Cyclodextrin–fullerenes: a new class of water-soluble fullerenes

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Received (in Cambridge, UK) 24th January 2000, Accepted 9th May 2000
Published on the Web 8th June 2000

Solubilization of fullerenes in water by nucleophilic addition of cyclodextrin-R-monoamines to C_{60}, where R represents iminoalkyl and iminoaryl residues, is reported and studies involving the host–guest characteristics, free radical scavenging and DNA-cleaving properties indicate that this class of compounds has potential for a number of biological and medical applications.

Fullerenes are extraordinarily susceptible to attack by a variety of chemical reagents.[1] They exhibit high reactivity towards multiple addition of organic free radicals[2] and have been demonstrated to cleave DNA in the presence of light.[3] It has been reported recently that certain mutagenic and carcinogenic properties, and a number of neurodegenerative diseases, are associated with free radicals.[4] Hence, water-soluble fullerene compounds are suitable for potential biomedical applications. Previous approaches to the preparation of water-soluble fullerenes,[5] such as inclusion complexation with cyclodextrin or cyclodextrin prepolymers, multi-hydroxylation, 1,3-dipolar cycloaddition, nucleophilic cyclopropanation, polymer embedding *etc.*, are characterized by significant alteration of the fullerene molecule by modification. Our approach involves covalent binding of fullerenes with cyclodextrins (CD) leading to high water solubility and biocompatibility.

Nucleophilic additions of primary and secondary amines are known reactions and have been investigated for functionalization of the fullerene cage.[6] Preparation of fullerenylated aminopolymers has also been reported.[7] Synthesis of the fullerene–cyclodextrin conjugate (**4**) (Scheme 1)† was achieved by adding small volumes of a cyclodextrin monoamine (**3**) solution to a C_{60} solution at intervals of six hours, until about 30% of the fullerene remained unreacted.[7] The CD-amine–fullerene was purified by subsequent membrane filtration.[8] Separation from any higher derivatives was achieved by using membranes with a molar mass cut-off (MMCO) of 2000 g mol^{-1} and low-molecular weight components, if any, by membrane of MMCO of 1000 g mol^{-1}. FT-IR spectra of a model compound [60]fullerene-deoxy-6-(1,4-diiminophenyl)-β-cyclodextrin (**4a**) display the distinct features of the fullerene component. The UV–Vis spectrum (Fig. 1) shows considerable peak broadening beyond 350 nm for **4a**. Several authors have ascribed this behavior to aggregation of the molecules in water, confirmed further from dynamic light scattering studies.[5f,g] The ^{13}C NMR spectrum showed resonances due to the fullerene carbons at *ca.* 140 ppm.

To rule out the possibility of a competitive host–guest complexation reaction typical of cyclodextrins,[9] we synthesized the β-CD-1,4-diaminobenzene host–guest complex and characterized the material by various spectral techniques. Though the IR of both the inclusion complex and the covalently bonded CD-diamine (**3a**) did not show any significant differences, there were typical distinguishing patterns in the UV–Vis, ^1H NMR, TGA, and DSC data.

Cyclodextrin cage compounds have been demonstrated to be useful for several medical applications involving the host–guest complexation strategy and controlled release of the guest drug molecule.[10] In order to prove that the CD ring is still available for formation of inclusion complexes, we used *p*-nitrophenol (PNP) as a model molecule. The reaction between **4a** and PNP was monitored and compared with the reaction between β-CD and PNP by recording the change in the UV–Vis absorption pattern. It was seen that, with time, the peak at 405 nm gradually

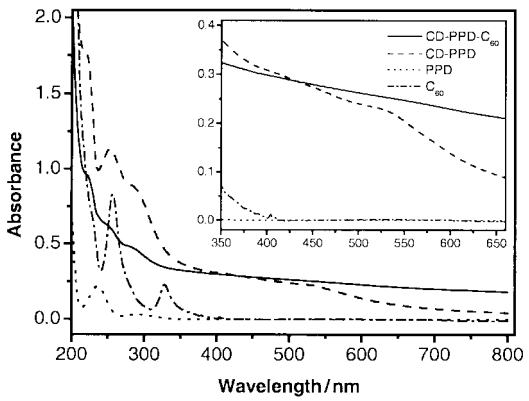
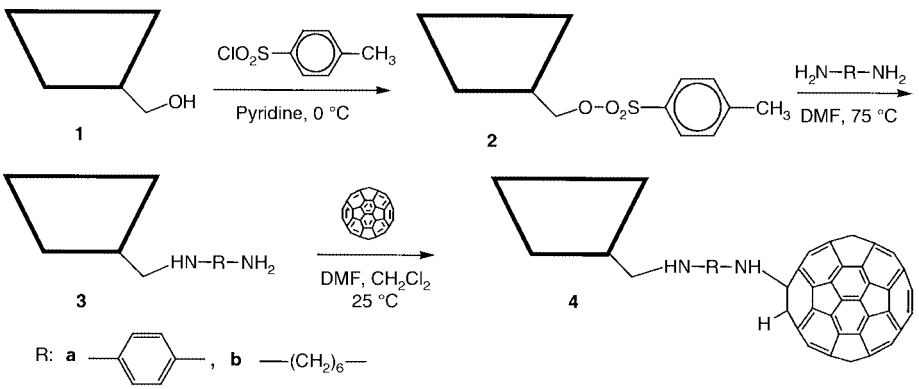


