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Trace elements and vitamins in membrane function

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The title of this article might well have sprung directly from an examination question, for example 'Discuss the role of trace elements and vitamins in membrane function'. One could imagine perplexed students, faced with this enigmatic problem, racking their memories for some clue to the thoughts of a perverse examiner. For of course, the question is meaningless. There is no systematic reason why any particular link should be drawn between membrane function and trace elements or vitamins.

Nevertheless, this question does raise a more general one for descriptive biology. What are the characteristics possessed by that assorted and disparate group of inorganic and organic molecules, the so-called vitamins and trace elements, which justify their collective classification as micronutrients? It is clear that they are environmental molecules required in 'small' amounts for specific biochemical functions. However, another characteristic they have in common is the fact that their functions, unlike those of most macronutrients, each depend on a particular intrinsic chemical property which is maintained in vivo but which can be readily demonstrated in simple, non-biological systems. In contrast the metabolic substrates, the macronutrients, provide the atoms for structural use as well as the energy from chemical bonds to power biochemical processing. The functional derivatives of the macronutrients can thus be very different from the actual food molecules absorbed by the gut.

Trace elements and vitamins are chemically-active substances having biological functions defined by their chemical reactivity. They are concerned with the management of biochemistry rather than being the substrates of biochemical reactions. As an example of this utilization of a specific chemical property in biology one can consider the micronutrient, pyridoxal phosphate. In vitro, the aldehyde group of this molecule forms a Schiff-base bond with the amino group of an amino acid. In vivo, when pyridoxal phosphate is associated with specific enzyme proteins, such Schiff-base complexes allow catalytic transamination or decarboxylation of amino acids to occur.

It is of interest when assessing the chemical roles of trace elements and vitamins in biology, to speculate about the evolutionary adoption of these molecules as essential components of biochemistry. Apart from the loss of pre-existing biosyntheses of these substances, there must have been stages in evolutionary development when biological systems benefitted from the incorporation of environmental molecules with particular chemical functions. Such a consideration might help to identify a feature of some micronutrients which could apply to the present topic: a relation to membrane function.

Of course one association which micronutrients have with membranes is as the cofactors of membrane-bound enzymes. Although the functions of these enzymes and, hence, of the cofactors as well, undoubtedly contribute to those of the membrane itself, there is no common feature which justifies a collective analysis of micronutrient cofactors in this way.

It is therefore apparent that only two general functions of cell membranes could be conceivably linked with micronutrients. Either these molecules have an effect on a selective permeability function or else they are concerned with membrane structure. No matter how hard one tries to find a link, it is clear that no general relation between micronutrients and membrane permeability can be identified. On the other hand, a few micronutrients may be concerned with the maintenance of the structure of membranes.

Throughout evolutionary history, living cells have been vulnerable to attack by spontaneously-formed free radicals. The polyunsaturated fatty acids of membrane phospholipids, in particular, are susceptible to peroxidation under the influence of free radicals or by reaction with hydrogen peroxide. This oxidative damage to membrane structure can be severe because a catalytic chain reaction of autoxidation follows from a single free radical attack, but a number of protective mechanisms have evolved to counter this destructive process.

To understand these protective mechanisms it is necessary here to define the various stages of the autoxidation of polyunsaturated fatty acids. The initial free radical may be produced by the abstraction of a hydrogen atom from any oxidizable molecule. This creates an unpaired electron in a resultant highly-reactive radical (R•). In the presence of oxygen, free-radical propagation then follows with the formation of the peroxyl radical (ROO•). This oxidized product can react with a further oxidizable substrate to yield a hydroperoxide (ROOH) and another reactive free radical (R•). Hence autoxidation is catalytically propagated: from one initial free radical a large number of hydroperoxides may be

