1. Structure and Biosynthesis

Tocopherols constitute a series of related benzopyranols (or methyl tocols) that occur in plant tissues and vegetable oils and are powerful lipid-soluble antioxidants. In the tocopherols, the C_{16} side chain is saturated, and in the tocotrienols it contains three trans double bonds. Together, these two groups are termed the tocochromanols. In essence, the tocopherols have a 20-carbon phytol tail (including the pyranol ring), with variable numbers of methyl groups attached to the benzene ring, and the tocotrienols a 20-carbon geranylgeranyl tail with double bonds at the 3', 7' and 11' positions, attached to the ring system. The side-chain methyl groups of natural tocopherols have $R,R,R$ stereochemistry. The four main constituents of the two classes are termed - alpha (5,7,8-trimethyl), beta (5,8-dimethyl), gamma (7,8-dimethyl) and delta (8-methyl). The tocotrienols have a single chiral centre.

These compounds are only synthesised by plants and other oxygenic photosynthetic organisms, such as algae and some cyanobacteria, but they are essential components of the diet of animals, and collectively they are termed ‘vitamin E’ (the individual tocopherols are ‘vitamers’). In plants, there is a great range of tocochromanol contents and compositions, and photosynthetic plant tissues contain from 10 to 50 µg tocochromanols per g fresh weight. Tocopherols are present in all photosynthetic organisms, but the tocotrienols are found only in certain plant families. α-Tocopherol is often the main tocochromanol in leaves. Seed oils are a major source for the human diet and the compositions of tocopherols in some unrefined oils are listed in Table 1. Sunflower and olive oils are good sources of α-tocopherol and palm oil of the tocotrienols. In general, tocotrienols tend to be abundant only in seeds and fruits, especially of monocots such as wheat.
rice and barley, though a major commercial source is palm oil. In leaf tissue, α-tocopherol is often the main isomer, while γ-tocopherol is the primary tocopherol of many seeds.

Table 1. Tocopherol and tocotrienol contents (mg/Kg) in some seed oils

<table>
<thead>
<tr>
<th></th>
<th>α-T*</th>
<th>β-T</th>
<th>γ-T</th>
<th>δ-T</th>
<th>α-TT*</th>
<th>β-TT</th>
<th>γ-TT</th>
<th>δ-TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>palm</td>
<td>89</td>
<td>-</td>
<td>18</td>
<td>-</td>
<td>128</td>
<td>-</td>
<td>323</td>
<td>72</td>
</tr>
<tr>
<td>soybean</td>
<td>100</td>
<td>8</td>
<td>1021</td>
<td>421</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>maize</td>
<td>282</td>
<td>54</td>
<td>1034</td>
<td>54</td>
<td>49</td>
<td>8</td>
<td>161</td>
<td>6</td>
</tr>
<tr>
<td>sunflower</td>
<td>670</td>
<td>27</td>
<td>11</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rapeseed</td>
<td>202</td>
<td>65</td>
<td>490</td>
<td>9</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Abbreviations: T, tocopherol; TT, tocotrienol

The mechanism of biosynthesis of tocopherols is well understood, and involves coupling of phytol diphosphate with homogentisic acid (2,5-dihydroxyphenylacetic acid), followed by cyclization and methylation reactions.

The plant chloroplast is the site of biosynthesis, and the aromatic amino acid tyrosine can be considered the basic precursor. This is oxidized to p-hydroxy-pyruvic acid, which in the first committed step is converted to homogentisic acid by the enzyme p-hydroxyphenylpyruvate dioxygenase. Homogentisic acid is condensed with phytol diphosphate in a reaction catalysed by a prenyl transferase to yield 2-methyl-6-phytyl-plastoquinol, which is first methylated to form 2,3-
dimethyl-5-phytyl-1,4-benzoquinol and then converted by the enzyme tocopherol cyclase to \( \gamma \)-tocopherol. A further methylation reaction produces \( \alpha \)-tocopherol, while modifications to the pathway produce \( \beta \)- and \( \delta \)-tocopherols and plastoquinones. Tocotrienols result from a similar series of reactions but with geranylgeranyl diphosphate as substrate in the condensation step.

