Mini Review

Novel Antioxidative Activity of Astaxanthin and Its Synergistic Effect with Vitamin E

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Summary Astaxanthin (Asx) is known to be a potent quencher of singlet oxygen and an efficient scavenger of superoxide anion. However, the scavenging activity of Asx toward the hydroxyl radical was currently unclear because the high lipophilicity of Asx prevents analysis of such activity in water. Liposomes containing Asx (Asx-lipo) were previously shown to be dispersed in water. Analysis of the hydroxyl radical scavenging activity of Asx-lipo demonstrated a dose-dependence in water, with the effect of Asx being more potent than the vitamin E α -tocopherol (α -T). Furthermore, liposomes co-encapsulating Asx and vitamin E derivatives, namely tocotrienols (T3), showed a synergistic elimination effect on singlet oxygen and hydroxyl radical, although the antioxidative activity of liposomes co-encapsulating Asx and α -T was lower than the calculated additive value of each independent activity. A calculation of the most stable structure of Asx in the presence of α -T or T3, suggested that only T3 was able to hydrogen bond with Asx, and the Asx polyene chain partially interacting with the α -T3 triene chain, which could explain the synergistic effect between Asx and T3, but not Asx and α -T. This review introduces the hydroxyl radical scavenging activity of Asx, and its synergistic effect with T3.

Key Words astaxanthin, vitamin E, tocotrienol, synergistic effect

Astaxanthin (Asx; Fig. 1) is more effective than β -carotene at quenching of singlet oxygen, as well as preventing lipid peroxidation in biomembranes (1–5). The direct radical scavenging activity of Asx has been confirmed using the synthesized radical 1,1-diphenyl-2-picrylhydrazyl (6). Moreover, it was reported that some Asx derivatives, such as disodium disuccinate astaxanthin and astaxanthin-amino acid conjugate, could scavenge the superoxide anion radical (7, 8). It was previously suggested that the potent antioxidative activity of Asx in biomembranes could be attributable to the conjugated polyene and terminal ring moieties of Asx that trap radicals at the interface and inner lipophilic regions of lipid membranes, respectively (4). Highly active reactive oxygen species (ROS), such as the superoxide anion radical and the hydroxyl radical, are thought to be causative agents of severe diseases, such as arteriosclerosis and ischemic reperfusion injury (9–11). Therefore, Asx is expected to prevent the pathogenesis of such ROS-related diseases by scavenging highly active ROS. However, there were no reports on the scavenging activity of Asx toward the hydroxyl radical; although, Asx was shown to inhibit radical generation in cultured cells induced by treatment with Fenton's reagent (12). The high lipophilicity of Asx would prevent analysis of the hydroxyl radical scavenging in water. Thus, a liposomal formulation of Asx was suggested to facilitate analysis of such activity in aqueous solution, because liposomes allow for homogeneous dispersion of lipophilic Asx. In

a previous report, it was suggested that Asx penetrates through the liposomal membrane, and that the terminal rings of Asx interact with the polar head groups of membrane lipids via hydrogen bonding (4). In such a system, Asx is available to react with extraneous free radicals in the aqueous phase, with the terminal ring moieties of Asx located at the membrane interface. Therefore, Asx-encapsulated liposomes (Asx-lipo) represent an ideal formulation for measurement of the hydroxyl radical scavenging activity of Asx in water.

1. Novel Antioxidative Activity of Astaxanthin Against the Hydroxyl Radical

Direct observation of the reaction of β -carotene with the hydroxyl radical was previously confirmed using a photo-Fenton reagent to generate hydroxyl radicals in acetonitrile/tetrahydrofuran solution (13), while similar measurements have not been performed in water. Asx-lipo was shown to decrease hydroxyl radical-dependent chemiluminescence intensity, which indicates hydroxyl radical production by the Fenton reaction, in a dose-dependent manner in water with respect to Asx in egg yolk phosphatidylcholine liposomes (EPC-lipo) (Fig. 2) (14). This result indicates that Asx encapsulated in EPC-lipo membranes can scavenge hydroxyl radicals in water. As mentioned above, the terminal rings of Asx are thought to be located at the membrane interface, while the polyene moiety would be embedded in the lipophilic region of the lipid membranes (4). Thus, the terminal rings of Asx at the interface of the liposomal membrane should be available to scavenge hydroxyl

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radicals. The IC_{50} value of Asx-encapsulated EPC-lipo (Asx-EPC-lipo) against the hydroxyl radical was lower than that of EPC-lipo encapsulating α -tocopherol (α -T) (14). This result indicates that Asx encapsulated in EPC-lipo is a more effective scavenger of hydroxyl radicals generated in water than the liposomal formulation of α -T. The potent hydroxyl radical scavenging activity of Asx may be due to dual radical trapping by the terminal ring, as well as the conjugated polyene moieties of Asx at the interface and within the lipid membrane (4). Asx-lipo comprised of dimyristoyl phosphatidylcholine (DMPC), of which the acyl chains were saturated, also decreased the hydroxyl radical-dependent chemiluminescence intensity in a dose-dependent manner (14). The Asx-encapsulating DMPC-lipo exhibited nearly the same scavenging effect on hydroxyl radicals as Asx-EPC-lipo. Thus, saturation of the lipid acyl chains of the liposomes did not affect the scavenging activity of Asx. Asx-

Fig. 1. Chemical structure of astaxanthin (Asx).

