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Review Article

Molecular mechanism of α-tocopherol action

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Abstract

The inability of other antioxidants to substitute for α-tocopherol in a number of cellular reactions, the lack of a compensatory antioxidant response in the gene expression under conditions of α-tocopherol deficiency, the unique uptake of α-tocopherol relative to the other tocopherols and its slower catabolism, and the striking differences in the molecular function of the different tocopherols and tocotrienols, observed in vitro, unrelated to their antioxidant properties, are all data in support of a nonantioxidant molecular function of α-tocopherol. Furthermore, in vivo studies have also shown that α-tocopherol is not able, at physiological concentrations, to protect against oxidant-induced damage or prevent disease allegedly caused by oxidative damage. α-Tocopherol appears to act as a ligand of not yet identified specific proteins (receptors, transcription factors) capable of regulating signal transduction and gene expression.

Contents

Definition

α-Tocopherol has been defined as a radical-chain breaker [1], which, due to its hydrophobic nature, operates in a lipid environment. The effects of α-tocopherol as an antioxidant are thus restricted to its direct effects in membranes and lipoprotein domains. Consequently, other definitions such as "secondary antioxidant," antioxidant as inhibitor of "enzymes that produce radicals," or activator of "genes coding for antioxidant enzymes" are confusing and do not help in understanding the molecular mechanism of α-tocopherol function in vivo. The possible exclusion of α-tocopherol from the category of radical-chain breaker in a hydrophobic environment as defined above has prompted the reactive suggestion that the antioxidant properties of α-tocopherol may be exerted within a micro-domain of a receptor or of an enzyme. These types of suggestions, however, go beyond the discussion of the molecular aspects of α-tocopherol action.

The chemical paradigm

The antioxidant properties of α-tocopherol are a very well-established chemical paradigm. Indeed, vitamin E can act as an

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antioxidant in the test tube, in lipid and phospholipid suspensions [1], in cell-free Hevea brasiliensis latex [2], or perhaps in plants, although in this case the alternative function of cellular signaling by modulating jasmonic acid levels has been also proposed [3]. There is little doubt that, in vivo, if given in pharmacological concentrations, possibly by parenteral administration to humans or animals, α-tocopherol must act as an antioxidant; however, this situation goes obviously beyond the concept of physiological function. Such an antioxidant function, that is, intrinsic part of the chemistry of the molecule, may in fact not be always desirable, similarly to the possible negative effects of the administration of other antioxidants in large amounts. It is known that the amount of the micronutrients, such as polyphenols, provided with an antioxidant function in vitro, which are accepted by the organism, is extremely low [4] and certainly below the amount needed to produce a significant antioxidant function. The emerging view is that polyphenols are likely to exert beneficial and/or toxic actions on cells not through their potential to act as antioxidants but rather through their modulation of protein and lipid kinase signaling cascades [5].

The unverified extension of the antioxidant concept from chemistry into biology

The argument that chemically tested antioxidants must have in vivo antioxidant properties is not tenable. Other in vitro "antioxidants" as ubiquinone [6] and carotenoids [7] have in vivo nonantioxidant properties. Also estrogens can be considered antioxidants [8], although not potent ones, and physiological levels of 17,β-estradiol binding to LDL are associated with enhanced resistance to their Cu^{2+}-mediated oxidation [9]; however, this effect is not the consequence of radical scavenging; 17β-estradiol enhances the resistance of LDL to oxidation by stabilizing apoB-100 conformation [10]. In any case, 17β-estradiol, the most potent mammalian estrogenic hormone, is not acting by virtue of its antioxidant properties, but by binding to specific cellular receptors. Retinal [11], polyphenols, phytoestrogens, and flavonoids [4,12] are other examples of micronutrients provided with in vitro antioxidant capacity; the concentration that they reach in vivo is of the order of μM or lower that is not compatible with a significant in vivo antioxidant function [13]. Rather, by directly modulating signal transduction events they modify cell functional parameters [4]. An intriguing conjecture (there are not yet data to back it up) can be made at this point that the concentration of plant polyphenols provided with in vitro antioxidant properties is kept in the human organism extremely low by limiting their absorption and by induction of phase 1 and phase 2 enzymes, responsible for their modification, conjugation, and efficient elimination [13–15]. It appears in general as if diet antioxidant uptake must be avoided and that antioxidant concentration must be kept very low. The only exceptions appear to be ascorbic acid and α-tocopherol. It may not be surprising that natural selection has developed mechanisms intended to protect the organism from excessive antioxidant intake since reactive oxygen species have evolved as signaling molecules [16–21]. The activity of nox's (NADPH oxidases, present not only in macrophages but in a large number of nonphagocyting cells) is tightly regulated by a number of enzymes [19,22,23] and is aimed at controlled production of superoxide and hydrogen peroxide. The latter is, for instance, capable of inhibiting protein tyrosine phosphatases with the consequent enhancement of the receptor tyrosine kinase signal [24–27]. Interference with such oxygen signaling by an antioxidant may not be desirable.

