Hypothesis

A function for the vitamin E metabolite α-tocopherol quinone as an essential enzyme cofactor for the mitochondrial fatty acid desaturases

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Abstract A critical analysis of the changes in fatty acid patterns and their metabolism elicited by vitamin E deficiency leads to the proposal that a major role of dietary *RRR*-α-tocopherol (α-TOC) is as an enzymatic precursor of α-tocopherolquinone (α-TQ) whose semiquinone radical functions as an essential enzyme cofactor for the fatty acid desaturases of the recently elucidated carnitine-dependent, channeled, mitochondrial desaturation-elongation pathway; a detailed mechanism for its function is proposed. Pathophysiological states produced by vitamin E deficiency and α-TOC transfer protein defects, such as ataxia, myopathy, retinopathy, and sterility are proposed to develop from the effects of impaired α-TQ-dependent desaturases and the resulting deficiency of their polyenoic fatty acid products.

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Key words: Docosahexaenoic; Peroxidation; Mitochondrion; Tocopherylquinone; Dystrophy; Infertility

1. Introduction

Among the repertoire of antioxidant defenses of biological systems [1,2], *RRR*-α-tocopherol (α-TOC) is a well established chain-breaking antioxidant [1,2]. However, increasing evidence suggests that tocopherols are involved in the control of cell proliferation and differentiation [3]; α-TOC also functions as a scavenger of active nitrogen species [4] and a γ-tocopherol metabolite (LLU-α) is a natriuretic factor [5]. In addition to its other functions, analysis of previous data suggested that α-TOC or its quinone derivatives are involved in fatty acid desaturation [6,7]. A critical analysis of additional experimental evidence leads to the proposal that a major role of dietary α-TOC in mammalian systems is as a precursor of its D-α-tocopherolquinone (α-TQ) metabolite whose semiquinone radical is required as an essential enzyme cofactor by the recently elucidated carnitine-dependent, channeled mitochondrial fatty acid desaturases; a detailed testable mechanism for this function is proposed.

2. Metabolic characteristics of the mitochondrial desaturation pathways

The metabolic characteristics of the mitochondrial carnitine-dependent channeled multifunctional desaturation-elongation pathway, and the conventional microsomal desaturases, have been described [7,8]. Briefly, 22:6$n-3$ is proposed to be synthesized solely by the mitochondrial pathway, while 20:4$n-6$, 20:5$n-3$ (and their elongation products 22:4$n-6$ and 22:5$n-3$, respectively) and 22:5$n-6$ can be synthesized by both the microsomal and mitochondrial pathways. Various lines of evidence indicate that the $n-6$ and $n-3$ fatty acids undergo desaturation-elongation reactions by independent n-series-specific enzyme systems, and without involvement of 24-carbon fatty acids as intermediates [7,8]. In addition to its carnitine requirements, the channeled nature of the mitochondrial pathway is characterized by lack of incorporation of the Δ^6-desaturation-elongation intermediates, such as 18:3$n-6$ and 20:3$n-6$ (or 18:4$n-3$ and 20:4$n-3$), into phospholipids; these intermediates would remain bound to the multifunctional desaturase and, therefore, would not be available to phospholipid acyltransferases [7,8]. Under normal physiological conditions both pathways appear to operate concomitantly. However, tracer studies suggest that the channeled mitochondrial pathway carries most of the biosynthetic flux for 20:4$n-6$ and 20:5$n-3$, since these fatty acids are labeled faster than their conventional microsomal Δ^6-desaturation-elongation precursors when cell cultures are incubated with labeled 18:2$n-6$ or 18:3$n-3$ in the presence of lactate (as a source of intramitochondrial acetyl-CoA via lactate and pyruvate dehydrogenases), and addition of unlabeled intermediates does not produce the isotopic trapping effects readily observed for open pathways; this channeling effect is increased by the addition of carnitine to the culture medium [7,8], further supporting the mitochondrial location of this channeled desaturation pathway, since fatty acids are transported into mitochondria as carnitine esters thus favoring the mitochondrial pathways. In vivo experiments have also shown a similar channeled synthesis of 22:6$n-3$; pulse chase studies with labeled 18:3$n-3$ show higher label incorporation into rat liver 22:6$n-3$ than into its conventional upstream precursors, i.e. 20:4$n-3$ and 22:5$n-3$, under a variety of dietary conditions [9]. A major role of the microsomal pathway may be to synthesize low concentrations of eicosanoid precursors, such as 18:3$n-6$ and 20:3$n-6$, as a consequence of its separate desaturation and elongation systems. Depending on the metabolic control design, these two pathways may be interregulated as compensatory-redundant systems for the synthesis of fatty acids such as 20:4$n-6$, 22:5$n-6$ and 20:5$n-3$ in some tissues. As deduced from the effects of lactate indicated above, another characteristic of the mitochondrial desaturation-elongation pathway is its independence from malonyl-CoA (in contrast to the microsomal system), with preferential incorporation of mitochondrially generated acetate (such as acetyl-carnitine), rather than exogenous acetate, into its fatty acid products, even though exogenous acetate is readily incorpo-

