Molecular Mechanisms of Vitamin E Transport

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Molecular Mechanisms of Vitamin E Transport

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ABSTRACT: Vitamin E is the most important lipid-soluble antioxidant in humans. Specific tocopherol-binding proteins favor the retention of the most potent vitamin E homologue, RRR-α-tocopherol (RRR-α-T) in man. The crystal structures of both the ligand-charged and the apo-forms of human α-tocopherol transfer protein (α-TTP) and of human supernatant protein factor (SPF) have been solved. The renewed interest in the biological function of tocopherol binders is based on the discovery of ataxia with vitamin E deficiency, a neurological disorder that is caused by genetic defects of the α-TTP gene and/or vitamin E deficiency. The analysis of the crystal structure of α-TTP provides the molecular basis of vitamin E retention in man. SPF has been reported to enhance cholesterol biosynthesis by facilitating the conversion of squalene to lanosterol. Nevertheless, the physiological role of SPF as well as its ligand specificity is not known. Investigations on the substrate specificity of SPF have uncovered binding of RRR-α-tocopherylquinone (RRR-α-TQ). RRR-α-TQ represents the major physiological oxidation product of RRR-α-T. The three-dimensional overlay of the ligand-charged structures of SPF and α-TTP indicates that ligand specificity in both proteins is mostly modulated by side-chain variations rather than by the backbone. Recent reports point towards the *in vivo* reduction of RRR-α-TQ to RRR-α-TQH_2 and its protective role in low-density lipoprotein oxidation. On the basis of these reports, it is proposed that SPF may enhance cholesterol biosynthesis indirectly by mediating the transfer of RRR-α-TQ to low-density lipoprotein, thus reducing oxidation of low-density lipoprotein and its subsequent cellular uptake by scavenger receptors.

KEYWORDS: α-tocopherol transfer protein; squalene; α-tocopherylquinone; crystal structure; SEC14-like; CRAL_TRIO; ataxia; vitamin E deficiency

VITAMIN E HISTORY

The term "vitamin E" describes an essential nutrient factor that was introduced in 1922 by Evans and Bishop,[1] who observed that normal reproduction of female rats was abolished by feeding them with rancid fat. The animals developed a severe deficiency syndrome in which fetal resorption was the most characteristic symptom. Adding fresh salad to the diet was reversing the symptoms, and so they concluded that plants contain a specific factor responsible for the observations. Consequently, fetal resorption in rodents was further used for testing the biological activity of vitamin E.[2]

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The physicochemical properties of the factor began to appear in 1936 when two compounds with vitamin E activity were isolated and characterized from wheat germ oil.[3] These compounds were designated α- and β-tocopherol, deduced from the Greek *tokos* (childbirth) and *phorein*(to bring forth). In the following years, two additional tocopherols, γ- and δ-tocopherol, as well as the tocotrienols, were isolated from edible plant oils, so that today, a total of four tocopherols and four tocotrienols are known to occur in nature.[4–6]

CHEMISTRY OF TOCOPHEROLS AND TOCOTRIENOLS

The tocopherols, as well as the tocotrienols, are derivatives of 6-chromanol. The first group derives from tocol, which carries a saturated isoprenoid C_{-16} side chain and three chiral centers with configuration R at positions 2, 4′, and 8′. The members of the second group have a triply unsaturated side-chain at positions 3′, 7′, and 11′. Within one group, the members are designated α, β, γ, and δ, depending on the number and the position of the methyl groups attached to the aromatic ring.[7]

Tocopherols are naturally occurring phenolic benzopyrans that display antioxidant activities *in vivo* and *in vitro*.[8] Since their initial discovery, they have been investigated in order to elucidate their mechanism of action and to identify potential metabolites. Much interest has been focused on their reactivity towards peroxyl radicals as well as on their remarkable regiospecificity towards oxidation and electrophilic substitution.[9–11] Burton and colleagues[12] have undertaken extensive studies on the effects of the chemical structure of phenolic compounds on their reactivity towards peroxyl radicals. By measuring the rate constant for hydrogen abstraction from tocopherols and related phenols, they found that α-tocopherol had the highest value ($k_1 = 2.35 \times 10^6$ M^{-1} s^{-1} at 30°C) of all compounds examined. It was concluded that the rate constant k_1 is determined primarily by the bond dissociation energy of the phenolic O-H bond, which is influenced by stereoelectronic effects as well as by constituent effects. They could demonstrate that the unpaired electron of the α-tocopheroxyl radical is resonance-stabilized over the heterocyclic chromanol ring and that the effect is increased by the electron-donating methyl groups.

