Stability and Synergistic Effect of Antioxidative Properties of Lycopene and Other Active Components

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INTRODUCTION

Carotenoids have drawn considerable interest, due to their role in preventing cardiovascular disease. In a large prospective study of female nurses, a 26% and 20% lower risk of coronary artery disease was observed among the subjects in the higher quintile of \( \beta \)-carotene and \( \alpha \)-carotene intake over a 12 year, follow-up period, respectively, compared with those in the lower quintile (Osganian et al., 2003). In vitro studies have indicated that carotenoids may prevent the initiating events in atherogenesis by directly inhibiting low density lipoprotein (LDL) oxidation, thereby decreasing the early formation of foam cells and plaque (Diaz et al., 1997; Maxwell and Lip, 1997; Naruszewicz et al., 1992; van de Vijver et al., 1997). The incubation of the \( \beta \)-carotene isomers with whole plasma resulted in substantial carotene binding to very low density lipoprotein (VLDL) and to LDL, although limited binding was observed in high density lipoprotein (HDL). Lipid peroxidation of VLDL and LDL was significantly decreased by \( \beta \)-carotene. In addition, the incubation of \( \beta \)-carotene also decreased macrophages mediated cellular degradation of oxidized lipoproteins by 18% (Lavy et al., 1993). The oxidatively modified form, but not native LDL, is preferably recognized by the receptor and could, therefore, be taken up by tissue macrophages to induce the fatty-streak lesion of atherosclerosis.

More than 600 carotenoids have been found in plants and microorganisms, and many of these carotenoids have been identified in human blood and tissues. The chemical and biological function of carotenoids is determined by their characteristic structure.

Lycopene is a member of the carotenoid family, and it is the naturally occurring compound that gives the characteristic red color to the tomato, watermelon, pink grapefruit, orange, and apricot. Carotenoids have important roles in photosynthesis. For example, they protect the photosynthetic system from photo-oxidative damage by quenching chlorophyll triplet states and the singlet oxygen generated, they also function as light-harvesting pigments in the antenna system of the photosynthetic organism. In addition to its function as a pigment, lycopene also is strong antioxidant, able to neutralize free radicals generated from oxygen.

The biological function of lycopene is determined by its structure. Lycopene is a lineal acyclic isomer of \( \beta \)-carotene. It contains 11 conjugated double bonds in the center part of the molecule and 2 unconjugated double bonds at each end (Figure 1). It is the longest carotenoid in the family. Since lycopene doesn’t contain \( \beta \)-ionone ring structure at the end of the molecule, it cannot be converted to vitamin A. Lycopene in raw
tomatoes is generally present as the all-trans geometric isomer, the most thermodynamically stable form. However, light, heat, or chemical reaction may introduce kink along the conjugated double bonds and transform all-trans lycopene into various cis-isomers.

Lycopene is the most abundant carotenoid in human plasma, which may explain its more important roles in the human body, compared with other carotenoids, such as β-carotene and lutein. In human serum, lycopene is generally bound to low-density lipoprotein and transported to various tissue sites, such as the liver, adrenal, testes, and prostate.

Cis-isomers of lycopene have altered physical and chemical properties compared with the all-trans lycopene. Absorption spectroscopy of cis-lycopene is characterized by a so-called “cis-peak” appearing at shorter wavelength than the primary visible absorption band of all-trans lycopene. Trans-cis isomerization also produces a slight decrease in the molar extinction coefficient of the primary peaks and a minor blue shift of the primary peaks to shorter wavelengths, which is referred to as a hypochromic effect, as a result of reversible spatial rearrangements of the chromophoric double bonds (Zechmeister, 1962). For example, the cis-peak for 9-cis-lycopene occurs at approximately 360 nm, and the primary visible absorption band changes from 444, 470, 502 nm for all-trans-lycopene to 438, 464, 495 nm. In general, the appearance of cis-peak is attributed to the lowered asymmetry of the molecule (Frank, 1993). Usually, each cis double bond can introduce 2–5 nm blue shifts of the primary peaks.

The lycopene metabolites in the human body are not yet clearly understood. The major metabolite of lycopene identified in human plasma is 5,6-dihydroxy-5,6-dihydrolycopene, probably due to the oxidation of lycopene via conversion from intermediate lycopene epoxides (Khachik et al., 1995).

**BIOAVAILABILITY AND STABILITY OF LYCOPENE**

There is mounting evidence to support the belief in the health benefits derived from lycopene and other carotenoids in the tomato. This belief is based on their apparent ability to provide protection against various cancers and many other chronic diseases (Agarwal and Rao 2000a, b). An important consideration in determining the efficacy of carotenoids is their bioavailability. However, the mechanism responsible for their intestinal absorption is not completely understood.

In humans, lycopene absorption is low, with only 10 to 30% of the consumed lycopene being absorbed, and the rest being excreted. Absorption of lycopene from fresh tomatoes and unheated tomato juice is less compared with processed tomato product such as tomato paste and tomato sauce (Stahl and Sies, 1992; van het Hof et al., 2000a; Gartner et al., 1997). In the pericarp tissue of ripe tomato fruits, lycopene is localized in the chromoplasts, in the form of fine crystals, which are associated with the membrane structures (Bouvier et al., 1998). Crystalline formation of lycopene is a major factor influencing the accessibility of lycopene and, hence, its bioavailability. Lycopene absorption was found to be apparently more efficient at low dosages than at higher dosages (Erdman et al., 1993; Stahl and Sies, 1992), possibly due to the low potential to form crystals at low dosages.

Absorption of lycopene also is influenced by many other factors, such as dietary lipid, fiber, presence of vitamins, minerals, and other carotenoids (Erdman et al., 1988). Since lycopene is highly hydrophobic, it is easily dissolved in oil. It is apparent that the presence of fat in the intestine will promote the formation of micelle; therefore, including fat in the diet has the potential to improve the absorption of lycopene and other carotenoids. It was reported that the consumption of fat, together with tomato, increased the absorption of lycopene. It seems that dietary fat might facilitate bile flow from the gall bladder (Prince et al., 1993; Erdman et al., 1993), which is necessary for the formation of micelles and incorporation of fat soluble lycopene into micelles in the intestinal lumen. Jayarajan et al. indicated that 5 g of fat in a meal significantly enhanced carotenoid uptake (Jayarajan et al., 1980). The absorption of lycopene also was influenced by the type of oil present in the diet. Clark et al. reported that the average recovery of lycopene was 6% from olive oil emulsions, but only 2.5% when infused with corn oil (Clark et al., 2000). However, the presence of different fibers in the diet decreased the bioavailability of carotenoids (Riedl et al., 1999), probably due to interference of fiber with micelle formation (Erdman et al., 1986). Lycopene-rich tomato extracts in the form of oleoresin or tablets are commercially available; however, the lycopene absorption from these forms is not satisfactory in human tissues, limiting their use for humans (Paetau et al., 1999). Richelle and colleagues recently demonstrated that when lycopene is embedding into whey proteins, its contents in both plasma and body tissues are significantly enhanced (Richelle et al., 2002).

