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Antioxidant activity of carotenoids

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Abstract

Carotenoids are pigments which play a major role in the protection of plants against photooxidative processes. They are efficient antioxidants scavenging singlet molecular oxygen and peroxyl radicals. In the human organism, carotenoids are part of the antioxidant defense system. They interact synergistically with other antioxidants; mixtures of carotenoids are more effective than single compounds. According to their structure most carotenoids exhibit absorption maxima at around 450 nm. Filtering of blue light has been proposed as a mechanism protecting the macula lutea against photooxidative damage. There is increasing evidence from human studies that carotenoids protect the skin against photooxidative damage.

1. Introduction

Carotenoids are among the most common natural pigments, and more than 600 different compounds have been characterized until now, with β-carotene as the most prominent (Olson and Krinsky, 1995). Carotenoids are responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish, and crustaceans. Only plants, bacteria, fungi, and algae can synthesize carotenoids, but many animals incorporate them from their diet. Carotenoids serve as antioxidants in animals, and the socalled provitamin A carotenoids are used as a source for vitamin A. Carotenoids attracted attention, because a number of epidemiological studies have revealed that an increased consumption of a diet rich in carotenoids is correlated with a diminished risk for several degenerative disorders, including various types of cancer, cardiovascular or ophthalmological diseases (Mayne, 1996). The preventive effects have been associated with their antioxidant activity, protecting cells and tissues from oxidative damage (Sies and

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Stahl, 1995). Carotenoids also influence cellular signaling and may trigger redox-sensitive regulatory pathways (Stahl et al., 2002).

2. Structures of carotenoids

The unique structure of carotenoids determines their potential biological functions and actions (Britton, 1995). Most carotenoids can be derived from a 40-carbon basal structure, which includes a system of conjugated double bonds. The central chain may carry cyclic end-groups which can be substituted with oxygen-containing functional groups. Based on their composition, carotenoids are divided in two classes, carotenes containing only carbon and hydrogen atoms, and oxocarotenoids (xanthophylls) which carry at least one oxygen atom.

The pattern of conjugated double bonds in the polyene backbone of carotenoids determines their light absorbing properties and influences the antioxidant activity of carotenoids. According to the number of double bonds, several *cis/trans* (E/Z) configurations are possible for a given molecule. Carotenoids tend to isomerize and form a mixture of mono- and poly-*cis*-isomers in addition to the all-*trans* form. Generally, the all-*trans* form is predominant in nature.

Carotenoids are lipophilic molecules which tend to accumulate in lipophilic compartments like membranes or lipoproteins. The lipophilicity of these compounds also influences their absorption, transport and excretion in the organism (Stahl et al., 1993).

3. Antioxidant activity—singlet oxygen quenching, peroxyl radical scavenging

As an attribute to aerobic life the human organism is exposed to a variety of different prooxidants capable to damage biologically relevant molecules, such as DNA, proteins, carbohydrates, and lipids (Sies, 1986; Halliwell, 1996). Among the various defense strategies, carotenoids are most likely involved in the scavenging of two of the reactive oxygen species, singlet molecular oxygen ($^1O_2$), and peroxyl radicals. Further, they are effective deactivators of electronically excited sensitizer molecules which are involved in the generation of radicals and singlet oxygen (Truscott, 1990; Young and Lowe, 2001).

The interaction of carotenoids with $^1O_2$ depends largely on physical quenching which involves direct energy transfer between both molecules. The energy of singlet molecular oxygen is transferred to the carotenoid molecule to yield ground state oxygen and a triplet excited carotene. Instead of further chemical reactions, the carotenoid returns to ground state dissipating its energy by interaction with the surrounding solvent. In contrast to physical quenching, chemical reactions between the excited oxygen and carotenoids is of minor importance, contributing less than 0.05% to the total quenching rate. Since the carotenoids remain intact during physical quenching of $^1O_2$ or excited sensitizers, they can be reused several fold in such quenching cycles. Among the various carotenoids, xanthophylls as well as carotenes proved to be efficient quenchers of singlet oxygen interacting with reaction rates that approach diffusion control (Foote and Denny, 1968; Baltschun et al., 1997; Conn et al., 1991; Di Mascio et al., 1989).

The efficacy of carotenoids for physical quenching is related to the number of conjugated double bonds present in the molecule which determines their lowest triplet energy level. β-Carotene and structurally related carotenoids have triplet energy levels close to that of $^1O_2$ enabling energy transfer. In addition to β-carotene, also zeaxanthin, cryptoxanthin, and α-carotene, all of which are detected in human serum and tissues, belong to the group of highly active quenchers of $^1O_2$. The most efficient carotenoid is the open ring carotenoid lycopene, which contributes up to 30% to total carotenoids in humans (Di Mascio et al., 1989).

For clinical use, β-carotene is applied to ameliorate the secondary effects of the hereditary photosensitivity disorder erythropoietic protoporphyria (Mathews-Roth, 1993). It is suggested that the carotenoid intercepts the reaction sequence that leads to the formation of singlet oxygen; the latter is thought to be the damaging agent responsible for the skin lesions observed in this disease.

Among the various radicals which are formed under oxidative conditions in the organism, carotenoids most efficiently react with peroxyl radicals. They are generated in the process of lipid peroxidation, and scavenging of this species interrupts the reaction sequence which finally leads to damage in lipophilic compartments. Due to their lipophilicity and specific property to scavenge peroxyl radicals, carotenoids are thought to play an important role in the protection of cellular membranes and lipoproteins against oxidative damage (Sies and Stahl, 1995). The antioxidant activity of carotenoids regarding the deactivation of peroxyl radicals likely depends on the formation of radical adducts forming a resonance stabilized carbon-centered radical.

