The location and function of vitamin E in membranes (Review)

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Summary

Vitamin E is a fat-soluble vitamin that consists of a group of tocols and tocotrienols with hydrophobic character, but possessing a hydroxyl substituent that confers an amphipathic character on them. The isomers of biological importance are the tocopherols, of which \( \alpha \)-tocopherol is the most potent vitamin. Vitamin E partitions into lipoproteins and cell membranes, where it represents a minor constituent of most membranes. It has a major function in its action as a lipid antioxidant to protect the polyunsaturated membrane lipids against free radical attack. Other functions are believed to be to act as membrane stabilizers by forming complexes with the products of membrane lipid hydrolysis, such as lysophospholipids and free fatty acids. The main experimental approach to explain the functions of vitamin E in membranes has been to study its effects on the structure and stability of model phospholipid membranes. This review describes the function of vitamin E in membranes and reviews the current state of knowledge of the effect of vitamin E on the structure and phase behaviour of phospholipid model membranes.

Keywords: Vitamin E, \( \alpha \)-tocopherol, model membranes, phospholipid bilayers, free radical oxidation.

Abbreviations: DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; ESR, electronic spin resonance; SAXS, small-angle X-ray scattering; WAXS, wide-angle X-ray scattering; \( \alpha \)-T, \( \alpha \)-tocopherol; RRR-\( \alpha \)-tocopherol; \( \gamma \)-T, \( \gamma \)-tocopherol; RRR-\( \gamma \)-tocopherol; \( \Delta \), lamellar liquid-crystalline phase; \( \Pi \), lamellar gel phase; \( \Lambda \), lamellar crystal phase; \( \Pi \)\( _h \), inverted hexagonal phase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DMPE, 1,2-dimyristoyl-sn-glycerol-3-phosphoethanolamine; DPPE, 1,2-distearoyl-sn-glycerol-3-phosphoethanolamine; DLPE, 1,2-dilauroyl-sn-glycerol-3-phosphoethanolamine; DPPC, 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine; DSPC, 1,2-distearoyl-sn-glycerol-3-phosphocholine; DOPE, 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine; DMPC, 1,2-dimyristoyl-sn-glycerol-3-phosphocholine; DPPC, 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine; DSPC, 1,2-distearoyl-sn-glycerol-3-phosphocholine; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; TBP, tocopherol binding protein.

Introduction

Vitamin E is a ubiquitous, albeit minor, component of biological membranes. It is classified as a fat-soluble vitamin, but, because it possesses a hydroxy group attached to the ring structure, it is weakly amphipathic. In some respects, the vitamin resembles cholesterol in its amphipathic character.

Despite its relatively low concentration compared to other membrane lipids, it is believed to play an important part in preserving the integrity of membranes. Foremost amongst its functions is its ability to protect polyunsaturated lipids of the lipid bilayer matrix of membranes against oxidation. Another important function in membranes is the formation of complexes between vitamin E and products of membrane lipid hydrolysis such as lysophospholipids and free fatty acids. The complexes thus formed tend to stabilize membranes and prevent the detergent-like actions of lipid hydrolytic products on the membrane.

This review summarizes recent evidence for the role of vitamin E in protecting membranes from free radical attack and the consequences of lipid oxidation in membranes. One of the key factors in understanding how vitamin E fulfils its functions in membranes is how it is localized in the lipid bilayer matrix and what effect does its presence have on the structure and stability of membranes. Studies with model membrane systems containing vitamin E which address these questions are described.

Biochemistry of vitamin E

Vitamin E is used as the generic description for all tocotrienol derivatives qualitatively exhibiting the biological activity of \( \alpha \)-tocopherol (IUPAC 1974). Tocopherols should be regarded as derivatives of tocol. The tocopherols possess a \( 4', 8 \), 12-trimethyltridecyl phytyl side chain and the tocotrienols differ by the presence of double bonds at the \( 3', 7 \) and \( 11' \) positions of the side chain. The \( \alpha -, \beta -, \gamma - \) and \( \delta - \) isomers of tocopherol and tocotrienol differ in the number and position of the methyl substituents attached to the chromanyl ring. The structures and names of these isomers are shown in figure 1. The structure of tocotrienol indicates that there are three centres of asymmetry; these are at \( C_2, C_4' \) and \( C_8' \). The tocotrienol possesses only one centre of asymmetry in \( C_2 \), in addition to sites of geometrical isomerism at \( C_3' \) and \( C_7' \). Thus, a number of stereo isomers of the tocol and tocotrienol can exist.

In nature, the most abundant form of vitamin E was shown conclusively to have the 2R, 4'R, 8'R configuration (Mayer et al. 1963, Sheppard et al. 1993), and was called RRR-\( \alpha \)-tocopherol. It has the highest biological activity and accounts for \( \sim 90\% \) of the vitamin E activity found in tissues (Machlin et al. 1982, Cohn 1997). Its melting point is 2.5-3.5°C, so that, at room temperature, it is a viscous oil. It is fluorescent with an emission maximum of \( \sim 325 \) nm in hydrophobic environments. Its infrared spectra show OH (2.8-3.0 \( \mu m \)) and CH (3.4-3.5 \( \mu m \)) stretching and a characteristic band at 8.6 \( \mu m \). The optical rotations of these tocopherols are very small and depend on the nature of the solvent.

