Food Sci. Biotechnol. Vol. 18, No. 1, pp. 1 ~ 11 (2009)

RESEARCH REVIEW

Food Science and Biotechnology

Bio-functions of Marine Carotenoids

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Abstract Carotenoids being most important pigments among those occurring in nature, have received increased interest owing to their beneficial effects on human health. An effort is made to review marine carotenoids as important bioactive compounds with reference to their presence, chemical, and biofunctional benefits they afford. The potential beneficial effects of marine carotenoids were particularly focused on astaxanthin and fucoxanthin, major marine carotenoids found in marine animals and aquatic plants, respectively. Both carotenoids show strong antioxidant activity which is attributed to quenching singlet oxygen and scavenging free radicals. The potential role of the carotenoids as dietary antioxidants has been suggested as being one of the main mechanism by which they afford their beneficial health effects such as anticancer activity and anti-inflammatory effect. Only recently, antiobesity effect and antidiabetic effect have been noted as specific and novel bio-functions of fucoxanthin. Nutrigenomic study reveals that fucoxanthin induces uncoupling protein 1 (UCP1) expression in white adipose tissue (WAT) mitochondria to lead to oxidation of fatty acids and heat production in WAT. Fucoxanthin improves insulin resistance and decreases blood glucose level, at least in part, through the down-regulation of tumor necrosis factor α (TNFα) in WAT of animals.

Keywords: carotenoid, astaxanthin, fucoxanthin, antioxidant, anticancer, anti-inflammatory, antiobesity, antidiabetes

Introduction

Carotenoids belong to the tetraterpenes family found principally in plants, algae, photosynthetic bacteria, and animals. They are the most important pigments among those occurring in the nature that are responsible for various colors of different fruits, vegetables, and plant parts. Animals including humans are incapable of synthesizing carotenoids and many are colored by carotenoids derived through their diets. The distribution of carotenoids in animal sources is primarily the result of specific dietary habits, absorption, and metabolic transformation.

Carotenoids have received considerable attention because of their various functions in human health. The best known biological function of carotenoids is their established role as provitamin A. In addition, dietary carotenoids have shown to reduce the risk of cardiovascular diseases, age related macular degeneration, and cancers (1,2). Further, epidemiological studies established a positive correlation between carotenoid consumption and a reduced risk of cancer (3,4).

Reactive oxygen species (ROS) and oxidative damage to bio-molecules have been widely postulated to be involved in the cause and progression of several chronic diseases, including cancer and cardiovascular diseases. Carotenoids have been implicated as important dietary nutrients having antioxidant potential, being involved in the scavenging ROS, singlet molecular oxygen and peroxy radicals generated in the process of peroxidation (5). The antioxidant properties of carotenoids have been suggested as being the main mechanism by which they afford their beneficial health effects (6-8).

In addition, the carotenoids also are capable of altering patterns of gene and protein expressions and cell function with a specific and important nutritional and bio-functional impact on the body (9). The nutritional functions of carotenoids depend on their chemical structures which differ depending on the length of the polyene, nature of the end group, and various substituents they contain. The specific regulation of a carotenoid on a particular bio-molecule will be responsible for the characteristic physiological effect of the carotenoid.

Carotenoids in general, especially those of terrestrial origin, have been thoroughly reviewed with respect to their occurrence, biological functions, and possible health benefits. However, there has been relatively little information on the physiological effects or beneficial applications of marine carotenoids. Hence, we have made an effort to review the published literature with respect to occurrence of marine carotenoids and their physiological benefits.

Distribution of Carotenoids in Marine Biota

In marine environments carotenoids are widely present in both plant and animal kingdom, the occurrence of them being species dependent. Palermo *et al.* (10) reported the presence of β-carotene, zeaxanthin, fucoxanthin, and fucoxanthinol (Fig. 1) in the red algae. In brown algae fucoxanthin is the dominant carotenoid (11). The metabolites of fucoxanthin, apo-9'-fucoxanthinone, and apo-13'-fucoxanthinone, were also isolated from brown seaweeds (12). Carotenoids are synthesized *de novo* by all plants and some microorganisms. The number of naturally occurring carotenoids reported continues to rise and has now reached more than 700. Among the carotenoids, fucoxanthin is the most abundant one and it contributes more than 10% of the estimated total production carotenoids in nature (13).

Carotenoids are responsible for the color of many important fish and shellfish products. The distribution of

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Received July 29, 2008; Accepted August 12, 2008

