Synthesis of a Water-Soluble Fullerene Derivative Nanoball and Its Biological Activity

Guichang Jiang,[1,2] Qixing Zheng,[1] Dazhi Yang[1]

[1]*Department of Biology, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China*
[2]*Department of Chemistry, Huainan Teachers College, Huainan, Anhui 232001, China*

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ABSTRACT: A novel fullerene–acrylamide copolymer was synthesized via radical polymerization. It is soluble in polar solvents such as water and dimethyl sulfoxide. The product was characterized by FTIR, UV–vis, and GPC. TEM analysis showed that the average particle diameter was about 46 nm. The in vitro antitumor activity of the fullerene–acrylamide derivatives was tested, and the results showed that the novel fullerene–acrylamide derivatives exhibited better antitumor activity in vitro against bone tumor cells. The cytotoxicity against the tumor cell mechanism of the fullerene copolymer nanoball was studied. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 99: 2874–2877, 2006

Key words: copolymer; water solubility; biological activity; fullerene; mechanism

INTRODUCTION

In the last few years many interesting biological properties of fullerene C_{60} and its derivatives have been investigated, such as DNA photocleavage and apoptosis.[1,2] However, upon UV irradiation, fullerene derivatives can cause the formation of singlet oxygen, hydroxyl radicals, and superoxide that then become cytotoxic to cells.[3,4,5] C_{60}, because of its antioxidant properties and high reactivity toward free radicals, shows promising behavior for application in the neuroprotective field.[6,7]

For the investigation of such biological properties, it is necessary that the C_{60} derivatives be soluble in polar solvents, particularly water. It is therefore quite important to synthesize fullerene derivatives with a versatile anchor group that can be used for conversion to polar functional addends.[8] Considering that acrylamide is a compound with highly significant biological activity, it can be foreseen that acrylamide derivatives of C_{60} might have important medicinal applications. In this study, a novel water-soluble fullerene–acrylamide copolymer was synthesized, and the biological activity of soluble fullerene copolymer was studied for the first time.

EXPERIMENTAL

Materials

Fullerene (C_{60}) was obtained from Wuhan university (purity > 99.99%). Acrylamide (AR) was obtained from Hubei University. Benzoyl peroxide was obtained from Hubei University. The other solvents were analytically pure reagents.

Preparation of fullerene–acrylamide copolymer

The fullerene–acrylamide copolymer was synthesized by typical free-radical polymerization. A solution of C60 in *o*-dichlorobenzene (*o*-DCB) or toluene was mixed with acrylamide. The mixture (dark red in color) was deoxygenated by bubbling dry nitrogen gas for ~25 min, followed by the addition of benzoyl peroxide. The polymerization reaction was carried out in a glass bottle at 70°C. The reaction products were precipitated from the *o*-DCB solution into methanol. The precipitation procedure was repeated several times, yielding a solid sample almost black in color. The solid sample was further purified by washing with dimethyl sulfoxide/CH_3OH and dimethyl sulfoxide/C_6H_{14}. After drying under vacuum, the final copolymer sample was obtained. Polymer of neat acrylamide was prepared under the same conditions to be used as a reference.

Cytotoxicity

In vitro cytotoxicity against bone tumor cells was evaluated by studying the inhibition of the growth rate. To examine the effect of light, incubation was carried out with or without irradiation of light for 20 min each day during the 3-day incubation period. The fullerene–acrylamide derivatives were dissolved in a small amount of DMSO, and these solutions were further

Correspondence to: G. Jiang (jiangguichang@sina.com).