Besides having radical trapping properties, α-tocopherol can act as a strong reductant and as an electrophilic agent in chemical reactions, depending on its environment. It has recently been shown that chemical oxidation of α-tocopherol (α-T) to α-tocopherylquinone (α-TQ) proceeds in two steps under retention of configuration R of the chiral center at the 2-position and is accompanied by the formation of a para-quinoid transition state and its mesomeric orthoquinone methide (FIG. 1).[11]

Investigations of the chemistry of the quinone methide indicate that the intermediate is reactive towards nucleophilic agents as well as towards protic solvents. Reactions of the orthoquinone methide that is formed by the phenolic OH group and a ring methyl group always occur in the 5-position, never by the 7-methyl group. The preference of the 5-position, the so-called Mills–Nixon Effect, has been calculated and ascribed to simple changes in conjugative effects, as discussed by Behan and colleagues.[13] Accordingly, tocopheryl quinones (TQs) can be divided in two classes, the nonarylating α-TQs and the arylating γ- and δ-TQs, the latter two lacking the 5-methyl group and being highly cytotoxic by stimulating apoptosis in mammalian cells.[14,15] The observation that partially substituted TQs form Michael adducts with

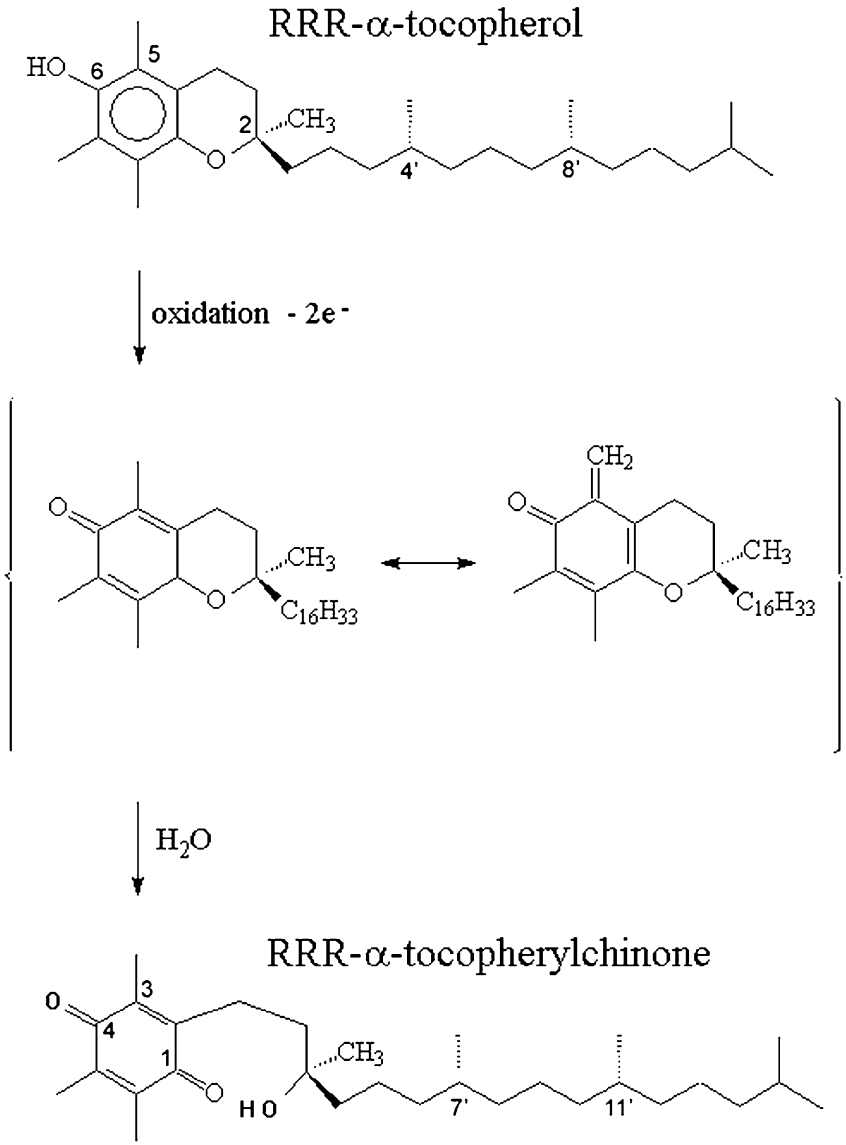

FIGURE 1. Flowchart describing the oxidation of RRR-α-T to RRR-α-TQ (atom numbering included).

high mutagenicity in mammalian cells has led to the hypothesis that the selective accumulation of RRR-α-T might represent an evolutionary advantage due to the relatively low toxicity of its corresponding oxidation product, RRR-α-TQ.[14] Consequently, alterations in the biological activity of tocopherols depend on specific structural alterations, including the presence or absence of ring methyl groups, the stereochemistry of the chiral carbon centers, and the branching or desaturation of the side-chain.

