

Biological functions and activities of animal carotenoids

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Abstract— Astaxanthin, one of the dominant carotenoids in marine animals, showed both a strong quenching effect against singlet oxygen, and a strong scavenging effect against free radicals. These effects are considered to be defence mechanisms in the animals for attacking these active oxygen species. The activities of astaxanthin are approximately 10 times stronger than those of other carotenoids that were tested, namely zeaxanthin, lutein, tunaxanthin, canthaxanthin and β -carotene, and 100 times greater than those of α -tocopherol. Astaxanthin also showed strong activity as an inhibitor of lipid peroxidation mediated by these active forms of oxygen. From these results, astaxanthin has the properties of a "SUPER VITAMIN E".

INTRODUCTION

Studies on biological functions and activities of carotenoids have been mainly performed in the fields of photosynthetic plants, algae and bacteria (ref.1), and two major functions have been revealed: 1) a light-harvesting role in the antenna complexes of the chloroplast in photosynthesis. 2) as protecting agents against the harmful photooxidative effects of bright light.

On the other hand, the well established biological function of carotenoids in animals is as vitamin A precursors (refs.2-4). β -Carotene or other carotenoids which possess a β -retinylidene or 3-dehydroretinylidene end-group can easily be bioconverted into retinoids through cleavage of the C-15,15' double bond by β -carotene-15,15'-oxygenase. Carotenoids without such an end-group show little or no pro-vitamin A activity, and any significance of these carotenoids remains unknown.

For the past ten years, our group has studied ovarian carotenoids in marine fish and shellfish, and astaxanthin was found to be the most widely distributed and major carotenoid (ref.5). This carotenoid, however, possesses neither β -retinylidene nor 3-hydroxyretinylidene end-group and shows little pro-vitamin A activity (ref.4). Moreover, it is not considered to be an effective protecting colorant because the color of astaxanthin, pink to red, is far different from that of the surroundings. In the case of the ovaries, astaxanthin usually exists both in the free form and bound to protein as astaxanthin-protein complexes, but not in esterified form (ref.5). These studies showed the possibility of another function of carotenoids, especially astaxanthin in the free form.

In the case of photosynthetic plants, β -carotene, lutein and other carotenoids play roles as quenchers or scavengers against active oxygen species for the purpose of photoprotection against bright sunlight. Our group has therefore searched for a similar role for carotenoids in animals, and has found interesting activities of these carotenoids, especially of astaxanthin.

In this article, I will describe the quenching or scavenging effects of animal carotenoids, especially astaxanthin, against active oxygen species, singlet oxygen(1O_2) and hydroxy-radical($\cdot OH$), and against organic free radicals, and compare the effects with those of other carotenoids and α -tocopherol(vitamin E).

QUENCHING EFFECTS AGAINST SINGLET OXYGEN

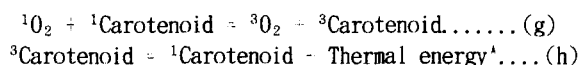
The strong quenching effect of β -carotene against singlet oxygen in plants has been known for over twenty years (ref.6), but, in the case of animal carotenoids, such activity still remains unknown. In this chapter, the activity of astaxanthin is described, and compared with those of the other carotenoids and α -tocopherol as controls (ref.7).

Singlet oxygen was produced by methylene blue under irradiation with white light, and the quantity of 1O_2 was measured by determining the peroxide value of linoleic acid dependence on 1O_2 by the TBA(thiobarbituric acid) method (ref.7). This *in vitro* model assay system was employed to check the inhibitory activities of astaxanthin, α -tocopherol and other carotenoids by measuring the increase in TBA value. The increase of the value in the absence of any carotenoids or other compounds was determined as a reference, and the carotenoid activities were calculated by comparing with this reference value. The ED_{50} of astaxanthin was approx. 80 nM by this calculation, and the relative inhibitory activities of the other samples are shown below (ref.8).

Astaxanthin >>> astaxanthin diester.....	(a)
Astaxanthin >> α -tocopherol.....	(b)
Astaxanthin > zeaxanthin > β -carotene.....	(c)
Astaxanthin > canthaxanthin > β -carotene.....	(d)
(3S, 3'S)-astaxanthin \approx (3S, 3'R)-astaxanthin \approx (3R, 3'R)-astaxanthin..	(e)
Zeaxanthin > lutein > tunaxanthin.....	(f)