TABLE I
Results of Fullerene-Acrylamide Copolymerization reactions[a]

ID	C_{60} : acrylamide (mg : g)	Ben.peroxide (mg)	Yield (%)[b]	C_{60} wt %	Solvent	Product[c]
I	30 : 4.0	200	~45	~1.7	Pyridine	No good
II	50 : 1.3	200	~65	~5.7	Dioxane	Good
III	70 : 1.1	200	~67	~8.9	Dichlorobenzene	No Good
IV	100 : 1.0	200	~70	~14	Toluene	Good
V	100 : 0.5	200	~60	~30	Toluene	Good
VI	100 : 0.1	200	~95	~53	Toluene	No Good

[a] Reaction temperature 70°C, reaction time of 10 h.
[b] Calculated without considering the contribution of initiator fragments.
[c] Solubility in water.

diluted with DMEM medium to afford solutions that were suitable for fullerene–acrylamide derivatives in the concentration range studied. Each solution was placed in a 96-well microplate (100 μL/well). Bone tumor cells were suspended in DMEM medium containing 10% of fetal calf serum at a concentration of 5 $\times 10^4$ cells/mL, and 100 μL of the solution was added to the 96-well plate. After 3 days of incubation at 37°C, MTT [3-(4,5-dimethy thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] solution was added to each well, and the plate was incubated at 37°C for 4 h. The mitochondrial dehydrogenase of viable cells reduced MTT to a blue formazan product that could be measured with a spectrometer.

RESULTS AND DISCUSSION

Fullerene–acrylamide copolymerization reactions

The results clearly showed that C_{60} and acrylamide could be copolymerized under different conditions (Table I). With a constant benzoyl peroxide amount, C_{60} content in the copolymers increased with an increasing initial C_{60} : acrylamide reactant ratio (Table I). There was no apparent reduction in the polymer yield at high initial C_{60} : acrylamide reactant ratios, probably because of the relatively small C_{60} : initiator values. In addition, the solubility in water of the copolymers prepared with different initial C_{60} : acrylamide reactant ratios did not significantly differ (Table I). In a classical mechanism of radical polymerization, propagation of $C_{60}\cdot$ radicals is critical to the formation of true C_{60}–acrylamide copolymers.[9] However, it has been suggested[9] that the propagation of $C_{60}\cdot$ radicals is slow in general because of their relatively high stability. It also has been suggested that C_{60} competes effectively with acrylamide for initiator radicals and, as a logical extrapolation, for AM $\cdot$ radicals as well. In this regard, C_{60} may act as a radical inhibitor in the copolymerization reaction. Evidence for the inhibiting effect includes the observation[9] that at high C_{60} : initiator ratios, polymerization yields are low in comparison with those in neat acrylamide polymerization. Thus, the mechanism of C_{60}–acrylamide copolymerization may be different from the classical mechanism of radical polymerization of acrylamide such that there is a significant population of C_{60} radicals and their propagation was relatively slow. However, even with the suggested inhibiting effect of C_{60} in the copolymerization reaction, the contributions of polyacrylamide structures are substantial, even in copolymers with high C_{60} contents, as shown in the observed FTIR spectra of the copolymers (Fig. 1). By assuming that C_{60} radicals participate in termination processes only, the average number of C_{60} cages per polymer (N_{C60}) should be 1–2. However, in the copolymers with high C_{60} contents (Table I), the estimated N_{C60} values were apparently larger. The high C_{60} populations might be explained by the possibility that functionalized C_{60} cages may also compete for initiator radicals. The results could used to support a proposed copolymer mechanism as follows:

