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Different hydroxyl radical scavenging activity of water-soluble β-alanine C_{60} adducts

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Abstract—Three C_{60} derivatives [C_{60} $(NHCH_2CH_2COONa)_n(H)_n$], $n = 1, 5, 9$] (A, B, C) with different additional number of β-alanine were synthesized by the control of relative amount of C_{60} and β-alanine added. Hydroxyl radical scavenging activity of the adducts was evaluated in a copper-catalyzed Haber–Weiss reaction by chemiluminescence technology. The 50% inhibition concentrations (IC_{50}'s) of A, B, and C were 147.2 μmol/L, 76.3 μmol/L, and 96.2 μmol/L, respectively. The difference should be closely related to the numbers of residual C=C bonds in C_{60}, steric effect and electron-withstanding effect of amino group especially.

The study of bioactivities of C_{60} has drawn extensive attention since its discovery.[1] Water-soluble C_{60} derivatives are a unique class of compounds with potent antioxidant properties. Recently, different kinds of water-soluble C_{60} derivatives were synthesized and their free radicals scavenging activities were reported.[2–9] These novel antioxidants are potential neuroprotecting agents for neurodegenerative disorders, including Parkinson's disease. Water-soluble β-alanine C_{60} adducts have excellent active oxygen radical scavenging ability and can prolong the life span of mouse thymus cells.[10,11]

In order to investigate the effect of functional groups on their radical scavenging ability, three C_{60} derivatives with different number of β-alanine were synthesized and their hydroxyl radical scavenging activity were estimated by chemiluminescence technique.

β-alanine C_{60} adducts were prepared according to the method described previously.[11,12] 2.5 g (80 mg, 40 mg) β-alanine and 3.0 g sodium hydroxide were dissolved in 6 mL deionized water, 40 mL ethanol was added, then the resulting solution was added dropwise to a stirred dry toluene solution (100 mL) containing C_{60} ($\geq$99.9%, supplied by Wuhan University, 110 mg). After stirring at room temperature for 5 days, the aqueous layer was separated from the organic layer, diluted with water to 10 mL, 80 mL absolute ethanol was then added to cause the precipitation of product A (B, C). The precipitation was purified several times by solving in water and precipitated from absolute ethanol, then dried under a vacuum to obtain products A, B, and C. Ninhydrin test showed no free β-alanine present in the products.

FT-IR and ^{1}H NMR (500 MHz) were used for the characterization and the results were the same as the previous report.[12] The incorporating of β-alanine group in C_{60} was confirmed by absorption bands (IR) at 1406 [γ_{sym} (CO_2)] and 1596 cm^{-1} [γ_{as} (CO_2)], and proton absorption (^{1}H NMR) at $\delta = 2.35$ and 2.89 ppm. Elemental analysis results showed that the average number of β-alanine in A, B, and C was 1 (1.15), 5 (5.18), and 9 (9.11), respectively.

Chemiluminescence technology has been widely used to evaluate free radical scavenging activity of C_{60} derivatives.[5,13,14] $^{\bullet}$OH was produced by a copper-catalyzed Haber–Weiss reaction, and zymosan was used as a chemiluminescence amplifier.[15] 0.4 mL $CuSO_4$ (2 mmol/L), 0.2 mL ascorbic acid (2 mmol/L), 0.6 mL phosphoric acid buffer solution (50 mmol/L) or β-alanine C_{60} adducts, and 0.2 mL zymosan (25 mg/mL) were mixed intensively and the background intensity was measured on a bio-chemical luminescence measuring instrument (SGH-1, made in Shanghai). 0.6 mL H_2O_2 (66 mmol/L)

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was added to initiate the luminescence reaction. The luminescence kinetics curve [chemiluminescence (CL)-*t*] was measured with an interval time of 15 s and accumulated continuously for 1500 s. The relative content of hydroxyl radical in the system was calculated as the abstraction value of the peak value of the CL-*t* curve and the background. The scavenging efficacy (SE) to hydroxyl radical was calculated according to the following formula: $SE/\% = (CL_0–CL_1)/CL_0 \cdot 100\%$. CL_0 and CL_1 represented the peak value in the CL-*t* curve of the control group and test group, respectively. Every data point was obtained from three parallel determinations. The tolerance was no more than 3%. The free radical produced in this system was proved to be hydroxyl radical $^{\bullet}$OH tested by superoxide dismutase (SOD), catalase, and mannitol. β-Alanine, thiourea, manitol and benzoic acid were used as a control.