A variety of products have been detected subsequent to oxidation of carotenoids, including carotenoid epoxides and apo-carotenoids of different chain length (Kennedy and Liebler, 1991). It should be noted that these compounds might possess biological activities and interfere with signaling pathways when present in unphysiologically high amounts (Wang and Russell, 1999).

The antioxidant activity of carotenoids depends on the oxygen tension present in the system (Burton and Ingold, 1984; Palozza, 1998). At low partial pressures of oxygen such as those found in most tissues under physiological conditions, β-carotene was found to inhibit the oxidation. In contrast, the initial antioxidant activity of β-carotene is followed by a prooxidant action at high oxygen tension. It has been suggested that prooxidant effects of β-carotene may be related to adverse effects observed under the supplementation of high doses of β-carotene.

4. Cooperative effects of carotenoids with other antioxidants

The antioxidant defense system of the organism is a complex network and comprises several enzymatic and non-enzymatic antioxidants (Sies, 1993). It has been suggested that interactions between structurally different compounds with variable antioxidant activity provides additional protection against increased oxidative stress. Vitamin C, for instance, the most powerful water-soluble antioxidant in human blood plasma, acts as a regenerator for vitamin E in lipid systems (Niki et al., 1995). β-Carotene might also play a role in such radical transfer chains (Truscott, 1996; Böhm et al., 1997). There is evidence from in vitro studies, that β-carotene regenerates tocopherol from the tocopheroxyl radical. The resulting carotenoid radical cation may subsequently be repaired by vitamin C. Synergistic interactions against UVA-induced photooxidative stress have been observed in cultured human fibroblasts when combinations of antioxidants were applied with β-carotene as main component (Böhm et al., 1998a,b). In comparison to the individual antioxidants, vitamins E, C and β-carotene exhibited cooperative synergistic effects scavenging reactive nitrogen species (Böhm et al., 1998a,b). The cooperative interaction between β-carotene and α-tocopherol was also examined in a membrane model (Palozza and Krinsky, 1992). A combination of both lipophilic antioxidants resulted in an inhibition of lipid peroxidation significantly greater than the sum of the individual inhibitions. Antioxidant activity of carotenoid mixtures was assayed in multilamellar liposomes, measuring the inhibition of the formation of thiobarbituric acid-reactive substances (Stahl et al., 1998). Mixtures were more effective than single compounds, and the synergistic effect was most pronounced when lycopene or lutein was present. The superior protection of mixtures may be related to specific positioning of different carotenoids in membranes.

5. Photoprotection in humans

In biological systems, light exposure leads to the formation of reactive oxygen species which are damaging to biomolecules and affect the integrity and stability of subcellular structures, cells and tissues (Stahl and Sies, 2001; Krutmann, 2000). Photooxidative processes play a role in the pathobiochemistry of several diseases of light-exposed tissues, the eye and the skin.

Age-related macular degeneration is a major cause for irreversible blindness among the elderly in the Western world and affects the macula lutea (yellow spot) of the retina, the area of maximal visual acuity (Landrum and Bone, 2001). Lutein and zeaxanthin are the pigments responsible for coloration of this tissue; other carotenoids such as lycopene, α-carotene or β-carotene are not found in the macula lutea. Epidemiological data support the concept that the macular pigment has a protective role (Beatty et al., 2001). Protection against photooxidative processes has been related to the antioxidant activities of the macular carotenoids and/or their light filtering effects.

The efficacy of carotenoids to filter blue light was investigated in unilamellar liposomes (Junghans et al., 2001). Liposomes were loaded in the hydrophilic core space with a fluorescent dye, excitable by blue light, and various carotenoids were incorporated into the lipophilic membrane. The fluorescence emission in carotenoid-containing liposomes was lower than in controls when exposed to blue light, indicating a filter effect. In this model, lutein and zeaxanthin showed a better filtering efficacy than β-carotene or lycopene. It was suggested that the more prominent efficacy of lutein and zeaxanthin is due to differences in the location of the incorporated molecules within the liposomal membrane. Such differences may also be a reason why lutein and zeaxanthin can be incorporated into membranes in higher amounts than other carotenoids like β-carotene or lycopene.

When skin is exposed to UV light, erythema is observed as an initial reaction. There is evidence from in vitro and in vivo studies that β-carotene prevents photooxidative damage and protects against sunburn (erythema solare) (Stahl and Sies, 2001). When β-carotene was applied alone or in combination with α-tocopherol for 12 weeks, erythema formation induced with a solar light simulator was significantly diminished from week 8 on (Stahl et al., 2000). Such protective effects were also achieved with a dietary intervention (Stahl et al., 2001): ingestion of tomato paste, corresponding to a dose of 16 mg lycopene/day over 10 weeks, led to increases in serum levels of lycopene and total carotenoids in skin. Erythema formation was significantly lower in the group that took tomato paste as compared to the control. Thus, protection against UV-light-induced erythema can be achieved by modulation of the diet.

6. Conclusion

Carotenoids are efficient antioxidants protecting plants against oxidative damage. They are also part of the antioxidant defense system in animals and humans. Due to their unique structure it can be suggested that they possess specific tasks in the antioxidant network such as protecting lipophilic compartments or scavenging reactive species generated in photooxidative processes. They may further act as light filters and prevent oxidative stress by diminishing light exposure. The possible role of carotenoids as prooxidants and the implication of their prooxidant activity in adverse reactions remains to be elucidated.

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