After ingestion, lycopene, like many other carotenoids, is generally incorporated into mixed micelles, due to their lipophilic property, and are absorbed by intestinal epithelial cell via passive diffusion. Carotenoids are further transported in chylomicrons to the lymphatic system or portal circulation. Carotenoids accumulate in the liver, where they are packaged and released into the circulation with VLDL particles. In blood plasma, carotenoids are mainly associated with LDL, which transports the carotenoids to various tissue sites, such as adrenal, prostate, lung, kidney, pancreas, and ovary (Schmitz et al., 1993; Erdman et al., 1993). Different tissues contain various levels of specific carotenoids, which may suggest specific biological functions of different carotenoids in some tissues but not in others.

Bierer and colleagues examined the absorption and transport of lycopene and some other carotenoids in preruminant calves. Single 20-mg oral dose of various carotenoids was added to the diet. The serum peak of lutein and canthaxanthin appeared earlier than lycopene and β-carotene; their clear rate from serum...
also was quicker compared with that of lycopene and β-carotene (Bierer et al., 1995). The half-life of lycopene is 7- to 14-d in human blood, and the consumption of a lycopene-free diet for 1 wk is accompanied by significantly lower plasma lycopene concentrations (Allen et al., 2002; Hadley et al., 2003; Schwedhelm et al., 2003).

In food processing operations, tomatoes undergo homogenization, extraction of juice, and steam sterilization. Lycopene appears to be stable during these processing steps. Agarwal and colleagues (Agarwal et al., 2001) also monitored the lycopene content during a series of stages of processing. Lycopene concentration was relatively stable during the process, except for the initial loss of 15.89% from fresh tomatoes to the scalded pulp. The storage of canned tomato juice samples at 4°C, 25°C, and 37°C for up to 12 m showed no effect on lycopene content. Although total lycopene content was not altered by heating treatment in the presence of oil, cis-isomer of lycopene was increased from 10% to 30%, and remained unchanged for at least 4 m of storage at 4°C (Agarwal et al., 2001).

Processed fruits and vegetables are usually considered less valuable than the fresh ones, because of the loss of nutritional components. However, bioavailability of lycopene was improved in processed tomato products. Lycopene in tomatoes are found in association with protein complex or membrane structure, which may prevent lycopene digestion and absorption. Harsh treatments during food processing, such as mechanical texture disruption and steam, may denature the lycopene-protein complex and release lycopene from the cellular matrix (Gartner et al., 1997; van het Hof et al., 2000b; Hadley et al., 2003; Boileau et al., 1999).

Lycopene is the predominant carotenoid present in tomatoes. Generally, all carotenoids are highly susceptible to light, heat, and oxygen. Different processing conditions for tomatoes may influence the stability and bioavailability of lycopene and other carotenoids to various degrees. Sharma and Le Maguer studied the kinetics of lycopene degradation during the heating of tomato pulp at 100°C under various conditions (Sharma and Le Maguer, 1996). They reported a pseudo first-order reaction of lycopene degradation; the lycopene degradation rate was higher for the concentrated sample than for the nonconcentrated sample, suggesting possible interaction with the acids and sugars in the tomato pulps. Moreover, the apparent degradation rate was higher in freeze-dried, fiber-rich tomato pulp compared with oven-dried samples; exposure to air, light, and high storage temperatures increased lycopene loss.

Interaction of various carotenoids also may affect the absorption of individual carotenoids (van den Berg, 1999). Carotenoid interactions during absorption and in postabsorptive metabolism have been investigated in both human and animal studies. For example, Kostic et al. (1995) reported that when co-ingested in the same dose, β-carotene significantly reduced the plasma concentration of lutein. The results of White et al. (1994) indicated that β-carotene inhibited the absorption of canthaxanthin, but canthaxanthin did not show the same inhibition on the absorption of β-carotene. The interaction of various carotenoids may be attributed to the competition of different carotenoids at the level of incorporation into micelle, uptake by intestine cells, incorporation into chylomicrons, or binding and transport by LDL, since they differ only slightly in structure (Tyssandier et al., 2002). Johnson and colleagues have investigated the serum response to a single oral dose of lycopene and β-carotene, alone or in combination, on the basis of 24-h areas under the curves (AUC) (Johnson et al., 1997). They found that when administered alone, the peak concentration time of lycopene is significantly earlier than that of β-carotene. Suggesting that after enterocyte uptake, the release of β-carotene is much slower than lycopene. This phenomenon is attributed to the faster uptake and release of lycopene, as well as the presence of the enzyme that binds β-carotene and delays its release from enterocyte. The authors also observed that simultaneous oral administration of 60 mg of β-carotene and 60 mg of lycopene significantly enhance lycopene serum concentration over the 24-h period, however, the serum concentration of β-carotene was not affected by the presence of lycopene. This observation suggested that serum response of lycopene may be improved in the presence of β-carotene. It was suggested that absorption of lycopene is affected by the presence and content of other carotenoids, and there may be independent pathways for β-carotene and lycopene absorption; the pathway for lycopene is subjected to mediation by β-carotene, and lycopene is absorbed to the same extent as the β-carotene when these two carotenoids are consumed together. Another explanation for the increased lycopene content in the presence of β-carotene is that β-carotene may enhance the solubility of lycopene and, therefore, increase the accessibility of lycopene. Other authors reported lower serum and tissue β-carotene content when it was concurrently absorbed with lycopene and canthaxanthin in ferrets (White et al., 1993).

Wahlqvist and colleagues have investigated the long term effect of β-carotene supplementation on the serum content changes of other major carotenoids with administration of 20 mg β-carotene/d or placebo over 24 mo (1994). Their result showed enhanced concentrations of lycopene (176% in men) and α-carotene (211% in men and 166% in women) after body mass index, baseline concentration, change in respective carotenoid intake, and other confounding factors were taken into account. These results suggest a biological interaction between β-carotene and lycopene, as well as between β-carotene and α-carotene.

The above findings are in contrast with the observation of Prince et al., who reported that a high dose of β-carotene (300 mg/d for 21 d) decreased the serum lycopene amount (Prince et al., 1991). Since both β-carotene and lycopene are hydrophilic, they are absorbed through incorporation micelles. Reduced serum lycopene concentration may be attributed to the competition of these two carotenoids in absorption (Borel et al., 1996). The different observations of the above two groups may be due to the different dosage of β-carotene in supplementation, since carotenoid absorption decreases with an increased dosage (Brown et al., 1989; Stahl and Sies, 1992).