VITAMIN E METABOLISM

Post-absorptive elimination of the various forms of vitamin E appears to play a major role in regulating tissue tocopherol concentrations.[16] This pathway involves ω-hydroxylation of the tocopherol phytyl side-chain by cytrochrome p450 enzymes such as CYP4F2 and CYP3A4,[17,18] followed by stepwise removal of two- or three-carbon moieties, ultimately yielding the carboxyethyl-hydroxychromane (CEHC) metabolite that is excreted largely as glucuronide conjugate in the urine.[19] In this way, the liver does not accumulate toxic levels of vitamin E. Administering high doses of RRR-α-T (335 mg/day) increases plasma RRR-α-T only to a threshold of 1.5–3.0-fold and instead increases plasma α-CEHC, the major metabolite of RRR-α-T, 15–30-fold.[20] Nevertheless, urinary α-CEHC excretion in normal control subjects is negligible, significant amounts being observed solely after supplementation of RRR-α-T at levels that exceed nutritional intake. In contrast, substantial proportions of estimated daily intake of all other vitamin E homologues such as RRR-γ-T are excreted in human urine as CEHC-glucuronide conjugates. All forms of vitamin E have been shown to activate gene expression via the pregnane X receptor.[21] This nuclear receptor is known to regulate drug-metabolizing enzymes such as CYP3A4. In conclusion, these findings again stress the importance of α-tocopherol transfer protein (α-TTP) in rescuing RRR-α-T from hepatic elimination by drug-metabolizing enzymes, a process that appears to be the "default" pathway of vitamin E in man.

RELATIONSHIP BETWEEN STRUCTURE AND BIOLOGICAL ACTIVITY OF VITAMIN E

Although the physiological process has remained obscure, vitamin E is thought to act mainly through its antioxidant properties in preventing damage caused by free radicals. Various independent studies confirm the outstanding role for RRR-α-T as the most potent chain-breaking antioxidant in membranes and the most biologically active of the eight vitamin E homologues.[22–26] Unlike naturally occurring RRR-α-tocopherol (RRR-α-T), the most common synthetic form of vitamin E, *all-rac*-α-tocopherol (*all-rac*-α-T), contains eight different stereoisomers arising from the three stereocenters of the tocopherol backbone. Although the *all-rac*-α-T form displays impaired biological activity, it is equally potent as an antioxidant.[27,28] This indicates that the stereochemistry of RRR-α-T is essential for its biological activity, but moreover that antioxidant activities of tocopherols do not necessarily match corresponding biological activities. Following uptake of dietary vitamin E, RRR-α-T is selectively retained in the body whereas other vitamin E homologues, including non-

natural stereoisomers, encounter rapid clearance.[29,30] Thus, the outstanding biological potency of RRR-α-T seems to be associated with a bio-discrimination that modulates its extracellular and intracellular abundance.

ABSORPTION OF VITAMIN E

In man, vitamin E is taken up passively by micellar absorption together with dietary fats through the brush border membrane of the intestine.[31] A combination of triglycerides, phospholipids, cholesterol, vitamin E, and apolipoproteins is then reassembled to chylomicrons in the mucosa cells.[32] The chylomicrons are stored as secretory granula in the mucosa and eventually excreted by exocytosis to the lymphatic compartment from where they reach the blood stream via the ductus thoracicus.[33] The high clearance rate (24 hr) of a bolus of vitamin E from plasma makes it likely that the transformation of chylomicrons to remnants triggers receptor-mediated endocytotis of the latter by hepatic receptors for apo-E and apo-B.[34–36] Intravascular degradation of the chylomicrons to remnants by the endothelial lipoprotein lipase (LPL) as well as the apolipoprotein exchange between chylomicrons (types AI, AII, and B_{48}) and HDL (types C and E) seem to be instrumental for this process. The plasma lipoproteins are transported to the endosomal compartment, undergo hydrolysis, and release the vitamin E associated with them.[37] Several lines of evidence indicate that after it reaches the liver endosomal compartment, dietary vitamin E encounters rapid elimination, with the exception of RRR-α-T, which is re-secreted into plasma in conjunction with very-low-density lipoprotein (VLDL).[33,38] It has been known for some time that plasma and tissue RRR-α-T concentrations are remarkably stable. This stability suggests that protein factors are involved in its regulation.

REGULATION OF VITAMIN E BY TOCOPHEROL-BINDING PROTEINS

α-Tocopherol Transfer Protein

Hepatic RRR-α-T levels have been proven to be under the control of α-TTP, a cytosolic 32-kDa protein first described by Catignani.[39,40] α-TTP selectively binds to RRR-α-T (100%) relative to RRR-β-T (38%), RRR-γ-T (9%), and RRR-δ-T (2%).[41] Of these four naturally occurring analogues, RRR-β-T is found in negligible amounts in food, whereas RRR-δ-T, RRR-α-T, and RRR-γ-T are abundant in different ratios in most edible oils,[42] stressing the prominent role of α-TTP as a carrier for food-derived RRR-α-T.[43]