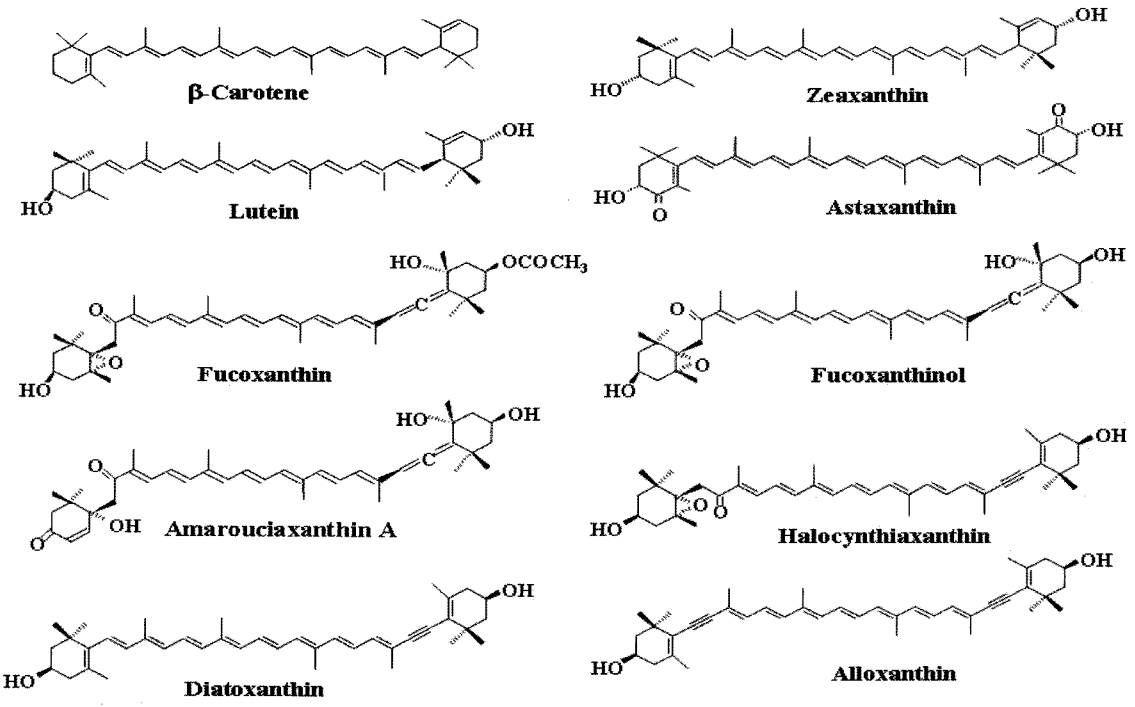

Fig. 1. Structures of some selected carotenoids.

carotenoids in these marine animals varies with species, habitat, and their food habits. Commonly found carotenoids from fish are tunaxanthin in yellow fish, astaxanthin (Fig. 1) in red fish, zeaxanthin in anchovies, flatfish, and shark, tunaxanthin, lutein, and zeaxanthin in brackish water fish and lutein and zeaxanthin in fresh water fish (13,14). Several other carotenoids have been isolated and characterized from fishes and new carotenoids are continuously being identified. Matsuno *et al.* (15) isolated 2 new carotenoids parsiloxanthin and dihydroparsiloxanthin from Japanese catfish. Yamashita *et al.* (16) reported the presence of a new apocarotenoid micropteroxanthin in black bass *Micropterus salmonides.* Two new carotenoids, salmoxanthin and deepoxysalmoxanthin, were isolated from fishes belonging to salmonidae (17).

Crustaceans such as shrimp, prawn, lobster, krill, and crab contain astaxanthin (Fig. 1) as their main pigment present in free forms, esterified or as bound form to macromolecules such as protein or chitin (13). Crustaceans absorb the pigments from the diet and deposit them as such or transfer them metabolically to keto or hydroxy derivatives (18). Astaxanthin and its esters are the main pigment in crustaceans irrespective of the species and the environment from which they are harvested (19). Astaxanthin was found to be present in both enatiomeric and meso forms in shrimp *Pandalus borealis* (20). Free and esterified carotenoids were also found as the main pigments in deep-sea shrimps (21). In crayfish along with astaxanthin other pigments such as idonirubin, idoxanthin, and canthaxanthin have been isolated (22). Overall astaxanthin is the main carotenoid pigment found in aquatic animals (23), while fucoxanthin is specifically abundant in aquatic plants.

Carotenoids from aquatic plants and microorganisms accumulate in animals either unchanged from the diet or are metabolically modified in the body. Modification is most apparent in the lower animals (13). Occurrence of various kinds of carotenoids has been observed in these aquatic animals such as mollusks, echinoderma, tunicates, sea anemone, marine sponges, etc (14). Presence of mytiloxanthin and isomytiloxanthin is reported from the mussel *Mytilus edulis* (24). Maoka and Matsuno (25) isolated pectanol and 4-hydroxyaloxanthin from Japanese sea mussel *Mytilus corscus*. Several other acetylenic carotenoids have been isolated from starfish (26). Occurrence of mactraxanthin (27), 2 representative fucoxanthin metabolites, amarouciaxanthin (28), and fucoxanthinol (29) (Fig. 1) is reported from clams. Amarouciaxanthin was also isolated from tunicate species (30,31). Fujiwara *et al.* (32) isolated crassostreoxanthin from the oyster *Crassostrea gigas*. In sea urchins β-echinenone was observed to be the major carotenoid (33). Cucumarioxanthin, a novel carotenoid has been identified in sea cucumber (34).

Antioxidant Activity of Astaxanthin

Carotenoids serve a protective role by effectively dissipating excess energy, preventing the formation of ROS, and by deactivating singlet oxygen generated during the photosynthetic process (35-39). In addition, dietary carotenoids react with a wide range of free radicals such as $CCl_3O_2^{\cdot}$, $RSO_2^{\cdot}$, $NO_2^{\cdot}$, and various arylperoxy radicals via electron transfer producing the radical cation of the carotenoid.

The quenching of singlet oxygen by carotenoids has been attributed mainly to physical mechanism, where the excess energy of singlet oxygen is transferred to carotenoid. The carotenoid with added energy is excited to triplet state and upon losing the energy as heat relaxes to singlet state without change in the structure as follows,