Because vitamin E is a fat-soluble vitamin, it tends to partition into tissue lipids, locate in hydrophobic domains like lipoproteins, or partition into the hydrophobic core of the various cell membranes. The distribution of vitamin E in the
Body changes from one tissue to another (Podda et al., 1996, Hidiroglou et al. 1997, Viana et al. 1999). Adipose tissue, liver and muscle represent the major sites of vitamin E in the body, with ~90% of the vitamin being contained in the

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(a). Tocol

(b). Tocotrienol

(c). Space-filling model of RRR-$\alpha$-tocopherol

Figure 1. Chemical structures of tocol (a) and tocotrienol (b), and a space-filling model (c) of RRR-$\alpha$-tocopherol.
adipose tissue (Traber and Kayden 1987). Of all the subcellular membrane fractions, the greatest concentrations of α-tocopherol were found in the Golgi membranes and lysosomes (Buttriss and Diplock 1988, Zhang et al. 1996). The molar ratio of α-tocopherol:phospholipid in these membranes was in the order of 1:65 phospholipid molecules, which is about an order of magnitude greater than found in the other subcellular membranes. In all tissues, α-tocopherol was found to be the major form of vitamin E.

Tocopherol binding protein (TBP) is believed to be involved in the transport and metabolism of α-tocopherol in tissues (Traber and Arai 1999). Three α-tocopherol binding proteins have been isolated from the cytosol of mammalian tissues and characterized (Dutta-Roy 1999). The first one is a hepatic protein of 30 kDa (Kuhlenkamp et al. 1993, Sato et al. 1993, Wolf 1994) and is responsible for discrimination between the homologues of vitamin E, by incorporating α-tocopherol from lysosomes to the endoplasmic reticulum for packaging in VLDL (Traber et al., 1994). This could explain why γ-tocopherol only accounts for 10–15% of plasma tocopherol although it is efficiently absorbed. The second α-tocopherol binding protein, which has a molecular weight of ~15 kDa has been identified in the cytosol of various tissues, including liver. It may be involved in the intracellular distribution and metabolism of α-tocopherol in tissues (Dutta-Roy et al. 1993a, b, 1994, Gordon et al. 1995, Dutta-Roy 1997). Unlike the 30 kDa TBP that only resides in hepatocytes, the 15 kDa TBP is present in all major tissues. The 15 kDa TBP specifically binds α-tocopherol, in preference to δ and γ-tocopherol, and may exclusively transport α-tocopherol to intracellular sites. The third one is a plasma membrane α-tocopherol binding protein (TBPpm). This is characterized in human erythrocytes and liver, and may regulate α-tocopherol levels in these cells (Dutta-Roy et al. 1994, Bellizzi et al. 1997a, b).

Functions of vitamin E in membranes

The vitamin E deficiency syndrome and the benefit of vitamin E to the body have been well known for many years, but the mode of action of tocopherols at a molecular level is not clearly understood. There is considerable literature on the subject, and some of the conclusions are conjectural. It is believed that the main function of vitamin E is its action as an antioxidant in membranes and lipoproteins (Fukuzawa et al. 1997, Surai et al. 1999), but more functions, such as stabilizing membranes, inhibiting platelet aggregation and maintaining the immune system, have been characterized recently.

Scavenging free radicals

α-Tocopherol and γ-tocopherol constitute essential components of cellular defense mechanisms against endogenous and exogenous oxidants. Unlike many other cellular antioxidants that are constituent enzymes or enzyme-dependent systems, the antioxidant reaction of α-tocopherol is non-enzymatic and fast. The principal role of α-tocopherol, as an antioxidant is believed to be in scavenging lipid peroxyl radicals, which are the chain-carrying species and propagate lipid peroxidation (Burton and Ingold 1986, Liebler 1993).

The reactions involved in the scavenging function of vitamin E are illustrated in figure 2. The oxidation of lipids is seen to proceed by a free radical mediated chain process, whereby a lipid peroxyl radical serves as a chain carrier. Chain propagation occurs by abstraction of a hydrogen atom from the target lipid by the peroxyl radical to produce a lipid hydroperoxide and a carbon-centred lipid radical. The carbon-centred lipid radical can react with molecular oxygen to generate another lipid peroxyl radical to produce further chain carrying radicals.

The principle role of α-tocopherol is to scavenge the lipid peroxyl radical before it is able to attack the target lipid substrate producing α-tocopheroxyloxyl radicals. Stereo electronic features of α-tocopherol contribute to the stabilization of tocopheroxy radical and account for the high first-order rate constant for hydrogen transfer from α-tocopherol to peroxyl radicals (Burton and Ingold 1981, Burton et al. 1985). α-Tocopherol reacts with a peroxyl radical either by concerted hydrogen transfer, or by sequential electron then proton transfer, to form a lipid hydroperoxide and the tocopherol radical. The stability of the tocopherol radical arises from delocalization of the unpaired electron about the fully substituted chromanol ring system, rendering the radical relatively unreactive (Kamal-Eldin and Appelqvist 1996). In vitro measurements of the relative rate of chain propagation to chain inhibition by α-tocopherol have indicated that α-tocopherol scavenges the peroxyl radicals considerably faster than the peroxyl radical reacts with lipid substrate.

The concentration of α-tocopherol in cell membrane is relatively low. In most cell membranes, the amount of α-tocopherol is less than 2 mol/mol phospholipid. The question arises as to how such small amounts of α-tocopherol protect
membrane lipids against sustained free radical attack. Redox cycles of \( \alpha \)-tocopherol are believed to occur in membranes (Lieber 1993). Regeneration of \( \alpha \)-tocopherol \textit{in vitro} from its tocopheroxyl radical form mediated by vitamin A (Bohm \textit{et al.} 1997, 1998), vitamin C (Kagan \textit{et al.} 1992, Bowry and Stocker 1993) and coenzyme Q (Ingold \textit{et al.} 1993, Stoyanovsky \textit{et al.} 1995) have been demonstrated. There is, however, some doubt about the significance of regeneration by vitamins A and C \textit{in vivo} (Lüre and Tescoive 1999).