The crystal structure of human α-TTP has been solved in two distinct conformations at 1.9 Å resolution, shedding light on its transfer mechanism and its function as a physiological carrier for RRR-α-T (FIG. 2).[44]

In the closed RRR-α-T-charged form of α-TTP, a mobile helical surface segment seals its binding pocket. The conformation of this lid affects ligand access to the lipid-binding site. The RRR-α-T molecule is deeply buried in a hydrophobic pocket that is closed by the lid. The hydrophobic side of the lid helix lies on the entrance of the pocket, whereas the more polar one faces the solvent. In the presence of detergent, α-TTP adopts a conformation with an open lid most likely representing the

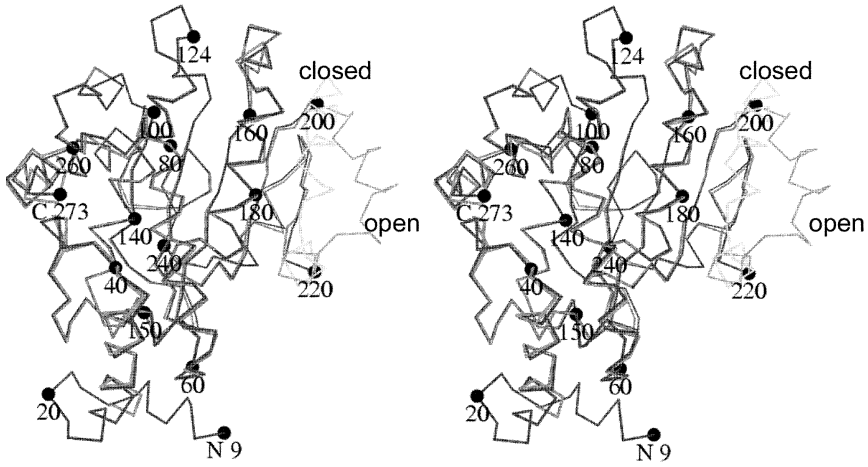

FIGURE 2. Stereoview of labeled Cα traces of the open end of the closed conformation of the α-TTP structure. Figures were prepared using the PYMOL program (Warren Delano, http://www.pymol.org).

membrane-bound form of the protein. The hydrophobic side of the lid helix now faces the solvent. The rotation of the lid by about 80° causes a shift of about 14 Å. The crystallographic evidence obtained from two distinct conformational states of α-TTP allows a rather precise prediction concerning the transfer mechanism of RRR-α-T.

In the closed conformation, a large hydrophobic area (Phe203, Val 206, Phe207, Ile210, and Leu214) of the lid is in direct contact with the side-chain of RRR-α-T. Opening of the lid shifts these residues towards the exterior, establishing new hydrophobic contacts with lipids, detergents, or hydrophobic surfaces. Bound tocopherol is then released into the membrane or, vice versa, is shuffled into the empty or water-filled binding pocket by lid closure. Water molecules in the cavity, if present, are probably squeezed out through a tunnel connecting the rear of the pocket with the hydrophilic solvent. The closed lid exposes a more polar face to the solvent, and the charged carrier–ligand complex can leave the membrane.

The preference of α-TTP for RRR-α-tocopherol can be explained semi-quantitatively from the observed van der Waals contacts in the lipid-binding pocket of the crystal structure (FIG. 3). The chromanol moiety of RRR-α-T is mostly surrounded by hydrophobic residues, with the exception of Ser 136, Ser140, and three water molecules. One of these connects the para hydroxyl group of the chromanol ring with the backbone carbonyl of Val182 through a hydrogen bond. The aromatic methyl group in 5-position of the chromanol moiety fits snugly into a niche formed by the side chains of residues Ile194, Val191, Ile154, and Leu183, the latter two being in van der Waals contact, with a distance of about 3.6 Å. On the other side of the chromanol ring, the two aromatic methyl groups in 7- and 8-position make contacts to Phe187, Phe133, and Leu137. The position and geometry of the pyran half-chair

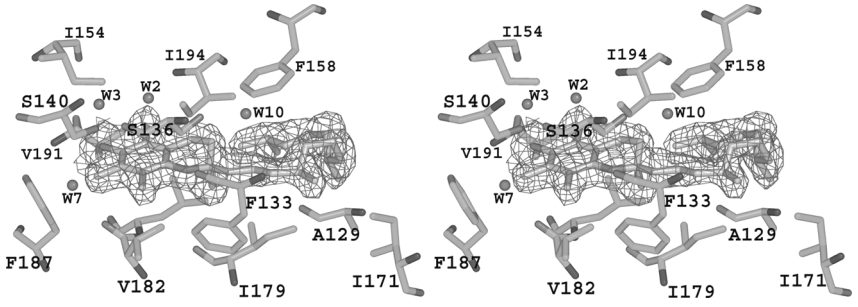

FIGURE 3. Stereoview of the ligand-binding pocket of α-TTP with electron density of bound RRR-α-tocopherol. Shown is a $2F_o$-F_c density map contoured at 1.0 sigma above the mean. The map was computed before the ligand was included in the model. RRR-α-T and residues within van der Waals distance are depicted as stick models, with internal water molecules shown as balls.

of the chromanol ring determine the relative positions of the substituents at the stereocenter in 2-position with the axial methyl group protruding into an indent of the cavity formed by residues Phe133, Val182, and Ile179. The prenyl side-chain is bent into a U-turn involving both stereocenters at the 4′- and at the 8′-position.

