Vitamin A and Carotenoids as Antioxidants in a Physiological Context

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Summary Under selected conditions, vitamin A and carotenoids can both accept and donate electrons, and carotenoids can also quench singlet oxygen. Thus both sets of compounds can theoretically participate in a biological antioxidant network. Under physiological conditions, vitamin A esters are transported and stored in a lipid matrix that contains other antioxidants, and retinol and its active metabolites are largely bound in clefts of specific retinoid-binding proteins. Thus, vitamin A seems to be protected in vivo by other antioxidants and proteins rather than protecting other molecules. Carotenoids are largely distributed in lipoproteins, membranes, and the lipid phases of intracellular structures, usually together with vitamin E. Carotenoids can interact with other antioxidants in vitro, but whether they play similar significant roles in vivo is not clear. Nonetheless, some genetic conditions and precancerous lesions respond to carotenoids, and the dietary intake of carotenoids has been associated with a reduced risk of several chronic diseases. Carotenoids seem to act per se in such systems rather than by their conversion into vitamin A.

Key Words vitamin A, carotenoids, antioxidants, chemical properties, physiologic ambient, therapy, chronic diseases

INTRODUCTION

In a chemical sense, an antioxidant inhibits oxidation, i.e. the removal of electrons from a compound. In a biologic context, the definition is less clear, in as much as the prevention of essential oxidative and free radical reactions causes the death of aerobic organisms. Cyanide, for example, is a highly effective antioxidant in vivo. The current intense interest in biological antioxidants therefore focuses on their potential beneficial effects, i.e. protecting biological systems from oxidative harm (1). But harm is also a subjective term; harm can be mild and readily reversible or can be extensive and potentially lethal. Furthermore, one physiological process can be benefited by a given antioxidant, whereas another in the same organism can be adversely affected. Thus, the
effectiveness of a given antioxidant must be defined in a specific physiologic context. Furthermore, antioxidants can also serve as prooxidants under specific condition; e.g. carotenoids in relation to oxygen tension (2) and ascorbic acid to the presence of ferrous ions (3). The key issue, therefore, is the specific physiological ambient in which a given antioxidant acts.

In aerobic organisms, the state of oxygen is of primary importance. The oxygen of air is in a triplet state, which is much less reactive than singlet oxygen. A variety of reduced forms of oxygen exist, with differing oxidative potential (Fig. 1). Antioxidants convert more active to less active forms of oxygen. A large number of biological antioxidants exist, including enzymes, e.g. superoxide dismutase, catalase and glutathione peroxidases; lipid-soluble compounds, such as the tocopherols and tocotrienols, carotenoids, vitamin A, quinones, and bilirubin; and water-soluble factors, such as ascorbic acid, uric acid, creatinine, bilirubin glucuronides, glutathione and other thiols, metal-binding proteins, and heme-binding proteins (1, 3).

Antioxidants not only interact with active oxygen species and with other free radicals, but also with each other. As an example, \( \alpha \)-tocopherol, in reducing the superoxide radical to \( \text{H}_2\text{O}_2 \), is converted to a chromanoxyl radical, which can accept electrons from ascorbate, NADH, NADPH and other donors to regenerate \( \alpha \)-tocopherol. Dehydroascorbate, in turn, can be reduced to ascorbate by dihydrolipoic acid, glutathione, and probably by other thiols (4). This sequential interaction of various antioxidants involved in maintaining \( \alpha \)-tocopherol in a reduced state is termed the “Vitamin E Cycle” (4).

CHEMICAL REDOX REACTIONS WITH VITAMIN A

Retinol (Fig. 2), which contains 5 conjugated double bonds, can transfer single electrons to acceptors, like chloranil, in polar organic solvents, to yield a chloranil radical anion and a retinyl radical cation (5). Retinol is also dehydrated by acids in polar organic solvents to yield a fluorescent retinyllic cation, which can

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\begin{array}{c|c}
\text{INACTIVE} & \text{HIGHLY ACTIVE} \\
\hline
\text{\( ^3\text{O}_2 \)} & \text{\( ^1\text{O}_2 \)} \\
\text{HOH} & \text{O}_{2}^* \quad \text{HO}_2^* \\
\text{OH}^- & \text{HOOH} \quad \text{HO}^* \\
\text{ROH} & \text{ROOH} \quad \text{ROO}^* \\
\text{R=O} & \text{RO}^* \\
\end{array}
\]

Fig. 1. Relative reactivity of oxygen species.
be stabilized at $-78^\circ$ (6). Retinol will also accept an electron from donors like tetramethyl-phenylene-diamine and N, N dimethyl-dodecylamine to form the transient retinyl radical anion, albeit not characterized, and the radical cation of the donor (7). Thus, in keeping with theoretical calculations, retinol can freely accept or donate electrons to appropriate donors. The key query, however, is whether or not these reactions occur to a significant degree in a physiologic context.