The interaction between lipophilic lycopene and hydrophilic lutein was investigated by Tyssandier et al. (2002). The authors
analyzed both the chylomicron incorporation and plasma content of lycopene and lutein when supplemented alone or together. It was shown that after a single co-ingestion, lutein diminished the content of lycopene in the chylomicron fraction. Lycopene displayed the same effect on the lutein content in the chylomicron fraction. It was possible that lutein and lycopene compete for the uptake and metabolize in the enterocyte or incorporation into chylomicrons. However, plasma response after 3 wk consumption of lycopene and lutein together contrasted with the observations in chylomicrons. The plasma-lycopene concentration increased by 92% after simultaneous intake of the tomato puree and lutein pills, compared with 16% increase of lycopene concentration after intake of tomato puree alone. The authors speculated that a possible explanation for the observed discrepancy obtained in chylomicrons and in plasma was that the presence of lutein induced an antioxidant-sparing effect, resulting in an enhanced content of lycopene in plasma. Their results also indicated that lycopene, lutein, and \( \beta \)-carotene did not affect the plasma concentrations of zeaxanthin, \( \alpha \)-carotene, and \( \beta \)-cryptoxanthin.

Lycopene exists in multiple isomeric forms. The all-trans form of lycopene is the most predominant geometric form present in fresh tomatoes and tomatoes products, comprising 79–91% of the total lycopene in raw tomatoes (Richelle et al., 2002). However, the predominant form of lycopene found in human serum was the cis-lycopene isomers. Moreover, cis-lycopene in some tissues, such as prostate, is particularly high, comprising more than 80% of the total lycopene (Clinton et al., 1996). It is interesting to note that the consumption of lycopene-low products for 1 wk resulted in a significant increase of cis-lycopene in human plasma, which is accompanied by a significant decrease in plasma all-trans-lycopene (Hadley et al., 2003). This observation suggested a possible biological conversion of trans-lycopene isomer to 5-cis-lycopene (Holloway et al., 2000). The mechanism of lycopene trans-cis conversion and the physiological importance of cis-lycopene are not yet elucidated.

Another important feature affecting lycopene bioavailability is its geometric configuration. Cis-lycopene exhibits a lower tendency to aggregate and, hence, to form crystals, so the lycopene crystal particle size is minimized; its absorption is enhanced (Britton, 1995). In addition, the solubility of cis-isomers is higher in the lipophilic phase; they are more efficiently incorporated into micelles and pass across the intestinal barrier (Böhm and Bitsch, 1999). Harsh thermal treatment during food processing converts lycopene from the all-trans configuration into the cis configuration, which is more easily absorbed by the human body. More studies are needed to understand the trans-to cis-isomer conversion of lycopene and to study their specific biological importance in human health.

**ANTIOXIDANT PROPERTIES OF LYCOPENE**

Free radical-mediated chain oxidative damage to proteins, DNA, and lipids in the human body is one of the major factors in the development of carcinogenesis, atherosclerosis, heart diseases, inflammation, cancer, and many other pathological changes in humans and animals (Cross et al., 1987; Frei et al., 1991; Kehrer, 1993; Diplock et al., 1998; Tsai et al., 1998). Several lipid peroxidation products were identified as cytotoxic and genotoxic and were shown to play an important role in the etiology of several chronic diseases (Boyd et al., 1991; Tsimikas et al., 2003). Oxidation of LDL is responsible for the increased presence of activated macrophages and is believed to contribute to the development of atherosclerosis and endometriosis (Santanam et al., 2002). Oxidative stress has been shown to induce lipid and protein oxidation in the cell membrane, and to cause DNA damage in lymphocytes (Yen et al., 2003). DNA damage caused by oxidative stress also is involved in the etiopathogenesis of Parkinson’s disease (Sharma et al., 2003).

Free radicals may be from exogenous sources, such as air pollutants, radiation, chemicals, environmental toxins, and deep fried food, as well as physical stress. A number of free radicals, including hydroxyl radicals (·OH), superoxide anions (O·2−), and nitric oxide (NO), as well as other active oxidant, such as hydrogen peroxide (H2O2), also are continuously generated by normal cellular metabolism and during acute or chronic immune responses (Ames et al., 1993; Lledias et al., 1999; Gate et al., 1999). Low levels of free radicals may be beneficial in intracellular signal transduction and defense against microorganisms (Sundaresan et al., 1995). Oxidative stress arises when there is an imbalance between the production and removal of reactive oxygen species. This imbalance may cause severe cellular malfunctions and contribute to the aging process and chronic diseases, such as cancer and coronary heart disease (Ames and Shigenaga, 1992; Benzie, 2000). The human body contains a wide range of endogenous cellular defense mechanisms, such as glutathione peroxidase, catalase, and superoxide dismutase, to prevent damage by oxidative stress (Lee et al., 2001). However, these endogenous defense mechanisms are insufficient or inefficient to protect the body from certain kind of oxygen species, such as singlet oxygen. In this case, food-based antioxidants are important to scavenge free radicals to maintain human health. Antioxidants scavenge radicals from the system either by reacting with them to yield stable and harmless products or by disrupting free radical chain reactions.

Carotenoids are one of the major groups of antioxidants found in fruits and vegetables. Currently, much work has been directed at characterizing the antioxidant capacity of carotenoids. The polyene chain is the structural feature that determines the chemical reactivity of carotenoids toward free radicals, and hence, its antioxidant properties. A number of studies have indicated the health benefits of consuming lycopene, a carotenoid antioxidant, found in tomato and tomato products. The consumption of lycopene-rich foods, such as tomato paste, chili sauce, and spaghetti sauce, has been demonstrated to prevent the occurrence of a number of chronic diseases. DNA damage was protected by lycopene supplementation, implying its role in lowering the risk of cancer (Agarwal et al., 2001).
Carotenoids, such as lycopene, are highly lipophilic and are generally incorporated within cell membrane or associated with lipoproteins. It is, therefore, believed that the interaction of carotenoids with free radicals is greater; therefore, antioxidant activity of carotenoids to scavenge free radicals is higher in a hydrophobic environment (Tsuchihashi et al., 1995). It appears that the localization of carotenoids in the lipophilic component of the cell provides a greater resistance of lipid and lipoproteins to oxidative damage (Clevidence and Bieri, 1993; Ribaya-Mercado et al., 1995a). The activities of antioxidants in plasma depend on the structure and hydrophobicity of antioxidants; therefore, their orientation in membrane lipid bilayer, and the localization of attacking radical species (Woodall et al., 1997a; Massaeli et al., 1999). Amphiphilic antioxidants, such as zeaxanthin (Sujak et al., 1999), span the lipid bilayer of the membrane, with the polar end groups interacted with the polar component of the membrane. α-tocopherol is located in the interface between the aqueous and lipid compartments at the surface of lipoprotein. The lipophilic antioxidants, such as β-carotene and lycopene, embed themselves entirely within the nonpolar inner-environment of the membrane and exhibit limited mobility. Ascorbic acid and uric acid are water-soluble antioxidants.