Nonantioxidant physiological function of α-tocopherol: Evidence at a cellular level

K.C.D Hickman wrote in 1946: "The cutting down of cell metabolism is a primary and intracellular function of vitamin E, and … it has a secondary and more general antioxidant role which may be taken by other substances" [28] as cited in [29]. This conclusion was reached on the basis of the differential effects exhibited by vitamin E relative to methylene blue in preventing oxygen toxicity in the rat.

Such a conclusion was, in subsequent years, ignored with some exceptions such as A.T. Diplock who wrote in 1983 "The results suggest that α-tocopherol is capable of exerting a controlling influence upon the linoleyl and arachidonyl residues within membrane phospholipids which cannot be explained on the basis of the antioxidant function of the vitamin…" [30].

In more recent years the mechanism of action of α-tocopherol has been thoroughly reinvestigated. In light of new experimental findings, the view of Tappel [31] that the chain-breaking antioxidant vitamin E is the main protector against in vivo lipid peroxidation and of Burton and Ingold [32] that vitamin E functions in living systems primarily as a lipid antioxidant and free radical scavenger had to be revised. Among the important discoveries that have brought to this new paradigm is the finding that of the eight vitamin E family members (α-, β-, γ-, δ-tocopherol and the homonymous tocotrienols) only α-tocopherol (and to a much lesser extent γ-tocopherol) appears to be retained in significant amounts [33] by the organism. This event is the consequence of the expression in the liver of a protein, α-TTP, with high selectively for α-tocopherol [34,35] and low or very low affinity for the other tocopherols with the implicit message of a particular evolutionary pressure exerted by α-tocopherol, which is not shared by other equally potent antioxidants. A second line of evidence comes from the experimental observations that α-tocopherol is able to modulate a number of cell functions in a unique way, not shared by any other antioxidants [36,37]. Our original observations were followed by a number of studies [38–44] indicating that a number of cell functions, such as inhibition of smooth muscle cell proliferation, preservation of endothelial integrity, inhibition of monocyte-endothelial adhesion, inhibition of monocyte reactive oxygen species and cytokine release, and inhibition of platelet adhesion and aggregation are controlled by the nonantioxidant properties of α-tocopherol. It is hard to imagine that such a fine regulation of cellular functions be mediated by noncontrollable free radical chain reactions [45]. After our original finding that α-tocopherol is able to modulate gene expression [46–50],

many other genes have been found to be under the control of α-tocopherol [51–56]. However, no genes expressing antioxidant enzymes appear to be up regulated in the absence of α-tocopherol as expected by an obvious compensatory mechanism. The different tocopherols and tocotrienols have effects, at a cellular level, that are independent of their relative antioxidant properties; for instance, the different tocopherols and the analogue compounds carbonitrile derivatives inhibit smooth muscle cell proliferation by a mechanism not correlated with the antioxidant properties of the molecules [57]. Moreover, the competition between α-tocopherol and β-tocopherol in inhibiting PKC or smooth muscle cell proliferation [58] suggests the existence of a common binding site for the two molecules and cannot be explained in terms of two antioxidants that, added together, have less effect than α-tocopherol alone. α-Tocotrienol has been also shown to act by the regulation of gene expression in an antioxidant-independent way [59]. γ-Tocopherol, less potent than α tocopherol as an antioxidant [60,61], has unique cellar functions, indicating again that their molecular structures and not their antioxidant properties determine the differential functions of tocopherols [62–72].