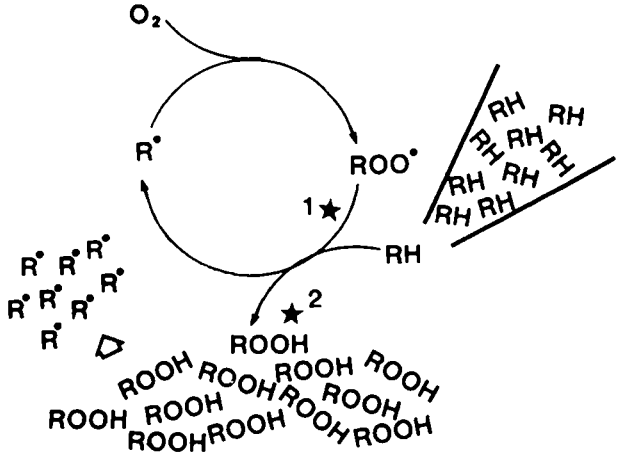

Fig. 1. Schematic representation of propagative autoxidation. Polyunsaturated fatty acids are represented by RH and their hydroperoxides by ROOH. •The two points of antioxidant action. Chain-breaking antioxidants act at point 1 and peroxide-decomposing antioxidants act at point 2.

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formed. Furthermore, the hydroperoxides themselves can give rise to more free radicals so that propagative autoxidation, once initiated, can expand in an overwhelming fashion (Fig. 1).

In principle, autoxidation can be terminated by the reaction of the peroxyl radical with another radical to form non-free-radical products. However, propagative autoxidation can also be interrupted at two points in the sequence (see Fig. 1) by the intervention of other reactive molecules, the antioxidants. The first type of antioxidant action breaks the chain of free-radical formation while the other prevents the generation of more free radicals by inhibiting hydroperoxide production.

A chain-breaking antioxidant reacts with the peroxyl radical to yield a hydroperoxide but in this instance the free radical product is derived from the antioxidant itself. In comparison with the usual autoxidative product the antioxidant radical is much less reactive with O_2 or with other oxidizable substrates. Thus, rather than generating more peroxyl radicals it combines with one to form a non-radical product, thereby preventing the generation of any new reactive species.

The other type, the preventive or hydroperoxide-decomposing antioxidants, convert the hydroperoxide product of oxidation to a hydroxyl molecule which is then unable to generate more free radicals.

Because some vitamin- and trace-element molecules act in vitro as either chain-breaking or preventive antioxidants, their possible role as antioxidants in vivo is considered here for the protection of membrane structure.

Vitamin E

Vitamin E (α-tocopherol) can be oxidized in vitro by peroxyl radicals to yield the free radical, tocopherol quinone (Fig. 2) (Green & McHale, 1965), which is an effective trap for a further peroxyl radical and thus terminates the oxidative chain. As vitamin E is distributed in the lipid phase of membranes it could be predicted that it would act in that site in vivo as a chain-breaking antioxidant to prevent peroxidation of membrane polyunsaturated fatty acids (Visser, 1980). Although cogent arguments against such a function for vitamin E have been put forward (Bunyan *et al.* 1968), it is now apparent that vitamin E deficiency is associated with increased lipid peroxidation in vivo (Hafeman & Hoekstra, 1977). The peroxidative decompositions of linolenic and linoleic acids yield respectively ethane and pentane (Riely *et al.* 1974). These volatile hydrocarbons are found in increased amounts in the exhaled air of vitamin-E-deficient rats and the production of these fragments is diminished by restoration of normal vitamin E status (Dillard *et al.* 1976).