**PHYSIOLOGY OF VITAMIN A**

Vitamin A in the diet is primarily present in two forms: retinyl esters and provitamin A carotenoids. The latter are converted, largely by central cleavage, to retinal, which is reduced to retinol. Primarily as retinyl esters, vitamin A is transported in chylomicra via the lymph to the systemic circulation, where chylomicra are rapidly converted to chylomicron remnants (8, 9). The latter are taken up by the liver and other tissues, and in large part stored in vitamin A-containing globules in stellate cells and parenchymal cells of various tissues (9, 10).

The metabolic transformations of vitamin A primarily involve retinoid-binding proteins as coligands (8–10). In these specific protein complexes vitamin A is imbedded in a $\beta$-barrel within the protein, which protects it from oxidation (8–10). Although the biologically active $\beta$-glucuronides of retinol and retinoic acid are water-soluble and are not bound to proteins, vitamin A is generally highly protected in vivo, first by significant amounts of the tocopherols and other lipid antioxidants in chylomicra and then by their association with specific carrier proteins. Thus, vitamin A is a sequestered molecule whose transport and metabolism are highly regulated. It clearly tends to be protected from oxidation in vivo by other antioxidants rather than serving as a part of the antioxidant network of cells.

**QUENCHING AND CHEMICAL REDOX REACTIONS OF CAROTENOIDS**

Carotenoids, unlike vitamin A, are members of a family of approximately
Polyenes and carotenoids commonly found in foods are depicted in Fig. 3. The most common carotenoids contain 11 conjugated double bonds, although the number can vary from 3 to 13 double bonds (Fig. 3).

Singlet oxygen (1O2), commonly produced chemically from 3O2 by use of photosensitizers, can be generated in eosinophils in vivo and in the skin of patients suffering from erythropoietic protoporphyria. Singlet oxygen reacts with carotenoids to yield triplet states of both reactants. The triplet state of carotenoids then is converted to the ground state by the release of energy through radiationless transfer to the solvent. Of various carotenoids, lycopene, \( \beta \)-carotene and astaxanthin are approximately twice as active as \( \beta \)-carotene, which in turn is approximately twice as active as lutein and cryptoxanthin (II). The rate constants for the tocopherols and glutathione are approximately \( 10^{-2} \) and \( 10^{-4} \) that of lycopene, although the approximate concentrations of the latter compounds in tissues are 20 and 50-fold greater than that of lycopene (II). Thus antioxidants other than carotenoids may play a role in singlet oxygen quenching in vivo, even though carotenoids are the most active in this regard.

Carotenoids may donate a single electron to, or accept an electron from, appropriate compounds to yield radical cations or anions of the carotenoid (I2). The resultant radical cation can then accept an electron from chlorophyll a, whereas the resultant radical anion can donate an electron to several chlorophylls or to oxygen (I3–I5). Interestingly, lycopene and the superoxide radical anion transfer an electron reversibly, whereas β-carotene seems to form an adduct with the superoxide radical (I5).

At low oxygen pressure (15 torr), β-carotene inhibits in a concentration-dependent manner the rate of oxidation of tetralin or methyl linoleate by azo-bis-isobutyronitrile in chlorobenzene at 30°C (2). As the oxygen pressure increases to 760 torr, however, β-carotene becomes less protective, and, indeed, can become a pro-oxidant (2). The antioxidant action of β-carotene at low oxygen pressure may well be due to the formation of a resonance-stabilized, carbon-centered radical adduct (2, I6). This adduct can react with another peroxyl radical to give nonradical products, or can reversibly add oxygen to yield a reactive peroxyl radical (2, I6). Because the rate of formation of the latter radical is dependent on the oxygen pressure, the antioxidant action of β-carotene decreases with increased pO2 (2, I6). Thus, carotenoids differ in their properties from other antioxidants, such as the tocopherols, that initially transfer a hydrogen atom to a free radical to yield a peroxide and a chromanoxyl radical that can be recycled (2, 4).

In its antioxidant actions, carotenoids are converted to a variety of products, including peroxides, epoxides, alcohols, ketones, cleavage products (such as the β-apocarotenals) and ill-defined polymers (I7–I9). Interestingly, ketocarotenoids and the xanthophylls, such as astaxanthin, canthaxanthin, and zeaxanthin, are much better antioxidants than β-carotene (I1–I3). Several xanthophylls, such as lutein, zeaxanthin, and cryptoxanthin, are present in significant concentrations in blood plasma and tissues.