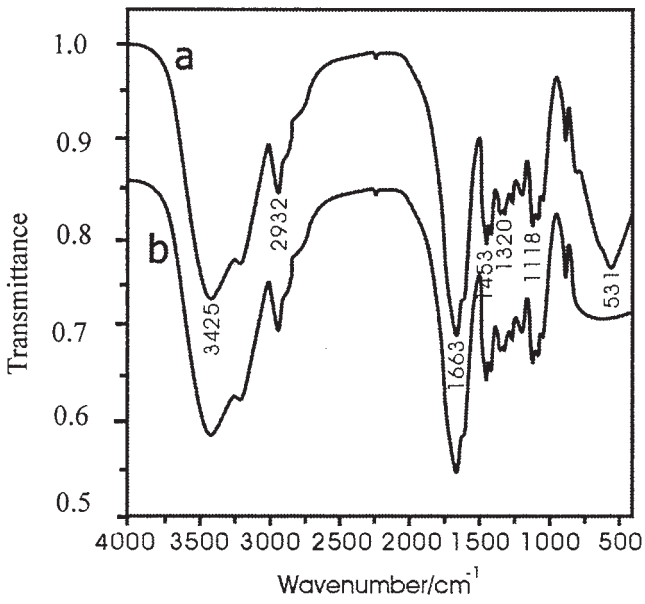
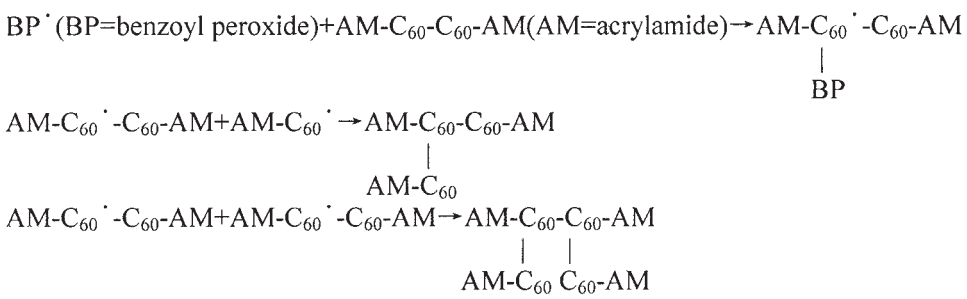
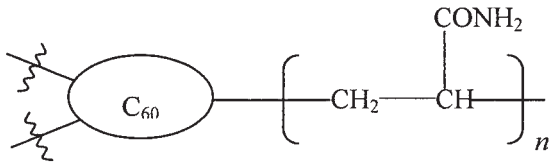


Figure 1 FTIR spectra of (a) C_{60}-acrylamide copolymer and (b) polyacrylamide.



Characterization of fullerene–acrylamide copolymer

The copolymer was characterized by FTIR, UV, and GPC. FTIR spectra were measured in KBr matrices, and the results were compared with those of neat polyacrylamide samples prepared under the same experimental conditions. As shown in Figure 1, the observed FTIR spectra of the copolymers with C_{60} contents were very similar to those of neat polyacrylamide. However, for the copolymer with C_{60} contents, the FTIR spectrum showed extra absorption in the 531 cm^{-1} region, which is typical with substituted C_{60}.[9] UV absorption spectra were measured in THF, and the results were compared with those of polyacrylamide. As shown in Figure 2, observed the UV absorption spectra of copolymer with C_{60} contents are very different from those of polyacrylamide. The somewhat structured absorption band of free C_{60} was replaced by a steadily decreasing curve, typical of substituted C_{60}.[9] The maximum UV absorption wavelength of copolymer with a C_{60} content was 252 nm, different from that of free C_{60} and polyacrylamide, which were typical of substituted C_{60}.[10,11] The emission can be attributed to the C_{60}-containing sites in the polyacrylamide structure. The average molecular weight (*MW*) was obtained by GPC (Voria 5060 model). The *MW* of the copolymer with a C_{60} content was 3360. The molecular structure of the copolymer may be a star polymer with fullerene as core and a grafting of one or several polyacrylamide chain segments. The results could be used to support a proposed copolymer structure as follows.



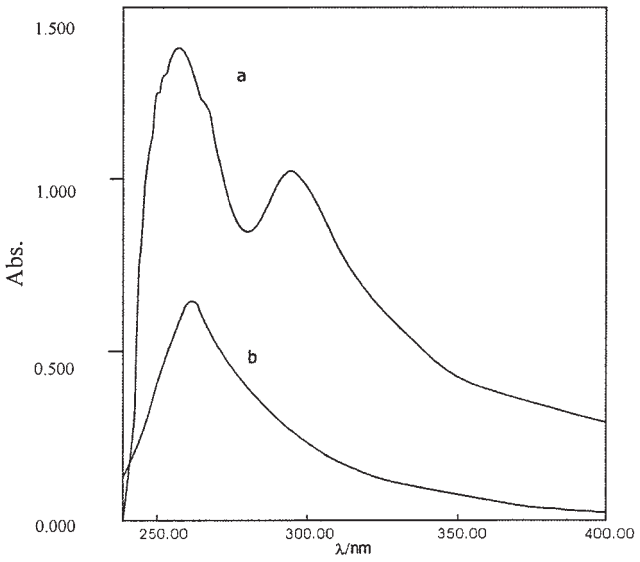


Figure 2 Comparison of (a) C_{60}-acrylamide copolymer UV absorbance with (b) polyacrylamide UV absorbance. All are in THF.