RRR-γ-T lacks one aromatic methyl group in 5-position and therefore fits into the cavity as well. However, the absence of one methyl group reduces the surface available for hydrophobic interactions and diminishes the packing density. Studies by Fersht and colleagues derived an average penalty of 1.3 ± 0.5 kcal/mol for the removal of a single methylene group from the hydrophobic main core of chymotrypsin inhibitor 2.[45] If this number is taken as a rough guide for the removal of a methyl group from the hydrophobic cavity of α-TTP, a binding ratio of 8.3 ± 6.7 (at 310 *K*) is obtained for $K^{\gamma-T}/K^{\alpha-T}$. This estimate fits the experimentally determined 10-fold reduction in RRR-γ-T binding.[41] Of course, the error is large because of the varying extent of hydrophobic contacts of a methyl group, which is reflected in the large uncertainty of the figure given by Fersht and colleagues. In the case of RRR-δ-T, where an additional methyl group is missing in 7-position, the computed ratio of $K^{\delta-T}/K^{\alpha-T}$ equals 92, a value that correlates reasonably well with the experimentally observed 50-fold reduction in binding, provided the large error is again considered.

The importance of RRR-α-T retention in man becomes evident by analyzing mutations in the gene of α-TTP in patients suffering from ataxia with vitamin E deficiency (AVED).[46,47] The AVED syndrome is characterized by deficient plasma vitamin E and by a progressive peripheral neuropathy with a specific dying back of the larger caliber axons and the sensory neurons, which finally results in ataxia.[48] Mapping the known AVED-causing amino acid substitutions onto the crystal structure indicates that the binding pocket is not affected in either case, indicating that these mutations seem to influence function rather by impairing the stability of the protein than by prohibiting ligand transfer (FIG. 4).

The six mutations (R59W, R221W, R192H, E141K, A120T, and H101Q) have been investigated by base substitution mutagenesis of recombinant wild-type α-TTP.[49] It was shown that variants associated with the severe version of AVED pa-

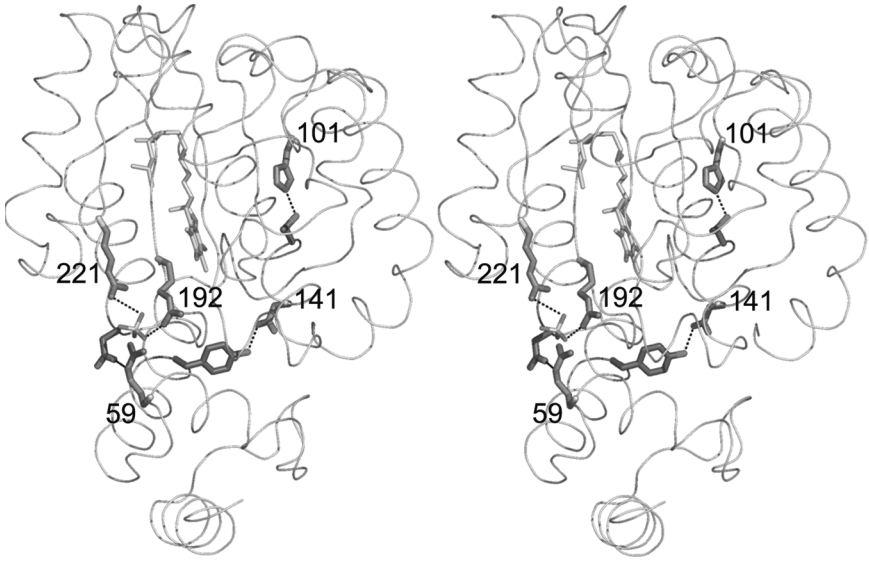

FIGURE 4. AVED-associated mutations in α-TTP. The clinically characterized mutations are mapped onto the three-dimensional structure. Residues undergoing mutation are depicted as stick models; the interacting amino acids described in the text are shown without labeling.