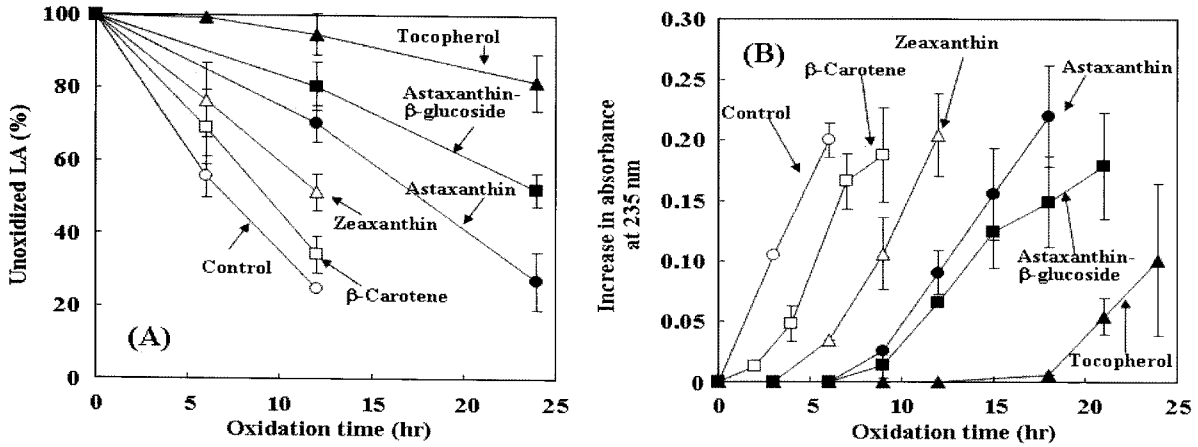

Fig. 2. Effect of carotenoids on oxidative stability of 1-palmitoyl-2-linoleoyl phosphatidylcholine in liposomes. Oxidative stability was measured by decrease in unoxidized lonoleate (LA) (A) and increase in conjugated dienes ($A_{235\ nm}$) (B). Each value is expressed as the mean±SD from 3 separate experiments. (From Ref. 47, with permission).

$^1O_2$*+^{1}Car → $^3O_2$+^{3}Car*
^{3}Car* → ^{1}Car+heat

The singlet oxygen quenching rates of carotenoids is characterized by the rate constant k_q, with larger the k_q values the faster being the quenching reaction. The quenching rate constant (kq- L/mol/sec) for astaxanthin (2.4×10^{10}) was found to be twice than that of β-carotene (1.4×10^{10}) and 80 times more than the antioxidant tocopherol (0.03×10^{10}) (40). Fukuzawa *et al.* (41) observed that singlet oxygen quenching of carotenoids is 40-80 times higher than that of α-tocopherol in ethanol, but only 6 times higher in liposomes. The authors concluded that the singlet oxygen quenching rates of carotenoids in a biological system depends on the factors such as, concentration of carotenoids in membranes, membrane localization of active groups, solubility of generation site of $^1O_2$ in membranes, and the mobility of carotenoids in membranes.

Another role of carotenoids as antioxidants is attributed to the scavenging of free radicles. The free radicals obtain the electron from other molecule or forms adducts with the other molecule. The electron rich status of carotenoids makes them more suitable for reaction with the free radicals thus avoiding the use of cellular components by the free radicals for reactions. Krinsky and Yeum (42) reviewed the carotenoid-radical interactions and suggested following 3 possible interactions,

1. Adduct formation:
   Car+R· → R-Car·
2. Electron transfer:
   Car+R· → Car·$^+$+R$^-$
3. Allylic hydrogen abstraction:
   Car+R· → Car·+RH

Mortensen *et al.* (43) demonstrated that the carotenoid-radical reactions not only depend on carotenoids but also on the nature of radical. With nitrogen dioxide radical, carotenoid forms a cationic radical and the reactivity was higher with lycopene and lutein than astaxanthin. With mercaptoethanol and glutathione thiyl radicals, astaxanthin showed higher order of reactivity than canthaxanthin, zeaxanthin, lycopene, and lutein.

Astaxanthin has been found to be more effective than β-carotene in preventing fatty acid preoxidation in chemical solutions (44) and delaying lipid peroxidation in membrane model (45). Goto *et al.* (46) demonstrated that the higher antioxidant activity of astaxanthin compared to β-carotene is due to trapping of radicals at the surface and inside the phospholipid membrane and the unique structure of the terminal ring moiety. We compared the antioxidant activity polar carotenoids including astaxanthin and astaxanthin-β-glucoside on phosphatidylcholine (PC) in liposomes and found that astaxanthin and astaxnathin-β-glucosides are highly active antioxidants (Fig. 2) (47). This result indicates that the antioxidant activity of carotenoids on PC liposomes depend not only to their ability to scavenge free radicals but also on their location and orientation in a PC liposome system and the extent of their incorporation into PC bilayers.

Further studies have confirmed that astaxanthin is a better agent to destroy free radicals than other carotenoids (48). In the study using fibroblast cells harvested from chicken embryos, astaxanthin effectively decreased the oxidative stress induced by addition of paraquat. Hill *et al.* (49) demonstrated that carotenoids except astaxanthin reacts with CCl_3O_2* radical to form cation radical and then the addition radical, whereas, astaxanthin behaves differently than other carotenoids, with its radical cation not forming initially but forming solely through the addition radical.

Antioxidant Activity of Fucoxanthin

Although it is most abundant carotenoid found in natural resources, investigations on antioxidative activity of fucoxanthin is limited. Nomura *et al.* (50) demonstrated the 1,1-diphenyl-2-picrylhydrazyl (DPPH) quenching activity of fucoxanthin isolated from the diatom *Phaeodactylum tricornutum*. Electron spin resonance (ESR) analysis showed the quenching ability of fucoxanthin against both organic radicals DPPH and 12-doxyl-steraic acid (12DS) (51). Yan *et al.* (52) demonstrated the strong DPPH radical scavenging activity of organic extracts from different edible seaweeds and fucoxanthin was identified as the active compound. Fucoxanthin has a unique structure including