Fig. 1 UV–Vis spectra of C_{60} in hexane and *p*-phenylenediamine (PPD), mono-6-deoxy-6-(1,4-diiminodiphenyl)-β-cyclodextrin (CD-PPD), and [60]fullerene-deoxy-6-(1,4-diiminodiphenyl)-β-cyclodextrin (CD-PPD-C_{60}, **4a**) in water.



Scheme 1 Synthesis of fullerene–cyclodextrin conjugates.

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increased in intensity for both the **4a**–PNP and β-CD–PNP experiments, whereas this absorption for PNP alone was not significant. The molar ratios **4a**:β-CD:PNP for **4a**–PNP and β-CD–PNP inclusion experiments were 1:2:8. It was observed that the rate of increase in the absorption intensity for β-CD–PNP complexation was nearly twice than that for the **4a**–PNP reaction, which means that the CD ring in **4a** is as active as that in free CD molecules.

It is known that C_{60} readily adds simple alkyl and aryl radicals[2] and undergoes radical polyreactions.[11] The stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been used to study the antioxidant activities of phenols and catechol.[12] We utilized this radical for the reaction with **4a** in ethanolic solution and monitored the absorbance at 517 nm. The reaction proceeded with a gradual decrease of absorbance at 517 nm, with a visual bleaching of the pink color of the radical solution, indicating that **4a** is an effective radical scavenger.

The interaction potential of fullerenes with biological molecules has been recently recognized, especially their ability to cleave DNA nucleotides[3b,c] *via* a singlet oxygen transfer at the guanosine base sequence.[3d] We conducted a screening experiment in which a sample of DNA oligonucleotide, which was purified by membrane filtration prior to use, was treated with **4a** in aqueous media. The UV–Vis spectrum of the solution mixture shows two peaks, one at 260 nm, characteristic of the DNA component, and another broad absorption with a maximum at 343 nm due to the fullerene compound. It was observed that the fullerene peak intensity gradually decreased, indicating that there had been a reaction with the DNA in which **4a** was gradually consumed. There was no noticeable decrease in the intensity of the DNA peak.

Upon diafiltration of the fullerene compound–DNA solution mixture (20 mL) through the membrane (MMCO 10 000 g l^{-1}, used for purification of crude DNA) with 800 mL water (filtration factor, $Z = 40$), the amount of DNA in the permeates sharply decreased and only a very small quantity of the DNA component was left in the retentate. This was ascertained from a rapid decrease in the characteristic DNA absorbance at 260 nm in the permeates. The molar mass of the DNA in these samples was measured by GPC. It was found that almost the entirity of the DNA had undergone cleavage, leaving behind no high molecular mass species.

We are grateful to KOFST and K-JIST, Korea, for partial financial support and to B. J. Choi and S. J. Choi for technical assistance.

Notes and references

† General procedure for synthesis of [60]fullerene-β-cyclodextrin conjugate (**4**): Amine **3** (0.02 mmol) dissolved in 10 mL DMF was added in small portions, keeping a gap of 6 h between each addition, to the fullerene (C_{60}) (14 mg, 0.02 mmol) dissolved in 100 mL methylene chloride, until 30% of the theoretical amount of fullerene remained unreacted. The mixture was stirred at 25 °C under N_2 (3–10 d), after which the organic solvent was removed. Then 200 mL water was added, the insoluble black residue filtered off and the filtrate freeze-dried. The crude sample was purified further by membrane filtration (details in the text). The yield and solubility of the fullerene–cyclodextrin conjugates varied, representing the characteristics of the amine component and the macrocycles, respectively.

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