Studies on Organo-functionalized Carbon Clusters

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The development of the method for functionalization of carbon clusters is one of the important research topics because the functionalized carbon clusters will obtain much potential for application. As described in my thesis new multiple addition reactions are developed and these reactions are good candidates for supply of useful functionalized carbon clusters in practical scale. The modified carbon clusters are attractive enough that we want to investigate by ourselves. While the functionalization of outer surface is efficient in preparation of material, that of inner space gave the opportunity to "see" single small organic molecule.

Carbon clusters, represented with [60]fullerene, carbon nanotube, carbon nanohorn aggregate, have been found to have unique properties due to its size, shape, and wide conjugated system. Carbon atom, consisting the entire skeleton of carbon clusters, is ubiquitous element, meaning that carbon clusters are free from the problem of supply. Carbon atom also offers opportunities for covalent functionalization. This functionalization can control the surface properties and introduce functional group. Obtaining this flexibility, we can enjoy the development of infinite number of useful functionalized carbon clusters. In this thesis, the development of synthetic method and application of carbon clusters are described.

Soon after the discovery of mass production of [60]fullerene, amination reaction was found as one of the oldest functionalized fullerenes. Some aminofullerene have already been found to have useful bioactive properties; tetraaminofullerene epoxide works as a vector of gene derivery. The research on application, however, hampered by the lack of efficient synthetic methods. After careful examination, the addition of DMSO into solvent under oxygen atmosphere was found to accelerate the generation of tetraaminofullerene epoxide. Easy scale up of the preparation could be demonstrated due to the simple method. When [60]fullerene and amine was mixed in mixed solvent under nitrogen, fullerene radical anion could be detected by NIR spectroscopy. These mechanistic study indicated that DMSO assists the single electron transfer from aliphatic amine to electron deficient [60]fullerene with its high donor-number and large electron permittivity.

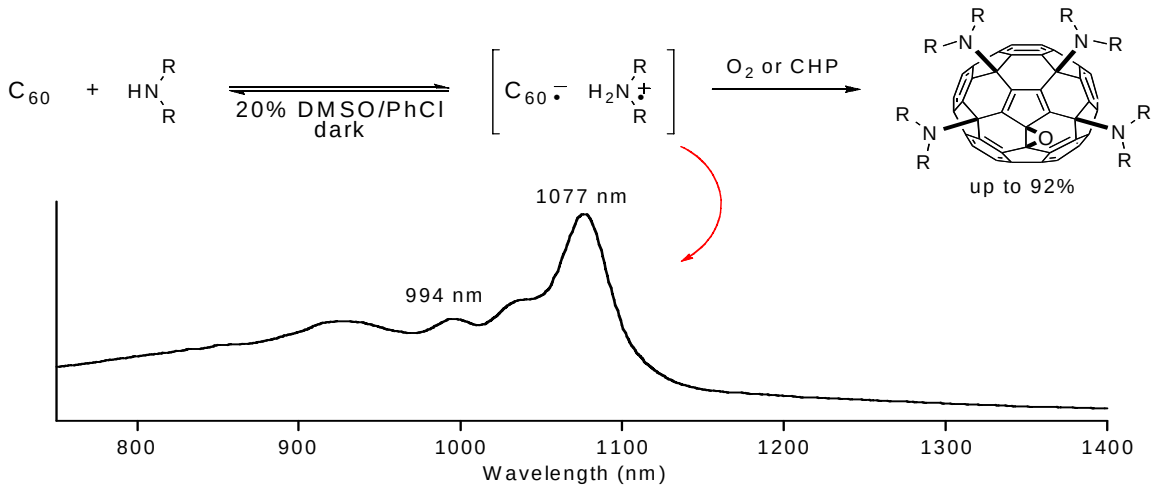

Figure 1. Reaction scheme of synthesis of tetraaminofullerene epoxide (top) and NIR spectrum of mixture of C_{60} and piperidine in deaerated 20% DMSO/PhCl (bottom). The absorption maxima at 994 nm and 1077 nm is characteristic of C_{60} radical anion.

The preparation method of tetraaminofullerene epoxide still have problem on the oxygen atmosphere. The contact of molecular oxygen and organic solvents leads explosion in the industrial synthesis and should be avoided. The precise investigation of previous reaction suggests that hydrogen peroxide generated in situ promotes the addition reaction. Optimization of the reaction showed that cumene hydroperoxide, one of the mild peroxide, gave the best result. This new reaction affords the efficient and safe preparation method of tetraaminofullerene epoxide. When this reaction was applied to higher fullerenes, DMSO, cumene hydroperoxide, and photo-irradiation assisted amination reaction with [70]fullerene gave monooxy diaminofullerene. The development of functionalization method of higher fullerene is expected to application to carbon nanotube, which have fullerene-like end caps.

