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Antioxidant action of sugar-pendant C_{60} fullerenes

Masanori Horie [a], Akiko Fukuhara [a], Yoshiro Saito [a], Yasukazu Yoshida [a], Hiroe Sato [b], Hiromi Ohi [c], Makoto Obata [b], Yuji Mikata [b], Shigenobu Yano [c], Etsuo Niki [a,*]

[a] Health Technology Research Center, National Institute of Advanced Industrial Science and Technology, 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan
[b] Nara Women's University, Kitauoyahigashi-machi, Nara 630-8506, Japan
[c] Kyoto University, Kyoto-Daigaku Katsura, Nishikyo-ku, Kyoto 615-8520, Japan

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ABSTRACT

The action of C_{60} fullerene and its derivatives as a radical-scavenging antioxidant has received much attention, but their reactivity toward free radicals and antioxidant capacity have not been well elucidated yet. In the present study, the reactivity of the two types of water-soluble, sugar-pendant C_{60} fullerenes, C_{60}-1S and C_{60}-2S, toward peroxyl radical and their effect against human plasma lipid peroxidation were measured. The rate constants for the reaction of C_{60}-1S and C_{60}-2S with peroxyl radicals were obtained from their effect on the bleaching of β-carotene in lipid-SDS micelle system as 4.6×10^3 and 8.0×10^3 M^{-1} s^{-1} at 37 °C, respectively. They inhibited the free radical-induced lipid peroxidation in human plasma in a concentration-dependent manner. These results suggest that the sugar-pendant fullerenes C_{60}-1S and C_{60}-2S act as a radical-scavenging antioxidant with the activity similar to the phenolic antioxidants.

It is now generally accepted that lipid peroxidation mediated by free radicals is involved in the pathogenesis of various diseases. Lipid peroxidation induces membrane damage and gives various products which are cytotoxic, mutagenic, and carcinogenic and capable of modifying biologically essential molecules such as proteins and DNA bases.[1] Consequently, the role and beneficial effects of free radical-scavenging antioxidants have received much attention.[2] Vitamins E and C are the most well known lipophilic and hydrophilic natural antioxidant, respectively. Various synthetic antioxidants have also been prepared, evaluated and applied to cope with the oxidative stress induced by free radicals.[3]

The discovery that C_{60} fullerene reacts with free radicals and is capable of acting as a 'radical sponge'[4,5] inspired numerous studies to evaluate the reactivity of C_{60} fullerene as a radical-scavenging antioxidant, both in vitro and in vivo. The C_{60} fullerenes have poor solubility in water and polar solvents, which limits bio-medical application. To overcome this disadvantage, the suspensions and/or emulsions of C_{60} have been prepared by using surfactants such as polyvinylpyrrolidone (PVP) and super-structures of cyclodextrin.[6,7] Furthermore, various water-soluble C_{60} derivatives have been prepared by chemical modification and it was found that they exert antioxidant effects against several types of oxidative stress. Dugan et al. have found that polyhydroxylated C_{60}, fullerenol,[8] and malonic acid C_{60} derivatives,[9–12] carboxyfullerenes, act as hydroxyl radical scavenger and superoxide dismutase and thereby protect neuronal cells from oxidative damage. Water-soluble C_{60} adducts of α-alanine[13] and cystine[14] were also reported to act as a scavenger of hydroxyl radical and superoxide. Polyhydroxylated fullerenols were reported to act as a radioprotector,[15] and carboxyfulleren was found to prevent iron-induced oxidative damage in rat brain.[16] Although other reports also show that fullerenols[17] and carboxyfullerene[18] scavenge superoxide, the rate constant for scavenging superoxide by C_{60} fullerene and derivatives is reported to be much smaller than that of natural SOD.[11] It is also unlikely that fullerene derivatives act as an efficient hydroxyl radical scavenger in vivo, since most biological molecules react with hydroxyl radical at near diffusion controlled rate. The paper by Lee et al.[19] clearly shows that hexasulfobutyl C_{60} fullerene acts as a peroxyl radical scavenger to suppress the oxidation of low density lipoprotein (LDL). However, as pointed out by Enes et al.[20] the reactivity of fullerene and water-soluble derivatives toward free radicals has not been clearly measured.