Nonantioxidant physiological function of α-tocopherol: In vivo evidence

In a number of in vivo situations, no antioxidant effect of α-tocopherol has been found. Only few of these observations, as examples, will be cited here. No effect of supplementation with vitamin E is seen on oxidative DNA damage as estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion [73], again indicating that in vivo tocopherol did not act as an antioxidant. The fact that also vitamin C and coenzyme Q have no effect on 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion [73] may raise a question regarding generalized antioxidant properties of these two molecules. Furthermore, antioxidants do not prevent muscle oxidative damage in response to an ultramarathon run [74]. Administration of vitamin E has been shown to trigger preconditioning via K(ATP) channels and cyclic-GMP without inhibiting lipid peroxidation [75]. Human atherosclerotic plaque contains both oxidized lipids and relatively large amounts of α-tocopherol and ascorbate, indicating that α-tocopherol is not able to act in the plaque, as an antioxidant [76]. Furthermore, human supplementation with α-tocopherol results in increased plasma and LDL tocopherol levels but the degree of protection against copper-catalyzed LDL oxidation is only evident at doses > or = 400 IU/day [73], thus indicating that, at physiological concentrations, no antioxidant effect can be demonstrated. α-Tocopherol does not have any protective effect against a number of pathologies, at the basis of which is presumably an excess of oxygen radical production such as on exercise-induced increases in muscle damage or recovery [74] or in carotid and aortic human lesions, where large amounts of oxidized lipids coexist with relatively normal α-tocopherol levels [76]. Also, recent mechanistic studies demonstrate that the role of α-tocopherol during the early stages of lipoprotein lipid peroxidation is complex and that the vitamin does not act as a chain-breaking antioxidant [77]. The poor performance of antioxidant strategies using α-tocopherol in preventing either atherosclerosis or cardiovascular events is an established problem [78]. Such a situation casts severe doubts either on the implication of oxygen radicals as pathophysiological important for the onset of atherosclerosis [79] or on the in vivo efficacy of α-tocopherol as an antioxidant or on both [80].

If α-tocopherol is not acting as an antioxidant what protects membrane phospholipid against oxidative damage?

A number of compounds produced physiologically in the body in a much regulated way have been shown to act in protecting membranes against lipid oxidation. Among them, bilirubin has been shown to be an antioxidant of physiological importance [81] whose production is regulated by the oxidant-inducible enzyme heme oxygenase.

Superoxide radicals can also reduce membrane damage by acting as radical chain breakers [82] as well as nitric oxide, which has been shown to react with lipid peroxyl radicals exhibiting great oxidant protection [83]. Finally, phospholipid-hydroperoxide glutathione peroxidase (GPx-4) is a well-established mechanism for phospholipid hydroperoxide repair [84].

A double role for α-tocopherol? Is DNA an antioxidant?

The fact that α-tocopherol plasma or tissue concentration may be diminished under conditions of high radical production (sepsis, smoking, etc) and its oxidation products may be excreted has been taken as evidence that α-tocopherol acts as an antioxidant. However, excretion of oxidized α-tocopherol products does not to imply that α-tocopherol has finalistically sacrificed itself to protect the organism against free radicals. In fact, similarly to DNA, α-tocopherol requires protection by other antioxidant systems to prevent its loss and with it, the loss of its regulatory properties. When the oxidative mechanisms are not compensated by sufficient protective mechanisms, burning up of α-tocopherol may take place with the appearance of its oxidation products; parallely, changes in the signaling effects of the molecule may take place. It is well known that an excess of free radicals can produce DNA single-strand and double-strand breaks and the appearance in the urine of base oxidation products such as 8-OH guanine. It is also known that this damage can be repaired, with great efficiency, by appropriate mechanisms. If the specific function of DNA and its hierarchal superiority relative to all other cell functions were not known, DNA could be considered a mechanism for free radical scavenging, equipped with a capable recycling mechanism.

It appears that the relationship between α-tocopherol and its oxidative environment is that of a sensor, monitoring the environment and, through its concentration changes, transferring information to the cell. Recycling phenomena and TAP (tocopherol-associated proteins) protection [85], as a consequence of the tight interaction between protein and α-tocopherol [86], may be mechanisms of preserving α-tocopherol from oxidative damage and degradation. Given the

specific functions of α-tocopherol, it is unthinkable to attribute to α-tocopherol a co-primary role as antioxidant: if α-tocopherol were an antioxidant, its concentration would diminish as a consequence of an increased radical production with abrogation of important physiological functions. As discussed above, α-tocopherol must be protected against free radical damage rather than be used to eliminate free radicals. "Recycling" is therefore intended to regenerate damaged α-tocopherol and not to reactivate a lost antioxidant.

Conclusion

A number of lines of evidence, evolutionary, genetic, biochemical, and functional, have indicated that the natural function of α-tocopherol is that of cell signaling. Such a property is not shared by any other antioxidant molecule. Recent experiments have indicated that α-tocopherol, but not other antioxidants, is the precursor of a more active form of vitamin E, α-tocopheryl phosphate; this species may be ultimately the molecule which specifically interacts with a receptor or transcription factor and modulates cell functions [87,88]. α-Tocopherol has been shown as well not to protect in vivo against oxidative damage or to prevent diseases which have at their basis an oxidative insult. Altogether, the conclusion can be drawn that α-tocopherol is not physiologically acting as an antioxidant.

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