4).

RRR- α -tocopherol is the active form of vitamin E, by far better retained in the human body and less rapidly degradable, relative to the other vitamins (β -, γ -, δ -tocopherols and all the tocotrienols) (4). The tocopherols and tocotrienols have been described to have tocopherol-specific cellular effects that are independent of their antioxidant potential and the exact mechanism for this is still unknown (4). Moreover, it is not clear how tocopherol uptake, storage and transfer take place and how α -tocopherol auto-oxidation with the generation of free radicals, is avoided or controlled. TAP proteins have been suggested to act as transport/chaperone proteins, but detailed events have still to be clarified (5,6). The unique gene

regulatory properties of α -tocopherol suggest the existence of a receptor, but, despite intensive search, such a protein has not been identified (7,8). An attempt has also been made to identify active metabolites, possibly involved in α -tocopherol signaling functions (9).

In the search of active cell derivatives of α -tocopherol, α -tocopheryl phosphate (TP) has been found to be present in tissues and foodstuffs (10) indicating that it is a natural derivative of α -tocopherol. TP, when tested with cells *in vitro*, has been shown to have more potent cellular effects than α -tocopherol itself in terms of inhibition of cell proliferation and regulation of gene expression (11). For instance, the transcriptional inhibition of the scavenger receptor CD36 in human coronary artery smooth muscle cells (HCASMC) after 24 hours incubation with 50 μ M α -tocopherol was not significant (3%) while TP, at the same concentration produced more than 40% inhibition (Fig. 1).

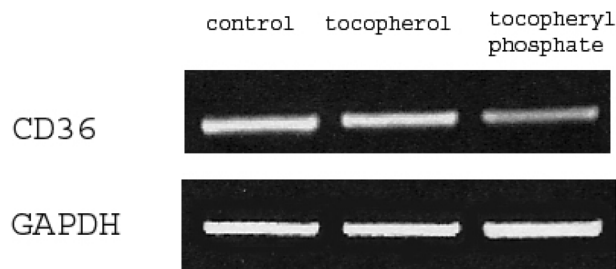


Figure 1. Inhibition of CD36 mRNA expression by tocopheryl phosphate and not by α -tocopherol in human aortic smooth muscle cells (HCASMC). The experiment was carried out as described in (7). α -tocopherol and α -tocopheryl phosphate concentration was 50 μ M.

Received 15 November 2004; accepted 16 December 2004

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The possibility that TP may be more efficient due to its better penetration through the plasma membrane and its easy hydrolysis by cellular esterases cannot be excluded *a priori*. However its presence in low amounts in cells indicates its possible synthesis and hydrolysis. An additional possibility that TP found in tissues may be a storage form of α -tocopherol appears to be unlikely, given the small amounts recovered from tissues of the order of 150–250 ng/g tissue, thus in the submicromolar range. These very low amounts found in tissues (of the same order of magnitude of the well known signaling molecule inositol phosphate) are compatible with the proposition that TP is a signaling molecule. Thus, we would like to propose the following hypothesis on the molecular function of α -tocopherol. Tocopherols and tocotrienols (but in particular α -tocopherol), with the chromanol hydroxyl group esterified by phosphoric acid, are molecules capable of modulating cell functions and that their enzymatic phosphorylation and de-phosphorylation are part of a cell signaling pathway.

The data of Fig. 2 show that TP can be synthesized in primary human coronary artery cells and mast cells, an important element needed to postulate a regulatory function of TP. TP synthesis must be catalyzed by a kinase, and, to avoid excessive accumulation of the compound, a competent phosphatase must be present in cells; a postulate confirmed by the finding that porcine intestinal alkaline phosphatase is capable of hydrolyzing TP (Table 1).

The activity of such a phosphatase must be low to prevent rapid and total hydrolysis of TP, an event that has been preliminarily verified (see Table 1) with a calculated phosphatase activity with TP as substrate of approximately 1 nmol/min/IU phosphatase, compared with the activity with 4-nitrophenyl phosphate of 1 μ mol/min/IU, under the same conditions. The phosphatase(s) and kinase(s), and their regulation, which may be involved in inter-converting α -tocopherol and TP should be thus an object of future investigations.