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rated by the malonyl-CoA-dependent microsomal elongation system [8]. Recent in vivo evidence has confirmed the role of acetyl-carnitine in the synthesis of brain 20:4$n-6$ and 22:6$n-3$ [10].

3. Effects of vitamin E deficiency on polyunsaturated fatty acid patterns

The relative rates of peroxidation of unsaturated fatty acids containing from 1–6 double bonds have been determined to be in the ratio of 0.16:1:1.4:2:2.4:2.8 [11]; therefore, vitamin E deficiency would be expected to produce decreases of unsaturated fatty acids following these ratios if vitamin E functions only as an antioxidant. However, vitamin E deficient diets are known to elicit changes in phospholipid fatty acids which do not follow the above peroxidation kinetics; these effects have been shown by in vitro and in vivo experimental approaches [12,13]. Rat testis phospholipids display a characteristic fatty acid pattern after sexual maturation; 22:5$n-6$ is increased several-fold after puberty, while levels of 22:6$n-3$ are low and remain unchanged [14,15]. Vitamin E deficiency is known to produce reduced sperm motility with eventual development of sterility in a variety of mammalian species [16]; in the rat, these physiological changes are accompanied by striking alterations in the fatty acid composition of testicular phospholipids, in which levels of 22:5$n-6$ are drastically decreased while 20:4$n-6$ and 22:4$n-6$ (the apparent $n-6\Delta^4$-desaturase precursor) are increased [17,18]. However, when vitamin E deficient diets contain 22:6$n-3$, the testicular concentration of this fatty acid is not decreased in rats [17,18], even though loss of this fatty acid in vitamin E deficiency would be expected due to its high peroxidation potential. These data clearly indicate that the decreases of testicular 22:5$n-6$ observed in vitamin E deficient rats are not primarily from peroxidation losses but from impaired de novo biosynthesis. While rat testis does not appear to synthesize 22:6$n-3$, this fatty acid is actively synthesized by skeletal muscle; when rats are fed a vitamin E deficient diet containing only the 18-carbon essential fatty acids, the resulting myopathic skeletal muscle shows striking decreases in 22:6$n-3$, while 20:4$n-6$ and the apparent $n-3\Delta^4$-desaturase precursor 22:5$n-3$ are increased [19]. Again, these fatty acid changes can not be explained by their relative peroxidation rates, which suggests that the observed decreases in muscle 22:6$n-3$ elicited by vitamin E deficiency are actually from impaired biosynthesis.