The present study was performed to measure the reactivity of water-soluble C_{60} derivatives toward peroxyl radicals and antioxidant activity against lipid peroxidation under well-defined reaction conditions. Peroxyl radical was chosen as a target free radical since it is the chain-carrying species in lipid peroxidation and more importantly it is the key radical that can be scavenged efficiently in vivo by radical-scavenging antioxidant.[21] Two types of sugar-pendant C_{60} fullerenes, C_{60}-1S and C_{60}-2S (Fig. 1) were used since they are water-soluble and their structure has been well characterized.[22,23]

* Corresponding author. Tel.: +81 72 751 9693; fax +81 72 751 9964.
E-mail address: etsuo-niki@aist.go.jp (E. Niki).

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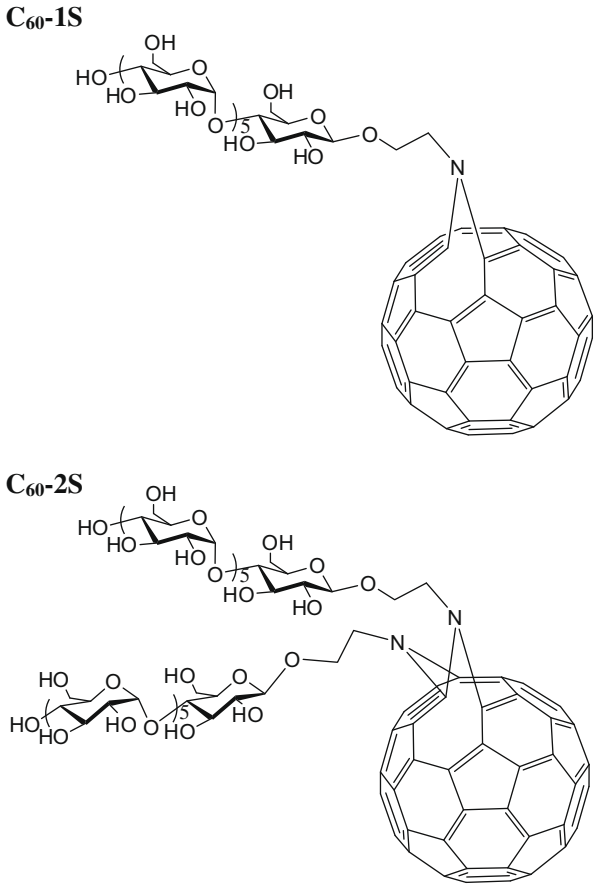

Figure 1. Structure of sugar-pendant C_{60} fullerenes used in this study.

The reactions of C_{60}-1S and C_{60}-2S toward peroxyl radicals were measured by a competition method using β-carotene as a reference compound as reported previously.[24] The peroxyl radicals were generated at a constant rate from the azo compound.[25] β-Carotene has its characteristic visible absorption spectrum and its reaction with peroxyl radicals can be followed by measuring a decrease in the absorption at 454 nm. In the presence of added antioxidant, β-carotene and the antioxidant compete to react with the peroxyl radicals as shown by the reactions (1) and (2):

$$\beta\text{-Carotene} + AO_2^{\cdot} \xrightarrow{k_{\beta C}} \beta\text{-carotene radical} \quad (1)$$

$$IH + AO_2^{\cdot} \xrightarrow{k_{IH}} AOOH + I \quad (2)$$

where $AO_2^{\cdot}$ and IH are peroxyl radical and antioxidant and $k_{\beta C}$ and k_{IH} are the rate constant for the reactions (1) and (2), respectively. The rates of consumption of β-carotene in the absence (R_0) and presence (R_{IH}) of antioxidant are given by (Eqs. 3 and 4), respectively.

$$R_0 = k_{\beta C}[AO_2^{\cdot}][\beta\text{-carotene}] \quad (3)$$

$$R_{IH} = \frac{k_{\beta C}}{k_{\beta C}[\beta\text{-carotene}] + k_{IH}[IH]} \times k_{\beta C}[AO_2][\beta\text{-carotene}] \quad (4)$$

