P-5 Carotenoids and Tocopherols as Antioxidants and Singlet Oxygen Quenchers

H.SIES

Institut für Physiologische Chemie I, Universität Düsseldorf,
Moorenstrasse 5, W-4000-Düsseldorf, Germany

I. INTRODUCTION

Biological systems can maintain a steady state against the influx of prooxidants by a pattern of antioxidant defense. When there is a disturbance in the prooxidant/antioxidant balance in favor of the former, leading to damage products, there is oxidative stress(1,1a). Antioxidant defense is enzymic and nonenzymic, and carotenoids and tocopherols constitute the major lipid-soluble nonenzymic antioxidants, being essential micronutrient vitamins. The present overview deals with some aspects of carotenoids and tocopherols.

II. CAROTENOIDS

Carotenoid pigments are widely distributed in nature(2), and they play an important role in protecting cells and organisms. The first association that comes to mind may be β -carotene in its function as a provitamin A. Indeed, all-trans-retinol and related retinoids as products generated from β -carotene fulfill biological functions in diverse processes as the visual process or differentiation. However, there also is an antioxidant function of carotenoids independent of the functions related to vitamin A. In this regard, the protective functions of carotenoids in the photosynthetic apparatus in the plant world are well-known as the quenching of electronically excited states generated by light. However, there also is an antioxidant function of β -carotene and other carotenoids in the animal cell (photochemistry in the dark). This field has emerged relatively recently(see (3) for review).

Antioxidant functions of carotenoids are mainly twofold, radical reactions or singlet oxygen quenching reactions. The latter has been studied more intensively than the former, and our own work also mainly looked at singlet oxygen quenching, as will be described below. These antioxidant properties of carotenoids may explain a possible role in the prevention of cancer(4), since the question has been raised whether β -carotene might have anticarcinogenic properties independent of its provitamin A activity(5). An inverse relationship between β -carotene intake and the incidence of certain types of cancer, such as lung and gastrointestinal tract cancer, has been observed(6,7), and animal experiments have revealed anticarcinogenic properties of carotenoids(8).

Although the role of carotenoids in protecting plants against the photosensitization induced by their own chlorophyll as well as in treatment of patients with photosensitivity diseases is established, the mechanism by which β -carotene may exert a

protective function against cancer remains unknown. Singlet oxygen has been shown to be capable of inducing DNA damage and to be mutagenic(9-11); see also a recent review(12).

In our recent work, we determined the quenching abilities of biological compounds by monitoring singlet oxygen by direct infrared photoemission at 1270 nm using a germanium diode photodetector and then plotting the ratio of the intensity of the signal in the absence and presence of quencher using Stern-Volmer plots(13,14). There were considerable differences in the rate constant of quenching, k_q , for various compounds. Lycopene showed the greatest quenching ability, double that of β -carotene, as deduced from the Stern-Volmer plot shown in Fig. 1.

FIG 1. Stern-Volmer plot of lycopene, β -carotene, and lutein. From Ref. (13).

Comparison of the structures of lycopene, gamma-carotene and β -carotene revealed that the opening of the β -ionone ring increased the quenching ability. Replacing the two ionone rings by carboxylate and a methyl ester group as in bixin, or decreasing the number of conjugated double bonds as in crocin, or addition of chemical groups on the β -ionone ring as in the xanthophylls(oxy-carotenoids) also modulated quenching ability(13),

suggesting that the quenching properties of carotenoids reside not only in the triplet energy state, i.e. the length of the conjugated double bond system, but also in the functional groups. In recent work on synthetic carotenoid-like compounds, polyene polyketones, even higher singlet oxygen quenching was observed than for lycopene(15).

Carotenoids also participate in free radical reactions, as mentioned above. This is evidenced by their high rate constants in the reaction with trichloromethyl peroxy radicals(16). Also, β -carotene and related carotenoids decreased the rate of formation of methyl linoleate hydroperoxides(17). An interesting study by Burton and Ingold(18) showed that β -carotene is an unconventional antioxidant, being neither a peroxide-consuming preventive antioxidant nor a classical chain-breaking antioxidant as the tocopherols(see below). It is likely that β -carotene forms a resonance-stabilized system with peroxy radicals which then rearranges to form oxidation products(18), mainly polyene carbonyls. Recently, β -carotene epoxides were identified as products of peroxy radical oxidation(19), as well as other breakdown products(20,21).