4. Effects of vitamin E deficiency on metabolism of polyunsaturated fatty acids

The increases in testicular 22:5$n-6$ observed during puberty in the rat [18] are accompanied by striking decreases in conventional microsomal Δ^6- and Δ^5-desaturase activities [18,20,21]. These observations have led to the proposal that an androgen-controlled, channeled, carnitine-dependent mitochondrial desaturation pathway, rather than the conventional microsomal desaturation-elongation pathway, is largely responsible for the synthesis of rat testicular 22:5$n-6$ [7]; several lines of evidence indicate that this pathway preferentially uses intramitochondrial acetate sources, while the conventional microsomal desaturation-elongation pathway has better access to exogenous acetate [8]. During vitamin E deficiency the channeled mitochondrial desaturation pathway appears to be severely impaired, while microsomal desaturation-elongation is activated as a compensatory response. Consistent with this proposal is the observation that incorporation of exogenous [^{14}-C]acetate into rat testicular 20:4$n-6$ and 22:4$n-6$ is increased during vitamin E deficiency [22]; similar increases of labeled acetate incorporation are observed in muscle 20:4$n-6$ and 22:5$n-3$, but not into 22:6$n-3$ [19]. Vitamin E deficient diets also increase liver microsomal Δ^6- and Δ^5-desaturases, with increased labeled acetate incorporation into 20:4$n-6$ [23–25]. These findings, taken together, are consistent with an activation of the microsomal vitamin E-independent pathway as a compensatory response to impairment of the vitamin E-dependent mitochondrial pathway in these tissues. Therefore, the decreases in rat testicular 22:5$n-6$ and muscle 22:6$n-3$ by vitamin E deficiency are predicted by impairment of the mitochondrial pathway, while the increases in liver 20:4$n-6$, testicular 20:4$n-6$ and 22:4$n-6$, and muscle 20:4$n-6$ and 22:5$n-3$, are expected as a result of activation of the microsomal pathway in these tissues. The net result of the interrelationship between these two pathways is an apparent impairment of the Δ^4-desaturation step of both $n-6$ and $n-3$ fatty acid series by vitamin E deficiency, as has been previously noted [6]; mitochondrial synthesis of 20:4$n-6$ would also be impaired, but this decrease appears to be compensated by up-regulation of the microsomal pathway in these rat tissues. In some instances, a small increase in 22:5$n-6$ has been observed in vitamin E deficient skeletal muscle [26], suggesting a compensatory up-regulation of microsomal synthesis of this fatty acid in type I fibers; however, in tissues such as rat testes where microsomal desaturation is dramatically down-regulated upon sexual maturation [20,21], the impaired mitochondrial synthesis of 22:5$n-6$ in vitamin E deficiency does not appear to be compensated for by up-regulation of microsomal synthesis of this fatty acid. The above data strongly argue for a coenzyme role of vitamin E in the mitochondrial desaturation pathways.

5. Proposed involvement of α-tocopherolquinone in fatty acid desaturation

It has long been known that both α-TQ and its hydroquinone (α-TQH_2) have vitamin E activity [27–29], even though these quinones are not converted to α-TOC [30,31]. Both α-TQ and α-TQH_2 are found in low concentrations in many tissues [32,33]; however, since they are known to be formed as one of the byproducts of α-TOC oxidation during peroxidation reactions in in vitro systems [34,35], it is commonly believed that this is their only origin. These quinones are, however, also known to be enzymatically synthesized by photosynthetic organisms via two routes: from α-TOC [36,37] and also by de novo synthesis [38,39]; the latter route must be the one utilized by non-photosynthetic microorganisms since they make α-TQ but not α-TOC [40]. There are some data showing that mammalian tissues are also able to synthesize α-TQ via the de novo pathway [31], and this quinone may also be enzymatically synthesized from α-TOC as it is rapidly formed from labeled α-TOC [41]. Since mammals do not appear to convert α-TQ to α-TOC [30,31], the significance of the above evidence has been overlooked. As mitochondria contain most of the α-TOC and α-TQ in mammalian tissues [31,42,43], with the outer membrane containing the majority of α-TOC [42,44,45], it is likely that α-TQ is also enzymati-

cally synthesized from dietary α-TOC by a mitochondrial enzyme system in the outer membrane. Interestingly, the mitochondrial outer membrane, which does not have the high oxidative reactions of the electron transport chain of the inner membrane, contains a significantly lower concentration of unsaturated phospholipids [13,44,46]; therefore the higher α-TOC concentration in the outer membrane is not likely to be due to higher antioxidant needs. Significantly, α-TQH_2 has been implicated as a hydrogen donor for fatty acid saturation by rumen bacteria [47]. Therefore, it is plausible that α-TQ might be involved in the reverse reaction, i.e. fatty acid desaturation, by mitochondrial desaturases since mitochondria evolved from endosymbiotic prokaryotes [48].