Fig. 2. Effect of Asx-EPC-lipo on hydroxyl radical-dependent chemiluminescence intensity. Chemiluminescence intensity is represented as a relative percentage of control EPC-lipo. Data are shown as means with SD from separate measurements ($n > 3$). Statistical significance relative to control EPC-lipo without Asx is represented as * $p < 0.05$ or ** $p < 0.01$.

lipo exhibits a red color due to absorption of the polyene chain. In particular, Asx-lipo gave an absorption peak at 470 nm, which is nearly the same as that previously reported for Asx in acetonitrile/tetrahydrofuran solution (13). Upon generation of hydroxyl radicals in the Asx-lipo suspension, the peak absorbance values for Asx decreased (14), suggesting that the conjugated polyene chain moiety is disrupted by hydroxyl radicals, and that Asx encapsulated in liposomes scavenges hydroxyl radicals via direct reaction with its polyene moiety.

2. Synergistic Antioxidative Effect of Astaxanthin with Vitamin E Tocotrienol

Vitamin E (tocopherols and tocotrienols; Fig. 3) has long been known as a lipophilic antioxidant (15). The various derivatives of vitamin E are designated as α -, β -, γ -, and δ -tocopherol (α -, β -, γ -, and δ -T, respectively) and -tocotrienol (α -, β -, γ -, and δ -T3, respectively). The phenolic hydroxyl group of the vitamin E chroman ring is the active site for ROS scavenging, and vitamin E is thought to prevent ROS attack of lipid membranes via localization of the hydroxyl group at the lipid membrane interface (16). Thus, vitamin E is recognized as a useful antioxidant for preventing various ROS-related diseases, such as atherosclerosis, as well as for food preservation. Since Asx and vitamin E are effective antioxidants, a combination of these compounds was expected to additively enhance their individual activities. The ability of a combination of Asx and vitamin E to prevent oxidative damage in a diabetic rat model was previously studied, and Asx was found to complement the antioxidative effect of vitamin E by preventing decreases in α -T caused by oxidation (17).

Based on the results of the previous in vivo study, it was hypothesized that co-existence of Asx and vitamin E within the same lipid membranes could facilitate functional complementarity. The in vitro antioxidative activities of liposomes co-encapsulating Asx with α -T or T3 toward singlet oxygen and hydroxyl radicals were evaluated. In particular, liposomes co-encapsulating Asx and vitamin E reduced singlet oxygen-dependent chemiluminescence intensity more effectively than liposomes encapsulating either Asx or vitamin E alone (18). For liposomes co-encapsulating Asx and α -T (Asx/ α -T-lipo), the actual quenching activity was lower than the combined values of Asx-lipo and liposomes containing α -T (α -T-lipo). Thus, an additive effect of both antioxidants

Fig. 3. Chemical structures of tocopherols and tocotrienols.

Fig. 4. Optimized structures of Asx and α -T3. The minimum energy conformation of Asx with α -T3 was calculated. Hydrogen bonding between a hydroxyl moiety or a carbonyl moiety in the terminal ring of Asx and the hydroxyl moiety in the chroman ring of α -T3. The dotted circle indicates a hydrogen bonding region between Asx and α -T3. The dotted arrows indicate intermolecular interactions between the Asx polyene chain and the α -T3 triene chain.

was not observed upon combination. In contrast, the actual measured values for liposomes co-encapsulating Asx with T3 were higher than the calculated combined values. These results suggest that co-encapsulation of Asx and T3 produces a synergistic effect on the singlet oxygen quenching activity of these compounds (18). Asx/ α -T3-lipo also showed a higher preventative effect on hydroxyl radical-dependent chemiluminescence than did Asx-lipo or α -T3-lipo (18). The actual measured scavenging activity of Asx/ α -T3-lipo was higher than the calculated combined value of Asx-lipo and α -T3-lipo, wherein the ratio of the actual value was 1.4-fold higher than the additive activity. This result indicates that, as with singlet oxygen, Asx/ α -T3-lipo showed a synergistic scavenging activity of the two antioxidants toward hydroxyl radicals (18).

To elucidate the reason for the synergistic effect of the combination of Asx and T3, but not Asx and α -T, on antioxidative activity, the minimum energy structure of Asx with α -T or α -T3 was calculated by computer simulation (18). The predicted distances between the Asx terminal ring and the α -T chroman ring or the α -T3 chroman ring were approximately 3.8 Å and 1.8 Å, respectively. This result suggests that the hydroxyl moiety or carbonyl moiety in the Asx terminal ring can undergo hydrogen bonding with the hydroxyl moiety in the α -T3, but not the α -T, chroman ring (Fig. 4). In addition, the small distance value (ca 4.2 Å) suggests that the Asx polyene chain partially interacts with a triene chain of T3, although the distance between lipophilic

regions, such as the polyene chain of Asx and the triene chain of α -T3, would gradually spread for the other end of the chain. On the other hand, the distance between the Asx polyene chain and the α -T phytol chain was large (ca 8.8 Å). The triene chain of T3 could affect the electron state of the Asx polyene chain through intermolecular interactions between π -conjugated systems, although no interactions should occur between the Asx polyene and the α -T phytol moieties. Thus, the simulation results suggest that the synergistic effect of Asx and T3, but not Asx and α -T, on antioxidative activity is due to changes in the Asx polyene electron state resulting from intermolecular interactions with the T3 trienol chain. Hydrogen bonding at the terminal ring moieties of Asx with T3 is also expected to affect the electron state of the carbonyl-conjugated polyene structure.

Conclusion

Herein, the antioxidative activity of Asx toward the hydroxyl radical was demonstrated. The hydroxyl radical scavenging ability of Asx is thought to be due to direct reaction of the hydroxyl radical with the polyene chain and terminal ring moieties of Asx. Furthermore, intermolecular interactions between Asx and T3 are suggested to be responsible for the recently discovered synergistic antioxidative effect of Asx with the vitamin E compound T3. Therefore, utility of Asx as a radical scavenger is expected to be improved in the future by combination with other functional compounds.

Disclosure of State of COI

No conflicts of interest to be declared.

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