Hence, the ratio of these rates, R_0/R_{IH}, is given by

$$\frac{R_0}{R_{IH}} = 1 + \frac{k_{IH}[IH]}{k_{\beta C}[\beta\text{-carotene}]} \quad (5)$$

R_0 and R_{IH} are measured experimentally and a plot of R_0/R_{IH} as a function of [IH]/[β-carotene] should give a straight line, whose slope corresponds to $k_{IH}/k_{\beta C}$.[24]

The example of the results of the competition between β-carotene and C_{60}-1S is shown in Figure 2. β-Carotene was consumed at a constant rate, which decreased with increasing concentrations of C_{60}-1S or C_{60}-2S. The plots of (Eq. 5) gave satisfactory straight line (Fig. 3) and the slope, $k_{IH}/k_{\beta C}$, was obtained as 0.147 and 0.257 for C_{60}-1S and C_{60}-2S, respectively. The rate constant k_{IH} was calculated as $k_{C60\text{-}1S} = 4.6 \times 10^3$ and $k_{C60\text{-}2S} = 8.0 \times 10^3$ M^{-1} s^{-1} for C_{60}-1S and C_{60}-2S, respectively, from the slope and $k_{\beta C} = 3.1 \times 10^4$ M^{-1} s^{-1}.[24]

Plasma has been used often as a substrate to assess the antioxidant capacity against lipid peroxidation. Cholesteryl ester hydroperoxides (CEOOH) are formed as a major product in the oxidation of human plasma together with phosphatidylcholine hydroperoxide (PCOOH) as minor product.[26] PCOOH is reduced by extracellular glutathione peroxidase (eGPx) to the hydroxide PCOH, while CEOOH is reduced to the corresponding hydroxide (CEOH) by eGPx more slowly.[27] In the present study, the effect of C_{60}-2S on the formation of CEOOH and CEOH in the oxidation of human plasma was measured by a method reported previously[26] and summarized briefly in the notes.[28] As shown in Figure 4, C_{60}-2S suppressed the formation of CEO(O)H in a concentration-dependent manner.

The above results clearly show that the sugar-pendant C_{60} fullerenes, C_{60}-1S and C_{60}-2S, have antioxidant properties. Their reactivity toward peroxyl radicals is similar to those of many phenolic

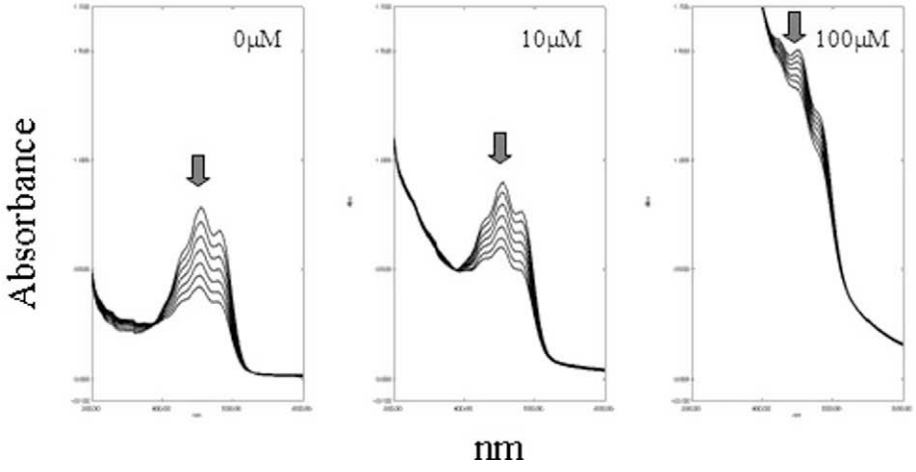

Figure 2. The bleaching of β-carotene induced by free radicals in the absence and presence of C_{60}-1S. The visible absorption spectrum was measured every 5 min in methyl palmitate/SDS micelles containing 10 μM β-carotene and 3 mM AIPH in the absence and presence of 10 and 100 μM C_{60}-1S.