III. TOCOPHEROLS

Vitamin E, a term that encompasses a small group of related tocopherols, is the major lipid-soluble antioxidant responsible for protecting the polyunsaturated fatty acids in membranes against lipid peroxidation (22-24). The normal intake of vitamin E in US diets ranges between 4 and 22 IU/d in adults not taking vitamin E supplements, with average values between 11 and 13 IU/d (24). This maintains an average blood level of 23 $\mu\text{mol/L}$ (24), although levels < 23 $\mu\text{mol/L}$ can be typical, depending on local diets (25).

The vitamin E content of membranes often determines the susceptibility of microsomal membranes, low-density lipoproteins, hepatocytes, or whole organs to damage by peroxidizing agents such as hydroxyl radicals, alkoxyl radicals, peroxy radicals, singlet oxygen, and perhaps a number of oxygen-metal complexes (26-29). These agents not only damage the lipids but produce secondary intermediates, lipid hydroperoxides, which can decompose into alkoxyl and organic peroxy radicals, and thus lead to a chain reaction of lipid peroxidation. Tocopherols protect lipids by scavenging peroxy radicals without reacting in further chain-propagating steps.

Using phosphatidylcholine liposomes as a model membrane, Niki and co-workers (30,31) found that when lipid peroxidation was initiated by the peroxy radical-generating compound 2,2'-azobis(2-amidinopropane) (AAPH), vitamin E protected the liposomes against rapid peroxidation until it was consumed. This contrasted with our recent observation that peroxidation initiated with Fe/ADP/NADPH began already after only 15% of the vitamin E was lost from microsomal membranes prepared from rat liver (32). This prompted further analyses of the role of this antioxidant, particularly with respect to its relative importance vs different types of prooxidants (33).

Figure 2, shows the temporal relationship between the loss of alpha-tocopherol and the onset of lipid peroxidation in the experiments with free metal ions. There was no lag period before either the chemiluminescence increase or the accumulation of thiobarbituric acid-reactive substances (TBARS) induced by

FIG 2. Time course of chemiluminescence, thiobarbituric acid-reactive substances (TBARS) accumulation, and vitamin E loss initiated by FeSO₄ (A) or CuSO₄ (B). Microsomal fractions were prepared as described. From Ref. (33).

500 μ mol FeSO₄/L (Fig 2A) indicating that lipid peroxidation started although vitamin E content was at initial levels. In contrast, a clear lag period preceded both chemiluminescence and TBARS accumulation induced by 100 μ mol CuSO₄/L (Fig 2B) whereas α -tocopherol was lost as quickly as with other prooxidants and without any apparent lag. The unique feature of this prooxidant is that there is still a period left before lipid peroxidation starts even following substantial vitamin E depletion. This relationship is similar to that noted during the CuCl₂-induced peroxidation of human low-density lipoproteins (29).

The site of formation, lipid-solubility, and oxidation potential of the peroxidizing agents all influence their reactivity with vitamin E and lipids. Based on these differences, it is concluded that the importance of vitamin E in protecting membranes depends on the type of prooxidant used. The relative importance of vitamin E is diminished when peroxidation is initiated by complexes containing Fe²⁺ and enhanced when peroxidation involves peroxy radicals, especially those generated in propagation reactions. Therefore, although the finding that vitamin E is nearly the exclusive peroxy-radical scavenging

compound detected in membranes (22) supports the contention that it is a major antioxidant (34), our results (33) suggest that other important antioxidant factors also protect membranes before or after the oxidation of vitamin E.

Ascorbic acid may be a physiologically relevant enhancer of the effectiveness of vitamin E. For example, it may regenerate vitamin E from the chromanoxyl radical and so prolong the lag phase (31,35,36). However, hydrogen transfer from ascorbate to vitamin E radicals, although directly observed by ESR in chemical model systems (37), has not yet been demonstrated using similar techniques in biological membranes because of the low endogenous level of tocopherols. In a recent overview (38), we have compiled further aspects (including the so-called vitamin E reductase activity involving GSH).

The biological aspects on this topic as related to human plasma antioxidant defense in plasma(39) and LDL(40) are fascinating and currently under intense investigation by many groups.