From result (a), astaxanthin diester showed no activity, but, this compound can be activated to astaxanthin in the free form by esterase enzymes after administration to animals, so that free astaxanthin and astaxanthin diester show the same activity when they are used in the diet. In the case of α -tocopherol, the ED_{50} is approx. 1 μM in this system, and this value is about one-hundredth of that of astaxanthin from result (b). α -Tocopherol has been used as an antioxidant in lipids, drinks and other foods, and this result shows that astaxanthin has the possibility to be used for this purpose instead of α -tocopherol. Results (c) and (d) showed that carbonyl and hydroxyl groups in carotenoids are important for the quenching activities, perhaps because of the affinities between carotenoids and free fatty acid (linoleic acid) or the hydrophobicities of the solvent used in the model experiments. From result (e), the activity is clearly independent of the steric configuration of the carotenoid. These results showed that the carotenoids and 1O_2 come in contact directly, and the energy transfer is very rapid. This phenomenon is considered to be almost the same as the reaction between β -carotene and 1O_2 in photosynthetic plants. Result (f) showed that the number of the conjugated double bonds is very important to the activities. This result also indicated that the magnitude of the quenching depends on the polyene chain, and the

effects are shown in equations (g) and (h). These equations are the same that Foote and Denny (ref.6) described in the case of β -carotene, and the quenching effects are suggested to be due to the vibration of the polyene C=C and C-C bonds. That is, the energy of the singlet state oxygen is quickly transferred to the carotenoid by direct contact, and the carotenoid in the triplet state relaxes to the singlet state very quickly by the transformation of the potential energy into thermal energy through the C=C and C-C vibration of the polyene chain.



Overall, from these results, astaxanthin is the best compound of the samples used and a role similar to that of β -carotene or lutein for photoprotection of plant chloroplasts from bright sunlight is presumed to exist in marine fish and shellfish. On the basis of these structure-activity relationships, idoxanthin, β -carotenetetrals, mactraxanthin and astacene also have a possible strong protective action against ${}^1\text{O}_2$.

SCAVENGER EFFECTS AGAINST FREE RADICALS

β Carotene has been revealed to be an efficient scavenger of free radicals, notably under low partial pressure of oxygen (ref.9). Terao's group (ref.10) examined the effects of carotenoids on peroxy radical-mediated lipid peroxidation in model systems, and reported that astaxanthin and canthaxanthin showed more efficient scavenging effects against free radicals than did β -carotene and zeaxanthin and they concluded that carotenoids which possess oxo groups were more efficient than those without oxo groups.

I have also investigated the effects of carotenoids as free radical scavengers using the TBA method with ferrous ion of hemeprotein as a free radical producer with heat and linoleic acid as an acceptor (ref.11). Astaxanthin, β -carotene, lutein, zeaxanthin, tunaxanthin and canthaxanthin were applied in this in vitro model assay system, and the most efficient scavenger was found to be astaxanthin, followed by zeaxanthin and canthaxanthin. The ED_{50} of astaxanthin was approx. 200 nM, and those of all the carotenoid samples used were in the range 200 - 1000 nM. In contrast, the ED_{50} of α -tocopherol was approx. 3 μM (Table 1). These results showed that the number of carbonyl and hydroxyl groups present was very important, and suggested that these groups had an affinity for free radicals, linoleic acid or the solvent (100 % ethanol, 50 % aqueous ethanol, or 50 % aqueous dimethylsulfoxide) used in this experiment. In these experimental conditions, idoxanthin, astacene or crustaxanthin could also have strong activity like astaxanthin. This structure-activity relationship is very similar to that for the quenching effect against singlet oxygen. Moreover, from these results, the important structural feature of carotenoids is again suggested to be the polyene chain.

Table 1. ED_{50} value of carotenoids and α -tocopherol as free radical scavengers

Scavenger	ED_{50} (nM)
Astaxanthin	200
Zeaxanthin	400
Canthaxanthin	450
Lutein	700
Tunaxanthin	780
β -Carotene	960
α -Tocopherol	2940

By these two model experiments, astaxanthin has been proved to be an efficient free radical scavenger. Further experiments to confirm this activity with red blood cells and mitochondria from rats (ref.12) are described below.

1) Free radicals were produced by the action of dialuric acid and ferric ion on rat red blood cells with heat, and this initiated lipid peroxidation in the membrane of the cells through chain reaction. The inhibitory activity of astaxanthin on the peroxy radical-mediated lipid peroxidation were investigated in comparison with a control which contained neither carotenoids nor other antioxidants. The results are shown in Fig. 1.

The ED_{50} of astaxanthin is approx. $2 \mu\text{M}$ and this activity is very strong compared with that of other commercially available antioxidants.

2) Free radicals were produced by action of ferrous ion (Mohr's salt) with heat on a homogenate of rat mitochondria, and lipid peroxidation in the homogenate was caused by chain reactions. The inhibitory activity of astaxanthin on the peroxy radical-mediated lipid peroxidation was investigated, compared with a control which contained neither carotenoids nor other antioxidants. The activity of α -tocopherol was also measured in the same manner. The results are shown in Fig. 2.

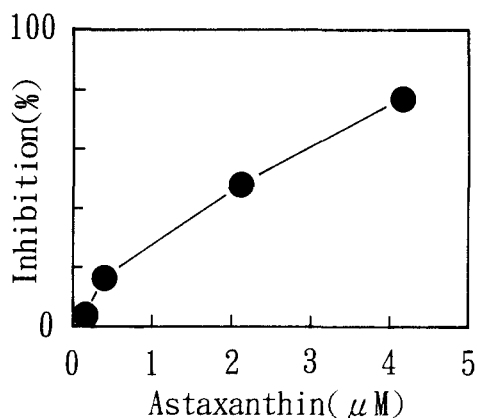


Fig. 1. The inhibitory activity of astaxanthin against the action of free radicals on red blood cells of rat.

