β-1,3-Glucan Schizophyllan Can Act as a One-dimensional Host to Arrange Icosahedral Carboranes

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We here demonstrate that schizophyllan (SPG: one kind of *β*-1,3-glucans)/*m*-carborane nanocomposites can be easily created in a supramolecular manner by utilizing the unique wrapping capability of SPG. TEM observations revealed that incorporated *m*-carborane molecules adopt continuous one-dimensional (1-D) arrangement in the SPG hydrophobic cavity. We believe that the present system can provide a novel concept not only for the separation of carborane isomers and the construction of one-dimensional architectures but also for the new formulation of boron neutron capture therapy (BNCT) agents.

Schizophyllan (SPG) is a natural *β*-1,3-glucan and is known to exist as a triple helix (t-SPG) in water but as a single random coil (s-SPG) in dimethyl sulfoxide (DMSO).[1] When water is added to its DMSO solution, s-SPG retrieves its original triple helix (renaturation). These specific structural characteristics have made s-SPG form the stable water-soluble complexes with certain polynucleotides,[2] single-walled carbon nanotubes,[3] conjugated polymers,[4] fibrous silica,[5] porphyrin,[6] and Au nanoparticles[7] during its renaturation process. In the present paper, our attention focuses on extending this concept toward the creation of 1-D array from carboranes, i.e., 1,2- and 1,7-dicarbadodecaborane (hereafter abbreviated as *o*- and *m*-carborane, respectively) (Figure 1). Carboranes are carbon-containing polyhedral boranes, which are extremely stable caged molecules known for over 30 years.[8] The size of carboranes is about 0.8 nm in diameter, which is almost consistent with that of polyhedral carbon cluster C_{60}. So far, carboranes and C_{60} have been regarded as attractive guest molecules in host–guest chemistry due to their unique morphologies; that is, the bowl-shape host molecules such as calixarenes,[9] cyclodextrins,[10] and cyclotriveratrylenes[11] exhibit the unique inclusion behaviors due to the specific interactions with these guest molecules. Recently, much attention has been directed toward carboranes not only due to their unique shapes acting as building blocks in supramolecular assemblies but also due to their potential applications to the biological fields. In particular, carboranes and their derivatives have great potential as boron neutron capture therapy (BNCT) agents.[12] Carboranes, however, are highly hydrophobic molecules by themselves, so that one has to bestow sufficient water solubility on BNCT agents based on carboranes. We report herein the intriguing preliminary results that SPG can accommodate carboranes into its 1-D hydrophobic cavity to create highly water-soluble nanocomposites, which would be readily applicable to BNCT of cancers. In addition, we have investigated the formation mechanism of SPG/carborane nanocomposites, where the unique inclusion behavior of SPG and the potential hydrogen-donating ability of carboranes play important roles in the composite formation.

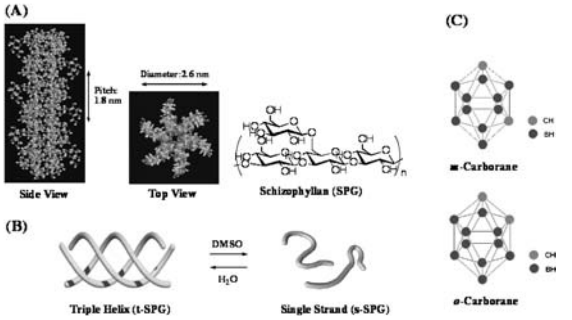

Figure 1. (A) Calculated models of SPG triple helix and repeating units of SPG; (B) renature and denature processes of SPG; (C) structural models of carborane isomers used here (*m*-carborane and *o*-carborane).

Firstly, we tried to prepare the SPG/carborane composite using *m*-carborane (see the Supporting Information).[15] The obtained aqueous solution was clear and unaggregated even after leaving it for several weeks. One can regard, therefore, that *m*-carborane would be incorporated into the hydrophobic 1-D cavity without forming the insoluble 3-D aggregate. On the other hand, when the initial *m*-carborane concentration was adjusted above $2.0\,\mathrm{mg\,mL^{-1}}$, uncomplexed *m*-carborane was precipitated out during the dialysis treatment. These results indicate that when the [*m*-carborane]/[s-SPG] ratio is adjusted below 1/5 (w/w), *m*-carborane can be entirely encapsulated into the SPG cavity without forming the 3-D aggregate or the precipitate. Based on these findings, hereafter, we adjusted the initial SPG/carborane ratio to [*m*-carborane]/[s-SPG] = 1/5 (w/w).

To obtain more quantitative data on how much *m*-carboranes is solubilized in water by the SPG wrapping, we carried out ICP (inductively coupled plasma)-mass measurements, expecting that it can provide the elemental analysis data for the SPG/*m*-carborane composite in aqueous solution. First of all, to remove *m*-carborane adsorbed onto the SPG surface or nonspecifically entrapped in the SPG cavity, the obtained SPG/*m*-carborane composite was washed with MeOH for several times. After the freeze-dry treatment, the obtained white powder was re-dispersed into pure water and subjected to ICP-

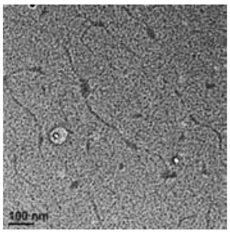

Figure 2. TEM image of SPG/*m*-carborane composite; The image was obtained without staining.