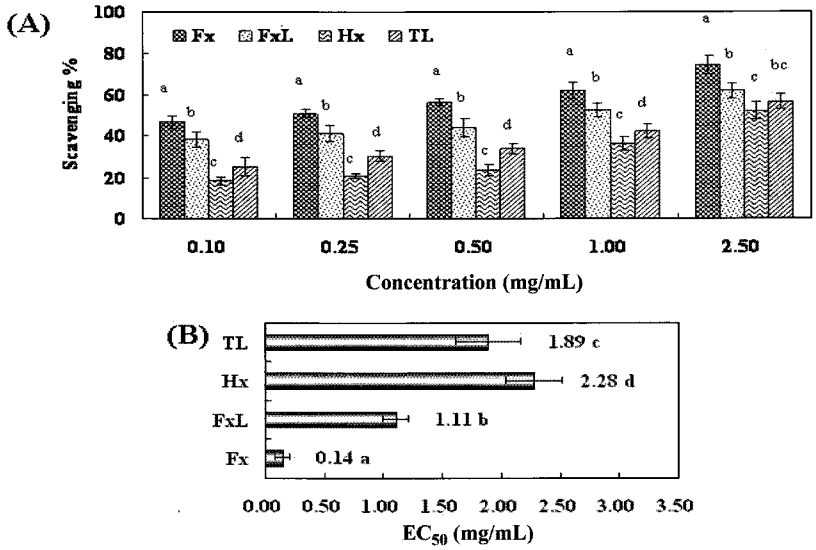

Fig. 3. Hydroxyl radical scavenging activity of carotenoids and α-tocopherol as measured by chemiluminescence technique. The activity of each compound was expressed as scavenging % (A) and concentration (μM) required for 50% scavenging (EC_{50}) (B). Fx, fucoxanthin; FxL, fucoxanthinol; Hx, halocynthiaxanthin; TL, α-tocopherol. (mean±SD; n=4); Bars with different superscripts for each concentration differ significantly (p<0.05). (From Ref. 57, with permission).

an unusual allenic bond and 5,6-monoepoxide in its molecule (Fig. 1). This structure will affect its antioxidant activity. The ability of carotenoid to quench singlet oxygen increase with increasing number of conjugated double bands (53), whereas antioxidant activity of carotenoids increases with the presence of functional group in terminal rings as seen in astaxanthin and fucoxanthin (54). Murakami *et al.* (55) screened 19 natural carotenoids for their structure-function relationship with respect to radical scavenging activity using human promyelocytic HL-60 cells. They found that presence of allenic bond as seen in fucocoxanthin and halocynthiaxanthin is an increasing factor for inhibition of superoxide and nitric oxide (NO) generation, while presence of 4-oxo-β-end group in their structure in astaxanthin and canthaxanthin enhances the NO generation.

Fucoxanthin is metabolized to other carotenoids in marine organisms (28-31). In sea squirt, *Halocynthia roretzi,* fucoxanthin is metabolized into fucoxanthinol and halocynthiaxanthin (Fig. 1) (31). Sugawara *et al.* (56) also demonstrated that fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human intestine cells and mouse. As many of the biological effects of carotenoids are related to their ability to scavenge ROS, recently, we analyzed the scavenging activity of fucoxanthin, fucoxanthinol, and halocynthiaxanthin against different radicals and also their ability to quench singlet oxygen (57).

DPPH radical and ABTS radical scavenging assay is one of the popular indirect methods of determining the antioxidative capacity of compounds (58,59). DPPH radical scavenging activity of fucoxanthin and fucoxanthinol were higher than alocynthiaxanthin with concentration (μM) required for 50% scavenging (EC_{50}) being 164.60, 153.78, and 826.39, respectively. ABTS radical scavenging activity of fucoxanthinol (EC_{50}-2.49 μM) was stronger than fucoxanthin (EC_{50}-8.94 μM). Further, hydroxyl radical scavenging activity as measured by chemiluminescnce technique showed that the scavenging activity by fucoxanthin was 7.9 times higher than fucoxanthinol, 16.3 times higher than halocynthiaxanthin and 13.5 times higher than α-tocopherol.

Similar trend was observed when the hydroxyl radical scavenging was assessed by ESR technique. In the ESR technique for analysis of hydroxyl radical scavenging the hydroxyl radical generated by Fenton system was trapped by the spin trapping agent 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) and the signal intensity of the resultant DMPO-OH· spin adduct was measured (Fig. 3). The signal intensity of DMPO-OH· spin adducts was lower in presence of fucoxanthin (56.6% of control) compared to fucoxanthinol (81.7%) and α-tocopherol (92.5%) at concentration of 2.5 mg/mL. This indicates a higher reduction in signal intensity in presence of fucoxanthin, and thus higher scavenging activity. The scavenging activity of fucoxanthin, fucoxanthinol, and α-tocopherol, each at 2.5 mg/mL corresponds to 43.4, 18.3, and 7.5%, respectively. Halocynthiaxanthin did not show any reduction in signal intensity indicating no scavenging activity. The ESR analysis of superoxide radical scavenging activity also showed the superiority of fucoxanthin over other 2 carotenoids tested. Singlet oxygen quenching ability of the 3 carotenoids was lower than that of β-carotene with quenching rate constants (k_Q)(10^{10}/M/sec) being 1.19, 1.81, 0.80, and 12.78 for fucoxanthin, fucoxanthinol, halocynthiaxanthin, and β-carotene, respectively.

The major structural difference in these 3 carotenoids is the presence of an allenic bond in fucoxanthin and fucoxanthinol and an acetylene bond in the terminal ring of fucoxanthin. Hence, it may be assumed that allenic bond is responsible for higher antioxidant activity of fucoxanthin and fucoxanthinol. Eventhough, halocynthiaxanthin showed very poor scavenging activity, it has been reported that halocynthiaxanthin shows highest suppressive effect on

generation of free radicals in stimulated leucocytes (55). The reaction of carotenoids with radicals also depends on factors such as oxygen pressure particularly in the biological systems, resulting in shift towards prooxidant behavior. Thus it will be important that *in vitro* antioxidant activity of carotenoids also be confirmed with *in vivo* experiments.