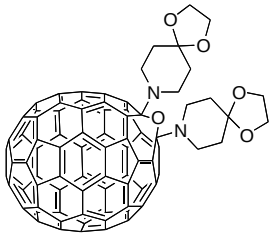

Figure 2. The structure of monooxy diaminofullerene.

During the research with fullerene, I got interested in carbon nanotubes. Carbon nanohorn aggregates, which are self-aggregate of short carbon nanotubes, have much number of reactive tips and thought to be more reactive than carbon nanotubes. Uniform size and well-controlled chemical composition are good properties of carbon nanohorn aggregate. Considering these properties, carbon nanohorn aggregates are suitable for preparation of standard sample of carbon nanotubes. Using this material accurate toxicity assessment of carbon nanotube, where the standardized recognition was not obtained, was conducted. We first prepared water-soluble carbon nanohorn aggregates, amino nanohorn aggregates, by treating with sodium amide in liquid ammonia. Taking advantage of solubility, all the physicochemical properties, which are suspected to affect toxicity of nano materials, could be determined using transmission electron microscopy, atomic force

microscopy, dynamic laser light scattering, and chemical reactions.

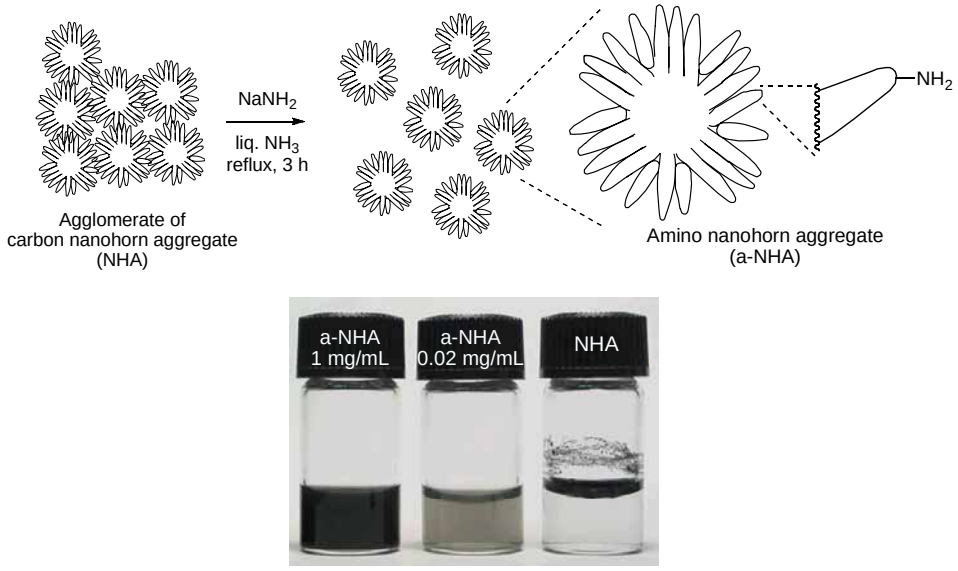

Figure 3. The preparation scheme of amino nanohorn aggregate (top). Picture of amino nanohorn aggregate and carbon nanohorn aggregate solution in water (bottom). Amino nanohorn aggregate dissolvs into water up to 1 mg/mL, while carbon nanohorn aggregate does not. The amino nanohorn aggregate makes monodispersed solution in water determined by dynamic laser light scattering.

To estimate the toxicity of nanomaterials, the definition of toxicity is important, because there is concern that nanomaterials show unusual properties coming from their size, shape, and compsits. We found out an important creterial and used quartz particles and TiO_2 particles are used as control. The cytotoxicity was estimated by total protein production, which is free from the affection of amino nanohorn aggregate. The result of cytotoxicity assessment showed the cytotoxicity of amino nanohorn aggregate was much lower than that of the quartz microparticles. Considering that quartz particles are widely used in load-marking paints, carbon nanotube can be said as safe material, however the celler uptake of amino nanohorn aggregate suggest the long term toxicity should be concerned.

