COMMUNICATIONS

Antioxidant Activity of β-Carotene-Related Carotenoids in Solution

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The effect of the antioxidant activity of β-carotene and related carotenoids on the free radical-oxidation of methyl linoleate in solution was examined by measuring the production of methyl linoleate hydroperoxides. Canthaxanthin and astaxanthin which possess oxo groups at the 4 and 4′-positions in the β-ionone ring retarded the hydroperoxide formation more efficiently than β-carotene and zeaxanthin which possess no oxo groups. The rates of autocatalytic oxidation of canthaxanthin and astaxanthin were also slower than those of β-carotene and zeaxanthin. These results suggest that canthaxanthin and astaxanthin are more effective antioxidants than β-carotene by stabilizing the trapped radicals.
Lipids 24, 659–661 (1989).

In recent years, epidemiological studies in humans (1–4) have suggested that β-carotene aids in cancer prevention. It was also implied that dietary β-carotene may exert an anticarcinogenic effect by a mechanism independent of its role as a vitamin A precursor (5). On the other hand, β-carotene is an effective singlet oxygen quencher (6), and we have found that β-carotene can prevent singlet oxygen-initiated oxidation of methyl linoleate in cooperation with α-tocopherol (7). Krinsky and Deneke (8,9) demonstrated that carotenoids including β-carotene are capable of inhibiting free radical-induced oxidation in liposomal lipids. Burton and Ingold (10) have shown that β-carotene belongs to a previously unknown class of biological antioxidants especially effective at low oxygen partial pressures such as those found in most tissues under physiological conditions. Therefore, the anticarcinogenic effect of β-carotene may be, at least partly, attributable to its antioxidant effect insofar as oxygen radicals are related to the process leading to human cancer (11).

However, little is known about the antioxidant activity of naturally occurring carotenoids other than β-carotene. We selected β-carotene (structure [1] in Fig. 1) and related carotenoids containing oxo groups and/or hydroxyl groups in the β-ionone rings as a common structural unit (that is, zeaxanthin [2], canthaxanthin [3], and astaxanthin [4] in Fig. 1) and examined their antioxidant effect upon the azo-initiated oxidation of methyl linoleate in solution. The results strongly suggest that the introduction of oxo groups at 4 and 4′ positions enhances the antioxidant activity of carotenoids.

MATERIALS AND METHODS

Materials. β-Carotene was obtained from E. Merck, Darmstadt. Canthaxanthin, astaxanthin and zeaxanthin were generously provided by Hoffmann-La Roche. The product of Nacalai Tesque Inc., Kyoto, Japan, was dl-α-tocopherol. Obtained from Wako Pure Chemical Industries, Osaka, Japan, was 2,2′-azo*bis*(2,4-dimethylvaleronitrile) (AMVN). Methyl linoleate (99%), supplied by Nacalai Tesque, was further purified by column chromatography with Florisil (100/200 mesh) (12). Other reagents and solvents were of analytical grade and used without purification.

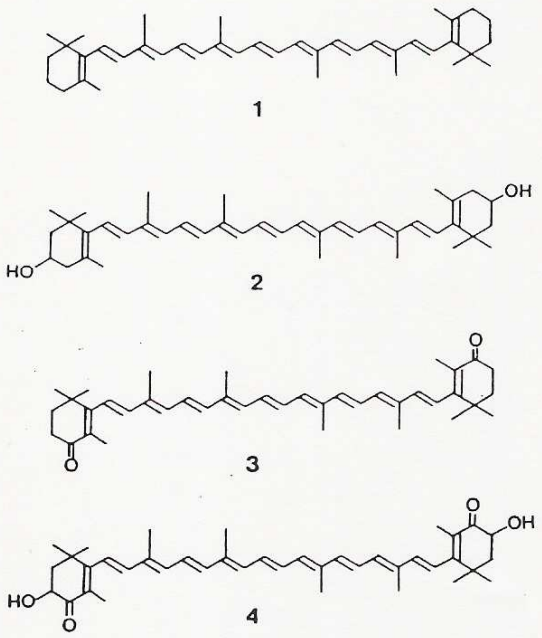

FIG. 1. Structures of carotenoids. (1) β-carotene, (2) zeaxanthin, (3) canthaxanthin, (4) astaxanthin.

Procedures. An appropriate amount of carotenoid in tetrahydrofuran (5 μmol/ml) was added to a mixture of hexane/isopropanol (1:1, v/v, 1.0 ml) containing methyl linoleate (100 μmol). Oxidation was initiated by adding a hexane solution of AMVN (10 μmol in 0.1 ml) and the mixture was incubated with continuous shaking under air in the dark at 37°C. At regular intervals, aliquots of the sample (10 μl) were withdrawn and injected into the HPLC column. The HPLC conditions employed and the procedure for the determination of methyl linoleate hydroperoxides have been described in a previous paper (12). Carotenoids and α-tocopherol were also quantified by HPLC using a column of YMC-Pack ODS (6 × 150 mm, 5 μm particle size, Yamamura Kagaku, Japan). The column was eluted with a mixture of acetonitrile/isopropanol (3:1, v/v). The flow rate was maintained at 3.0 ml/min and the effluent was monitored at 470 nm for carotenoids and 290 nm for α-tocopherol.

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Abbreviations: AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); HPLC, high performance liquid chromatography.