Anticancer Activity of Astaxanthin

One of the important health benefits of carotenoids, which have received the greater attention, is their anticancer activity. In addition to the investigations on the development of curing agents for cancer, studies were also directed towards dietary supplements, which can act as cancer preventing agents. As the involvement of free radicals and oxidant stress in carcinogenesis was demonstrated, the antioxidant properties of carotenoids were predicted to be responsible for their anticancerous activity (35,60). With the finding that high doses of retinoids, synthetic analogs of vitamin A, could inhibit carcinogenesis in animal models, studies were then directed towards the use of vitamin A and provitamin A carotenoids in cancer prevention.

β-Carotene is the commonest provitamin A carotenoid found mainly in vegetable, fruits, and other plants. Apart from its ability to convert into vitamin A β-carotene is also used as a 'gold standard' model to study the relationship between carotenoid intake and cancer prevention over a several decades. On the contrary evidences suggest increased incidence of lung cancer in smokers when β-carotene was supplemented at pharmacological levels (61); and, increased mortality due to cardiovascular disease smokers, former smokers and asbestos exposed individuals in the β-carotene and retinol efficiency trial (62). These contra effects have lead researchers to focus on different carotenoid as alternatives.

The chemopreventive effect of astaxanthin against chemically induced urinary bladder and oral carcinoma in animal models have been reported (63,64). In addition, dietary treatment with astaxanthin during post initiation period of chemically induced colon carcinoma in rats was found to have chemopreventive effect (65). Administration of astaxanthin prior to treatment with restraint stress in mice resulted in reduction of metastatic nodules and lipid peroxidation and thus improving the antitumor response (66).

The mechanism of antitumor activity of astaxanthin was suggested to be partly due to the antioxidative property as it was found to suppress the ROS potentiated invasive capacity of rat ascites hepatoma cells in culture (67). Jyonouchi *et al.* (68) postulated that astaxanthin exerts antitumor activity by modulating the immune response against tumor cells. Other possible mechanisms suggested for the anticarcinogenic activity of astaxanthin are prevention of oxygen mediated cytotoxicity and genotoxicity and, induction of xeno-biotic metabolizing enzymes.

Anticancer Activity of Fucoxanthin

Revelation that, dietary supplementation of algal powders or extracts suppresses the carcinogenesis in rats (69,70) led

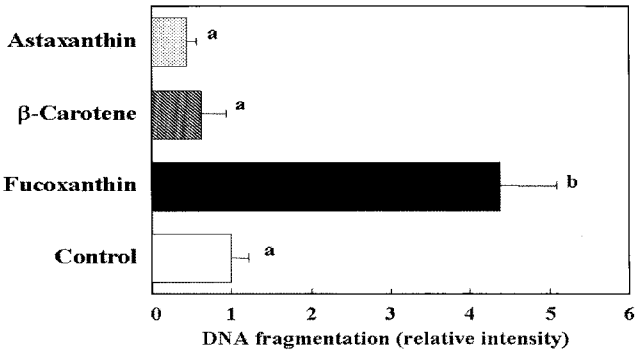

Fig. 4. Cell viability and DNA fragmentation in Caco-2 cells treated with carotenoids. From Ref. 76, with permission.

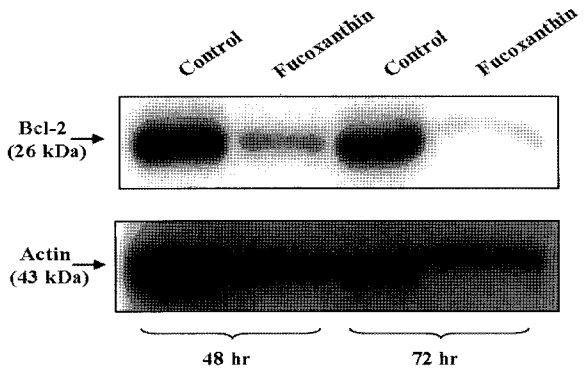

Fig. 5. Expression of Bcl-2 protein in Caco-2 cells treated with fucoxanthin. From Ref. 76, with permission.

to continued investigations on the effective anticancerous components in seaweeds. Fucoxanthin isolated from the brown algae, showed inhibitory effects on expression of *N-myc* genes and the growth of GOTO cells, a human neuroblastoma cell lines (71). Administering fucoxanthin in drinking water resulted in inhibition of chemically induced duodenal carcinoma in mouse (72).

The anticancerous effects of fucoxanthin have been established using different cancer line studies and possible mechanism suggested. The antiproliferative effect of fucoxanthin against human prostate cancer lines was observed (73) and it was attributed to the induction of apoptosis through caspace-3 activation (74).

We have demonstrated the antiproliferative activity of fucoxanthin against human leukemia cells and Caco-2 colon cancer cells. Fucoxanthin induced apoptosis in human leukemia cell (HL-60) lines (75) and colon cancer cell (Caco-2, HT-29, and DLD-1) lines (76). Fucoxanthin reduced the viability of Caco-2 cells at higher levels compared to astaxanthin and β-carotene and induced apoptosis by a dose dependent increase in cellular DNA fragmentation (Fig. 4). As the caspaces are know to play an important role in inducing the apoptosis (77), the involvement of caspase pathway was assessed by using broad spectrum caspace inhibitor, Z-VAD-fmk with fucoxanthin. It was also observed that apoptosis signaling in Caco-2 cells by fucoxanthin is mediated both by Caspace-dependent and -independent pathways as the inhibitor diminished DNA fragmentation by only 40%. In addition, as the level of apoptosis suppressing protein Bcl-

