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Dendritic Physical Gel: Hierarchical Self-Organization of a Peptide-Core Dendrimer to Form a Micrometer-Scale Fibrous Assembly

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Dendrimers are nanoscopic hyperbranched macromolecules with well-predictable three-dimensional shapes and potential building blocks for the construction of organized functional materials.[1,2] Recently, self-assembly of dendrons and dendrimers has attracted great attention, where hydrogen-bonding,[3] metal-ligating,[4] van der Waals,[5] and electrostatic interactions[6] are utilized as the driving forces. Herein, we report the first examples of a dendritic physical gel formed by self-assembly of dipeptide-core poly(benzyl ether) dendrimers in organic solvents,[7,8] and we highlight a "hierarchical" self-organization to form a micrometer-scale fibrous assembly of nanoscale dendritic fibrils.

A series of poly(benzyl ether) dendrimers carrying *tert*-butoxycarbonyl-(L)-tyrosinyl-(L)-alanine (**2**−**4**),[9] *tert*-Boc-Tyr-(L*n*)-Ala (L*n* = *n*-layered dendritic wedge, *n* = 2−4, Figure 1), was synthesized by an alkaline-mediated coupling reaction of Fréchet's dendron bromides (L*n*Br; *n* = 2−4)[10] with *tert*-Boc-(L)-Tyr-(L)-Ala[11] in DMF at 70 °C, and unambiguously characterized by ^{1}H NMR and MALDI-TOF-MS analyses.[9] When *tert*-Boc-Tyr(L3)-Ala (**3**) was dissolved in acetonitrile (1.0 mM), and the solution after sonication for 1 min was allowed to stand overnight at 20 °C, it formed a transparent gel, where about 20 000 solvent molecules are estimated to be gelled by one dendrimer molecule. The gelation was highly dependent on the concentration of **3**. As the concentration of **3** was higher than 1.0 mM, the time required for the gelation was considerably shorter (e.g., 5 min at 1.9 mM), whereas no gelation was observed even after one week when the concentration of **3** was lower than 1.0 mM (e.g., 0.7 mM). The gelation also took place in other organic solvents such as acetone, ethyl acetate, $CHCl_3$, CH_2Cl_2, benzene, and $CHCl_3$/benzene (1/1), where acetone and $CHCl_3$/benzene, similar to acetonitrile, were particularly effective for the gelation (critical concentrations for gelation; 4.6 and 1.9 mM, respectively). Interestingly, the gelation was also dependent on the size of the dendritic wedge: A non-dendritic benzyl-substituted dipeptide *tert*-Boc-Tyr(Bn)-Ala (**1**) and the smallest dipeptide-core dendrimer *tert*-Boc-Tyr(L2)-Ala (**2**) were highly soluble in acetonitrile and did not form gels even at a much higher concentration such as 130 mM. On the other hand, the largest dendrimer *tert*-Boc-Tyr(L4)-Ala (**4**) formed a gel in acetonitrile with a critical concentration for gelation of 1.4 mM, and also in acetone (2.2 mM) and $CHCl_3$/benzene (1.3 mM). Therefore, the dendritic wedge as well as the dipeptide core must be involved in the gelation process.

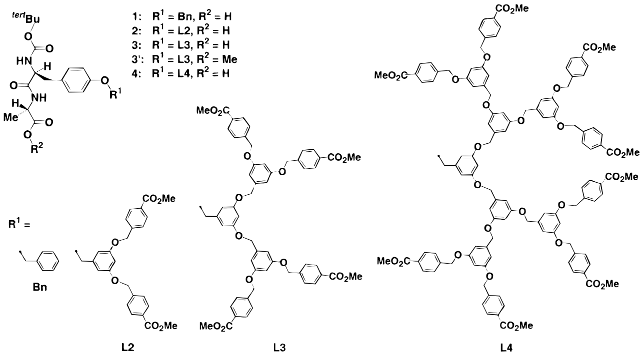

Figure 1. Schematic structures of dipeptide-core poly(benzyl ether) dendrimers, *tert*-Boc-Tyr(Bn)-Ala (**1**), *tert*-Boc-Tyr(L*n*)-Ala (**2**−**4**, *n* = 2−4), and *tert*-Boc-Tyr(L3)-Ala-OMe (**3′**).