The shielding of the OH group of α -tocopherol by three methyl groups makes it sterically difficult to have α -tocopherol phosphorylated by a tyrosine kinase. However such a kinase may be still a good candidate, especially in view of the fact that in some situations δ -tocopherol and γ -tocopherols (with a lower number of methyl groups in the chromane ring) have been described to be more potent than α -tocopherol *in vitro*. One may speculate that the cellular concentration of their phosphate esters reaches higher values due to their higher affinity for the kinase or that they may better bind to a putative receptor. Analogously, the extremely high *in vitro* potency of tocotrienols can hardly be explained by their unsaturated side chain and can be rather considered the consequence of their faster phosphorylation.

Furthermore, tocopheryl succinate and even more tocopheryl maleate (12) or the non-hydrolysable ether analog of

α -tocopherol, α -TEA (2,5,7,8-tetramethyl-2R-(4R, 8R-12-trimethyltridecyl) chroman-6-yloxyacetic acid) (13) have been shown to have cell properties far stronger than α -tocopherol. As working hypothesis it can be suggested that the non-hydrolysable tocopheryl succinate and tocopheryl maleate may mimic and substitute for TP causing a permanent activation of cellular signals.

If considered a signaling molecule the obvious target of TP can be enzymes, receptors or transcription factors. The scheme

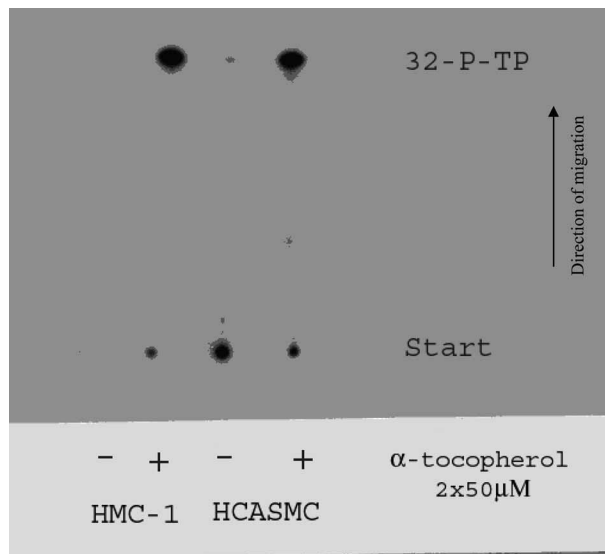


Figure 2. Synthesis of [32 P]-tocopheryl phosphate detected by TLC. Digitonin permeabilized [as described in (14)] mast cells and human primary coronary artery smooth muscle cells synthesis of TP from [32 P]-ATP. Incubation time 10 min. Analysis was carried out by thin layer chromatography after acidic extraction of TP in hexane according to (10). The arrow indicates the direction of TLC migration. 32-P-TP, [32 P]-tocopheryl phosphate.

Table 1
Hydrolysis of α -tocopheryl phosphate by alkaline phosphatase

Time (min)	α -tocopherol produced (nmol/ml)
0	0
10	16
20	35

The incubation medium contained: 976 μ mol/ml TP (Sigma), 1 M diethanolamine buffer pH 9.8, 20 IU alkaline phosphatase (Calf intestine, EC 3.1.3.1., Roche Diagnostic GmbH), 37°C. The determination of α -tocopherol was made by HPLC (mobile phase, methanol) after hexane extraction.

shown in Fig. 3 suggests that TP, in order to exhibit its cell modulatory effects, must be recognized by a protein, (possibly a receptor) capable of transducing the ligand information into cell responses.