Tsuchihashi et al. reported that β-carotene inhibited free-radical-mediated oxidation of methyl linoleate oxidation in benzene solution. The antioxidant activity of β-carotene, however, was much lower compared to α-tocopherol. When both β-carotene and α-tocopherol were added to a homogeneous solution and the radicals were formed in the aqueous environment, degradation of α-tocopherol was faster than that of β-carotene. However, when both β-carotene and α-tocopherol were incorporated into dimyristoyl phosphatidylcholine liposomal membrane bilayer, and the radicals were generated within the hydrophobic membrane environment, β-carotene degraded faster than α-tocopherol. The authors speculated that β-carotene and α-tocopherol competed for the reactive oxygen species in a homogenous solution, and α-tocopherol protected β-carotene from degradation (Tsuchihashi et al., 1995). Aldini and colleagues have introduced a selective fluorescence method to distinguish the lipophilic and hydrophilic components of the total antioxidant capacity of plasma using fluorescent probes, 2′,7′-dichlorodihydrofluorescein (DCFH) and C11BODIPY581/591 (BOD-IPY), as the markers of the oxidation reaction in aqueous and lipid compartments of plasma, respectively (Aldini et al., 2001). They reported that when the radicals were generated in the lipid compartment, β-carotene and α-tocopherol, which were hydrophobic, were degraded faster than ascorbic acid and uric acid; when the radical species were generated in the aqueous environment of the plasma, the order of consumption was ascorbic acid > α-tocopherol > uric acid > β-carotene, corresponding to the decreasing order of hydrophobicity. Further more, even when the free radicals were generated in the lipid phase of the plasma, ascorbic acids, a hydrophilic antioxidant, were rapidly degraded, indicating that there was an active interaction between ascorbic acid and hydrophilic radicals or hydrophilic antioxidants. In another experiment in the same lab, using MeO-AMVN as the lipophilic radical generator, they found similar results (Yeum et al., 2003). Several studies have found that ascorbic acid is a relatively strong water-soluble antioxidant and may regenerate the carotenoids radical cations, suggesting a direct interaction of carotenoid radical cations with ascorbic acid, even though the parent carotenoid is located in the hydrophobic membrane environment (Burke et al., 2001a; Mortensen et al., 2001). The regeneration of β-carotene by ascorbic acid and α-tocopherol and regeneration of α-tocopherol by green tea polyphenols also have been observed and addressed by other authors (Yeum et al., 2000). Therefore, when evaluating the antioxidant properties of antioxidant nutrients, the active interactions between the individual antioxidant should be included in the consideration.

The ability of antioxidants to prevent lipid peroxidation is apparently dependent on the concentration of antioxidant (Stahl et al., 1998). For example, β-carotene antioxidant activity was reduced at a concentration of 160 μM/g compared to a lower concentration of 80 μM/g, probably due to epoxide formation and β-carotene peroxy radical formation that enhanced the chain oxidation (Anguelovea and Warthesen, 2000). Park et al. reported that, at lower concentrations of dietary lutein supplementation, tumor lipid peroxidation activity was suppressed; however, higher dietary levels of lutein resulted in increased lipid peroxidation activity (Park et al., 1998). These observations may be caused by the pro-oxidation of lutein at high concentrations. Lowe and colleagues have investigated the inhibitory effect of lycopene on oxidative DNA damage in HT29 cells, as measured by comet formation (Lowe et al., 1999). Oxidative DNA damage caused by xanthine/xanthine oxidase was protected by relatively a low concentration of lycopene (1–3 μM). But lycopene at higher concentration (4–10 μM) promoted the oxidative damage toward DNA.

Oxidation reactions of carotenoids with free radicals may occur at different parts of the molecule involving cleavage of the polyene chain by addition of peroxyl radicals, electron capture by the molecule, and hydrogen abstraction from allylic positions (Burton et al., 1984; Packer et al., 1981; Woodall et al., 1997a). Moreover, the oxidation reaction was influenced by oxygen concentration. At low oxygen concentrations, carotenoids act as effective antioxidants to prevent the oxidation of lipids, while at high oxygen concentrations, carotenoids apparently enhance oxidation (Burton et al., 1984; Liebler, 1993; Palozza et al., 1997).

With its acrylic structure, large array of conjugated double bonds, and important hydrophobicity, lycopene exhibits a range of unique and distinct biological properties. Of these properties, its antioxidant activities continue to arouse substantial interest. The system of conjugated double bonds allows lycopene molecules to efficiently quench the energy from very deleterious forms of oxygen (singlet oxygen) and to scavenge a large spectrum of free radicals. Of all naturally occurring carotenoids, lycopene is the most efficient quencher of singlet oxygen (Di Mascio et al., 1989; Conn et al., 1991).
The antioxidant activities of lycopene and other carotenoids are related to their abilities to quench singlet oxygen (O$_2^*$) and to trap peroxyl radicals (ROO$^*$) (Foote and Denny, 1968; Burton and Ingold, 1984). Lycopene has been reported to deactivate an array of free radicals, such as hydrogen peroxide, nitrogen dioxide, thyl, and sulphonyl (Bohm et al., 1995; Lu et al., 1995; Mortensen et al., 1997). There are a number of investigations demonstrating that lycopene is a more potent ROS scavenger than many other dietary carotenoids and other antioxidants, including vitamin E, and the rate constant of quenching singlet oxygen for lycopene is almost double that of $\beta$-carotene (Di Mascio et al., 1989; Devasagayam et al., 1992; Levy et al., 1995; Miller et al., 1996). Woodall et al. also reported that among all the tested carotenoids lycopene reacted most efficiently with peroxyl radicals generated by the thermal decomposition of azo-compounds (Woodall et al., 1997b). It is generally believed that an increased number of conjugated double bonds and the opening of the $\beta$-ionone ring increase the quenching ability towards singlet oxygen (Di Mascio et al., 1991).

Lycopene, $\alpha$-carotene, and $\beta$-carotene were studied for their inhibitory effects on hydroperoxide formation during oxidation of methyl linoleate. Lycopene displayed a higher antioxidant effect compared to $\alpha$-carotene and $\beta$-carotene, which is in accordance with the results of increased reactivity of lycopene with singlet oxygen and free radicals (Anguelovea and Warthesen, 2000). Further more, $\beta$-carotene antioxidant activity was reduced at a concentration of 160 $\mu$M/g, compared to the lower concentration of 80 $\mu$M/g, probably due to the peroxide formation and $\beta$-carotene peroxyl radical formation that enhanced the chain oxidation. However, a higher concentration of lycopene showed similar antioxidant activity, as did the lower concentration of lycopene. The degradation rate of lycopene was the fastest among the three tested carotenoids, which was consistent with the results reported by Ribaya-Mercado and colleagues. They showed that UV irradiation caused greater decrease in the concentration of skin lycopene than that of skin $\beta$-carotene (Ribaya-Mercado et al., 1995b).