ACKNOWLEDGEMENTS

The contribution by the colleagues from this Institute as indicated in the references is gratefully acknowledged. Supported by National Foundation for Cancer Research, Bethesda, and by Ernst-Jung-Stiftung für Wissenschaft und Forschung, Hamburg.

REFERENCES

- (1) Sies, H., ed. (1985): Oxidative Stress, Academic Press, London
- (1a) Sies, H., ed. (1991): Oxidative Stress: Oxidants and Antioxidants, Academic Press, London
- (2) Pfander, H., ed. (1987): Key to Carotenoids, 2nd ed., Birkhäuser Verlag, Basel
- (3) Krinsky, N.I. (1989): Antioxidant functions of carotenoids. Free Rad. Biol. Med. 7, 617-635
- (4) Ames, B.N. (1983) Dietary carcinogens and anticarcinogens. Science 221, 1256-1264
- (5) Pung, A., Rundhaug, E., Yoshizawa, C.N., Bertram, J.S. (1988) β -Carotene and canthaxanthin inhibit chemically and physically-induced neoplastic transformation in 10T1/2 cells. Carcinogenesis 9, 1533-1539
- (6) Peto, R., Doll, R., Buckley, J.D., Sporn, M.B. (1981) Can dietary β -carotene materially reduce human cancer rates? Nature(London) 290, 201-208
- (7) Connett, J.E., Kuller, L.H., Kjelsberg, M.O., Polk, B.F. (1989) Relationship between carotenoids and cancer. Cancer 64, 126-134
- (8) Mathews-Roth, M.M. (1985) Carotenoids and cancer prevention --experimental and epidemiological studies. Pure Appl.Chem. 57, 717-722
- (9) Di Mascio, P., Wefers, H., Do-Thi, H.P., Lafleur, M.V.M., Sies, H. (1989) Singlet molecular oxygen causes loss of biological activity in plasmid and bacteriophage DNA and induces single-strand breaks. Biochim. Biophys. Acta 1007, 151-157

- (10) Di Mascio, P., Menck, C.F.M., Nigro, R.G., Sarasin, A., Sies, H. (1990) Singlet molecular oxygen induced mutagenicity in a mammalian SV40-based shuttle vector. *Photochem. Photobiol.* 51, 17-20
- (11) Decuyper-Debergh, D., Piette, J., Laurent, C., Van de Vorst, A. (1989) Cytotoxic and genotoxic effects of extracellular generated singlet oxygen in human lymphocytes in vitro. *Mutation Res.* 225, 11-14
- (12) Epe, B. (1991) Genotoxicity of singlet oxygen. *Chemico-biol. Interact.*, in press
- (13) Di Mascio, P., Kaiser, S., Sies, H. (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* 274, 532-538
- (14) Di Mascio, P., Sundquist, A.R., Devasagayam, T.P.A., Sies, H. (1991) Assay of lycopene and other carotenoids as singlet oxygen quenchers, in *Methods in Enzymology, Carotenoids*, ed. by Packer, L., Academic Press, San Diego, in press
- (15) Devasagayam, T.P.A., Werner, T., Ippendorf, H., Martin, H.D., Sies, H. (1991) Synthetic carotenoids, novel polyenepolyketones and new capsorubin isomers as efficient quenchers of singlet molecular oxygen, *Photochem. Photobiol.*, submitted
- (16) Packer, J.E., Mahood, J.S., Mora-Arellano, O., Slater, T.F., Willson, R.L., Wolfenden, B.S. (1981) Free radicals and singlet oxygen scavengers: Reaction of a peroxy-radical with beta-carotene, diphenyl furan and 1,4-diaza-bicyclo(2,2,2)-octane. *Biochem. Biophys. Res. Commun.* 98, 901-906
- (17) Terao, J. (1989) Antioxidant activity of β -carotene-related carotenoids in solution. *Lipids* 24, 659-661
- (18) Burton, G.W., Ingold, K.U. (1984) β -Carotene: An unusual type of lipid antioxidant. *Science* 224, 569-573
- (19) Kennedy, T.A., Liebler, D.C. (1991) Peroxyl radical oxidation of β -carotene: Formation of β -carotene epoxides. *Chem. Res. Toxicol.* 4, 290-295
- (20) Handelman, G.J., Van Kuijk, F.J.G.M., Chatterjee, A., Krinsky, N.I. (1991) Characterization of products formed during the autoxidation of β -carotene. *Free Rad. Biol. Med.* 10, 427-437
- (21) Mordì, R.C., Walton, J.C., Burton, G.W., Hughes, L., Ingold, K.U., Lindsay, D.A. (1991) Exploratory study of β -carotene autoxidation. *Tetrahedron Lett.*, in press
- (22) Burton, G.W., Joyce, A., Ingold, K.U. (1983) Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch. Biochem. Biophys.* 221, 281-290.
- (23) Horwitt, M.K. (1986) The promotion of vitamin E. *J. Nutr.* 116, 1371-1377.
- (24) Machlin, L.J. (1989) Use and safety of elevated dosages of vitamin E in adults, in *Elevated dosages of vitamins*, eds. by Walter, P., Brubacher, G., Stähelin, H., Hans Huber Publishers, pp. 56-68.
- (25) Gey, K.F., Brubacher, G.B., Stähelin, H.B. (1987) Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. *Am. J. Clin. Nutr.* 45, 1368-1377.
- (26) Cadenas, E., Ginsberg, M., Rabe, U., Sies, H., Evaluation of alpha-tocopherol antioxidant activity in microsomal lipid peroxidation as detected by low-level chemiluminescence. *Biochem. J.* 223, 755-759.
- (27) Reed, D.J., Fariss, M.W., Pascoe, G.A. (1986) Mechanisms of chemical toxicity and cellular protective systems. *Fund. Appl. Toxicol.* 6, 591-597.

