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Active Oxygen Radical Scavenging Ability of Water-Soluble b-Alanine C_{60} Adducts

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Abstract: Water-soluble β-alanine C_{60} adducts were synthesized, and the scavenging ability to superoxygen anion radical O_2^- and hydroxyl radical·OH were studied by autoxidation of pyrogallol and chemiluminescence, respectively. It was found that β-alanine C_{60} adducts showed an excellent efficiency in eliminating superoxygen anion radical and hydroxyl radical. The 50% inhibition concentration (IC_{50}) for superoxygen anion radical and hydroxyl radical were 0.15 mg/mL and 0.048 mg/mL, respectively. The difference should be mainly attributed to the different scavenging mechanisms.

Keywords: β-alanine C_{60} adducts, superoxygen anion radical, hydroxyl radical, autoxidation of pyrogallol, chemiluminescence.

The bioactivity study of C_{60} and its relative derivatives is of considerable importance. Functional fullerenes have peculiar biological activities, such as enzymatic inhibition, anti-HIV activity, neuroprotection, antibacterial activity, DNA cleavage, and photodynamic therapy *et al.*[1]. Neuroprotective properties are based on the facts that functional fullerenes have antioxidant properties and high reactivity toward free radical[2]. Active oxygen radical in biological system mainly includes superoxygen anion radical and hydroxyl radical. Their generation and removal are in an equilibrium status. When this equilibrium broken, the concentration of active oxygen radical will exceed the normal physiological limit and injure the organism; finally some pathological changes will take place[3]. Among various active oxide species the chemical activity of hydroxyl radical was the strongest, which can easily react with biomolecules such as proteins and DNA[4]. As known, under the presence of free Fe^{2+} ions, superoxygen anion radical can be reacted to produce hydroxyl radical. Therefore, it is very important to remove the active oxide radical in time. In this note, the active oxygen radical scavenging ability of water-soluble β-alanine C_{60} adducts was investigated for the first time.

Water-soluble β-alanine C_{60} adducts were prepared according to the method reported previously[5]. FT-IR and ^{1}H NMR (500 MHz) were used for the characterization and the results were the same as the previous report[5]. The FT-IR (KBr) spectrum showed a strong broad bands at 3421 cm^{-1}, corresponding to –OH and –NH, and two strong bands at 1569 cm^{-1} and 1406 cm^{-1}, corresponding to COO^- and C–N, respectively, and middle-strong band at 1620cm^{-1}, corresponding to C-C. ^{1}H NMR (D_2O) spectrum exhibited two triplets at 2.22 and 2.71 ppm (relative to DOH), corresponding to the two

methylene groups of β-alanine, and the three peaks centered at 1.7, 3.1, 3.5 ppm respectively can be due to protons on the C_{60} molecule. The elemental analysis showed a carbon:nitrogen ratio of 10.7:1.2, which correlated to an average of eight β-alanine moieties per C_{60} molecule.

0.3 mL 3 mmol/L pyrogallol, 4.5 mL 100 mmol/L buffer solution (Tris-HCl, pH= 8.2), and 4.2 mL redistilled water were mixed intensively after preequilibration at 25°C. The absorbency of the above mixture at 325 nm was measured every 30 seconds on an ultraviolet-visible spectrophotometer (WFZ 800-D 2C). The autoxidation rate of pyrogallol, calculated from the change of absorbancy at 325 nm, was controlled about 0.070 by adjusting the concentration of pyrogallol[6]. The autoxidation rate of pyrogallol represented by the increment of absorbancy every minute in the linear range. The scavenging ability of β-alanine C_{60} adducts for superoxygen anion radical was calculated as the following formula:

$$S=(K_0-K_1)/K_0*100\% \quad (1)$$

where K_0, K_1 were autoxidation rate of pyrogallol without and with β-alanine C_{60} adducts, respectively.