The dependence of scavenging effect for hydroxyl radical $^{\bullet}$OH on the concentration of three β-alanine C_{60} adducts is studied. As shown in Figure 1, the contents of scavenging efficacy gradually increase with the increasing of the concentration of three β-alanine C_{60} adducts. The 50% inhibition concentrations (IC_{50}'s) of A, B, and C are 147.2 μmol/L, 76.3 μmol/L, and 96.2 μmol/L, respectively. The IC_{50}'s of thiourea, mannitol, and benzoic acid in the system are 1200 μmol/L, 13,800 μmol/L, and 16,600 μmol/L, respectively. The result indicates that three C_{60} derivatives are good scavengers to hydroxyl radical $^{\bullet}$OH.

C_{60} has 30 carbon–carbon double bonds to which free radicals can add, and thus is called as a 'radical-sponge'.[16] If a C=C bond is opened, two hydroxyl radical $^{\bullet}$OH will be added.[3] This radical-absorbing character is closely correlated with the electron-defect property of C_{60} molecule. During the preparation of β-alanine C_{60} adducts, parts of C=C bonds of C_{60} are opened by β-alanine group additions, but other residual C=C bonds still make β-alanine C_{60} adducts have electrophilic ability. On the other hand, β-alanine shows no radical scavenging activity in this system. Therefore, the C=C bonds are mainly the origin of radical scavenging ability of water-soluble C_{60} derivatives including β-alanine C_{60} adducts.

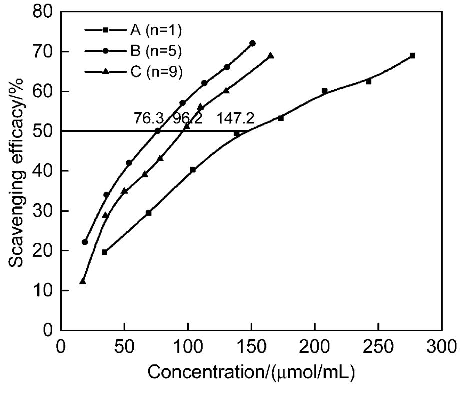

Figure 1. Hydroxyl radical scavenging activity of water-soluble β-alanine C_{60} adducts.

Hydroxyl radical scavenging effect of oligoadducts of malonic acid C_{60} showed that monoadduct had the highest scavenging effect, 40%, at the concentration of 2×10^{-4} mol/L, and tetraadduct had no obvious activity at the concentration of 8×10^{-4} mol/L.[4] The reports of Krusic[16] and Guldi[17] also showed a higher number of addends on the fullerene moiety should decrease its radical scavenging properties. Cusan reported that a new multi-charged monoadduct C_{60} pyrrolidine derivative showed no significant reaction with $O_2{\cdot}^{-}$.[18] The above facts indicated that radical scavenging ability of C_{60} derivatives may be related to multiple factors.

The derivatives with different number of β-alanine have different IC_{50}'s. The hydroxyl radical scavenging ability sequence is shown as: B $(n=5) > C(n=9) > A$ $(n=1)$. The difference should be due to the numbers of residual C=C bonds, electron-withstanding effect of amino group, and steric effect.

β-Alanine is incorporated with C_{60} by –NH group, which is a strong electron-withstanding group. The strong electron-withstanding ability of –NH group will probably increase electron cloud density of π-bonds in C_{60} molecule, and thus the residual C=C bonds will be easily attacked by electrophilic reagent hydroxyl radical $^{\bullet}$OH. With increasing of addition number, electron cloud density of π-bonds in C_{60} moiety will increase, and the adducts will be easier to react with hydroxyl radical $^{\bullet}$OH. So the scavenging ability of β-alanine C_{60} adducts to hydroxyl radical $^{\bullet}$OH increases with the increasing of the numbers of β-alanine added. The above general mechanism can proved by the fact that the scavenging ability of derivative B $(n=5)$ to hydroxyl radical $^{\bullet}$OH is better than that of derivative A $(n=1)$.

The number of π-bonds in the fullerene moiety becomes smaller with increasing number of β-alanine group, this is, the active sites that are attacked by hydroxyl radical $^{\bullet}$OH reduces. On the other hand, the steric effect increases with the increase in addition number. In the organic reaction, specially in free radical reaction, the reaction rate is controlled by the steric effect in the some way. The increasing steric effect makes $^{\bullet}$OH reach to C=C bonds more difficultly, which means the reaction chance between C=C bonds and $^{\bullet}$OH decreases. Therefore, when the addition number increases from 5 to 9, the radical scavenging ability of derivative B $(n=5)$ to hydroxyl radical $^{\bullet}$OH is better than that of derivative C $(n=9)$. Derivative A $(n=1)$ has the highest IC_{50}, which indicates that the electron-withstanding effect of –NH group is a key factor to the hydroxyl radical scavenging ability of water-soluble β-alanine C_{60} adducts.

Many of the biological functions of fullerene derivatives are believed to be due to their antioxidant properties. This result will be very helpful for expanding the research and applications of β-alanine C_{60} adducts in biomedical fields including as potential neuroprotecting agents.

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