An antioxidant role of coenzyme Q itself is likely to be subsidiary to its main function as a mobile redox proton carrier in the energy-transducing membranes of mitochondria and chloroplasts (Navarro \textit{et al.} 1998). Antioxidant mechanisms of vitamin E and coenzyme Q can be envisaged to operate individually or in tandem. In the first instance, independent reactions with peroxyl radicals take place in which each antioxidant acts as a phenolic scavenger of radicals (Barclay \textit{et al.} 1985). In the second case, there is redox cycling in which vitamin E acts as the primary scavenger of peroxyl radicals, and coenzyme Q recycles vitamin E from its phenoxy radical (Maguire \textit{et al.} 1992, Mukai \textit{et al.} 1992, Kagan \textit{et al.} 1994). These reactions are shown:

\[
\begin{align*}
\text{TO} & 
\rightarrow \text{QH}_2 
+ \text{QH} \rightarrow \text{TO} + \text{QH}_2 
\end{align*}
\]

where \( \text{QH}_2 \), \( \text{QH} \), and \( \text{Q} \) represent ubiquinol, ubisemiquinone and ubiquinone, respectively.

Electron carriers in rat liver microsomes, mitochondria, and submitochondrial particles have been demonstrated to regenerate vitamin E from its phenoxy radical by a ubiquinone-dependent reduction (Maguire \textit{et al.} 1992, Mukai \textit{et al.} 1992). This suggests that vitamin E/coenzyme Q interactions may be important in the antioxidant protection of electron transport membranes. In experiments with mitochondria and submitochondrial particles devoid of vitamin E, Ernster \textit{et al.} (1992) found that reduced coenzyme Q was able to effectively inhibit lipid peroxidation. This indicates that direct antioxidant action of coenzyme Q in mitochondria is also possible. Neuzil and Stocker (1994) showed that coenzyme Q can act as an effective peroxyl and oxygen radical scavenger in LDL, and that this reaction is essential for preventing chain-propagating pro-oxidant action of phenoxyl radicals of vitamin E in LDL.

In contrast to \( \alpha \)-tocopherol, \( \gamma \)-tocopherol is a powerful nucleophile that traps electrophilic mutagens in lipophilic compartments (Cooney \textit{et al.} 1993, 1995, Christen \textit{et al.} 1997). It thus, complements glutathione, which similarly scavenges electrophilic mutagens in the aqueous phase of the cell. An electrophilic mutagen prone to react with \( \gamma \)-tocopherol is peroxynitrite. Thus, \( \gamma \)-tocopherol may protect lipids, DNA, and proteins from peroxynitrite-dependent damage.

**Membrane stabilization**

There is some evidence to indicate that the formation of complexes of \( \alpha \)-tocopherol with certain membrane compo-

tents tends to stabilize bilayer structures, while other evidence suggests that tocopherol destabilizes membranes and promotes fusion. An early observation by Ahkong \textit{et al.} (1972) showed that when \( \alpha \)-tocopherol was added to a suspension of avian erythrocytes, the cells were induced to fuse, presumably because \( \alpha \)-tocopherol destabilized the membrane lipid bilayer. Similar studies with chromaffin granules aggregated with synexin, however, showed that the addition of \( \alpha \)-tocopherol did not induce the granules to fuse (Creutz 1981). Another series of studies in which the stability of model phospholipid bilayer membranes was determined in the presence of different amounts of \( \alpha \)-tocopherol also supported the notion that \( \alpha \)-tocopherol stabilizes lipid bilayers and prevents fusion. For example, the aggregation rate of phosphatidylcholine vesicles containing free oleic acid is decreased (Ortiz and Gómez-Fernández 1988) and Ca\( ^{2+} \)-induced fusion of large unilamellar vesicles of phosphatidylserine (Aranda \textit{et al.} 1996) is inhibited by \( \alpha \)-tocopherol. One explanation for the inhibitory effect of \( \alpha \)-tocopherol on Ca\( ^{2+} \)-induced fusion is that \( \alpha \)-tocopherol reduces the binding of Ca\( ^{2+} \) to phosphatidylserine vesicles (Sanchez-Migallon \textit{et al.} 1996).

\( \alpha \)-Tocopherol is believed to form complexes with membrane lipid components that have a tendency to destabilize the bilayer structure, thereby countering their effects and rendering the membrane more stable. Kagan (1989) has used paramagnetic resonance spectroscopy to demonstrate the formation of complexes between free fatty acids and \( \alpha \)-tocopherol in phospholipid bilayer membranes. Urano \textit{et al.} (1988, 1993) have investigated the interaction of unsaturated fatty acids with \( \alpha \)-tocopherol, by using fluorescence and NMR methods, which showed that \( \alpha \)-tocopherol decreased the fluidity of phospholipid liposomes that were perturbed by the presence of free fatty acids with more than one double bond. Evidence was also obtained for the formation of complexes between lysophosphatidylcholine and \( \alpha \)-tocopherol, that was said to explain the protective effect of \( \alpha \)-tocopherol against the action of products of phospholipase A\( _2 \) hydrolysis on biological membranes (Mukherjee \textit{et al.} 1997). Porcine pancreatic phospholipase A\( _2 \) hydrolyses phosphatidylcholine when in lamellar and micellar states. Grau and Ortiz (1989) have found that \( \alpha \)-tocopherol is able to inhibit the phospholipase A\( _2 \) activity only toward lamellar fluid membranes, thus protecting phospholipids toward this lytic enzyme. This compound decreased both the initial rate and the extent of hydrolysis. The inhibition is of the non-competitive type and the evidence strongly suggests that it is due to an effect of \( \alpha \)-tocopherol on the substrate, i.e. the membrane. Other tocopherols, such as \( \beta \)-, \( \gamma \)- and \( \delta \)-tocopherols, also displayed the phospholipase A\( _2 \) inhibition, but consecutively to a lower extent (Grau and Ortiz 1989). Cholesterol does not inhibit the phospholipase A\( _2 \) activity at concentrations even higher than those of tocopherols, indicating that the effect of tocopherols is not due to alteration of membrane fluidity (Grau and Ortiz 1989).