The antioxidant activity of lycopene also depends on the type of oxidants involved in the oxidation reaction. In a recent study by Yeh and Hu, lycopene activity was examined on lipid peroxidation induced by various oxidants in human foreskin fibroblasts (HS68 cells) (Yeh and Hu, 2000). It was reported that lycopene significantly inhibited lipid peroxidation induced by ferric nitrilotriacetate (Fe$^3+/NAT$) in a concentration-dependant manner, while lipid peroxidation induced by a water-soluble radical generator (2,2'-azobis(2-aminopropane)dihydrochloride) was not significantly changed by lycopene. However, lipid peroxidation induced by a lipid-soluble radical generator (2,2'-azobis[2,4-dimethylvaleronitrile]; AMVN) was enhanced by the presence of lycopene, also in a concentration-dependant manner. A similar phenomenon was observed when $\beta$-carotene was used. The theory of enhanced lipid peroxidation in HS68 cells by lycopene and $\beta$-carotene is not well understood. One possible explanation may be because lycopene and $\beta$-carotene are both extremely hydrophobic, and they react easily with lipid peroxyl radicals and form radical cation. This may explain the quicker and greater loss of lycopene and $\beta$-carotene by the lipid-soluble radical generator than by water-soluble radical generator.

Enhanced blood lipid peroxidation is reported to be associated with gastric cancer. Velmurugan and colleagues have conducted an animal study to examine lycopene antiperoxidation effect on the gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a potent carcinogen producing toxic and highly diffusible reactive oxygen species. Significantly reduced lipid peroxidation in both plasma and erythrocytes was observed in the animal group that was administrated with lycopene and accompanied by enhanced levels of glutathione (GSH) and biotransformation enzymes, such as glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) (Velmurugan et al., 2002). This is consistent with their previous observations that the ingestion of lycopene significantly decreased the lipid peroxides and enhanced the activities of hepatic biotransformation enzymes in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis (Bhuvaneswari et al., 2001; Bhuvaneswari et al., 2002). Based on the above evidences, the authors speculated that lycopene may exert its chemopreventive effects by modulating lipid peroxidation as an antioxidant agent and enhance the activities of the enzymes in the glutathione redox cycle.

Edge and colleagues have reported a pulse radiolysis study to determine the electron-transfer rate constant of various pairs of carotenoids, including astaxanthin, $\gamma$-apo-8'-carotenal, canthaxanthin, lutein, zeaxanthin, $\beta$-carotene, and lycopene. Lycopene was found to be the strongest reducing agent, therefore, the most easily oxidized (Edge et al., 1998). This result was supported by their later study of reduction potentials of carotenoid radical cations, using the one-electron oxidation of carotenoids by amino acid radicals. They measured the equilibrium constants for the one-electron transfer processes between TrpH and carotenoids, and the difference in reduction potential between TrpH$^+$; the carotenoid radical cations were obtained. Their results indicated that the reduction potential of the radical cation of lycopene is lower than that of the other tested carotenoids, implying that more lycopene was oxidized (Burke et al., 2001b).

Lycopene is known to be the most efficient quencher of singlet oxygen and free radicals. Until very recently, very few mechanistic studies have been reported on the radical scavenging properties. The quenching activity of the different carotenoids depends essentially on the number of conjugated double bonds. It is modulated by end groups or the nature of the substituents in the carotenoids that contain cyclic end groups (Foote and Denny, 1968; Stahl et al., 1993). This explains the important differences in the quenching rate constants ($K_q$) of the different carotenoids (Table 1). Lycopene’s superior ability to quench singlet oxygen vs. that of $\gamma$- and $\beta$-carotene is related to the opening of the $\beta$-ionone ring. Miller and colleagues have determined the hierarchy of the antioxidant capacity of various carotenes and xanthophylls in scavenging the ABTS$^{+}$ radical cations, by comparing the mean Trolox equivalent antioxidant capacity (TEAC).
Table 1  Comparison of the antioxidant activities (quenching of singlet oxygen) of different carotenoids

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Rate constant for quenching of singlet oxygen $K_q \times 10^9$ (mol$^{-1}$s$^{-1}$)</th>
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<tbody>
<tr>
<td>Lycopene</td>
<td>31</td>
</tr>
<tr>
<td>γ-Carotene</td>
<td>25</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>19</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>14</td>
</tr>
<tr>
<td>Lutein</td>
<td>8</td>
</tr>
<tr>
<td>Astaxanthin</td>
<td>24</td>
</tr>
<tr>
<td>Bixin</td>
<td>14</td>
</tr>
<tr>
<td>Canthaxanthin</td>
<td>21</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>10</td>
</tr>
</tbody>
</table>

Data adapted from Di Mascio et al. (1989, 1991), Conn et al. (1991, 1992), and Miller et al. (1996).

Lycopene was found to be the most effective antioxidant among all the tested carotenes and xanthophylls, as shown in Table 2. Comparing the molecular structures of lycopene, α-carotene, and β-carotene, the authors speculated that the ability of carotenes to scavenging the ABTS$^+$ radical cations increased with extension of the chromophore and maximum overlap of the double bond molecular orbits. In β-carotene, the steric hinderance between the methyl substituent at C-5 of the ring and the hydrogen atom at C-8 of the chain reduce the planarity of the molecule and the rings; C-5,6 and C-5$'$,6$'$ double bonds twisted out of the plane. In α-carotene, the molecule contains only 9 conjugated double bonds, making it less susceptible to the free radicals. Their results also indicated that the presence of functional groups with increasing polarities in the terminal rings of carotenoids also is associated with more efficient radical scavenging activity. Khachik et al. (1999) studied the dietary carotenoids and their metabolites as potentially useful chemoprotective agents against cancer in an anti-inflammatory study using a mouse ear model.

Little knowledge is available about the mechanism of lycopene antioxidant property and its oxidized products. Reaction of lycopene with hydroxyl radical (·OH) generated from hydrogen peroxide have been found to yield lycopene-1,2-epoxide and lycopene-5,6-epoxide (Lu et al., 1995). Khachik and colleagues have conducted extensive studies of lycopene oxidation with m-chloroperbenzoic acid (MCPBA), followed by acidic hydrolysis. They used $^1$H and $^{13}$C NMR spectroscopy to analyze the oxidation products (1998a). Actually, lycopene 1,2-epoxide and lycopene 5,6-epoxide were found to be the major products. Lycopene 1,2-epoxide is stable, while lycopene 5,6-epoxide is unstable and able to undergo rearrangement to form 2,6-cyclolycopene-1,5-epoxides A and B. Based on these observations, the authors proposed a metabolic pathways in human serum and tissues (Khachik et al., 1998b; Khachik et al., 2002). It was hypothesized that 2,6-cyclolycopene-1,5-epoxides A and B were converted to 2,6-cyclolycopene-1,5-diols A and B by acid or enzyme in human body. 2,6-cyclolycopene-1,5-diols A and B have indeed been identified in human serum and milk. 2,6-cyclolycopene-1,5-diols A and B also were found in tomato and tomato products in trace amount (Khachik et al., 1997) and would not be responsible for the presence of these compounds in human serum and milk. The metabolic pathway of lycopene and lycopene oxidation in humans was proposed by Khachik et al., as shown in Figure 2.