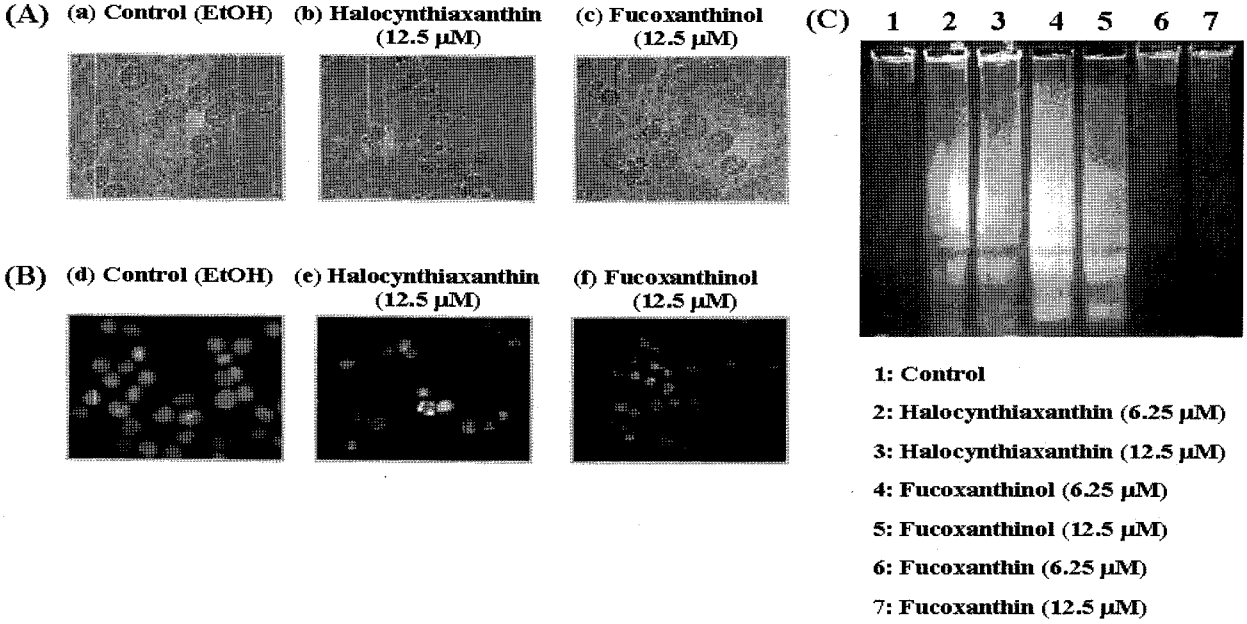

Fig. 6. Apoptosis induction in HL-60 cells by halocynthiaxanthin and fucoxanthinol. (A) Morphological changes, (B) fluorescence dye staining observation, and (C) agarose gel electrophoresis of DNA. (From Ref. 78, with permission)

2 was also reduced (Fig. 5), contribution of the down regulation of Bcl-2 protein in fucoxanthin induced apoptosis in Caco-2 cells was suggested.

As fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 cells and mice (56), the anticancerous activity of 2 its metabolites, fucoxanthinol, and halocynthiaxanthin (Fig. 1), isolated from the sea squirt *Halocynanthia roretzi* was assessed against different cancel cells (78). Both the metabolites were found to inhibit the growth of HL-60 human leukemia cells, MCF-7 human breast cancer cells and Caco-2 human colon cancer cells. The apoptosis inducing effect of the metabolites against HL-60 cells was higher than that of the fucoxanthin (Fig. 6).

These studies have indicated the potential of marine carotenoids as chemopreventive agents against some types of cancers. Further, investigations are needed to assess the efficacy of these carotenoids against different types of cancers as their effect may vary with the type of cancer cell lines used. In addition, studies with animal models may provide more insight into the anticarcinogenic effect of these carotenoids.

Antiobesity and Antidiabetic Effects of Fucoxanthin

Mitochondrial uncoupling proteins (UCPs) are the key molecules for metabolic thermogenesis and are the physiological defense against obesity (79) and their dysfunction leads to the development of obesity (80). Studies by Serra *et al.* (81) indicated that carotenoids can positively affect UCP1 expression in brown adipose tissues (BAT). However, as in adult humans the content of BAT is low and most of the fat is stored in white adipose tissue, we investigated the effect of seaweed lipids on UCP expression and accumulation of fat in white adipose tissues (WAT) of rat and mice (82). Feeding of lipids from the brown seaweed, *Undaria pinnatifida*, reduced the abdominal WAT weights in both in rats and mice and resulted in expression of UCP1.

As *Undaria* lipids mainly consists of glycolipids and fucoxanthin, we further examined the effect of these 2 constituents for their antiobesity effect (82). Feeding with fucoxanthin resulted in reduction of WAT weight and clear expression of UCP1 protein, but not with glycolipid feeding (Fig. 7). The results indicated the clear antiobesity effect of fucoxanthin by upregulating the expression of UCP1 in WAT resulting in reduced WAT weight.

UCP1 expression was found in WAT of 0.2% purified fucoxanthin-fed KK-A^y mice (83), although there was little expression in that of control mice. Expression of UCP1 mRNA was also found in WAT of 0.2% fucoxanthin-fed mice, but little expression in that of control. The finding that fucoxanthin induces both protein and mRNA expressions of UCP1 in WAT will give a clue for new dietary anti-obesity therapy. An enormous amount of data has been collected on thermogenesis in BAT through UCP1 expression. However, there had been little information on UCP1 expression in WAT induced by a dietary component until our report had appeared. Direct heat production by fat oxidation in WAT, therefore, will reduce risk of these diseases in humans.

KK-A^y mice in this study not only developed obesity but also hyperleptinemia and hyperinsulinemia along with insulin resistance. Therefore, glucose levels of mice fed the control diet reached levels higher than 400 mg/dL. On the other hand, mice fed the 0.1 and 0.2% purified fucoxanthin diets had significantly lower blood glucose concentrations of around 220 and 170 mg/dL, respectively (83). Furthermore, plasma insulin levels decreased in a dose-dependent manner after purified fucoxanthin intake (83).