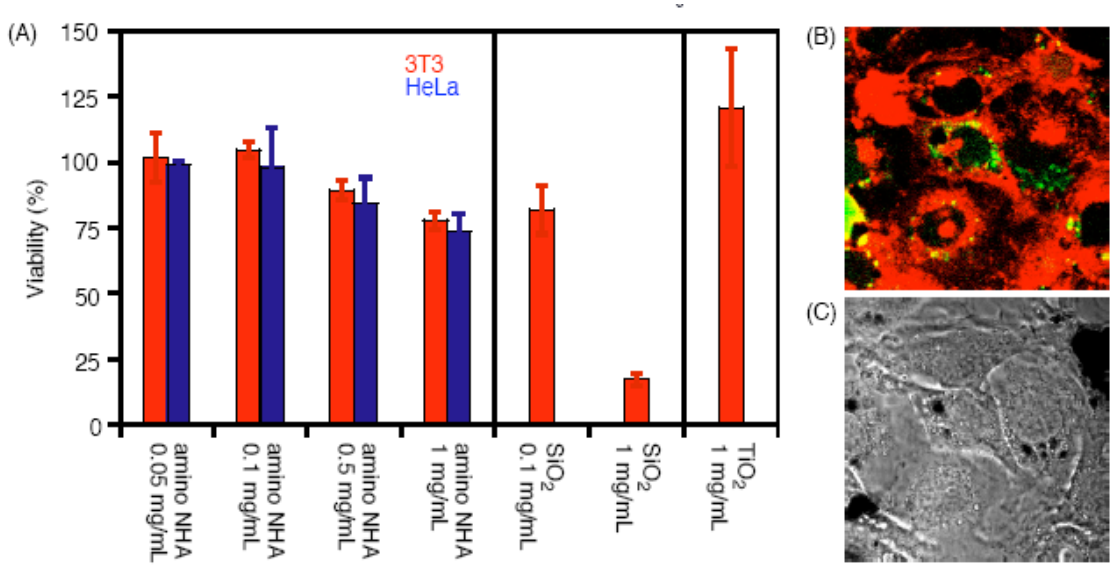

Figure 4. (A) Cytotoxic assessment of amino nanohorn aggregate. Viability of 3T3 (red) and HeLa (blue) cells after incubation with particles for 24 h. Viability was determined by Bradford assay. The viability of amino nanohorn aggregate at 1 mg/mL dose is almost same value as that of quartz particle at 0.1 mg/mL dose. (B and C) Confocal microscope images of 3T3 cells after incubation with fluorescent-labeled amino nanohorn aggregate for 20 h. Scale bars are 20 ºm. (B) Fluorescent microscope image. Amino nanohorn aggregate was observed with the fluorescence colored with green, and the cell membrane was stained with FM4-64 colored with red. Amino nanohorn aggregate (green) can be seen in the cell membrane (red). (C) Phase contrast image. Big black dot indicate the aggromerate of amino nanohorn aggregate.

When we concern the outside of carbon nanotube, a good material could be obtained. On the other hand, when we looked at inner space, we could obtained the images of molecules trapped in carbon nanotubes. Single-walled carbon nanotubes can contains molecules like fullerenes and the image of [60]fullerene can be obtained their images by transmission electron microscopy. We noticed that carbon nanotubes could be used as a container for trapping single organic molecule for observation, where molecules are moving around in vacuumed space. Experts, however, though that organic molecules will be decomposed by electron irradiation. Anyway, alkylcarborane, which contains carborane moiety for detectable tag, was designed and synthesized. After introduction of alkylcarborane into hole-opened single-walled carbon nanotube, images of molecules could be obtained by transmission electron microscopy. The shape, contrast, and spectroscopic analysis supported that the images were target molecules. The design of the molecules is key of this success. Why the molecules were not decomposed? The reason may be the molecules are isolated from surrounding molecules, except carbon nanotubes, and activated molecules cannot react with others inducing decomposition.