While an antioxidant mechanism may be suggested to explain the protective role of vitamin E against lipid peroxide formation in vivo, this does not have to be the only biological role of vitamin E. Many studies indicate that the hydrocarbon chain of vitamin E contributes to the structure and stability of membranes (e.g. Diplock, 1982) and this could well be a major biological function. Nevertheless, because the

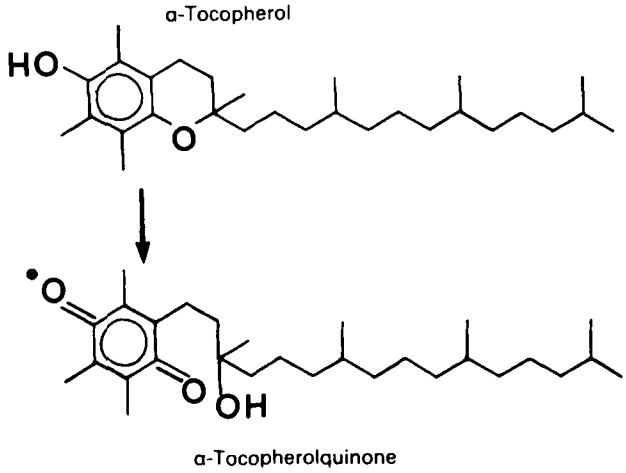

Fig. 2. The in vitro oxidation of α-tocopherol.

chain-breaking antioxidant property of vitamin E can be demonstrated in vitro, it is reasonable to propose that its protective effect against lipid peroxidation in vivo can be directly attributed to this property.

Selenium

Just as vitamin E has a demonstrable antioxidant property so inorganic Se, as selenite, is an effective catalyst in vitro in the oxidation of thiol groups (Jocelyn, 1972):

$$4RSH + SeO_2 \longrightarrow RSSR + RS.SeSR + O_2,$$

$$RS.SeSR + O_2 \longrightarrow RSSR + SeO_2.$$

This property is maintained when Se is incorporated into the enzyme glutathione peroxidase (*EC* 1.11.1.9). In each enzyme molecule there are four atoms of Se as the selenoamino acid residue, selenocysteine (Sunde & Hoekstra, 1980). Glutathione peroxidase catalyses two types of reaction: one specific, the other being applicable over a wider range of substrates. The primary action is in the reduction of H_2O_2 to water with the oxidation of the co-substrate, glutathione (GSH):

$$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$$

The other substrates for this selenoenzyme are various hydroperoxides:

$$2GSH + ROOH \longrightarrow GSSG + ROH + H_2O$$

Therefore, Se in glutathione peroxidase is able to protect membrane lipids against peroxidation either by removing the oxidant, H_2O_2, or by acting as a preventive

antioxidant and removing radical-producing hydroperoxides. Such actions, while not ruling out other biological functions of Se, demonstrate the use in biology of a fundamental chemical property of a trace element, here protecting against noxious peroxidation: an action clearly shown under hyperoxic atmospheric conditions (Forman *et al.* 1983).

β-*Carotene*

The carotenoid precursors of retinol are not conventionally regarded as vitamins even though they, rather than preformed retinol, are the main determinants of vitamin A status in most land vertebrates. Because β-carotene is found in various tissues as well as in the circulation, it is possible that it has a biological role which is independent of that of vitamin A. One such role could be as an antioxidant for β-carotene in vitro is a potent inactivator of singlet oxygen (Foote & Denny, 1968). However, it has recently been discovered that β-carotene has a novel antioxidant property, even in the absence of singlet oxygen, and this may well have biological significance (Burton & Ingold, 1984).

β-Carotene is thought to react with a peroxyl radical to form a resonance-stabilized, carbon-centred radical (Fig. 3) which might act as a chain-breaking antioxidant. However, when β-carotene was incubated under standard oxidizing conditions of 30° and 100 kPa (760 Torr) O_2 pressure, it was found to have no inhibitory action on the autoxidation of a polyunsaturated fatty acid. Here β-carotene may be combining with O_2 and promoting further free-radical formation (Fig. 4(*a*)). When, however, the O_2 pressure was lowered to 2 kPa (15 Torr), Burton & Ingold (1984) found that β-carotene was an effective antioxidant of a special chain-breaking type (Fig. 4(*b*)). It appears that molecular O_2 and peroxyl radicals compete for reaction with the resonance-stabilized β-carotene radical. When O_2 pressure is high, formation of the peroxyl-β-carotene radical is favoured with consequent further free-radical production (Fig. 4(*a*)). At low O_2 pressure the β-carotene radical can inactivate peroxyl radicals in a termination reaction and thus break the autoxidation chain (Fig. 4(*b*)).