The gelation of dendrimers **3** and **4** is most likely induced by hydrogen-bonding interactions of the dipeptide core. In fact, upon addition of even a few drops of DMSO, the gelled samples of **3** and **4** in acetonitrile rapidly became fluid. Likewise, an inorganic salt such as LiCl, which is known to interact with amide functionalities, was found to prohibit the gelation.[9] Infrared spectrum of a nongelled $CHCl_3$ solution of **4** (4.5 mM)[12] displayed relatively broad absorption bands at 3420 and 1680 cm^{-1}, characteristic of stretching vibrations of N−H and C=O bonds, respectively. On the other hand, the gelled sample of **4** in benzene/$CHCl_3$ (1/1) (2.6 mM) showed the corresponding absorption bands at 3,320 (N−H) and 1650 cm^{-1} (C=O), which are red-shifted by 100 and 30 cm^{-1} from those of the nongelled $CHCl_3$ solution of **4**. In relation to these observations, *tert*-Boc-Tyr(L3)-Ala-OMe (**3′**), an end-capped derivative of **3** having an esterified *C*-terminal, did not form gels but precipitated in acetonitrile and acetone, suggesting a participation of the terminal carboxylic acid functionality of the dipeptide core in the gelation process.

Cross-polarized microscopy of the gelled sample of **4** in acetonitrile showed that it contains bundles of micrometer-scale fibers. To obtain further structural information, the gelled sample of **4** in $CHCl_3$/benzene (1/1) was dried under reduced pressure

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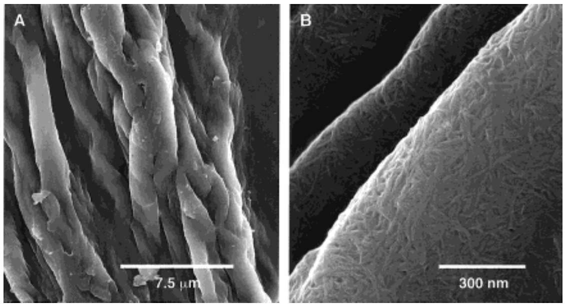

Figure 2. Scanning electron microscope (SEM) images of a dried gel of **4**. (A) 4000×, (B)100000×.

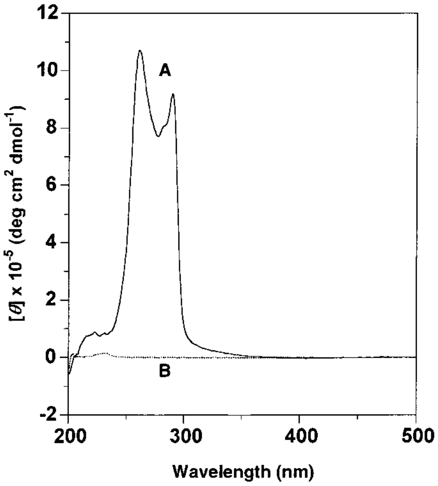

Figure 3. CD spectra of *tert*-Boc-Tyr(L3)-Ala (**3**) under gelled (A: 1.2 mM, 0.01-cm quartz cell) and nongelled (B: 0.18 mM, 0.1-cm quartz cell) conditions in acetonitrile at 20 °C.

and subjected to scanning electron microscopy (SEM).[13] From the SEM image of the dried gel (Figure 2A), the diameter of the bundled fibers was evaluated to be 1−2 μm. Of further interest is the fact that each fiber consists of a bundle of much thinner fibrils with a diameter of approximately 20 nm (Figure 2B). From a computer-aided calculation of the molecular structure of **4**, it is assumed that each elementary fibril includes at least 15 dendritic wedges in the cross section. Such a hierarchical self-organization from a molecular-level association to nanoscale and further to micrometer-scale assemblies is highly interesting, since it is common to biological organisms.