This possibility is open to experimental analysis and is an obvious alternative to the elusive search for α -tocopherol receptors. In conclusion, the discovery of the synthesis and hydrolysis of α -tocopheryl phosphate in tissues and cells is not only an interesting observation but it has now been rationalized in an experimentally testable hypothesis.

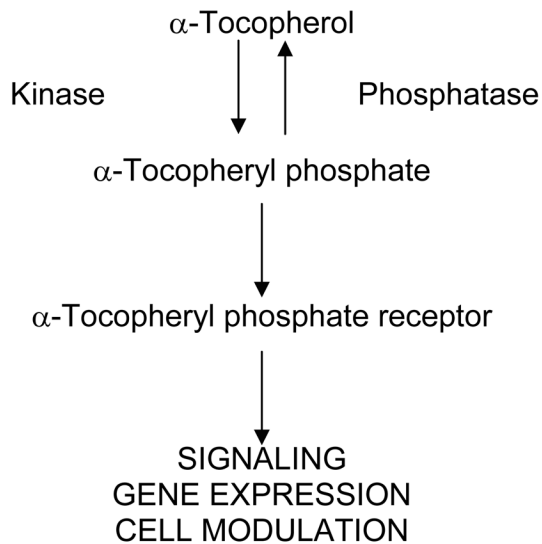


Figure 3. Hypothetical scheme of α -tocopherol phosphorylation.

Acknowledgements

The present study has been performed thanks to the support of the Swiss National Science Foundation, The Swiss Foundation for Nutrition Research and Phosphagenics Ltd.

References

- Burton, G. W., Joyce, A. and Ingold, K. U. (1982) *Lancet* **2**, 327.
- Bowry, V. W., Ingold, K. U. and Stocker, R. (1992) *Biochem. J.* **288**, 341–344.
- Azzi, A. and Stocker, A. (2000) *Prog. Lipid. Res.* **39**, 231–255.
- Brigelius-Flohe, R., Kelly, F. J., Salonen, J. T., Neuzil, J., Zingg, J. M. and Azzi, A. (2002) *Am. J. Clin. Nutr.* **76**, 703–716.
- Zimmer, S., Stocker, A., Sarbolouki, M. N., Spycher, S. E., Sassoon, J. and Azzi, A. (2000) *J. Biol. Chem.* **275**, 25672–25680.
- Kempná, P., Zingg, J. M., Ricciarelli, R., Hierl, M., Saxena, S. and Azzi, A. (2003) *Free Radic. Biol. Med.* **34**, 1458–1472.
- Ricciarelli, R., Zingg, J. M. and Azzi, A. (2000) *Circulation* **102**, 82–87.
- Gysin, R., Azzi, A. and Visarius, T. (2002) *FASEB J.* **16**, 1952–1954.
- Galli, F., Stabile, A. M., Betti, M., Conte, C., Pistilli, A., Rende, M., Floridi, A. and Azzi, A. (2004) *Arch. Biochem. Biophys.* **423**, 97–102.
- Ogru, E., Gianello, R., Libinaki, R., Smallridge, A., Bak, R., Geytenbeek, S., Kannar, D. and West, S. (2003) in: *Free Radicals and Oxidative Stress: Chemistry, Biochemistry and Pathophysiological Implications*, pp. 109–113 (Galaris, D., Ed.) Medimond, Bologna.
- Munteanu, A., Zingg, J. M., Ogru, E., Libinaki, R., Gianello, R., West, S., Negis, Y. and Azzi, A. (2004) *Biochem. Biophys. Res. Commun.* **318**, 311–316.
- Birringer, M., EyTina, J. H., Salvatore, B. A. and Neuzil, J. (2003) *Br. J. Cancer* **88**, 1948–1955.
- Zhang, S., Lawson, K. A., Simmons-Menchaca, M., Sun, L., Sanders, B. G. and Kline, K. (2004) *Breast Cancer Res. Treat.* **87**, 111–121.
- Wang, Q., Theriault, A., Gapor, A. and Adeli, K. (1998) *Biochem. Biophys. Res. Commun.* **246**, 640–643.