**PHYSIOLOGY OF CAROTENOIDS**

Hydrocarbon carotenoids, such as β-carotene, are absorbed moderately well from the intestine, but highly polar carotenoids, like neoxanthin, are poorly absorbed. Absorbed provitamin A carotenoids are cleaved in part in the intestinal mucosa, primarily by oxidative central cleavage, but also by asymmetric cleavage, ultimately to yield retinal, which can be reduced to retinal and then esterified (8). Carotenoids that are not metabolized by the intestinal mucosa are transported in chylomicra via the lymph into the systemic circulation. Carotenoids are taken up by various tissues from chylomicron remnants. In the steady state, carotenoids are mainly present in low-density and high-density lipoproteins (8). Of various tissues, the adrenal gland has the highest concentration, whereas the liver and adipose tissue contain the largest amounts. Various carotenoids tend to be found in similar ratios in tissues and in the plasma (I4, I5).
Specific binding proteins for oxocarotenoids have been identified and characterized in crustacea. In vertebrates, however, specific binding proteins for carotenoids have not been found. Thus, carotenoids seem to associate primarily, if not solely, with lipid-rich entities in body fluids and tissues. Because of their length and inflexibility, hydrocarbon carotenoids in bilayer membranes are probably distributed randomly within the lipid matrix and may even lie flat between the bilayers. Thus, unlike tocopherols and phospholipids, hydrocarbon carotenoids do not seem to be fixed in an orientation perpendicular to the membrane surface. How xanthophylls are distributed in membranes is less clear. Nonetheless, these differences in orientation between most carotenoids and other lipid antioxidants in lipoproteins and membranes must influence their possible interactions and activity.

The concentrations and patterns of carotenoids in plasma and tissues reflect dietary intake. When the amounts and types of carotenoids in the diet are significantly changed, a new steady state is reached only after several weeks. The mechanisms underlying the distribution of carotenoids between tissues and the plasma are not yet defined.

EPIDEMIOLOGIC ASSOCIATIONS

Since the mid-1960s, the possible beneficial actions of retinoids, including vitamin A, in reducing the risks of developing cancer of several tissues, and particularly that of the lung, have been voiced. In 1975, a dietary vitamin A index, based on the estimated average daily intake of vitamin A from all dietary sources, was defined (26). In 1981, however, the risk of lung cancer in a 19-year prospective study was shown to be inversely associated with the ingestion of dietary carotenoids but not of preformed vitamin A (27). Most subsequent epidemiological studies have shown similar inverse relationships between carotenoid intake and lung cancer risk, but little or no relationship with preformed vitamin A intake (28–31).

Because these associations are based on the intake of foods rather than of specific components of the diet, any protective effect may relate to a mixture of components, e.g. carotenoids, vitamin E, vitamin C, and fiber, rather than to any single component (28–31). Furthermore, increased intake of fruits and vegetables is usually accompanied by a decrease in the intake of animal products, a relationship that has not always been amply explored. Finally, the importance both of lifestyle, which includes diet, stress, smoking, medical attention, and exercise, and of genetics have not been fully explored relative to their relationships with cancer risk. Thus, the nature of the protective effect of dietary carotenoids per se against cancer risk is not clear.

Although most attention has been focused on \(\beta\)-carotene as a readily available and major dietary component, the relative activities of various carotenoids in a biological context have not been well defined. Thus, although \(\beta\)-carotene has become a reference carotenoid for such potential actions, other
carotenoids should also be considered. Indeed, different carotenoids are absorbed at different rates and metabolized differently (32). Generalizations about carotenoids, therefore, should be presented with due caution.

INTERVENTIONS

β-Carotene and canthaxanthin have been effectively used in treating erythropoietic protoporphyria (33). Although retinoids are often more effective, carotenoids do protect animals against UV-induced and many chemically-induced tumors (34). β-Carotene, administered with or without vitamins C and E, also induced the regression of oral leukoplakia in a significant number of human subjects (35). On the other hand, β-carotene supplements in vivo did not protect human low-density lipoproteins from copper-mediated oxidation in vitro (36) nor from the development of skin cancer in humans (37). Thus, the chemopreventive action of carotenoids, although most closely related to their antioxidant properties (38), seems to be highly selective. Further studies on the activity of different carotenoids against peroxidative stress in model systems and in various chronic diseases, as well as clarification of the role of metabolism in their actions, are well warranted.

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