thology (R59W, E141K, and R221W) are impaired in both binding and transfer activities. On the other hand, variants associated with the milder forms of AVED (H101Q, A120T, and R192H) were strikingly similar to the wild-type protein. In accordance with these results, our preliminary attempts to overexpress in *Escherichia coli* and isolate the mutants R59W and R221W turned out to be unsuccessful because of the formation of insoluble protein aggregates. Only the mutant H101Q could be isolated as soluble protein. The comparison of the transfer activities, in accordance with the work of Hosomi and colleagues,[41] did not reveal significant differences between wild-type α-TTP and freshly prepared mutant H101Q, although the latter showed a rapid loss of transfer activity even when kept at 4°C. This indicates that helix 9 (residues 129–143) represents an important element of the α-TTP fold, forming one wall of the tocopherol-binding cavity. The hydrogen bond between T139 and the semi-conserved H101 most likely is not completely abolished by the replacement of histidine with glutamine and thus may explain why this mutation exhibits less severe phenotypes than, for example, the E141K mutation on the opposite site of helix 9.[50]

Not only does the liver express α-TTP (with a high expression rate), the mammalian brain seems to be able to express its own α-TTP. Accordingly, the neurological phenotype of α-TTP$^{-/-}$ mice has been found to be even more severe and shows an earlier onset than that of wild-type mice when maintained on an α-T-deficient diet,[51] the severe phenotype being unable to walk straight forward. Moreover, an uterine form of α-TTP has been reported to be essential for embryogenesis by supplying the

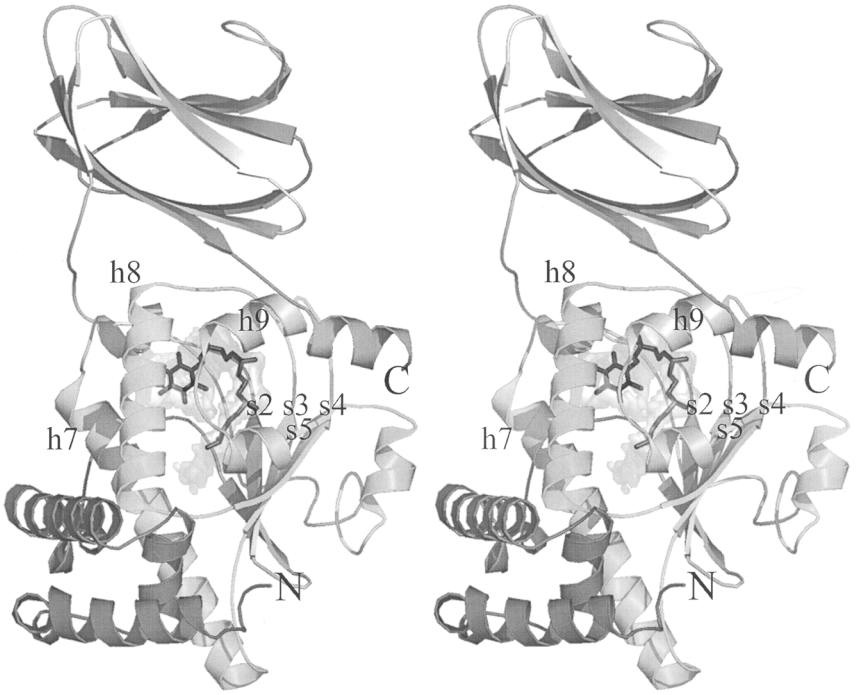

FIGURE 5. Stereoview of the SPF-RRR-α-TQ complex showing the three-helix coil (N-terminus), CRAL_TRIO lipid-binding domain with cavity, jellyroll (C-terminus).

labyrinth region of the placenta with RRR-α-T during development.[52] Both tissues are known to be exposed to high rates of oxidative stress and therefore seem to be specifically protected by α-TTP-mediated tocopherol delivery.

The function of α-TTP in mediating the incorporation of RRR-α-T into VLDL has remained elusive. Its re-secretion into plasma has been shown to be independent from Golgi-associated processes, and therefore seems not to depend on the secretion of VLDL into plasma. It has been suggested that the process of tocopherol re-secretion is linked to cellular cholesterol transport, which would implicate an involvement of the endosomal compartment.[53,54] The most recent report that α-TTP transiently localizes to late endosomes provides evidence for this hypothesis, showing that RRR-α-T is transiently removed by α-TTP from the endosomal compartment separating it from the other homologues of vitamin E that are destined for elimination.[37]

Supernatant Protein Factor

In 1999, a novel binder of vitamin E, termed tocopherol-associated protein (TAP), was discovered in the cytosol of bovine liver with the use of radioactively labeled α-tocopherol as tracer.[55] Subsequently, from the partial amino acid sequence of the bovine protein, its human homologue was identified. The human homologue

