J|A|C|S
COMMUNICATIONS

Published on Web 07/24/2004

Dendronized Protein Polymers: Synthesis and Self-Assembly of Monodisperse Cylindrical Macromolecules

Michael A. Zhuravel,[†,‡] Nicolynn E. Davis,[†] SonBinh T. Nguyen,[‡] and Ilya Koltover*[,†]

Materials Science and Engineering and Chemistry Departments, Northwestern University, Evanston, Illinois 60208

Received May 23, 2004; E-mail: ilya@northwestern.edu

Self-assembly of ordered molecular patterns is a powerful route towards new materials. Success of this synthetic strategy depends on the availability of well-defined molecular building blocks with nanoscale dimensions. Dendrimers, highly branched macromolecules consisting of dendritic wedges (dendrons) attached to a small core, are one type of such nanoscale building blocks.[1] Although most dendrimers have spherical architectures, recently the preparation of dendrimers with rodlike, cylindrical shapes has been described. Percec[2] and Schluter[3] have shown that the polymerization of dendronized monomers can give rise to dendritic polymers with elongated shapes, while Tomalia demonstrated that rod-shaped dendrimers can be prepared by stepwise growth of dendritic side chains from a polymeric initiator backbone.[4] Dendrimers prepared by these pioneering strategies possess well-defined diameters (*D*) and rigid shapes, but have a statistical distribution of lengths (*L*). New synthetic approaches are needed to prepare molecules with defined *L and D* that could self-assemble into nanostructures having long-range order controlled by the exact molecular dimensions.

Current synthetic methods do not allow for the generation of large polymeric molecules with strictly defined lengths. However, there are several natural examples of monodisperse macromolecules, such as rod-shaped viruses[5] and biosynthetic polypeptides,[6] that self-assemble into structures with order directly templated by their well-defined lengths. Motivated by these observations, we have developed a synthetic approach that combines tools of biology and chemistry to prepare cylindrical dendrimers with controlled dimensions (Figure 1a). A monodisperse, α-helical polypeptide backbone is expressed in a bacterial host (*Escherichia coli*) from engineered DNA template (gene), thereby defining the length and overall rod shape of the target cylindrical macromolecule. Synthetic dendrons are then grafted to the reactive amino acid side chains along the peptide backbone, covering it with a dendritic shell whose thickness is controlled by the generation and chemical structure of the dendrons. The resulting dendronized protein polymer (DPP) has length *L* and diameter *D* defined by the biological and chemical synthetic components, respectively. These DPPs self-assemble to form highly ordered liquid crystalline (LC) structures unique to molecules with absolutely defined shape and size.

Genetically engineered poly-L-glutamic acid was used as the DPP backbone in the first experimental realization of our synthetic scheme. We hypothesized that grafting dendritic wedges to every glutamic acid residue of the homopeptide would maximize the steric stabilization of the DPP shape and test the efficiency of the dendron-grafting chemistry. Synthetic genes encoding polyglutamic acids glu_n with three discrete lengths $n = 58$, 76, and 94 were cloned, transformed into *E. coli* cells, and expressed to yield the target-purified monodisperse polypeptides.

We chose to conjugate the glu_n peptides with hydrophobic dendrons since complete dendronizatition would stabilize α-helical

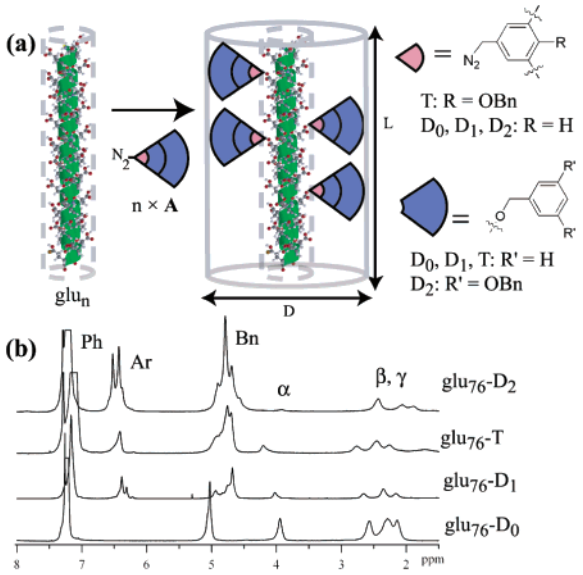

Figure 1. **(**a) Modular synthesis of cylindrical DPPs. Dendritic wedges **A** are grafted to the *n* reactive amino acid side chains (red) along a monodisperse α-helical peptide backbone (green), generating a cylindrical molecule with defined nanoscale length L and diameter D. For clarity, only four of the grafted dendrons are shown here. **(b)** ^{1}H NMR spectra of DPPs with glu_{76} cores and four different dendritic shells. Note the increase in the intensity of the dendritic shell resonances relative to that of the peptide backbone with increasing dendron size.