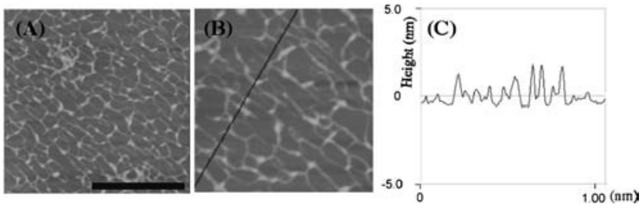

Figure 3. (A) AFM image of SPG/*m*-carborane composite (bar 1 µm); (B) magnified image of (A); (C) height profile obtained by tip scan along the black line in (B).

mass measurements. ICP-mass spectroscopic data revealed that the SPG/*m*-carborane composite contains 12.4 wt % of boron atom. From this result, we can conclude that one repeating unit of SPG (three-glucoses unit in main chain) interacts with 0.7 unit of *m*-carborane. Considering the helical pitch of natural SPG (1.8 nm/six-glucoses unit in main-chain) and the diameter of *m*-carborane, entrapped *m*-carboranes are continuously aligned in the SPG hydrophobic cavity to form 1-D array.

TEM images of the composite support the view that the *m*-carborane array is created in the SPG cavity (Figure 2). Although the images were obtained without staining, one can recognize many shadowed fibers. Furthermore, the contrast, which would arise from *m*-carborane, appears continuously along the SPG fiber, suggesting that continuous one-dimensional *m*-carborane array would be constructed in the SPG cavity. We also carried out AFM observation for the SPG/*m*-carborane composite. Figures 3A–3C show AFM images and a height profile of the composite after casting on mica, respectively. In Figures 3A and 3B, one can see many fibrous aggregates, the diameter of which can be estimated to be ca. 1.9 nm from Figure 3C. Considering the diameter of *m*-carborane (0.8 nm) and s-SPG chain (ca. 0.5 nm), *m*-carboranes would be incorporated into the SPG cavity without forming the three-dimensional aggregate, leading to the creation of the continuous one-dimensional *m*-carborane array.

To investigate whether the direct hydrogen-bonding interaction between *m*-carborane and SPG exists or not, we carried out IR spectroscopic measurements for the SPG/*m*-carborane composite. IR spectra (Figure 4) thus obtained show that *m*-carborane itself gives characteristic IR peaks at 2597 and 3064 cm^{-1}, which are assignable to a B–H stretching band and a C–H stretching band, respectively. It was found that upon mixing with SPG, the B–H stretching peak shifted from 2597 to 2605 cm^{-1}. On the other hand, the C–H stretching peak could not be identified due to the peak overlap with that arising from SPG. It is already known that the B–H hydrogen in carboranes bears a hydridic character to serve as a hydrogen-bond acceptor. In fact, the existence of B–H···H–O type hydrogen bonds was proposed by several research groups.[13] According to this view, the observed peak shift in the IR spectra would be attributed to the formation of the B–H···H–O type bond between *m*-carborane and SPG O–H groups in the interior hydrophobic cavity of SPG.[14] These results allow us to conclude that the B–H···H–O type interaction between *m*-carborane and SPG would play an important role for the composite formation and also for the stabilization of the resultant composite in aqueous solution.

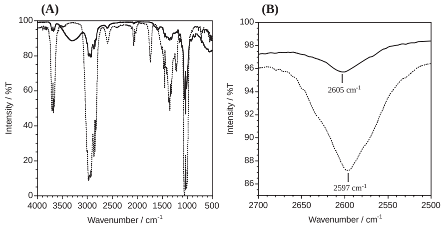

Figure 4. IR spectra of SPG/*m*-carborane composite (solid line) and *m*-carborane only (broken line).

In summary, we have demonstrated that SPG can entrap *m*-carboranes into its one-dimensional cavity with the aid of cooperative C–H···O and B–H···H–O interactions, in addition to the generally operating hydrophobic interaction. It was already shown that β-1,3-glucans modified with functional groups also act as one-dimensional hosts for hydrophobic guest molecules. Therefore, when β-1,3-glucans bearing cell-affinity groups are used as hosts for carboranes, the resultant modified SPG/carborane composites are readily applicable to the BNCT of cancer agents.[12] We believe that the present system can open a new way to create functional bio- and nanomaterials based on carboranes.

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14 Harada et al., also reported the similar peak shift of B–H bonds in the carborane/cyclodextrin complexes (see: Ref. 10b). In the present system, C–H···O type interaction would exist and it also results in strengthening of B–H bonds (see: Refs. 8b and 9).

15 Supporting Information is available electronically on CSJ-Journal Web site: http://www.csj.jp/journals/chem-lett/.

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