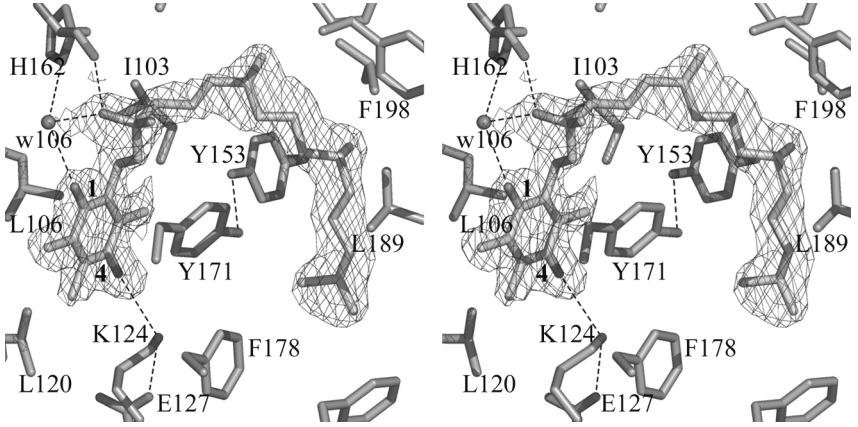

FIGURE 6. Electron density map of the RRR-α-TQ molecule embedded in the ligand-binding pocket of SPF. Shown is a $2F_o$-F_c density map contoured at 1.0 sigma above the mean. The map was computed before RRR-α-TQ was included in the model. RRR-α-TQ and residues within van der Waals distance to the quinone molecule are depicted as stick models (atom numbering included), with internal water molecules shown as balls.

of bovine TAP (hTAP) was cloned into *E. coli*, and its tissue-specific expression was assessed by Northern blot analysis.[56] On the basis of dot-blot analysis, it was concluded that the major mRNA transcript of hTAP is widely expressed in human tissues, with the highest levels being found in liver, brain, and prostate. In order to establish if hTAP interacts directly with tocopherol, a biotinylated α-tocopherol derivative was synthesized and used as a ligand for binding measurements performed using an IASys-resonant mirror system. The measured dissociation constant of 4.6×10^{-7} M suggested that hTAP is expressed with a functional lipid-binding domain and that it is able to bind to biotinylated tocopherol well within physiological concentrations.

Recently, hTAP has been re-identified as Bloch's supernatant protein factor (SPF), which has been reported to stimulate the conversion of squalene to squalene-epoxide.[57] Therefore, the TAP designation has not been maintained. SPF is considered to be essential to human cholesterol production, though its precise function and ligand specificity have remained obscure.[57–59] The crystal structures of human apo-SPF and of its complex with RRR-α-TQ, the major physiological oxidation product of RRR-α-T, have been solved at resolutions of 1.90 Å and 1.95 Å, respectively.[60,61] The structure of apo-SPF consists essentially in two structural entities, an N-terminal CRAL_TRIO domain and a C-terminal jellyroll β fold (FIG. 5).

The N-terminal CRAL_TRIO lipid-binding motif defines SPF as a member of the family of SEC14-like lipid-transfer proteins, including phosphatidylinositol/phosphatidylcholine transfer protein (SEC14) from *Saccharomyces cerevisiae*, cellular retinaldehyde-binding protein (CRALBP), and α-TTP.[56] Database sequence analysis indicates that the jellyroll domain of SPF represents the structural prototype of the recently discovered GOLD (Golgi dynamics) domain.[62] The GOLD domain has

been identified as an obligatory element of the widespread p24 protein superfamily serving as an anchor in protein recruitment and the formation of membrane-associated protein complexes at the Golgi apparatus. This hints towards an adaptor role of SPF in the assembly of membrane-associated complexes or in regulating assembly of cargo into membranous vesicles.

The structure of the complex reveals how SPF sequesters RRR-α-TP in its protein body and permits a comparison with the structure of human α-TTP in complex with RRR-α-TP. The overall structure of the SPF-RRR-α-TQ complex does not reveal significant differences compared with apo-SPF. The binding pocket of the SPF-RRR-α-TQ complex is mostly lined by hydrophobic amino acid side chains. The methyl groups at positions 5 and 6 of the quinone head group are in van der Waals distance to Val167, Val108, and Leu120, whereas the methyl group in position 3 at