The morphology and size of the fullerene copolymer in water were measured using a JEM 100CXII model transmission electron microscope (TEM) with a voltage of 200 kV. The synthesized fullerene copolymer is soluble in water, giving a clear brown solution. TEM analysis indicates that the copolymer presents an ideal spherical shape in water with a diameter of about 40–80 nm, as shown in Figure 3. For comparison, the appearance of the polyacrylamide in water was lamellar shaped. The physical structure of the fullerene copolymer nanometer tiny balls may be described as follows: the core is very hard fullerene, and the shell is polyacrylamide, which may be relatively soft but very elastic.[11]

Cytotoxicity against tumor cells

The fullerene–acrylamide derivatives were shown to exhibit cytotoxicity against a bone tumor cell line upon irradiation of light. No inhibition (fullerene–acrylamide derivatives concentration > 100 $\mu g/mL$) was observed in total darkness. In the present studies we compared the in vitro cytotoxicity for fullerene–acrylamide derivatives of different concentrations by studying the inhibition of growth rate, as shown in Table II. The results showed that the new fullerene–acrylamide derivatives exhibited better antitumor activity in low concentrations (fullerene derivative con-

centration of 60 μg/mL), and the inhibition of growth rate of fullerene derivatives was dose dependent ($p < 0.05$). On the basis of the above experimental results, the cytotoxicity against the tumor cell mechanism of the fullerene copolymer nanoball can be deduced. We believe that cell death mainly resulted from phospholipid and protein damage within the cell membrane. When irradiated with light, fullerene derivatives became excited to a singlet excited state and though intersystem crossing passed to an excited triplet state. Subsequently, they could be quenched by O_2 to produce singlet oxygen, a species that reacts readily with amino acids, nucleic acids, and membrane phospholipids, leading to cell damage and death.[12]

CONCLUSIONS

A novel fullerene–acrylamide copolymer was prepared. It was completely soluble in water, yielding a clear brown solution. TEM analysis showed that it presented an ideal spherical shape in water, with a diameter ranging from 40 to 80 nm. In vitro antitumor activity of the fullerene–acrylamide derivatives was tested, and the result showed that the fullerene–acrylamide derivatives exhibited better antitumor activity in vitro against bone tumor cells. Fullerene–acrylamide derivatives may become a potential medicine for antitumor uses.

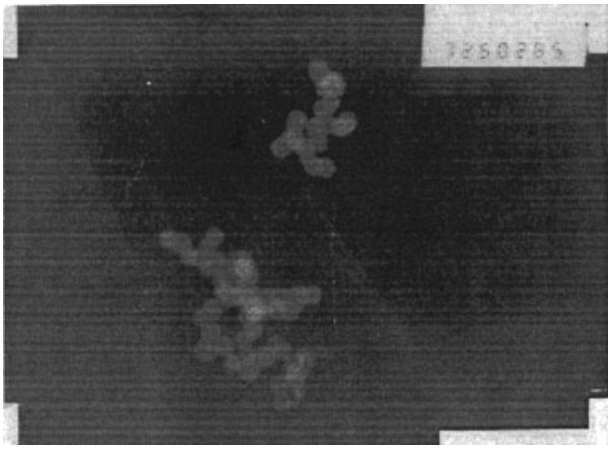


Figure 3 Morphology of nanometer balls of the fullerene copolymer in water. Magnification: 72,000×.

TABLE II
Inhibition of Growth Rate of Fullerene Derivatives of Different Concentrations

Concentration (μg/mL)	20	40	60	80	100
Inhibition of growth rate (%)	36.80	47.39	57.81	79.98	91.93

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