- (28) Pascoe, G.A., Reed, D.J. (1987) Vitamin E protection against chemical-induced cell injury. II. Evidence for a threshold effect of cellular alpha-tocopherol in prevention of adriamycin toxicity. Arch. Biochem. Biophys. 256, 159-166.
- (29) Quehenberger, O., Koller, E., Jürgens, G., Esterbauer, H. (1987) Investigation of lipid peroxidation in human low density lipoprotein. Free Rad.Res.Comm.3, 233-242
- (30) Yamamoto, Y., Haga, S., Niki, E., Kamiya, Y. (1984) Oxidation of lipids. V. Oxidation of methyl linoleate in aqueous dispersions. Bull. Chem. Soc. Jpn. 59, 1260-1264.
- (31) Niki, E., Kawakami, A., Yamamoto, Y., Kamiya, Y. (1985) Oxidation of lipids. VIII. Synergistic inhibition of oxidation of phosphatidylcholine liposome in aqueous dispersion by vitamin E and vitamin C. Bull. Chem. Soc. Jpn.58, 1971-1975.
- (32) Murphy, M.E., Scholich, H., Wefers, H., Sies, H. (1989) Alpha-tocopherol in microsomal lipid peroxidation. Ann. NY. Acad. Sci. 570, 480-486.
- (33) Scheschonka, A., Murphy, M.E., Sies, H. (1990) Temporal relationship between the loss of vitamin E, protein sulfhydryls and lipid peroxidation in microsomes challenged with different prooxidants. Chem. Biol. Interact. 74, 233-252
- (34) Burton, G.W. and Ingold, K.U. (1986) Vitamin E: application of the principles of physical organic chemistry to the exploration of its structure and function. Acc. Chem. Res. 19, 194-201.
- (35) Wefers, H., Sies, H. (1988) The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. Eur. J. Biochem. 174, 353-357.
- (36) Tappel, A.L. (1968) Will antioxidant nutrients slow the aging processes? Geriatrics 23, 97-105.
- (37) Lambelet, P., Saucy, F., Löliger, J. (1985) Chemical evidence for interactions between vitamins E and C. Experientia 41, 1384-1388.
- (38) Sies, H., Murphy, M.E. (1991) Role of tocopherols in the protection of biological systems against oxidative damage. J.Photochem.Photobiol.B:Biolog. 8, 211-224
- (39) Stocker, R., Frei, B. (1991) Endogenous antioxidant defences in human blood plasma, in Oxidative Stress: Oxidants and Antioxidants, ed. by Sies, H., Academic Press, London, pp.213-243.
- (40) Gebicki, J.M., Jürgens, G., Esterbauer, H. (1991) Oxidation of low-density lipoprotein in vitro, in Oxidative Stress: Oxidants and Antioxidants, ed. by Sies, H., Academic Press, London, pp.371-397.