Fish contain an unusual tocopherol that has been termed marine-derived \( \alpha \)-tocomonoenol that is found together with \( \alpha \)-tocopherol in a wide range of marine fish species. It appears to be a more efficient scavenger of free radicals at low temperatures. A related isomer with a \( \Delta 11 \) double bond has been found in palm oil and kiwi fruit. Pumpkin seeds contain both \( \alpha \)- and \( \gamma \)-tocomonoenols.

\( \alpha \)-Tocopheryl phosphate has recently been detected at low levels in liver and adipose tissue, and it is possible that it may be a common constituent of animal and plant tissue.

\( \alpha \)-Tocopherol is a minor but ubiquitous component of the lipid constituents of animal cell membranes with estimates ranging from one molecule of tocopherol to from 100 to 1000 molecules of phospholipid, depending on the membrane. The hydrophobic tail lies within the membrane, as might be expected, and the polar head group is orientated towards the surface but probably below the level of the phosphate moieties of the phospholipids. There may be some limited hydrogen bonding between the hydroxyl groups and phosphate depending on the degree of hydration of the membrane. On the other hand, there is a strong affinity of \( \alpha \)-tocopherol for polyunsaturated fatty acids, where the chromanol unit may interact with the double bonds, suggesting that tocopherol is located deep within the membrane.

During the refining of vegetable oils, much of the natural tocopherols are lost or destroyed. Most commercial vitamin E is therefore prepared by chemical synthesis with trimethylhydroquinone and phytol bromide as the precursors. The resulting product is a mixture of eight stereoisomers (from \( R,R,R \) to \( S,S,S \) methyl groups) of \( \alpha \)-tocopherol, with the various stereoisomers differing by a factor of two in biologic activity, as a consequence of the stereochemistry of the 2 position (i.e. 2S-\( \alpha \)-compared to 2R-\( \alpha \)-tocopherol). It is usually administered as the acetate derivative \textit{in vivo}.

Tocopherols are not usually regarded as effective antioxidants in the polyunsaturated seed oils of commerce, and at higher concentrations can even act as pro-oxidants, although the reasons for this are not understood.

2. Biological Functions of Tocochromanols in Plants

In plants, tocochromanols are found almost exclusively in the chloroplasts, where they were long believed to be the most important of the antioxidant molecules, limiting the damage from photosynthesis-derived reactive oxygen species during conditions of oxidative stress, including high-intensity light stress. However, recent studies seem to suggest that they are just one of a number of different components that are involved in photo-protection. Certainly, any tocochromanol peroxo radicals formed must be converted back to the original compounds by the concerted action of other plant antioxidants, for example by ascorbate and glutathione. On the other hand, there is no doubt that tocopherols are essential for the control of non-enzymatic lipid peroxidation during seed dormancy and germination of seedlings. In their absence, elevated levels of malondialdehyde
and phytoprostanes are formed, and there can be inappropriate activation of plant defence responses.

There is evidence that tocopherols also play a part in intracellular signalling in plants in that they regulate the amounts of jasmonic acid (see our web page on plant lipoxins) in leaves, via modulating the extent of lipid peroxidation and gene expression, and so influence plant development and stress responses. Thus, by controlling the degree of lipid peroxidation in chloroplasts, they limit the accumulation of lipid hydroperoxides required for synthesis of jasmonic acid, which in turn regulates the expression of genes that affect a number of stress conditions. In addition, tocopherols are required for the development of the cell walls in phloem transfer cells under cold conditions. It appears that α- and γ-tocopherol and the tocotrienols may each have distinct functions.

3. Tocopherols Metabolism in Animals

In animals, all tocopherols are absorbed to a similar extent in the intestines by mechanisms that are still obscure, and they are transported to the liver in chylomicrons mainly, but α-tocopherol is preferentially utilized and re-exported. This process is mediated by a specific tocopherol-binding protein (the α-tocopherol transfer protein) in the liver that has a marked affinity for α-tocopherol, transferring it to the plasma lipoproteins (mainly the VLDL (and thence to LDL) and HDL in humans) for transport to other tissues (together with much smaller amounts of γ-tocopherol). The “α-tocopherol salvage pathway” results in a 20- to 30-fold enrichment of α-tocopherol in plasma (average concentration 22-28 µM) relative to the other tocopherols.