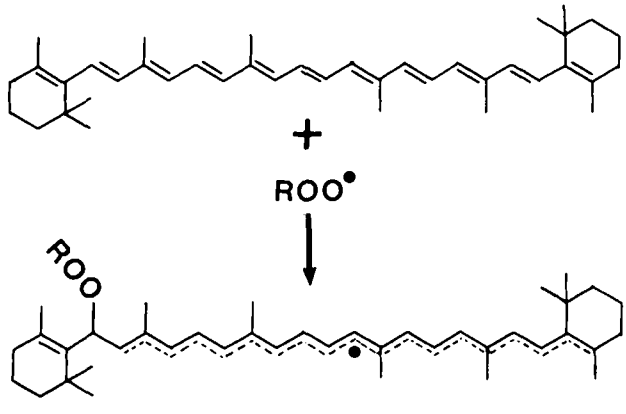

Fig. 3. Peroxidation of β-carotene in vitro to form a resonance-stabilized, carbon-centred radical.

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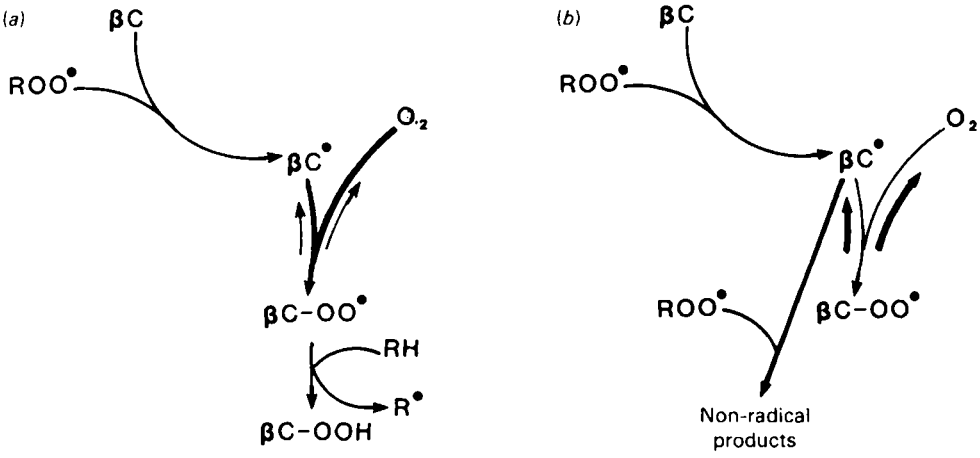

Fig. 4. Peroxidation of β-carotene (βC) under conditions of (*a*) high and (*b*) low oxygen pressures. With high O_2 pressure the peroxy-β-carotene radical acts as a pro-oxidant. Where O_2 pressure is low the β-carotene radical can terminate autoxidation by reacting with a peroxyl radical to form non-radical products.

Conclusions

The antioxidant properties of environmental molecules may have been adopted in biology to protect membrane lipids against peroxidation. Vitamin E and Se deficiencies are both associated with increased oxidative destruction of polyunsaturated fatty acids which can be prevented by correcting the deficiency. Therefore, the known chemical properties of this vitamin and trace element can help to explain their protective role. A newly-discovered antioxidant property of β-carotene at low O_2 pressure suggests that this molecule too might have a function in preventing oxidative damage to membranes. Furthermore, these three micronutrients might protect different tissues of the body according to the O_2 partial pressure. In sites of high O_2 pressure, as in erythrocytes or in the lung alveoli, the antioxidant functions of Se (in glutathione peroxidase) and of vitamin E may be particularly important. Where O_2 pressure is low, as in the vicinity of intracellular membranes, the special chain-breaking antioxidant function of β-carotene may have a protective role which was not previously suspected.

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