Lycopene also can be converted into peroxyl radicals capable of acting as pro-oxidants and of undergoing auto-oxidation

Table 2  Comparison of trolox equivalent antioxidant capacity (TEAC) values of various carotenoids

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>TEAC value (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotenones</td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>2.9 ± 0.15</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>1.3 ± 0.04</td>
</tr>
<tr>
<td>Xanthophylls</td>
<td></td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>2.0 ± 0.02</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>1.4 ± 0.04</td>
</tr>
<tr>
<td>Lutein</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Astaxanthin</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>Canthaxanthin</td>
<td>0.02 ± 0.02</td>
</tr>
</tbody>
</table>

Data adapted from Miller et al. (1996).
themselves. The proposed pathway for lycopene oxidative degradation is shown in Figure 3. Oxygen is introduced into lycopene in at least two ways: (a) oxidation of a methyl or methylene group, and (b) addition to a carbon-carbon double bond. Oxidative degradation can occur at either end of the C40-carbon skeleton.

**SUMMARY OF VARIOUS METHODS OF TESTING ANTIOXIDANT ACTIVITY**

Several methods have been developed to measure the antioxidative activity of biological samples and foods of plant origin. A great deal of effort has been focused on elaborating fast, sensitive, and easy-to-use techniques to assess the capacity of various antioxidants to scavenge free radicals. Monitoring hydroperoxide formation from the oxidation of methyl linoleate (ML) provides a simple way to test the effect of antioxidants on lipid peroxidation (Tsuchihashi et al., 1995). HPLC analysis has been applied to determine the formation of methyl linoleate hydroperoxides by measuring the UV absorbance at 234 nm. Hydroperoxide formation followed a first-order kinetic model. The apparent first-order rate constant was reduced in the presence of antioxidants, this parameter was used to compare the relative capacities of various antioxidants.

The thiobarbituric acid test (TEA) or thiobarbituric acid reactive substance (TBARS) assay is a widely used method for monitoring MDA (malondialdehyde) formation from lipid peroxidation. MDA is the colorless product derived from peroxidation of polyunsaturated fatty acids. MDA reacts with TBA and produces a red compound with an absorbance at 532 nm. This assay has been used to analyze the capacity of different carotenoids to protect microsomal lipids from peroxidation by metal ions (Guillen-Sans and Guzman-Chozas, 1998). In the presence of carotenoids, lower levels of MDA were formed. Antioxidant activity of carotenoids was calculated as a percentage of the inhibition of MDA formation.

Lavelli and colleagues studied the antioxidant activity of tomato products using 3 reaction models: (1) the xanthine oxidase (XOD)/xanthine system, which generates superoxide radical and hydrogen peroxide; (2) myeloperoxidase (MPO)/NaCl/H2O2 system, which generates hypochloric acid; (3) linoleic acid/CuSO4 system, which induces lipid peroxidation (1999). The hydrophobic and hydrophilic component of tomato products were analyzed by the above 3-model system, α-keto-γ-methiolbutyric acid (KMB) and 1-aminocyclopropane-l-carboxylic acid (ACC) were included in the (XOD)/xanthine system and (MPO)/NaCl/H2O2 system, respectively. The release of ethane was measured by GC. In the linoleic acid/CuSO4 system, the formation of conjugated dienes was measured at 234 nm. Trolox was used as the positive control. The antioxidant activity was assayed by two parameters, I50, the amount of antioxidant sample that causes 50% inhibition of reaction rate, and Trolox equivalents, the ratio of the I50 of Trolox to the I50 of antioxidant samples. Both XOD and MPO are enzymatic sources causing oxidative stress in vivo, and are involved in oxidative hazards in various diseases. Transition metal ions are catalysts for oxidative reactions and promote lipid peroxidation.

The application of these model systems may provide important information on the in vivo antioxidant capacity of food components.

The determination of antioxidant activity of a sample is dependent on which free radical generator was used in the measurement, and it is affected by the localization of free radical species (Yeum et al., 2003). Antioxidant activity of a sample was studied by examining its ability to scavenge the ABTS·+ radical cations. ABTS·+ radical cation absorbs light at 734 nm and can be generated by mixing ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) with manganese dioxide. Miller and colleagues used this method to test the relative antioxidant activity of different carotenes and xanthophylls (Miller et al., 1996). Various concentrations of Trolox were used as standards to generate a dose-response curve: absorbance at 734 nm as a percentage of the absorbance of the uninhibited radical cation. The mean Trolox equivalent antioxidant capacity (TEAC) value of different antioxidant was obtained from the Trolox dose-response curve.

Many laboratories use AMVN (2,2′-azoelbis(2,4′-dimethyl-valeonitrile), a lipid-soluble radical generator to produce peroxyl radicals. Lipid oxidation product, the PCOOH, was separated by HPLC and quantified at 234 nm, using a molar extinction coefficient of 30,000. In the presence of an antioxidant, such as
carotenoid, decreased absorbance at 234 nm was observed, the concomitant degradation of carotenoids also was monitored by absorbance measurement (Woodall et al., 1997a).

AAPH (2,2-azobis(2-amidinopropane)dihydrochloride) and Fe(NO3)3/NTA (nirilotriacetic acid) were used as water-soluble free radical generators (Yen and Hu, 2000). Lipids incubated with AAPH or Fe/NTA, in the presence or absence of antioxidants. Lipid peroxidation was measured as thiobarbituric acid-reactive substance (TBARS) using malondialdehyde (MDA) as the standard.

Some other generally used methods for testing antioxidant activity include oxygen radical absorbance capacity (ORAC) assay and Ferric-reducing antioxidant power (FRAP) assay. The ORAC assay tests the activity of antioxidants to protect R-phycoerythin, a fluorescent indicator protein, against attack by free radicals. AAPH was used as the peroxyl radical generator, and Trolox was the control standard. The fluorescence of R-phycoerythin was measured at 565 nm with excitation at 540 nm. The results were calculated using the difference of areas under the R-phycoerythin decay curves between the blank and the sample with antioxidants. The activity of antioxidant was expressed as ORAC unit, where 1 ORAC unit equals the net protection of 1 μM Trolox (Cao et al., 1998). FRAP was initially introduced to measure the antioxidant capacity of human plasma (Benzie and Strain, 1996). This assay depends on the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant, such as antioxidants. (Fe(II)-TPTZ) has an intense blue color with maximum absorbance at 593 nm. The activity of antioxidants was measured by comparing with the standard Trolox. Since FRAP assay provides an easy and convenient procedure, it has been used to test the potential antioxidant effect of tea and medicinal herb samples (Benzie et al., 1999).

Since there are many methods available for antioxidant activity determination, investigators need to choose carefully to meet their specific goals. Each method has its limitations in terms of sensitivity, test time, and interference with matrix. For example, FRAP assay cannot test the SH-containing antioxidant. ORAC assay testing time is very long; usually, it will take 60 min. In the TBARS assay, some chemicals other than MDA also will react with TBA and give a similar color. It has been reported that different methods resulted in different antioxidant capacity (Ou et al., 2002). In some situations, work needs to be done to compare the results of different methods.