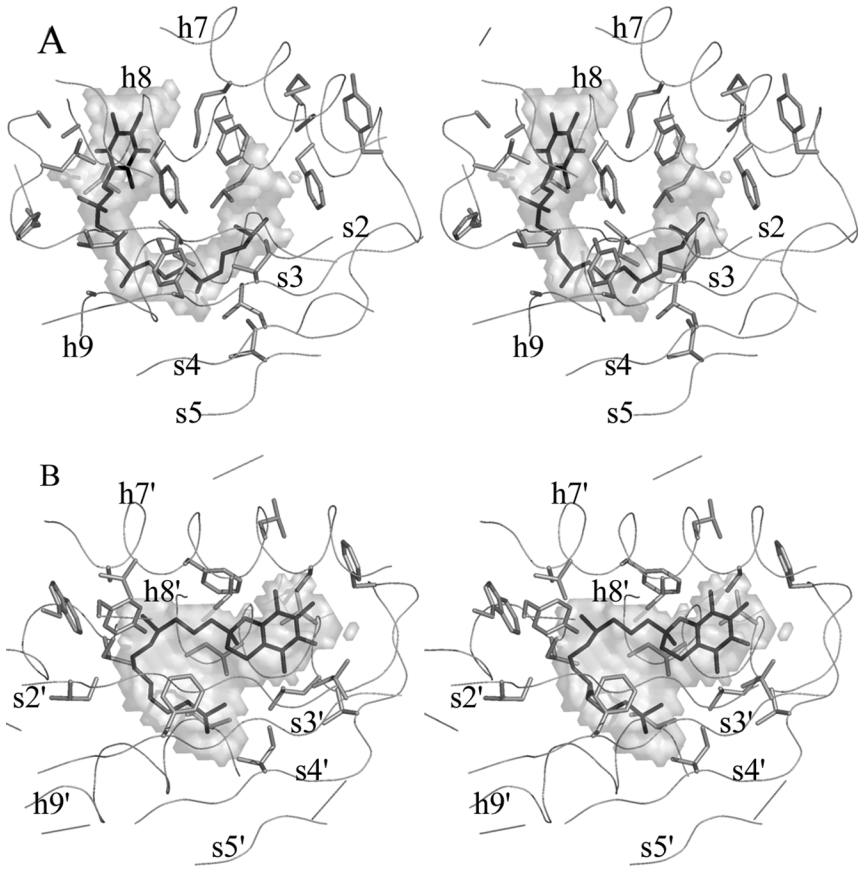

FIGURE 7. Stereoview of the ligand-binding pockets of (**A**) SPF with RRR-α-TQ and (**B**) α-TTP with RRR-α-T. Cavities are shown after least-squares superimposition of the Cα traces of the protein structures.

the opposite side of the ring forms van der Waals contacts to Leu106, Ile103, and Tyr171 (FIG. 6).

The side chain is embedded between Tyr153 and Phe198 and ends between Phe178 and Leu189. In contrast to the rather hydrophobic cavity of α-TTP that lacks charged residues, the RRR-α-TQ molecule of SPF is in vicinity to Lys124, which forms on one side a salt bridge (distance: 2.9 Å) with Glu127, and is on the other side hydrogen-bonded (distance: 3.4 Å) to the carbonyl oxygen in position 4 of the quinone head group. The second carbonyl oxygen in position 1 of the quinone forms a hydrogen bond via a water molecule to His162, the backbone carbonyl oxygen of which is hydrogen-bonded to the C3′ hydroxyl group of the quinone, which itself forms a second hydrogen bond (distance: 2.4 Å) to the water molecule mentioned earlier (FIG. 6). In analogy to the complex of α-TTP with RRR-α-T, the RRR-α-TQ molecule is sitting in the cavity of SPF with the phytyl tail facing the cavity entrance and the quinone head pointing towards the interior, as can be expected for a lipid being pulled out of a micelle or bilayer (FIG. 7).

In contrast to the Cα-traces, the overall positions of α-TQ in SPF and α-T in TTP are grossly different in their respective binding pockets, which indicates that the ligand position in both proteins is mostly modulated by side-chain variations rather than by the backbone.

The physiologic role of RRR-α-TQ is not known yet. Nevertheless, RRR-α-TQ levels have been found to be remarkably low in the cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis.[63] It has been reported by several groups that RRR-α-TQ is reduced *in vivo* to RRR-α-TQH_2.[64–67] In addition, oral supplementation of humans with RRR-α-T has been shown to result in micromolar plasma levels of both RRR-α-T and RRR-α-TQH_2.[68] Thus, plasma-bound RRR-α-TQH_2 has been proposed to serve together with other lipophilic antioxidants as a defense in blocking LDL (per)oxidation.[69] The concept that RRR-α-TQH_2 may act as co-antioxidant in LDL is based on the assumption that a specific carrier exists that mediates the transfer of RRR-α-TQ or RRR-α-TQH_2 to plasma lipoproteins in analogy to α-TTP that transfers RRR-α-T to plasma VLDL.[29,70] Blocking LDL oxidation by RRR-α-TQH_2 would diminish the uptake of oxidized LDL by the scavenger receptor CD36, with a resulting reduction in cellular cholesterol uptake.[71] Low levels of intracellular cholesterol would then subsequently induce cholesterol synthesis via known cellular feedback mechanisms.[72,73] Interestingly, recent studies from independent research groups have shown that overexpression of SPF in hepatoma cells increases cholesterol synthesis by two-fold and have suggested that SPF may have a role in regulating cholesterol synthesis *in vivo*.[57,74] Investigations on the role of RRR-α-TQH_2 as natural antioxidant[75] further support the idea of a putative link between the carrier function of SPF for RRR-α-TQ and its regulatory role in cellular cholesterol synthesis.

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