Molecular model studies have been used to infer formation of stable complexes with phospholipids comprised of polyunsaturated fatty acyl substituents (Lucy 1972, Dipllock and Lucy 1973). It was concluded from such studies that the pockets created by the stereoconfiguration of the \textit{cis} double
bonds of arachidonyl residues, for example, accommodate the methyl groups at position C4' and C8' of the tocopherol phytol side-chain, producing a strong association between the two molecules. It was rationalized that such complexes would be ideally suited to protect the vulnerable fatty acids of membrane lipids from oxidation, reduce the passive permeability of membranes containing high proportions of polyunsaturated fatty acyl residues, and decrease the susceptibility of such phospholipids to hydrolysis by endogenous phospholipases.

The effect of the association of α-tocopherol with single chain or very asymmetric phospholipids has been studied using \(^{31}P\) NMR, Fourier-transfer infrared spectroscopy and light microscopy (Salgado et al. 1993a, b). It is suggested that the complementary shapes of α-tocopherol and asymmetric phospholipids may be the reason for the stabilization of the bilayer structure. The rationale for the stabilizing effect of α-tocopherol is that, by formation of a molecular complex with molecules such as free fatty acids and lysophospholipids, the amphiphatic balance of the complex is restored to that which approximates a bilayer-forming phospholipid. This is illustrated in figure 3, that shows space-filling molecular models of diacyl phosphatidylcholine and compares this with complexes of α-tocopherol and lysophosphatidylcholine and palmitic acid.

Modulation of eicosanoid metabolism

Vitamin E up-regulates the activities of cytosolic phospholipase A\(_2\) and cyclooxygenase (Chan et al. 1998). The enhanced activity of these two rate-limiting enzymes in the arachidonic acid cascade provides a mechanism for the observation that vitamin E dose-dependently enhances release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation (Pyke and Chan 1990, Tran and Chan 1990).

α-Tocopherol modulates the \textit{in vitro} expression of some significant proteins/enzymes in various cell types involved in atherogenesis (Chan 1998). Vitamin E enrichment of endothelial cells down-regulates the expression of intracellular cell adhesion protein and vascular cell adhesion molecule-1, thereby reducing the oxidized LDL-induced adhesion of white cells to the endothelium (Cominacini et al. 1997). Recent advances in the area of arachidonic acid cascade have also demonstrated that α-tocopherol can regulate these pathways, and its effect is not always shared by other isomers of vitamin E (Chan 1998).

Regulation of protein kinase C

The effect of α-tocopherol on protein kinase C, has been studied (Cachia et al. 1998, Azzi and Stocker 2000). α-Tocopherol inhibits smooth muscle cell proliferation (Boscoboinik et al. 1991a), decreases protein kinase C activity (Boscoboinik et al. 1991b), increases phosphoprotein phosphatase A\(_2\) activity (Ricciarelli et al. 1998), and inhibits protein kinase Ca phosphorylation state and its activity (Azzi and Stocker 2000). These functions are not related to vitamin E antioxidant action because β-tocopherol, which has a similar antioxidant activity, does not perform any of these functions. α-Tocopherol effects on protein kinase C inhibition have also been reported in human platelets (Freedman et al. 1996), diabetic rat kidney (Koya et al. 1997, 1998), and human monocytes (Devaraj et al. 1996). The mechanism of protein kinase C inhibition by α-tocopherol may be attributable, in part, to its attenuation of the generation of membrane-derived diacylglycerol, a lipid that activates protein kinase C translocation and activity (Kunisaki et al. 1994, Tran et al. 1994). Cachia et al. (1998) also suggested that the inhibition of protein kinase C activity is not due directly to the antioxidant capacity of α-tocopherol, but requires the integration of α-tocopherol into a membrane structure. Addition of α-tocopherol to recombinant protein kinase C in the test tube does not result in inhibition of protein kinase C, suggesting that the inhibition of α-tocopherol is not caused by a direct interaction with protein kinase C (Azzi and Stocker 2000).

Maintenance of the immune system

Although vitamin E is a relatively minor constituent of all cellular membranes, it is found in high concentrations in membranes of cells of the immune system. It is possible that such membranes are at especially high risk of oxidative damage (Coquette et al. 1986, Machlin 1992, Beharka et al. 1997). The oxidant–antioxidant balance is an important determinant of cell function in immune systems, including maintaining the integrity and functionality of membrane lipids, cellular proteins, nucleic acids, and gene expression in cells of immune system. Vitamin E, as an antioxidant, is said to play an important role in the maintenance of the immune system (Meydani and Tengerdy 1991).

Effects of vitamin E on model membrane systems

In order to determine how α-tocopherol functions in cell membranes, it is necessary to know how it is arranged in the membrane and what influence it has on membrane structure.
and stability. Codispersions of \( \alpha \)-tocopherol with phospholipids in aqueous media have often been chosen as a model to examine the effect of vitamin E on the lipid matrix of cell membranes. A number of studies have been reported using phospholipids with particular acyl chain composition in codispersion with vitamin E.

**The effect of \( \alpha \)-tocopherol on phase behaviour of model membranes**

Many studies have shown that \( \alpha \)-tocopherol influences the phase behaviour of phospholipid model membranes. Examination of the thermal properties of mixed aqueous dispersion of \( \alpha \)-tocopherol with DSPC, DPPC or DMPC by DSC, showed that increasing proportions of \( \alpha \)-tocopherol cause a progressive broadening of the gel to a liquid-crystalline phase transition and a decrease in enthalpy change of the transition (DeKruiff et al. 1974, Missey et al. 1982, McMurchie and McIntosh 1986, Villalón et al. 1986, Ortiz et al. 1987, Quinn 1995). Similar results were obtained by Fourier transform infrared and Raman spectroscopy research (Lefèvre and Picquart 1996). Proportions of \( \alpha \)-tocopherol greater than \(~20\) mol\% resulted in almost complete loss of transition enthalpy.