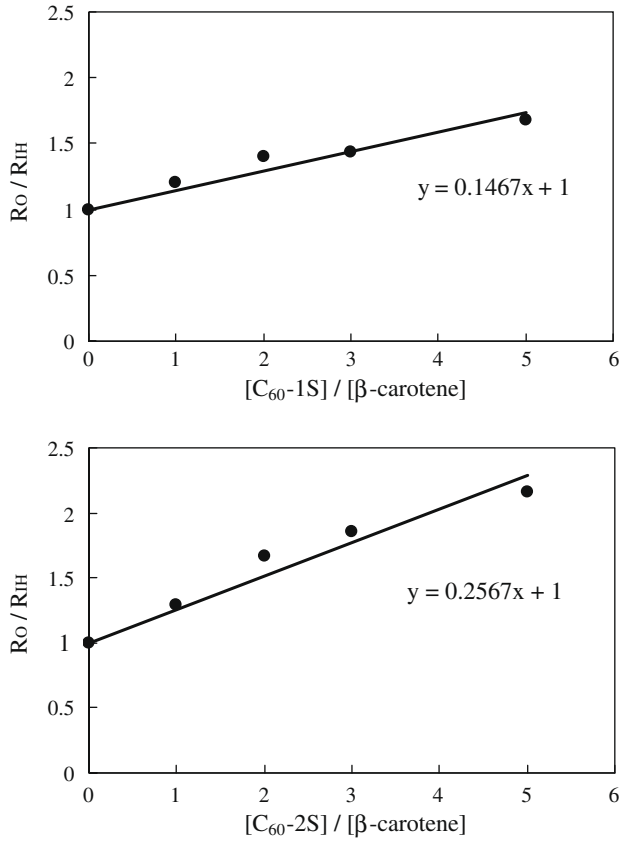

Figure 3. Ratio of rate constants $k_{IH}/k_{\beta C}$ for C_{60}-1S and C_{60}-2S against β-carotene. The experimental conditions are the same as in Figure 2.

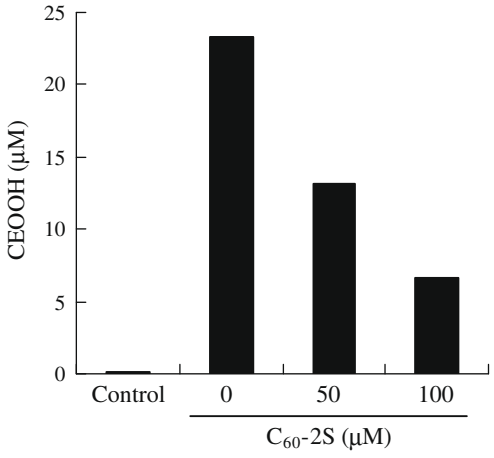

Figure 4. Effect of C_{60}-2S against lipid peroxidation in human plasma. The dialyzed human plasma (10 volume% in PBS) was oxidized in the presence of 20 mM AAPH and C_{60}-2S (0, 50, 100 μM) for 4 h at 37 °C and CEO(O)H was measured as described in the text. The control experiment was performed in the absence of AAPH.

antioxidant compounds, but smaller than those of vitamins E and C and β-carotene. The antioxidant activity of PVP-entrapped and CD-bicapped C_{60} fullerene was reported by Takada et al.[29] but unfortunately only the first order rate constants were measured and the active species were not clearly defined, making the results of little quantitative value. More recently, Yin et al.[30] reported that C_{60} fullerrenol and carboxyfullerene inhibited lipid peroxidation of egg PC liposome by measuring the variation of electron paramagnetic resonance spectra of spin probe which was ascribed to the decrease in oxygen concentration of the solution.

It is assumed that the C_{60} fullerene derivatives scavenge peroxyl radicals by either electron transfer or addition to the double bond mechanism, but the identification of the products is an important subject of a future study to elucidate the underlying mechanism. In any event, the present study gave the absolute rate constants for scavenging of peroxyl radicals by two water-soluble, sugar-pendant C_{60} fullerene derivatives and showed that they exerted moderate capacity for inhibition of lipid peroxidation in human plasma. It may be noted that their characteristic physical properties may render these sugar-pendant C_{60} fullerenes an important function as biological antioxidant.

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