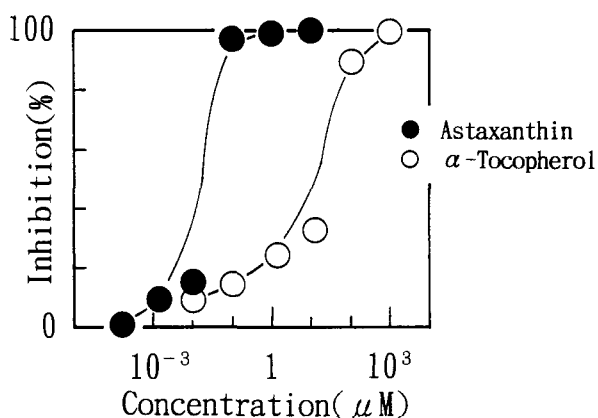


Fig. 2. The inhibitory activity of astaxanthin and α -tocopherol against the action of free radicals on rat mitochondria.

The ED_{50} of astaxanthin was approx. 40 nM , whereas that of α -tocopherol was approx. $10 \mu\text{M}$. Astaxanthin thus showed activity more than 100 times greater than α -tocopherol in this experiment. No other compound showed such a strong activity. This result suggested that the affinity between the scavenger and the membrane of the mitochondria was very important for the activity, and this affinity was assumed to be based upon both hydrophobic binding and hydrogen bonding. In the case of astaxanthin, the polyene chain and the 3-hydroxy, 4-keto substituents of the cyclohexene ring, respectively, will play these roles.

SCAVENGER EFFECTS AGAINST HYDROXY RADICALS

Active oxygen species are classified into two types; 1) those with long lives and less strong effects. 2) those of short lives and strong effects. In the first case, the scavengers are usually high molecular mass compounds, i.e. proteins and enzymes, and the time scale for scavenging is very long.

The type 1 active oxygen species are superoxide, hydrogen peroxide and others. In the second case, which includes singlet oxygen and free radicals, the quenchers and scavengers are almost all low molecular mass substances, and our group (ref. 12) examined the activity of astaxanthin against hydroxy radicals($\cdot\text{OH}$) which are classified in group 2) and are very important active oxygen species. I will review the scavenging effect of astaxanthin in experiments with rat red blood cells.

Male rats, six to seven weeks old, were divided into three groups: ① Given regular diet. ② Given a vitamin E deficient diet. ③ Given a vitamin E deficient diet supplemented with 1 mg/ 100 g astaxanthin. The rats in the three groups were reared for four weeks. The ghosts of the red blood cells were obtained by the method of Dodge *et al.* (ref. 13), and the hydroxy radical-mediated lipid peroxidations were produced by superoxide (xanthine and xanthine oxidase) and ferric ion. Malondialdehyde produced through this procedure in each group was measured by the TBA method. The results are shown in Fig. 3.

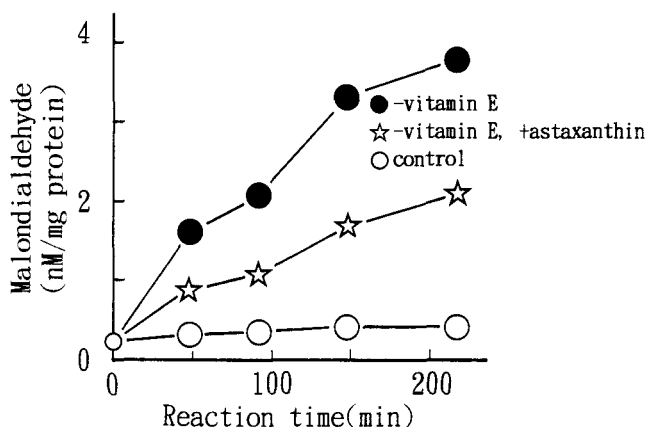


Fig. 3. The inhibitory activity of astaxanthin and α -tocopherol against the action of hydroxy radicals on ghosts of rat red blood cells.

As shown in Fig. 3, the ghosts of the cells obtained from the rats fed the control diet suffered almost no lipid peroxidation, whereas those from the rat fed a vitamin E deficient diet apparently produced a high concentration of malondialdehyde. In the case of those from the rats fed vitamin E-deficient diet supplemented with astaxanthin, the production of malondialdehyde was clearly depressed. These results indicate that astaxanthin plays a role as an inhibitor against lipid peroxidation, not only in *in vitro* model systems but also *in vivo*, and it is supposed that in this case the reaction targets that are protected are phospholipids in the membrane.

CONCLUSION

Astaxanthin is revealed to be either a quencher or a scavenger of active oxygen species in these studies, and the kind of the activity is very similar to that of vitamin E. Generally speaking, carotenoids showed strong activities, and astaxanthin was the strongest of the carotenoids examined. This suggests a role for astaxanthin as a "SUPER VITAMIN E".

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