It has been consistently observed in the thermal studies of mixtures of \( \alpha \)-tocopherol with saturated phosphatidylcholines, that the presence of \( \alpha \)-tocopherol apparently eliminates the pre-transition enthalpy. This does not mean that the ripple structure itself is eliminated. Evidence from synchrotron X-ray diffraction and freeze-fracture electron microscopy have shown that different ripple structures are induced in mixed aqueous dispersions of \( \alpha \)-tocopherol and PC (Wang et al. 2000). Figure 4(a) shows the SAXS patterns of codispersions of DSPC containing up to 10 mol\% \( \alpha \)-tocopherol at 25°C. It can be seen that the number and intensity of the additional peaks increase with increasing proportions of \( \alpha \)-tocopherol, suggesting that these peaks originate from \( \alpha \)-tocopherol enriched domains. Electron micrographs of freeze-fracture replicas prepared from these dispersions at 25°C showed areas with a ripple structure (figures 4(b) and (c)), suggesting that the \( \alpha \)-tocopherol enriched domain forms a ripple phase. The primary effect of \( \alpha \)-tocopherol on phosphatidylcholine might be to form disordered ripples of large periodicity of \(~50\)-150 nm (figure 4(b)). When the proportion of \( \alpha \)-tocopherol is increased, the ripples of large periodicity were replaced by the ordered ripples with a periodicity of 16 nm (figure 4(c)), which produce at least six reflections in the SAXS region (figure 4(a)). A further increase in the proportion of \( \alpha \)-tocopherol induces planar bilayers with a worm-like surface texture (figure 4(c), asterisk). A similar ripple phase has also been observed in codispersions of \( \alpha \)-tocopherol and unsaturated phosphatidylcholine (Wang 2000).

The effect of \( \alpha \)-tocopherol on phase behaviour of heteroacid PCs is complex, with evidence of domain formation. Sanchez-Mgallon et al. (1996b) examined the thermal behaviour of \( \alpha \)-tocopherol with 1,2-dil8-PCs having 18:0 acylated in the sn-1 position and 18:0, 18:1, 18:3 or 20:4 acylated at the sn-2 position of the glycerol. They constructed partial phase diagrams of the mixed acyl dispersions, and concluded that there was fluid phase immiscibility between the phospholipids and \( \alpha \)-tocopherol. The magnitude of the effect of \( \alpha \)-tocopherol on transition enthalpy was found to be dependent on the extent of unsaturation of the
hydrocarbon chain located at the sn-2 position of the glycerol backbone of the phospholipid. It was suggested that \( \alpha \)-tocopherol served to reduce the differences between gel and fluid states of the phospholipids that, in turn, are related to the molecular shape of the unsaturated phosphatidylcholines.

Unlike the bilayer-forming phospholipids, the effect of \( \alpha \)-tocopherol on the phase behaviour of non-bilayer forming lipids appears to be different. Ortiz et al. (1987) examined the effect of different proportions of \( \alpha \)-tocopherol in DPPE and DLPE and found that \( \alpha \)-tocopherol induces the formation of multiple peaks in differential scanning calorimetric curves which were interpreted as evidence of phase separation within the bilayer. Similar results were observed in the mixture of DMPE and \( \alpha \)-tocopherol (Micol et al. 1990). \( \alpha \)-Tocopherol has also been found to decrease the temperature of the lamellar to \( H_{II} \) phase transition in dielaidoyl PE, as well as to induce phase separations of isotropic and \( H_{II} \) phases in the gel phase phospholipid as deduced from \(^{31}\)P-NMR spectroscopy (Micol et al. 1990).

X-ray diffraction experiments have given strong evidence about the phase separation in the codispersion of \( \alpha \)-tocopherol and PEs (Wang and Quinn 1999a, b; Wang et al. 1999). There are three interesting aspects for the \( \alpha \)-tocopherol rich domain formed in the mixture of \( \alpha \)-tocopherol and phosphatidylethanolamine. First, they form a lamellar crystal phase during low temperature equilibrium. Secondly, they form an \( H_{II} \) phase at temperatures much lower than that of \( L_{c} \) to \( H_{II} \) transition of the pure PE, even below the main transition of the pure PE. Finally, they could form a \( Pn3m \) cubic phase at higher temperatures. Increasing the hydrocarbon chain length favours the formation of the lamellar crystal phase, but not the formation of the \( Pn3m \) cubic phase. Moreover, the cubic phase was only observed in mixtures of PE containing \( \alpha \)-tocopherol between 5–20 mol%. In the mixture containing 20 mol% \( \alpha \)-tocopherol, however, only the \( H_{II} \) phase was observed, which is independent of the hydrocarbon chain length of the phospholipid. Figure 5 shows static diffraction patterns of mixtures of DLPE containing 7.5, 10 and 15 mol% \( \alpha \)-tocopherol recorded at designated temperatures. It can be seen that cubic phases begin to dominate the diffraction patterns at 90°C in the mixture containing 7.5 mol% \( \alpha \)-tocopherol (figure 5(a)), and represent the only phase at 99°C in the mixture containing 10 mol% \( \alpha \)-tocopherol (figure 5(b)). It is noteworthy that in the mixture containing 15 mol% \( \alpha \)-tocopherol, the \( Pn3m \) phase already dominates the diffraction pattern at 81°C and completely replaces the \( L_{c} \) phase at 92°C; there was no evidence of any transformation of this cubic space group, even after heating to 101°C (figure 5(c)).