The process of conservation of one specific tocopherol appears to determine the relative vitamin E activities of the tocopherols and tocotrienols in vivo, rather than their individual potencies as antioxidants as measured in model systems in vitro. Only α-tocopherol (including synthetic material) or natural mixtures containing this can be sold under the label ‘Vitamin E’. However, the tocotrienols are more potent antioxidants, in vitro at least, while γ-tocopherol (which is relatively abundant in skin) has some specific biological properties that are distinct from those of α-tocopherol.

Transfer of tocopherols from the lipoproteins to peripheral tissues is promoted by the enzyme lipoprotein lipase. Concentrations of tocopherols can vary appreciably amongst tissues, with most in adipose tissue and adrenals, less in kidney, heart and liver, and least in the erythrocytes.

Most of the tocochromanols other than α-tocopherol, together with any excess of the latter, are selectively metabolized in the liver. The unwanted surplus may be excreted in the urine in the form of the so-called ‘Simon metabolites’, α-tocopheronic acid and α-tocopheronolactone, after oxidative cleavage of much of the phytol tail. However, these are normally in the form of conjugates as sulfate or glucuronidate esters.
The first step in catabolism is ω-hydroxylation by cytochrome P450 (CYP4F2) at the 13′ carbon to form a 13′-hydroxychromanol, followed by stepwise β-oxidation to cut off two or three carbon moieties from the phytyl chain in each cycle. Various carboxychromanol intermediates have been identified for all of the tocopherols together with sulfated forms of these in human cell cultures in vitro.

### 4. Tocopherols as Antioxidants

Although the syndrome associated with a lack of vitamin E in the diet of animals has been well known for decades, the mode of action and specific location of tocopherols in cell membranes are not clearly understood. Several theories have been proposed to explain their functions. It is evident that their primary task is to act as antioxidants to prevent free radical damage to unsaturated lipids or other membrane constituents and thence to tissues, although it has been suggested somewhat controversially that this may be secondary to more important biological functions (see below). That said there is no doubt that tocopherols are powerful antioxidants in vitro and in vivo. They are certainly extremely useful as antioxidants in non-biological systems, including foods, cosmetics, pharmaceutical preparations and so forth.

Because of their lipophilic character, tocopherols are located in the membranes or with storage lipids where that are immediately available to interact with lipid hydroperoxides. They react rapidly in a non-enzymic manner unlike many other cellular antioxidants, which are dependent on enzymes, to scavenging lipid peroxyl radicals, i.e. the chain-carrying species that propagate lipid peroxidation. In model systems in vitro, all the tocopherols (α > γ > β > δ) and tocotrienols are good antioxidants, with the tocotrienols being the most potent.

In general, the oxidation of lipids is known to proceed by a chain process mediated by a free radical, in which the lipid peroxyl radical serves as a chain carrier. In the initial step of chain propagation, a hydrogen atom is abstracted from the target lipid by the peroxyl radical as shown -

\[
\text{LOO}^\cdot + \text{LH} \rightarrow \text{LOOH} + \text{L}^\cdot \quad (1)
\]

\[
\text{L}^\cdot + \text{O}_2 \rightarrow \text{LOO}^\cdot \quad (2)
\]

- where LH is a lipid, LOO^\cdot is the lipid peroxyl radical and LOOH is the lipid hydroperoxide

The main function of α-tocopherol is to scavenge the lipid peroxyl radical before it is able to react with the lipid substrate as –

\[
\text{LOO}^\cdot + \text{TOH} \rightarrow \text{LOOH} + \text{TOO}^\cdot \quad (3)
\]

- where TOH is tocopherol and TOO^\cdot is the tocopheroxyl radical

It thus prevents propagation of the chain reaction. The potency of an antioxidant is determined by the relative rates of reactions (1) and (2). Studies of the relative rates of chain propagation to chain inhibition by α-tocopherol in model systems have demonstrated that α-tocopherol is able to scavenge peroxyl radicals much more rapidly than the peroxyl radical can react with a lipid substrate.