conformation of the peptide core by forcing intramolecular hydrogen bonding and produce DPPs that are soluble in organic solvents. Frechet-type[7] poly(benzyl ether) dendrons with an aldehyde function at the focal point were converted to tosylhydrazones, which afforded the corresponding diazo-functionalized dendrons (**A**, Figure 1a) upon treatment with an aqueous base. Reaction of excess **A** with the carboxylic acid side chains of the glu_n cores in DMSO efficiently positioned the wedge-shaped benzyl ether dendrons around the rodlike helical peptide core to complete the DPP cylinder. The large molecular weight difference between monodisperse DPPs (25−81 kDa, depending on peptide length and dendron type) and unreacted dendron byproducts (0.1−1 kDa), allowed us to purify DPPs in a single step using size-exclusion chromatography. Purified DPPs were isolated as white solids and were completely soluble in the same common organic solvents as monomeric dendrons, indicating complete sequestration of the polar peptide cores within the dendritic jackets.[8] To expand the range of similar dendritic architectures, we synthesized first-generation dendrons with two (D) and three (T) peripheral benzyls, with dendron sizes increasing in the order $D_0 < D_1 < T < D_2$.

The ^{1}H NMR spectra of DPPs dissolved in $CDCl_3$ (Figure 1b) show the expected α, *β*, and *γ* proton signals of the glutamic acid residues, as well as the benzyl (Bn), aryl (Ar), and phenyl (Ph) proton signals of dendron side chains. Remarkably, comparison of

[†] Materials Science and Engineering Department.
[‡] Chemistry Department.

10.1021/ja046965q CCC: $27.50

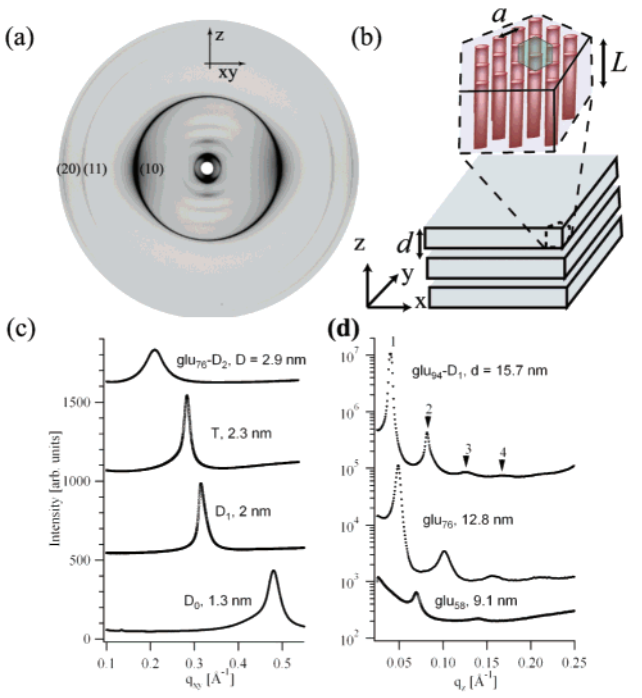

Figure 2. **(a)** SAXD pattern of glu_{76}-D_1 DPP dissolved in *m*-cresol (40 wt % solvent) showing lamellar (along the *z*-axis) and hexagonal (along the *xy*-plane) reflections. **(b)** Schematic illustration of the self-assembled structure of DPP solutions. **(c)** SAXD data of dry films prepared from DPPs with glu_{76} cores and varying dendron shells showing hexagonal packing peaks. **(d)** SAXD data of glu_n-D_1 samples at constant 40 wt % *m*-cresol concentrations showing lamellar stacking peaks.

the integrated signal intensities of the backbone and side-chain protons reveals essentially quantitative dendron-conjugation for all synthesized DPPs, regardless of the size of the dendritic wedges and despite the high density (1.5 Å/residue) of reactive glutamic acid side chains. MALDI-TOF analyses also confirmed >95% derivatization of the carboxylic acid groups. Importantly, the success of our synthetic approach does not depend on 100% efficiency in the grafting reaction: the *L* and *D* of the DPPs are fixed even if a few of the glutamic acid side chains remain unreacted, as long as the peptide is mostly covered by the dendrons.