0.4 mL 2 mmol/L $CuSO_4$, 0.2 mL 2 mmol/L ascorbic acid, 0.6 mL 50 mmol/L phosphoric acid buffer solution (or β-alanine C_{60} adducts), and 0.2 mL 25 mg/mL zymosan were mixed intensively and the background intensity was measured on a bio-chemical luminescence measuring instrument (SGH-C). 0.6 mL 66 mmol/L H_2O_2 was added to initiate the luminescence reaction, and the chemiluminescent emission from the resulting mixture was counted at an interval of 15 s for 100 times[7]. The amount of hydroxyl radical in the system was represented by the chemiluminescence intensity. The scavenging ability to hydroxyl radical was calculated according to the following formula:

$$S=(CL_0-CL_1)/CL_0*100\% \quad (2)$$

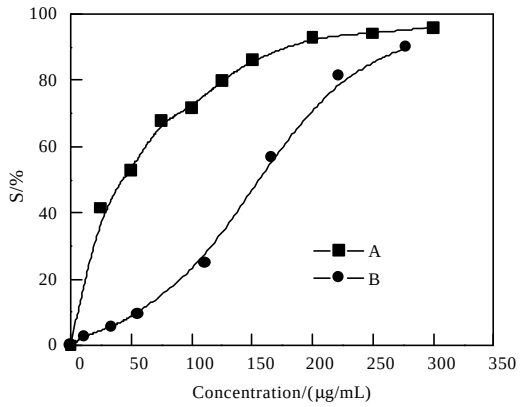
where CL_0, CL_1 were the chemiluminescence intensity in the system without and with scavenger, respectively.

The free radical produced in this system was proved to be hydroxyl radical tested by superoxide dismutase (SOD), catalase, and mannitol.

The dependence of scavenging effect on the concentration of β-alanine C_{60} adducts is shown in **Figure 1**. **Figure 1** (B) shows that the scavenging ability for superoxygen anion radical in the system gradually increases with the increasing of β-alanine C_{60} adducts concentration. At an applied β-alanine C_{60} adducts concentration of 0.15 mg/mL in the final solution, a superoxygen anion radical scavenging efficiency of approximately 50% was achieved, that is, the 50% inhibition concentration (IC_{50}) is 0.15 mg/mL, and the scavenging efficiency is 80% when its concentration is 0.23 mg/mL. The hydroxyl radical scavenging effects of β-alanine C_{60} adducts with different concentrations is shown in **Figure 1** (A). After 0.048 mg/mL β-alanine C_{60} adducts are added, about 50% hydroxyl radical is removed, and at the concentration of 0.12 mg/mL the scavenging efficiency is 80%. As shown above, β-alanine C_{60} adducts have excellent scavenging

ability for superoxygen anion radical and hydroxyl radical. The scavenging ability can be compared with those of some commonly used bioradical scavengers, such as green tea polyphenols (the 50% inhibition cocentration of green tea polyphenols in the experiment is 0.087 mg/mL and 0.029 mg/mL for superoxygen anion radical and hydroxyl radical, respectively). It is known that the β-alanine C_{60} adducts have better scavenging ability to hydroxyl radical than to superoxygen anion radical. The difference should be ascribed to their different amounts of free radicals in the two systems and different scavenging mechanisms. It is well known that C_{60} reacts with free radical mainly by the multiaddition of C=C double bonds[8]. During the preparation of β-alanine C_{60} adducts, parts of C=C double bonds of C_{60} are opened, but other residual C=C bonds still make β-alanine C_{60} adducts have electrophilic ability. The scavenging of hydroxyl radical is perhaps by the way of two hydroxyl radical added in one C=C double bond. But the scavenging of superoxygen anion radical is similar to the oxidation of C_{60}, that is, a superoxygen anion radical changes into an active oxygen intermediate, and the later is added on a C=C double bond. So a C=C double bond can react with two hydroxyl radicals but only one superoxygen anion radical. It may explain that why the β-alanine C_{60} adducts are more effective for scavenging hydroxyl radicals.

Figure 1 The dependence of scavenging effect on the concentration of β-alanine C_{60} adducts



So far the applications of C_{60} in biology field are impeded because of its poor water solubility and biotoxicity[9]. β-alanine C_{60} adducts have good water solubility. In addition, the reduced chemical reactivity of the remaining conjugated double bonded moieties in β-alanine C_{60} adducts certainly reduces their inherent biological toxicity comparing with the parent C_{60}. The above results suggest the potential uses of β-alanine C_{60} adducts in the biochemical or pharmaceutically related investigations.

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