**BIOACTIVE PROPERTY**

A number of human epidemiological and clinical studies have increased our understanding of the health benefits of tomato and processed tomato products (Klipstein-Grobusch et al., 2000; Rao and Agarwal, 2000). Although tomatoes contain many healthful nutrients, most of the studies attributed the beneficial effect of tomato-based products to the high content of carotenoids, such as β-carotene and lycopene (Flagg et al., 1995). However, several studies have found that high β-carotene intake has no significant effect on heart disease and breast cancer (Hennekens et al., 1996; Verhoeven et al., 1997); moreover, β-carotene consumption may increase the incidence of lung cancer in the high-risk group of smokers (Omenn et al., 1996b; Albanes et al., 1995). Animal model experiments showed that a high dosage supplementation of β-carotene and exposure to cigarette smoke caused an elevation of activator protein-1 (AP-1) expression and reduction in retinoic acid receptor β (RARβ) expression (Wang et al., 1999). AP-1 is a leucine zipper transcription factor that regulates the expression of a diverse range of genes involved in cell growth, proliferation, and transformation (Karim et al., 1997). RARβ plays an important role in lung mophogenesis (Malpel et al., 2000). It is hypothesized that smoking enhances β-carotene oxidation, generating central cleavage oxidative metabolites that interact with retinoid signalling and cause the mutagenesis. Thus, the hazardous effects of β-carotene on lung cancer are apparently attributed to its oxidative metabolites rather than the intact β-carotene (Wang et al., 1999).

Lycopene is a major carotenoid found in tomatoes, and has been reported to decrease the growth of carcinoma cells and the development of cancer (Kim et al., 2002). Lower serum lycopene is associated with incidence of bladder cancer (Helzlsouer et al., 1989). A study of a cohort of 47,894 subjects initially free of diagnosed cancer for a 1-year period revealed an inverse association between prostate cancer and a high consumption of lycopene and tomato products (Giovannucci et al., 1995). Much work has been employed to study the anticarcinogenic function of lycopene in an animal model and tissue culture. In a rat tumor model induced by 7,12-dimethyl-benz[a]anthracene (DMBA), pre-injection of lycopene before the tumor induction by DMBA significantly reduced the number of tumors in the rat and the size of the tumor (Sharoni et al., 1997). In tissue culture cells, lycopene was more effective at inhibiting mammary cancer cell proliferation than β-carotene and α-carotene (Levy et al., 1995). The mechanism of lycopene’s effect on the induction and development of cancer is not yet clearly understood. It is generally believed that lycopene may exert its anticancer action through its antioxidant activity. Lipoprotein was less susceptible to oxidative stress in the subjects fed carotenoid-rich tomato products, suggesting a protective role of carotenoid in vivo.

Although the antioxidant activity of lycopene is believed to be the primary mechanism for its health promoting ability, lycopene may also involve with mechanisms, such as cell growth control and intercellular communication.

A higher consumption of lycopene via a fruit- and vegetable-rich diet is associated with a decreased risk of lung cancer in human studies. Higher plasma levels of insulin-like growth factor 1 (IGF-1) and/or lower levels of IGF-binding protein 3 (IGFBP-3) are risk factors in breast, prostate, and lung cancers. Insulin-like growth factors also regulate the growth of mammary and endometrial cancer cells (Levy et al., 1995). In vitro experiments have shown that physiological concentrations of lycopene
greatly reduced the IGF-I simulated growth of MCF7 mammary cancer cells (Karas et al., 2000). The inhibitory effects of lycopene on MCF7 cell growth not associated with apoptotic or necrotic cell death. However, lycopene treatment markedly reduced the IGF-I stimulation of tyrosine phosphorylation of insulin receptor substrate 1 and binding capacity of the AP-1 transcription complex, suggesting lycopene interaction with the IGF signaling pathways. Using ferrets supplemented with lycopene and exposed to smoke, Liu et al. (2003) recently reported that both low dosages and high dosages of lycopene significantly increased plasma IGFBP-3 levels (p < .01) and decreased IGF-I/IGFBP-3 ratio (p < .01), indicating that lycopene may exert its the protective effects against smoke-induced lung carcinogenesis in ferrets through up-regulating IGFBP-3, which negatively regulates IGF-I receptor activation.

Interaction between neighboring cells via gap junction communication is considered to be a key factor involved in cell proliferation and tissue homeostasis; its deficiency is associated with the formation of cancer (Trosko and Chang 2000). An increased gap junctional communication is accompanied by up-regulation of connexin protein expression (Zhang et al., 1995). The connexin proteins are assembled to form hexamer channels to connect the cytosol of neighboring cells and to provide a pathway for diffusion of ions and signalling molecules (Goodenough et al., 1996). A number of studies have shown that lycopene inhibited the proliferation of various cell lines via an increased expression of the connexin43 protein. It was shown that lycopene inhibited KB-1 human oral tumor cell proliferation in a concentration dependent manner. Also, treatment of 3 μM lycopene significantly enhanced connexin43 expression on the cell membrane, as measured by immunocytochemistry, and increased the level of connexin43 mRNA (Livny et al., 2002). These results are in agreement with the previous observation of Stahl et al. (2000) and Zhang et al. (1991, 1992), who used human fetal skin fibroblast cell line and mouse C3H/10T1/2 cells.

Hypercholesterolemia is considered to be a major risk factor in heart disease, causing damage to the coronary artery and the aortic valve; it plays an important role in the development of malignant atherosclerosis (Kawaguchi et al., 2003).

Lycopene, also has been reported to promote recovery of the liver after radiation injury. Animal studies have indicated that body exposure to gamma irradiation resulted in lipid peroxidation of the organelle membrane of the liver cells and released mitochondrial glutamate dehydrogenase (GDH), lysosomal beta-glucuronidase, and microsomal glucose 6-phosphatase into the cytosol. Supplementation with lycopene significantly reduced liver lipid peroxidation and ameliorated the decrease in enzyme activity in the subcellular structure (Saada et al., 2001). In cultured rat liver cells, malondialdehyde formation from lipid peroxidation, caused by exposure to carbon tetrachloride, was inhibited by addition of lycopene (Kim, 1995).

A rat model of colitis, induced by 2,4,6-trinitrobenzene-sulfonic acid (TNBS), indicated that the addition of lycopene (300 micrograms/rat/day) in the diet significantly reduced the inflammation status as measured by colonic thickness, colonic weight, total area of inflammation, as well as the level of myeloperoxidase (MPO) (Reifen et al., 2001). The beneficial effects of lycopene against inflammation were much higher than that of β-carotene.

The varied distribution of isomers of lycopene in various tissues suggested that different lycopene isomers have different biological functions. Understanding the biological mechanisms for lycopene isomerization is essential for determining the physiologic role of cis-lycopene isomers in vivo.