The gelled sample of **3** in acetonitrile (1.2 mM) at 25 °C displayed strong circular dichrosim (CD) bands at the absorption bands of the dendritic wedge ($[\theta]_{261} = +1.06 \times 10^6$ and $[\theta]_{290} = +9.14 \times 10^5$), in addition to an enhanced CD band due to the dipeptide unit ($[\theta]_{231} = +8.26 \times 10^4$) (Figure 3A). This is in sharp contrast with the CD profile of **3** in a nongelled acetonitrile (0.18 mM,[12] Figure 3B) or dioxane (1.2 mM) solution, which displayed only a weak band due to the dipeptide unit without any detectable CD bands due to the dendritic wedge. These observations may suggest an interesting possibility that the dendritic wedges of **3** in the fibrils are arranged in a helical manner.[14]

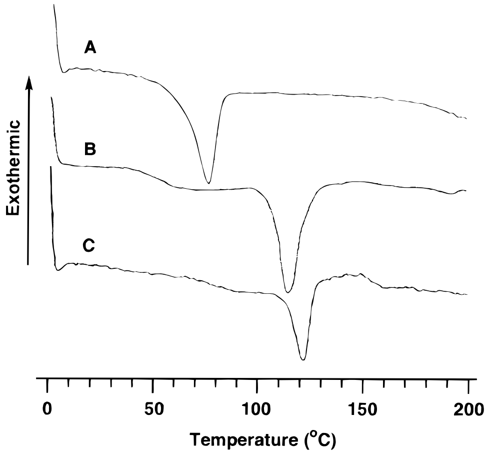

Figure 4. DSC profiles of dipeptide-core dendrimers; (A) *tert*-Boc-Tyr-(L2)-Ala (**2**), (B) *tert*-Boc-Tyr(L3)-Ala (**3**), and (C) *tert*-Boc-Tyr(L4)-Ala (**4**), in the solid state. The samples were prepared by evaporation of a nongelled acetonitrile solution of **2** and gels of **3** (15.4 mM, acetone) and **4** (10.4 mM, CHCl$_3$/benzene) on a piece of aluminum foil.

Thermal behaviors of the dried gels[15] were studied by differential scanning calorimetry (DSC) and temperature-variable infrared spectroscopy. Upon heating at a rate of 10 °C min^{-1} from 0 °C, the dried gels of **3** and **4** exhibited endothermic peaks at 112 (40.3 kJ mol^{-1}) and 122 °C (51.9 kJ mol^{-1}), respectively (Figure 4, B and C). Optical microscopy of the dried gels showed complete melting of the bundled fibers at these particular temperatures. Upon cooling at a rate of −10 °C min^{-1}, the melted samples were glassified, where the glass transition temperatures of **3** and **4** were observed at 55 and 76 °C, respectively. As described above, **2** does not form a gel in any of the above solvents. However, a solid sample of **2**, prepared by evaporation of an acetonitrile solution, showed characteristic stretching vibrations of hydrogen-bonded N−H and C═O functionalities (3320 and 1650 cm^{-1}, respectively) and also displayed a melting behavior (Figure 4A) and a glass transition at much lower temperatures 74 (27.0 kJ mol^{-1}) and 45 °C, respectively. In relation to these observations, infrared spectra of the dried samples of **2**−**4**, upon elevating the temperature, all showed complete destruction of the hydrogen bonds at their melting temperatures. For example, the dried gel of **4** at 130 °C displayed stretching vibrational bands of the N−H and C═O bonds at 3420 and 1680 cm^{-1}, respectively, just like those observed for the nongelled CHCl$_3$ solution of **4**. Therefore, it is likely that the bulky dendritic wedges can stabilize the hydrogen bonds among the dipeptide cores via van der Waals interactions.

In conclusion, through the present studies on the gel-forming behaviors of **1**−**4** and **3′** in organic solvents, we have demonstrated that large poly(benzyl ether) dendrimers carrying a dipeptide core (**3**, **4**) are hydrogen-bonded to form fine elementary fibrils (approximately 20 nm in diameter), which are further assembled via van der Waals forces to give bundles of micrometer-scale dendritic fibers. Such a hierarchical self-organization not only is interesting from a biomimetic point of view, but also may provide a new strategy for the design of micrometer-scale superstructured dendritic materials with nanometric precision.

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Supporting Information Available: Details for (1) synthetic procedures of **2**−**4** and **3′**, and (2) results of gelation experiments of **3** in the presence of LiCl (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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