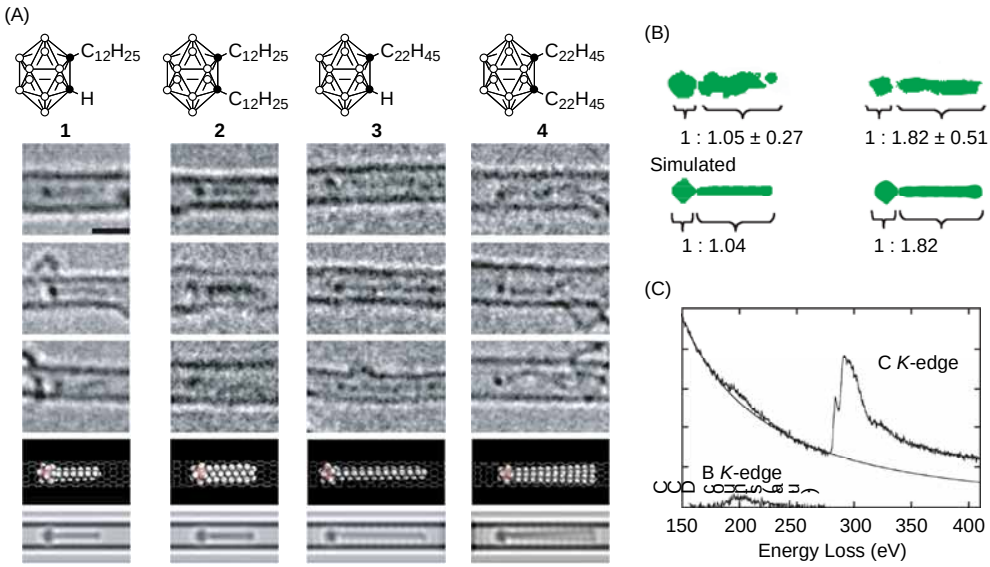

Figure 5. (A) Experimental transmission electron microscope images of the alkylcarboranes. Top three images are observed images. The bottom two images are molecular model of alkylcarboranes in single-walled carbon nanotubes and its simulated images. Scale bar shows 1 nm. In the strucure, black circle and white cricle represent BH and C atoms, repectively. (B) Contrast analysis of alkylcarborane 1 (left) and 2 (right). Top images are averaged contrasts estimated from (A) and bottom images are contrasts from simulated images. (C) EELS spectrum of 2 in carbon nanotubes. Peaks at about 200 eV and 300 eV come from boron and carbon atoms, respectively.

Further observation visualized the conformational and translational motion. These data will be applied for structural conformational study. So far such study is investigated by the analysis of ensembles. The images of not only alkyl chains but amide bonds could be seen locating both inside and outside of carbon nanotubes increase the expectation of strong analytical tool for the study of the structure and internal rotation of a wide range of organic and organometallic molecules.

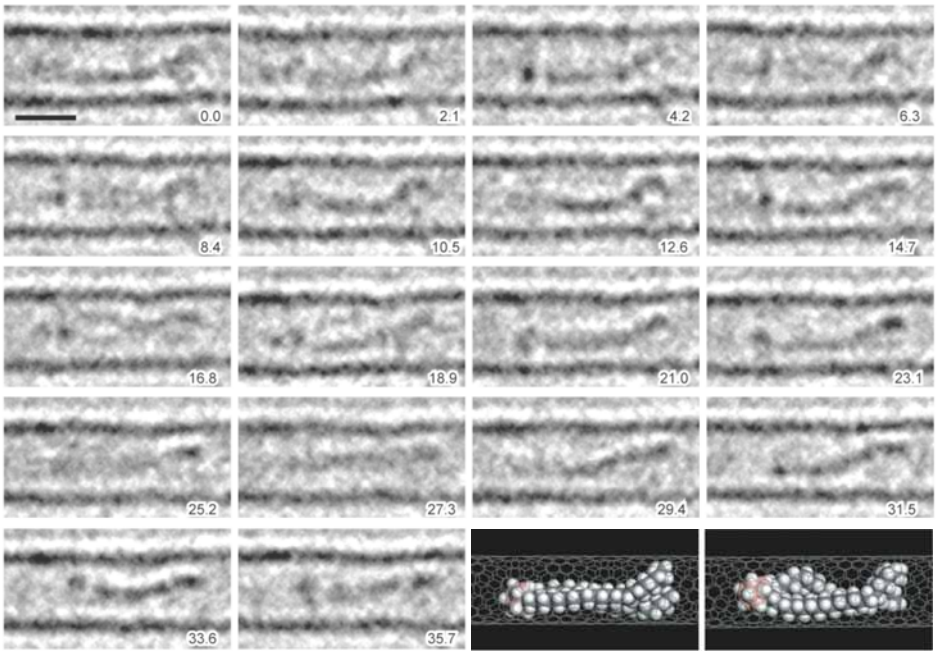

Figure 6. Transmission electron microscope images of the conformational change of **4** (see Figure 4) in a carbon nanotube of 1.2 nm diameter. (A) Experimental images showing that two alkyl chains change their conformation as the molecule wobbles. The images were obtained over ca. 40 s (from top left to bottom right). The numbers at the bottom of each image show the time of the observation in seconds. The scale bar is 1 nm. (B and C) A 3D model of the molecule in a carbon nanotube at times 6.3 s and 8.4 s, respectively. Hydrogen: white, boron: pink, carbon: gray.

In this thesis was described the development of functionalization method of carbon clusters and application of the modified them. Though this study, I, as a young chemist, learned that synthesis of molecule can open a wide range of research field.