SYNERGISTIC EFFECTS OF LYCOPENE AND OTHER ACTIVE COMPONENTS

Increasing interest in the carotenoids in tomatoes has surfaced recently due to their possible protective effect against various diseases. Although certain carotenoids found in tomatoes and tomato products appear to be specifically responsible for potential health benefits, interaction between carotenoids and the combined effects of each carotenoid and other bioactive compounds present in the diet, such as vitamin C and vitamin E, may contribute to the beneficial effects. The protective effect of a high intake of tomato products against the risk of a range of degenerative/chronic diseases may not rely on the action of a single antioxidant, but rather on the concerted action of several antioxidant nutrients.

Information on antioxidant interactions at the molecular level is very limited. There is some evidence indicating that α-tocopherol prevents β-carotene and lycopene degradation during lipid oxidation (Anguelova and Warthesen 2000). A few research groups have observed synergistically improved antioxidant activity against lipid oxidation when vitamin C and vitamin E were used together (Lambelet et al., 1985; Kagan et al., 1992; Chan et al., 1991). It was suggested that ascorbic acid was able to interact with α-tocopherol radicals in the initial stage of lipid oxidation and regenerate α-tocopherol. Steady-state radiolysis experiments of Mortensen and colleagues showed that α-tocopherol was able to protect β-carotene from oxidation by hexane radicals. Furthermore, the study of laser flash photolysis indicated that α-tocopherol reacted with β-carotene radical cations to regenerate β-carotene (Mortensen et al., 1998). An earlier study by the same group found that α-, β-, and γ-tocopherol all reduced the carotenoid radical cations tested, whereas the δ-tocopheroxyl radical was reduced by lycopene and β-carotene (Mortensen et al., 1997). Lutein or β-carotene, in combination with γ-tocopherol, was found to be able to enhance the activity of γ-tocopherol in preventing triglyceride oxidation (Haila et al., 1996). Palozza and Krinsky reported in 1992 that β-carotene and α-tocopherol interacted cooperatively to provide greater inhibition of lipid peroxidation than the additive activity of the individual antioxidant in a membrane system (Palozza and Krinsky, 1992).

Only a few studies have been done to investigate the association and synergistic effect of carotenoids, especially lycopene. Many epidemiologic studies have shown that the consumption of
lycopene-rich tomato products or plasma lycopene concentration had an inverse relationship with the risk of prostate cancer, while the other carotenoids found in tomatoes, such as \( \alpha \)-carotene, \( \beta \)-carotene, and lutein, did not show the same effect (Giovannucci et al., 1995). Cell culture studies have shown that lycopene was able to inhibit the growth of a range of tumor cells, including breast and lung cancer cells. The antiproliferation effect of lycopene was reinforced by the evidence that lycopene suppressed the insulin-like growth factor-I-stimulated growth (Levy et al., 1995). The dietary intake of \( \alpha \)-tocopherol, another common antioxidant, also has been associated with a low incidence of prostate cancer (Albanes et al., 1995). Lycopene and \( \alpha \)-tocopherol have been reported to inhibit the proliferation of different prostate carcinoma cell lines. However, the effective concentration of lycopene, when used alone, is above 50 \( \mu \)M, much higher than the physiological concentration of lycopene in human plasma or prostate, which is about 1 \( \mu \)M (Clintone et al., 1996). Pastori and colleagues reported that lycopene, when used alone, at low concentrations did not efficiently inhibit prostate carcinoma cell proliferation. However, the addition of low concentrations of lycopene, together with \( \alpha \)-tocopherol, significantly increased the inhibition effect of the latter (Pastori et al., 1998). The use of various concentrations of lycopene from 1 to 5 \( \mu \)M together with \( \alpha \)-tocopherol showed a synergistic phenomenon, rather than a simple additive effect. It is interesting to note that, the simultaneous addition of lycopene with \( \beta \)-tocopherol and ascorbic acid did not show a synergistic effect. The addition of ascorbic acid and \( \alpha \)-tocopherol did not exhibit the same stimulation as did lycopene. The inhibition of proliferation by lycopene in association with \( \alpha \)-tocopherol was not potent in other cell lines, as it was in the prostate cell, suggesting a specific function of lycopene in prostate tissue.

Amir et al. have investigated the inhibition effect of lycopene and 1,25-dihydroxyvitamin \( \alpha \)D \(_2\) (1,25(OH)\(_2\)\( \alpha \)D \(_2\)) on the proliferation of HL-60 promyelocytic leukemia cell line. Both lycopene and 1,25(OH)\(_2\)\( \alpha \)D \(_2\) inhibited cell growth by slowing cell cycle progression at the G0/G1 phase. After a 2-day incubation, a low concentration of lycopene and 1,25(OH)\(_2\)\( \alpha \)D \(_2\) alone produced only a small effect on cell proliferation. However, the simultaneous administration, of low concentrations of these two compounds showed a significant synergistic inhibitory effect on cell proliferation (Amir et al., 1999).

Limited information is available so far on the interaction between lycopene and other antioxidants. Some studies have shown that, although water-soluble vitamin C was less reactive with lipid radicals than hydrophobic lycopene, it was able to interact with the oxidized forms of lycopene and lycoxanthin to regenerate lycopene and lycoxanthin in a time-course reaction (Biacs et al., 2000). The inhibitory effect of vitamin C on \( \beta \)-carotene oxidation is low compared with that on lycopene oxidation, probably due to the lower exchange of \( \beta \)-carotene radicals to the aqueous phase or the carbon-centered radical product of \( \beta \)-carotene not being reactive with vitamin C. \( \alpha \)-tocopherol also inhibits the oxidation of tomato carotenoids, but in a different manner than with ascorbic acid. Interaction of \( \alpha \)-tocopherol with \( \beta \)-carotene is stronger than with lycopene. Although the combination of ascorbic acid and \( \alpha \)-tocopherol synergistically inhibited the oxidation of lycopene, the inhibition of \( \beta \)-carotene oxidation by ascorbic acid and \( \alpha \)-tocopherol was worse when used in combination, compared to their use individually. Thus, precaution is needed to choose the right combination of antioxidants.

There is additional evidence indicating that mixtures of carotenoids were more effective at protecting liposome from oxidation than an individual carotenoid. Results of various combinations of carotenoid mixtures showed that lycopene and lutein apparently contributed to the synergistic antioxidant activity of the mixture, while \( \alpha \)-carotene, \( \beta \)-carotene, and other carotenoids exerted limited synergistic effects (Stahl et al., 1998). It was recently reported that tomato oleoresin was more effective than pure lycopene in suppressing the formation of thiobarbituric acid reactive substances and in preventing oxidative damage to LDL, suggesting a synergistic effect of antioxidant components in tomato oleoresin (Fuhrman et al., 2000). This was supported by the observation that the use of a combination of pure lycopene and vitamin E inhibited LDL oxidation induced by copper ions or by the radical generator AAPH.

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