In biological systems, oxidant radicals can spring from a number of sources, including singlet oxygen, alkoxyl radicals, superoxide, peroxynitrite, nitrogen dioxide and ozone. α-Tocopherol is most efficient at providing protection against peroxyl radicals in a membrane environment.
When a tocopheroxyl radical is formed, it is stabilized by delocalisation of the unpaired electron about the fully substituted chromanol ring system rendering it relatively unreactive. This also explains the high first order rate constant for hydrogen transfer from α-tocopherol to peroxyl radicals. Reaction of the tocopheroxyl radical with a lipid peroxyl radical, as illustrated, yields 8α-substituted tocopherones, which are readily hydrolysed to 8α-hydroxy tocopherones that rearrange spontaneously to form α-tocopherol quinones. In an alternative pathway, the tocopheroxyl radical reacts with the lipid peroxyl radical to form epoxy-8α-hydroperoxytocopherones, which hydrolyse and rearrange to epoxyquinones. Tocopherol dimers and trimers may also be formed as minor products.

In plant and animal tissues, tocopherols can be regenerated from the tocopheroxyl radicals in a redox cycle mediated by a number of endogenous antioxidants, including vitamins A and C and coenzyme Q, and this must greatly extend their biological potency. Vitamin C (ascorbate) may be especially important in aqueous systems, although it may also act at the surface of membranes. However, it has been argued that data on the effects of vitamin E on biomarkers of oxidative stress in vivo are inconsistent, while oxidized metabolites of vitamin E, i.e. which have reacted as an antioxidant, are barely detectable in tissues. Thus, suggestions that dietary supplements of vitamin E may reduce the rate of oxidation of lipids in low-density lipoproteins and thence the incidence or severity of atherosclerosis now appear to be unfounded, although benefits in some conditions have been claimed. Indeed, there are suggestions that excessive vitamin E supplementation may even be harmful. A recent study has suggested that relatively high doses of natural α-tocopherol over a long period are required to demonstrate a significant reduction in the levels of F2 isoprostanes in the urine, which are considered to be the most reliable marker for oxidative stress in vivo. This subject is highly contentious and I prefer to leave it to the clinical experts.

5. Other Biological Functions of Tocochromanols

With the discovery that the antioxidant effects of various tocopherols and tocotrienols have little relation to their vitamin E activities in vivo has come a belief that they may have other functions in tissues, most of which are specific to α-tocopherol. There are many fat-soluble antioxidants in the diet but only α-tocopherol is a vitamin. Indeed, it has even been suggested that tocopherol may be protected from functioning as an antioxidant in some tissues in vivo through a network of cellular antioxidant defences. Only when other antioxidants are exhausted are the tocopherols utilized. However, there is no experimental proof of this hypothesis. While it is certainly true that most other
vitamins are essential cofactors for specific enzymes or transcription factors, no receptor that binds specifically to vitamin E has yet been discovered.

Other proposed functions not generally accepted include a role as a regulator of genes connected with tocopherol catabolism, lipid uptake, collagen synthesis, cellular adhesion, inflammation and cell signalling. It may also modulate the activity of several enzymes involved in signal transduction, and it has been suggested that it may have secondary roles in stabilizing the structure of membranes, in regulating haem biosynthesis, in modulating the immune response, and as a participant in electron transport chains. Some non-antioxidant effects of γ-tocopherol in tissues in relation to reactive nitrogen oxide species have been observed, but the specificity of these is not yet certain.

Tocotrienols have been shown to have neuroprotective effects, to inhibit cholesterol synthesis and to reduce the growth of breast cancer cells in vitro. These properties are largely distinct from those of the tocopherols, and the pharmaceutical potential of tocotrienols against cancer, bone resorption, diabetes, and cardiovascular and neurological diseases are currently being studied.

The biological function of α-tocopheryl phosphate is not known, but it has been suggested that it may be a storage or a transport form or it could be involved in cellular signalling. Synthetic phosphate derivatives of γ-tocopherol and α-tocopheryl succinate are known to have potent anti-cancer properties.

6. Analysis

Tocopherols can be analysed by gas chromatography, both with flame-ionization and mass spectrometric detection, but the methods that are usually recommended involve high-performance liquid chromatography with fluorescence detection (see our webpage on this topic). Related methods are used for ubiquinones and the isoprenoid alcohols.

Recommended Reading

Tocopherols and tocotrienols – structure, composition, biology and analysis


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