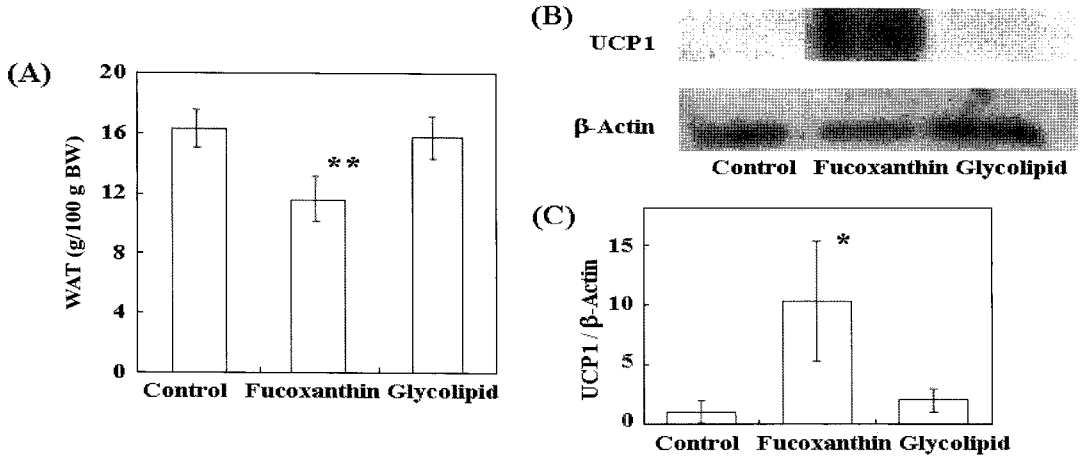

Fig. 7. White adipose tissue (WAT) weight (A), Western blot analysis of UCP1 (B), and UCP1 protein expression (C) in mice fed fucoxanthin and glycolipids from *Undaria*. Significant different from control at $*p<0.05$; $**p<0.01$. (From Ref. 82, with permission)

Anti-inflammatory Effect of Novel Marine Carotenoids

Inflammation is a normal protective response of human body to tissue damage or infection. However, excessive (chronic) inflammation often adversely affects health and rises in incidence of many diseases. It has been reported that persistence of chronic inflammation exposure increases the risk of obesity, insulin resistance, type 2 diabetes, cardiovascular, or cancer (84,85).

The inflammation process is initiated by the synthesis and secretion of pro-inflammatory cytokines, such as tumor nuerosis factor (TNF)α, interleukin (IL)-1β, and IL-6. The excessive production of inflammatory cytokines and the subsequent increase in reactive oxygen and nitrogen species are recognized as character of inflammation. This events associated with the acute inflammation is usually regulated by the secretion of anti-inflammatory cytokines. The intracellular anti-oxidants also regulate the development of inflammation.

Dietary fucoxanthin attenuates the body weight gain and the development of WAT in the diabetes/obesity mouse KK-A^y (82). Further, fucoxanthin improved blood glucose and insulin levels in KK-A^y mice (83). Adipose tissue is a major site of energy storage and plays an important role in fat and glucose metabolism. In addition, adipose tissue is now recognized as a major endocrine and secretary organ, releasing biologically active mediators termed adipocytokines (86,87). These adipocytokines have been shown to affect insulin sensitivity, glucose and lipid metabolism in the body. For example, TNFα is elevated in obesity and plays an important role in the development of insulin resistance and type 2 diabetes (88,89). In addition, resistin has been reported to affect insulin sensitivity (90), while adiponectin is known to play an important role in maintaining insulin sensitivity and glucose homeostasis (91).

Fucoxanthin down regulated TNFα mRNA level in WAT compared to that of control group, while adiponectin mRNA were not affected by fucoxanthin (Fig. 8) (83). Resistin mRNA level in WAT of the mice fed fucoxanthin also tended to be lower, but not significantly, than that of control group. This data suggests that fucoxanthin improves insulin resistance and decreases blood glucose level, at least in part, through the down-regulation of TNFα mRNA in WAT of diabetes/obesity KK-A^y mice.

Further, fucoxanthin suppressed leucoyte and protein infiltration, and productions of NO, PGE2, and TNFα induced in rat aqueous humour by injection of lipopolysaccharide (LPS) (92). The expression of induciblenitric oxide synthase (iNOS) and COX-2 protein in RAW264.7 cells induced by LPS also reduced significantly by fucoxanthin treatment. LPS injection is known to induce endotoxin-induced uveitis, which is an acute anterior segment intraocular inflammation (93). On the other hand, COX-2 and iNOS gene possess binding sites for NF-κB. These results indicate that fucoxanthin attenuates the inflammation of EIU by the inhibition of iNOS and COX-2 expression through the suppression of NF-κB activation, and exhibits anti-inflammatory effects on eye *in vivo*.

Sea squirts *Halocynthia roretzi*, being familiar to Japanese and Korean people as seafood, contains acetylenic carotenoids such as halocynthiaxanthin, alloxanthin, and diatoxanthin (Fig. 1) (94). We have found that all-*trans* alloxanthin and all-*trans* diatoxanthin isolated from *H. roretzi* suppressed the LPS-induced expression of IL-1β and IL-6 mRNA and protein in RAW264.7 cells (95). Furthermore, 9-*cis* isomers of alloxanthin and diatoxanthin also down-regulated IL-1β and IL-6 mRNA in RAW264.7 cells (Fig. 9). IL-1β has been shown to be an important cytokine in chronic inflammatory diseases (96). Although LPS stimulation is an acute inflammatory model (97), our results provide useful information regarding the effectiveness of alloxanthin and diatoxanthin with acetylenic structure for preventing both acute phase and chronic inflammation.