In light of the data discussed above, it is proposed that α-TQ is involved as an enzyme cofactor in the channeled mitochondrial desaturation pathway located in the mitochondrial outer membrane; therefore, a major function of α-TOC in mammalian tissues would be as a precursor of α-TQ for this coenzyme function. The essential steps of this mechanism are depicted in Fig. 1; the initial reaction starts with the binding of the fatty acid substrate to the desaturase active site followed by the simultaneous NADH-driven one-electron reductions (via cytochrome b_5) of two aligned enzyme-bound α-tocopherolquinones, i.e. NADH-quinone oxidoreductase reactions, to form the respective stabilized semiquinone radicals, which would then stereospecifically abstract two hydrogen atoms from the fatty acid substrate in a concerted reaction to form the *cis*-unsaturated product, plus two α-tocopherolhydroquinones which eventually transfer their four hydrogens to molecular oxygen to form two molecules of water, thus regenerating the respective α-tocopherolquinones for the next enzyme cycle. The initial electron flow may occur through intermediate steps; for instance, the transfer of NADH-originated electrons from cytochrome b_5 to the desaturase and to the two α-tocopherolquinones may occur via

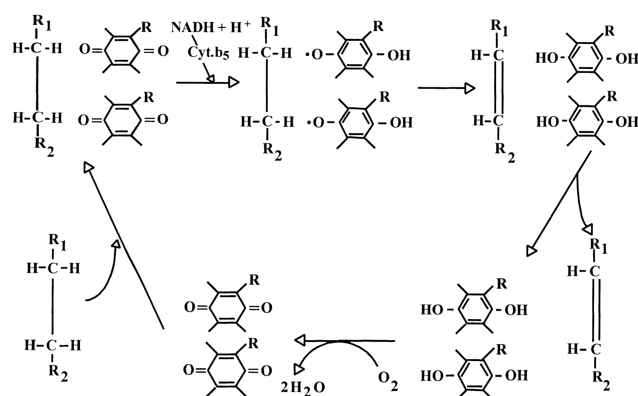

Fig. 1. Proposed mechanism of action for α-tocopherolquinone (α-TQ) as a specific cofactor for the mitochondrial desaturases. The reaction sequence is initiated by binding of the fatty acid substrate to the enzyme active site followed by the simultaneous NADH-driven one-electron reductions of two aligned enzyme-bound α-tocopherolquinones to form the respective stabilized semiquinone radicals, which then stereospecifically abstract two hydrogen atoms from the fatty acid substrate in a concerted reaction to form the *cis*-unsaturated fatty acid product and two α-tocopherolhydroquinones; these reduced quinones eventually transfer their four hydrogens to molecular oxygen to form two molecules of water, thus regenerating the respective α-tocopherolquinones for the next catalytic cycle. Other details and possible intermediates of this mechanism are described in Section 5.

$FADH_2$ and iron-sulfur or iron-selenium clusters using a similar mechanism to that of the NADH-ubiquinone oxidoreductase of the respiratory chain [49]; if so, the α-TQ semiquinone anion radical may be the active species, and protonation may occur downstream in the reaction sequence. The details of these reactions would have to be determined experimentally. Participation of these iron-sulfur or iron-selenium clusters would account for the known interaction between vitamin E and sulfur- and selenium-containing amino acids. The involvement of cytochrome b_5 is consistent with its presence in the outer mitochondrial membrane [50,51] and with the high cytochrome b_5 reductase activity (also referred to as rotenone-insensitive cytochrome C reductase) present in this membrane [50,52].