Static X-ray diffraction experiments were performed to investigate the relationship between the different phases observed in the codispersion of DPPE with 20 mol% \( \alpha \)-tocopherol, and the results are shown in figure 6. Two lamellar crystal phases (\( L_{C1} \) and \( L_{C2} \)) are clearly distinguished in this experiment. The \( L_{C1} \) is characterized by a relatively sharp diffraction at 6.4 nm in the SAXS, with a group of peaks in the WAXS region (46.4°C). The \( L_{C2} \) phase is characterized by a broader lamellar diffraction centred at 5.0 nm in the SAXS region, with another set of peaks in the WAXS region (52°C). Below 52°C, only one phase exists, suggesting that the stoichiometry of phospholipid: \( \alpha \)-tocopherol is about 4:1. It is also noteworthy that the appearance of the \( H_{II} \) phase at 56.9°C coexists with the \( L_{C2} \) phase, suggesting that the \( H_{II} \) phase originates directly from the \( L_{C2} \) phase and a phase transition sequence of the \( \alpha \)-tocopherol-rich domain in the initial heating scan of \( L_{C1} \rightarrow L_{C2} \rightarrow H_{II} \) can be inferred. There is a change in lamellar d-spacing from 6.1 nm in the \( L_{C1} \) phase to 5.0 nm in the \( L_{C2} \) phase. Assuming that there is no change in hydration between the two crystal phases, the d-spacing could be accommodated by a change in tilt of the hydrocarbon chains from the vertical in \( L_{C1} \) to 35° with respect to the bilayer normal in the \( L_{C2} \) phase. The

![Figure 5. Static small-angle X-ray scattering intensity profiles versus reciprocal spacing of fully hydrated codispersions of (a) 7.5, (b) 10, and (c) 15 mol% \( \alpha \)-tocopherol in DLPE at designated temperatures. Each diffraction pattern represents scattering accumulated in 5 min.](image-url)
tentative structural arrangements of the \( \alpha \)-tocopherol enriched domain are illustrated in figure 6(c).

**Location of \( \alpha \)-tocopherol model membranes**

The location of \( \alpha \)-tocopherol in model membranes has been the subject of considerable interest (Gómez-Fernández et al. 1989, Quinn 1998). ESR probe experiments have shown that the phytol chain of \( \alpha \)-tocopherol anchors the molecule firmly within the phospholipid bilayer (Niki et al. 1985). This is further supported by the results from \( ^{13} \)C and \( ^{2} \)H-NMR (Perly et al. 1985, Urano et al. 1987, Ekiel et al. 1988, Salgado et al. 1993a, Suzuki et al. 1993), \( ^{15}F \)-NMR (Urano et al. 1993) and fluorescence studies (Aranda et al. 1989). It was concluded that the molecule rotates about an axis perpendicular to the plane of the phospholipid bilayer, and the motion of the rigid chromanol ring is more constrained than the phytol chain in which the frequency of gauche \(^{\circ}\) rotamers increases with distance towards the centre of the bilayer. \( ^{13} \)C-NMR (Srivastava et al. 1983) and \( ^{2} \)H-NMR (Wassall et al. 1986) relaxation measurements of \( \alpha \)-tocopherol in phospholipids in the fluid phase both indicate a restriction in the motional freedom of both components of the mixture. Fourier-transform infrared studies of mixed aqueous dispersions of \( \alpha \)-tocopherol and DPPC have shown that \( \alpha \)-tocopherol does affect the acyl chain conformation. Earlier studies by Villalain et al. (1986) showed that the number of gauche \(^{\circ}\) rotamers in the acyl chains of the phospholipid in the gel phase was reduced by the presence of \( \alpha \)-tocopherol. This, however, was not confirmed by subsequent studies that showed that the number of gauche \(^{\circ}\) rotamers increased (Severcan and Cannistraro 1990). The latter is consistent with the observations from ESR (Wassall et al. 1991, Suzuki et al. 1993, Severcan 1997) and NMR (Wassell et al. 1986, Suzuki et al. 1993).

Different biophysical methods have been used to study the orientation of \( \alpha \)-tocopherol in phospholipid bilayer membranes. Some suggested that \( \alpha \)-tocopherol locates in the region of the aqueous interface of the structure (Srivastava et al. 1983, Perly et al. 1985). Some suggested it is \( \sim 1 \) nm from the aqueous interface (fragata and Bellamare 1980). Because the excitation and fluorescence emission wavelengths of \( \alpha \)-tocopherol are appropriate for fluorescence energy transfer with certain probes, several experiments using fluorescence quenching of probes have been performed to determine the location of the chromanol ring of \( \alpha \)-tocopherol (Ohyashiki et al. 1986, Kagan and Quinn 1988, Bisby and Ahmed 1989). These experiments led to the conclusion that the chromanol moiety of \( \alpha \)-tocopherol is oriented towards the lipid–water interface of the phospholipid bilayer, but does not extend into the lipid–water interface.

A number of studies on the hydrogen bonding between the \( \alpha \)-tocopherol and phospholipid have been done. Several Fourier-transfer infrared spectroscopy measurements showed evidence of H-bonding of the phenoxyl hydroxyl group to either the carbonyl or phosphate oxygen of the phospholipid molecules (Gómez-Fernández et al. 1991, Salgado et al. 1993b). Bilayer permeability experiments reported by Urano et al. (1990) concluded that the phenoxyl group of \( \alpha \)-tocopherol is hydrogen bonded to the carbonyl

![Figure 6. Static X-ray scattering intensity patterns in SAXS (a) and WAXS (b) from aqueous codispersions of DPPE containing 20 mol% \( \alpha \)-tocopherol recorded at different temperatures. (c) Schematic representation of the different structures of \( \alpha \)-tocopherol rich domain, which have a stoichiometry of 4:1 for DPPE: \( \alpha \)-tocopherol. Symbols and indicate DPPE and \( \alpha \)-tocopherol molecules, respectively.](image-url)
group of the ester carbonyl bond of the phospholipids arranged in a bilayer configuration. For further confirmation of this conclusion, time-dependent changes in the fluidity of liposomes caused by the addition of Pr$^{3+}$ were measured at the surface and inner region of liposomes using a fluorescence polarization technique (Urano et al. 1993). The fluidity increased in the surface region and decreased in the inner region of liposomes containing $\alpha$-tocopherol. These results show that when Pr$^{3+}$ passes through the liposome membrane, the hydrogen bonds between $\alpha$-tocopherol and the ester carbonyl of PC may be broken, leading to the formation of an $\alpha$-tocopherol/Pr$^{3+}$ complex, thus causing the fluidity to increase.