Alloxanthin and diatoxanthin also attenuated the expression of COX-2 and iNOS mRNA in RAW264.7 cells stimulated by LPS (95). Therefore, alloxanthin and diatoxanthin suggest showing anti-inflammatory effects through the down-regulation of the mRNA for COX-2 and iNOS as well as pro-inflammatory cytokines in the activated macrophages. Down-regulation of IL-1β mRNA by β-carotene was intended to be weaker compared to those of alloxanthin and diatoxanthin. Zeaxanthin did not suppress expression of pro-inflammatory cytokine mRNA

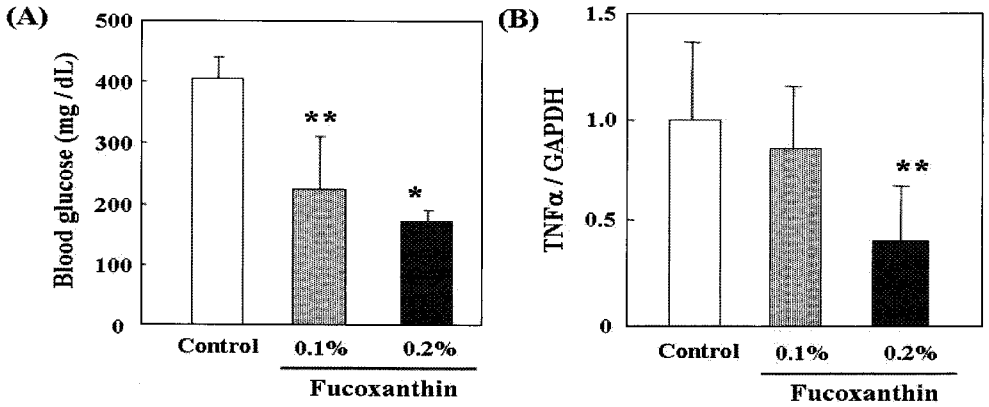

Fig. 8. Effect of fucoxanthin blood glucose (A) and TNFα (B) mRNA levels in white adipose tissue of diabetes/obesity KK-*A*^{y} mice fed fucoxanthin. Significant different from control at *p<0.05; **p<0.01. (From Ref. 83, with permission)

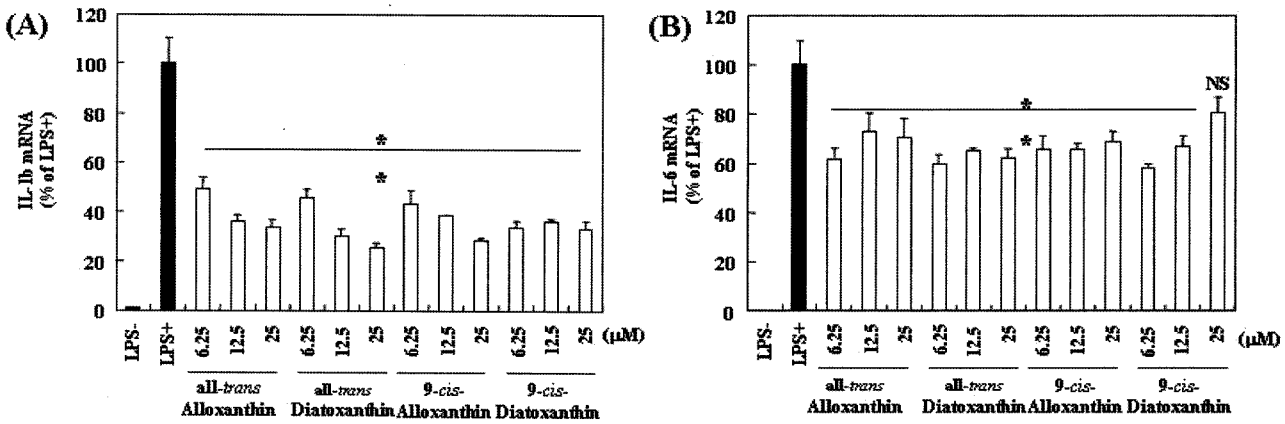

Fig. 9. Effect of alloxanthin and diatoxanthin on LPS-induced expression of inflammatory cytokine mRNA in RAW264.7 cells. *Significant different from control (LPS+) (p<0.01). (From Ref. 95, with permission)

in RAW264.7 cells treated with LPS in our study. In addition, down-regulation of COX-2 and iNOS mRNA by β-carotene and zeaxanthin were also weaker than those of alloxanthin and diatoxanthin. Although the mechanism involved has not been elucidated in detail, these results indicate that the actual structure of the carotenoids, in especially acetylene bond, is the key to their anti-inflammatory effects.

Conclusion

With the increasing knowledge of bio-functional properties associated with marine products, utilization of these materials has accelerated. Marine oils, proteins, and carbohydrates are known as major marine functional and nutraceutical resources. Marine oils serve as a rich source of long-chain omega-3 polyunsaturated fatty acids. Marine proteins and oligosaccharides or their hydrolyzates are the other major contributors of the bioactivites. In addition, marine carotenoids have received considerable attention because of their various functions in human health. Many studies showed that marine carotenoids have higher biological activity than those from terrestrial origin. These higher nutritional activities of marine carotenoids depend on their specific chemical structures. The anti-obesity effect of edible seaweed carotenoid, fucoxanthin, is very interesting, as its molecular mechanism has been made clear and its activity depends on the protein and gene expressions of UCP1 in WAT, although UCP1 is usually only found in BAT.

However, the nutrigenomic approach for beneficial physiological effects of other carotenoids has been a little. Investigations are needed to evaluate the mechanisms with special reference to their regulations on relative gene and protein expressions. Unless human intervention studies are conducted it is difficult to predict the bigger role of marine carotenoids in disease prevention. It is hoped that further studies will provide a positive results with respect to the application of these carotenoids as neutraceuticals.

Acknowledgment

This work was supported by 'Research and Development Program for New Bio-industry Initiatives' of Bio-oriented Technology Research Advancement Institution, the Grant-in-Aid for Scientific Research and the 21st Century COE Program of the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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