An essential feature of the above proposed scheme is that reduction of molecular oxygen, probably through di-iron centers [53], occurs downstream of the desaturation step. However, the transfer of hydrogens to molecular oxygen may not necessarily occur at the desaturase active site; the two α-TQH_2 products might be released from the desaturase and re-oxidized by a hydroquinone oxidase. Conversely, the two α-TQH_2 may transfer their hydrogens to mobile carriers such as free α-TQ or ubiquinone; the reduced form of these carriers may shuttle their electrons to the inner membrane mitochondrial respiratory electron transport chain through the inner-outer membrane contact sites. The postulated hydrocarbon oxidizing properties of the semiquinones is plausible in view of the fact that α-TOC radicals appear to engage in such hydrogen withdrawal reactions, which accounts for the pro-oxidant activity of α-TOC observed at high concentrations (reviewed in [54]). The oxidizing activity of the α-TOC radical may be due to the stability conferred by its semiquinone-like resonance structure [55]; other semiquinones which participate in oxidation-reduction reactions are known to be stabilized at the active site of these enzymes [56–59]. These observations lend further support to the postulation that α-TQ semiquinone radicals are the active cofactor species involved in the desaturation reaction.

The proposed site of synthesis of α-TQ in the same membrane where the desaturase is postulated to be located (mitochondrial outer membrane) is dictated by the known instability of quinones; this would explain the finding that this quinone can substitute for α-TOC when administered in frequent and small concentrations [28,29]. The known resistance to vitamin E deficiency by some species, such as mice and some rats [60,61], is proposed to occur by efficient up-regulation of endogenous de novo α-TQ synthesis; because of recurrent periods of starvation the structural and regulatory genes for its enzymatic machinery may have been conserved in these facultative omnivore species. Enzymatic synthesis of α-TQ from α-TOC is also likely to be up-regulated by vitamin E deficiency as a compensatory response. Consistent with this proposition is the observation that this conversion is increased in vitamin E deficient rats [62]. However, in herbivora the ability to make α-TQ from de novo synthesis was probably lost by genetic drift because of the abundant supply of α-TOC in their natural leafy diet; therefore, these species may only be able to make α-TQ from dietary α-TOC, explaining their known high susceptibility to vitamin E deficiency [63,64], a fact difficult to explain if α-TOC were to function only as an antioxidant.

The above proposed stereospecific hydrogen abstraction

mechanism accounts for the concerted formation of *cis*-unsaturated bonds, an exquisite power of fatty acid desaturases [65], for which a mechanism has not been previously proposed. Since the microsomal fatty acid desaturases are also known to introduce *cis*-double bonds, they may operate via a similar concerted mechanism. If so, since these desaturases do not appear to be dependent on α-TQ, their active site may contain other quinones, such as ubiquinone; the identity of these proposed alternative quinones remains to be elucidated. Their di-iron centers [53] may only participate in the final step of electron transfer to molecular oxygen.

6. Conclusion

Vitamin E was discovered more than 75 years ago [66]. Soon after the structures of the various tocopherols were elucidated, it was realized that this vitamin or its quinone derivatives would likely be involved in oxidation-reduction reactions [28,67]. A search for an enzyme cofactor function involving such reactions was sought but never substantiated. It is hoped that the above proposed involvement of α-TQ in fatty acid desaturation represents one of the long-sought enzymatic functions of this vitamin. The pathophysiological states of ataxia, myopathy and retinopathy in metabolic diseases associated with vitamin E deficiency [68,69], such as abetalipoproteinemias, cholestasis, cystic fibrosis, and genetic defects in the synthesis of α-TOC transfer protein [70,71], are likely to be due to deficiency of the main enzymatic products of the channeled mitochondrial α-TQ-dependent desaturation-elongation pathway, such as $20{:}4n-6$ and $22{:}6n-3$. Phospholipids containing these fatty acids have been proposed to play essential roles as tightly bound conformational cofactors for myelogenesis, ion pumps and other membrane proteins [7,8,72]; recent findings [73] have confirmed the postulated role of $22{:}6n-3$ in myelin synthesis. Therefore, administration of these fatty acids may have therapeutic value for patients with these devastating disorders. Because impaired lipid absorption is associated with some of these diseases, providing these fatty acids esterified to phospholipids, such as phosphatidylcholine, might improve their absorption.

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