Preferential interactions of vitamin E with particular phospholipids

The effect of $\alpha$-tocopherol on the phase behaviour of mixed diacylphosphatidylcholines to determine whether any preferential interactions take place has been reported. Ortiz et al. (1987) have studied the effect of $\alpha$-tocopherol on equimolar mixtures of DPPC/DS PC and DMPC/DSPC. The equimolar DPPC/DS PC mixture only showed a single endotherm which was modified by the presence of $\alpha$-tocopherol in a similar manner to the individual PCs. The equimolar DMPC/DSPC mixture, however, showed monotectic behaviour. With increasing proportions of $\alpha$-tocopherol, the highest temperature endotherm was broadened and shifted towards lower temperatures, while the lowest temperature endotherm was weakened and disappeared in the mixture containing 20 mol% $\alpha$-tocopherol. This was interpreted such that $\alpha$-tocopherol preferentially affects the lower melting component which corresponds to DMPC. Increasing proportions of $\alpha$-tocopherol in an equimolar mixture of 18:0/18:1- and 18:0/22:6-PC also results in a two component transition enthalpy (Stillwell et al. 1996). The lower temperature transition is progressively broadened as the $\alpha$-tocopherol content of the mixture increases, but the higher temperature component is virtually unaffected by the presence of up to 10 mol% $\alpha$-tocopherol. This was interpreted as a phase separation of $\alpha$-tocopherol within the mixed phospholipid dispersion, with a preference for an association with the more unsaturated molecular species of phospholipid.

The inverted hexagonal phase induced by $\alpha$-tocopherol in PEs can be used as a marker for the PE+$\alpha$-tocopherol domain in PC/PE mixtures (Wang and Quinn 2000a, b). An X-ray diffraction study of mixtures of $\alpha$-tocopherol with DOPC and DOPE showed that the effect of $\alpha$-tocopherol on unsaturated phospholipids follows a similar pattern to that observed for their unsaturated counterparts. However, in the X-ray diffraction study of mixed aqueous dispersions of $\alpha$-tocopherol with DOPE/DOPC (1:1) and DOPE/DMPC (1:1), it was found that lamellar gel and liquid-crystalline phases dominated the phase structure. This is consistent with the fact that $\alpha$-tocopherol does not preferentially interact with PE. The changes in the SAXS patterns are similar to those of mixtures of $\alpha$-tocopherol and phosphatidylcholines or of equimolar mixtures of PE/PC (Wang and Quinn 2000a). This again suggests that $\alpha$-tocopherol preferentially interacts with the PC in the mixture or distributes randomly in domains containing both PE and PC, regardless of the saturation and the length of hydrocarbon chains of the two phospholipids.

The effect of $\alpha$-tocopherol on equimolar mixtures of saturated PE and PC has been investigated using DSC (Ortiz et al. 1987) and X-ray diffraction (Wang and Quinn 2000b). The DSC results were interpreted that $\alpha$-tocopherol was preferentially partitioned in the most fluid phase, irrespective of whether this was PE or PC. However, the conclusions drawn from the X-ray data appear to be different (Wang and Quinn 2000b). The change in the WAXS intensity of the sharp peak at 0.43 nm to temperature plotted in figure 7(a) was of the equimolar mixtures of DMPC and DPPE containing up to 20 mol% $\alpha$-tocopherol. The curves show maxima corresponding to the midpoint of the phase transition.

![Figure 7](https://example.com/figure7.png)

Figure 7. Rate of normalized X-ray scattering intensity $I$ of the WAXS peak $d/dt$ recorded from heating scans at 2°/min of codispersions of DMPC/DPPE (a) and DLPE/DSPC (b) containing indicated mol% $\alpha$-tocopherol plotted as a function of temperature. Inset shows a plot of the relative heights, Peak 2: Peak 1, plotted as a function of $\alpha$-tocopherol in the mixture.
of the DMPC (Peak 1) and DPPE (Peak 2) components of the mixture. The ratio in height, Peak 2:Peak 1, is found to increase with increasing \( \alpha \)-tocopherol in the mixture. This is consistent with a preferential partition of \( \alpha \)-tocopherol into DMPC, so as to reduce the contribution of change in the intensity of the wide-angle reflection due to DMPC relative to DPPE. A similar analysis of the WAXS intensity data of mixtures of DLPE and DSPC containing different proportions of \( \alpha \)-tocopherol are presented in figure 7(b). The inset to the figure shows the relationship between the relative heights of Peaks 2 and 1, and the mol\% \( \alpha \)-tocopherol in the mixture. This shows that as the proportion of \( \alpha \)-tocopherol in the mixture increases the contribution to the change in scattering intensity of the WAXS peak from DSPC decreases relative to that from DLPE. This can be interpreted as a preferential partitioning of \( \alpha \)-tocopherol into the high melting point phospholipid component of the mixture, which is again phosphatidylcholine, DSPC.

**Vitamin E enriched domains in membranes**

According to the fluid mosaic model of membrane structure (Singer and Nicolson 1972), phospholipids, together with some other lipids, such as cholesterol, form a fluid bilayer where the incorporated proteins and lipids themselves are free to diffuse laterally. The idea of a free lateral diffusion implies that generally both lipids and proteins would be more or less randomly distributed. However, a considerable number of experimental and theoretical data have accumulated recently, indicating that membranes are not laterally homogeneous, but domains with distinct lipid and protein composition exist (Brown and London 1997, Simons and Ikonen 1997, Brown 1998, Rietveld and Simons 1998). Therefore, biological membranes are likely to be comprised of a mosaic of lipid domains (Vaz and Almeida 1993). In fact, several types of cholesterol-enriched domains have been proposed in plasma membranes (Bretscher and Munro 1993, Murata et al. 1995, Harder et al. 1998, Keller and Simons 1998, Rietveld and Simons 1998).

In a model of a mosaic of lipid domains, where does vitamin E locate? What is the evidence that vitamin E forms enriched domains in membranes? As has been noted already, preferential interactions between \( \alpha \)-tocopherol and phospholipids, and indirect evidence for the formation of specific complexes, have been obtained from a number of different experimental approaches. The presence of two signals from spin label studies of dispersions of DPPC containing relatively high proportions of \( \alpha \)-tocopherol (20 and 30 mol\%) was reported at temperatures over the range 30–50°C (Serecan and Cannistraro 1988). A relatively immobile component was attributed to an \( \alpha \)-tocopherol enriched domain coexisting with bilayers of pure phospholipid. Measurements of intrinsic fluorescence of 7 mol\% \( \alpha \)-tocopherol in bilayers of DPPC also led to the conclusion that \( \alpha \)-tocopherol was closely associated in particular domains of the phospholipid bilayer (Kagan and Quinn 1988, Aranda et al. 1989). Information about the forces that may operate between phospholipids and \( \alpha \)-tocopherol have come from Fourier transform infrared spectroscopy of mixed dispersions of DPPC and \( \alpha \)-tocopherol (Villalain et al. 1986). It was found from measurements of bandwidth and frequencies of the CH\(_2\) antisymmetric and symmetric stretch vibrations of the phospholipid acyl chains that the presence of \( \alpha \)-tocopherol causes a decrease in the average number of gauche\(^+\) chain conformers at temperatures above the main phase transition. Interestingly, the bandwidth and frequency of maximum absorbance does not appear to change in concert with changes in temperature that were interpreted as evidence for the coexistence of two phases. Evidence for complex formation between \( \alpha \)-tocopherol and phospholipids has also been obtained from synchrotron X-ray diffraction studies (Wang and Quinn 1999a, b, Wang et al. 1999). Codispersions of \( \alpha \)-tocopherol and a group of heteroacid PCs have been detected by the DSC method (Sanchez-Migallon et al. 1996a), and lateral phase separations containing different amounts of \( \alpha \)-tocopherol were suggested in the fluid phase. Phase separation was also found in the mixture of \( \alpha \)-tocopherol and PEs (Ortiz et al. 1987).

**Conclusions**

The function of vitamin E divides into those associated with its role as an antioxidant and those which rely on its presence as a constituent of biological membranes (Brigellis-Flohe and Traber 1999, Wang and Quinn 1999c). Because redox reactions require collisions between the reactants for electron transfer to occur, the efficiency of action depends, in part, on the location of vitamin E in the proximity of membrane constituents that are susceptible to oxidation. The evidence for location of \( \alpha \)-tocopherol in proximity to membrane phospholipids with polyunsaturated fatty acyl substituents is based on model predictions, and no direct experimental evidence for such complexes has yet been reported. Formation of complexes between \( \alpha \)-tocopherol and long-chain fatty acids and lysophospholipids, the hydrolytic products of phospholipase A action, have been characterized. Stabilizing membranes by restoring an appropriate amphipatic balance on formation of these complexes is believed to be the mechanism underlying this action of \( \alpha \)-tocopherol.

Membrane phospholipids with polyunsaturated acyl substituents would tend to create domains of relatively high fluidity in membranes. Evidence mainly from calorimetric studies of mixed aqueous bilayer dispersions of saturated molecular species of phospholipids has suggested that \( \alpha \)-tocopherol tends to partition into fluid phases in mixtures exhibiting gel-fluid phase immiscibility, irrespective of the phospholipid class segregating into the respective phase. This result has been challenged by evidence from direct structural examination of mixed aqueous dispersions of phospholipids. This X-ray data was consistent with the formation of complexes between \( \alpha \)-tocopherol and phosphatidylcholines which phase separate from pure phosphatidyl-lethanolamines, irrespective of whether the phosphatidylcholine is in a fluid- or gel-phase configuration. Further work will be required to reconcile these opposing views.

Thus, the evidence that \( \alpha \)-tocopherol is not randomly distributed throughout the lipid bilayer matrix of biological membranes, but instead forms complexes with specific membrane constituents, is emerging. The evidence for
location of α-tocopherol at membrane sites most susceptible to oxidation is not direct. The formation of complexes with membrane destabilizing agents is more convincing, suggesting that, although present as a relatively minor membrane component, it may exert a significant structural role via a localized effect. A preferential association with phosphatidylcholine, as opposed to phosphatidylethanolamines, may result from a more stable bilayer structure created by phosphatidylcholine–α-tocopherol complexes. Studies of α-tocopherol–phosphatidylethanolamine mixtures indicates that α-tocopherol is largely excluded from bilayer phases of phosphatidylethanolamine, but phase separates into domains of inverted hexagonal phase of the phospholipid which would clearly have a destabilizing effect on the membrane lipid bilayer matrix. Failure to form stable complexes with phosphatidylethanolamine may be explained on the same basis as that used to explain why α-tocopherol readily forms complexes with free fatty acids and lysophospholipids, namely, the amphiphatic balance within the complex is conducive to bilayer formation. The predominantly hydrophobic character of phosphatidylethanolamine is matched by that of α-tocopherol and a combination of the two can only exist in non-bilayer phases.

There is clearly compelling evidence that, although present in relatively minor proportions in biological membranes, α-tocopherol segregates in membranes and forms complexes with specific lipid constituents. These reactions have the effect of stabilizing the lipid bilayer matrix. The wider significance of these preferential interactions, especially in regard to the putative antioxidant function of α-tocopherol, remains to be established.

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