The monodispersity and the α-helical conformation of the glu_n peptide backbones establish the three discrete lengths $L = n \times 1.5$ Å = 8.7, 11.4, and 14.1 nm for the synthesized DPPs, with four diameters corresponding to the different types of dendron side chains.[9] The self-assembly of the DPPs was investigated in *m*-cresol. Concentrated DPP/*m*-cresol solutions exhibit optical anisotropy (birefringence) that increases with the DPP concentrations, indicating formation of ordered LC structures. Small-angle X-ray diffraction (SAXD) patterns of DPP/*m*-cresol solutions, sealed between mica windows and oriented by gentle shearing, show two orthogonal sets of sharp diffraction peaks (Figure 2a). Reflections with ratios of $1{:}\sqrt{3}{:}\sqrt{4}$ along the *xy*-plane indicate hexagonal packing of DPP cylinders with well-defined spacing *a* between the cylinder axis. Along the *z*-axis, reflections with ratios 1:2:3:4 reveal a layered structure with periodicity $d \gg a$. The diffraction pattern is consistent with the structure shown in Figure 2b: cylindrical DPPs form periodically stacked layers with thickness equal to *L*, and hexagonal packing of molecules within each layer; solvent occupies the space between the molecules in each layer ($a > D$) and between the layers ($d > L$).

The observed long-range ordered self-assembly of DPP materials is driven by excluded volume interactions between rigid DPP cylinders.[10] In dry DPP films, where $a = D$, SAXD scans show the expected increase of cylinder diameters *D* with increasing size of the dendron side chains (Figure 2c). Remarkably, the measured *D* values agree very well with dimensional estimates that are based on simple geometrical computer models of helical peptide backbones surrounded by dendritic side chains (12.9 and 29 Å for glu_n-D_0 and -D_2, respectively). Comparison of SAXD scans of the three glu_n-D_1 molecules dissolved in *m*-cresol at the same concentration (Figure 2d), shows that the periodicity of DPP stacks increases with *L*. Knowing the amount of solvent in each sample, we estimate that the thicknesses of DPP layers are 8.7, 11.4, and 14.1 nm, identical to the expected lengths *L* of the respective DPP molecules.

In conclusion, we have developed a combined biosynthetic route toward a new class of cylindrical dendrimers with predictable size, shape, and solubility. Our DPP materials self-assemble into highly ordered LC phases with ordering length-scales (*a*, *d*) controlled by the dimensions of the DPP molecules. The modular nature of our biosynthetic strategy can be used to expand the range of accessible DPP interactions and shapes through the variation of peptide sequences, dendron functionality, and dendron architectures. These studies, as well as complete investigations of the unique liquid crystalline phases formed in DPP solutions, are currently in progress.

Acknowledgment. This work was supported by the NSF (Grants DMR-0134874, -0094347, and -0076097) and the ACS-PRF (Grant 37669-G7). X-ray diffraction experiments were carried out at the DND-CAT beam line of the Advance Photon Source (ANL), supported by the DOE Contract No. W-31-102-Eng-38.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Newkome, G. R.; Moorefield, C. N.; Vogtle, F. *Dendritic Molecules. Concepts, Synthesis, Perspectives*; VCH: Weinheim, 1996.
(2) (a) Percec, V.; Ahn, C. H.; Ungar, G.; Yeardley, D. J. P.; Moller, M.; Sheiko, S. S. *Nature* **1998**, *391*, 161−164. (b) Hudson, S. D.; Jung, H. T.; Percec, V.; Cho, W. D.; Johansson, G.; Ungar, G.; Balagurusamy, V. S. K. *Science* **1997**, *278*, 449−452.
(3) (a) Stocker, W.; Karakaya, B.; Schurmann, B. L.; Rabe, J. P.; Schluter, A. D. *J. Am. Chem. Soc.* **1998**, *120*, 7691−7695. (b) Stocker, W.; Schurmann, B. L.; Rabe, J. P.; Forster, S.; Lindner, P.; Neubert, I.; Schluter, A. D. *Adv. Mater.* **1998**, *10*, 793−797.
(4) Yin, R.; Zhu, Y.; Tomalia, D. A.; Ibuki, H. *J. Am. Chem. Soc.* **1998**, *120*, 2678−2679.
(5) (a) Lee, S. W.; Wood, B. M.; Belcher, A. M. *Langmuir* **2003**, *19*, 1592−1598. (b) Wen, X.; Meyer, R. B.; Caspar, D. L. D. *Phys. Rev. Lett.* **1989**, *63*, 2760−2763.
(6) (a) Yu, S. J. M.; Conticello, V. P.; Zhang, G. H.; Kayser, C.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Nature* **1997**, *389*, 167−170. (b) Cormack, P. A. G.; Moore, B. D.; Sherrington, D. C. *Chem. Commun.* **1996**, 353−355.
(7) Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638−7647.
(8) DPPs with the smallest D_0 side chains are chemically identical to the poly(γ-benzyl L-glutamic acid). They required addition of CF_3COOH (ca. 1 vol %) for complete solubilization in nonpolar solvents, such as chloroform. This is consistent with simple geometric models, which suggest that the single benzyl groups are too small to completely shield the polar peptide backbone from solvent, while higher-generation dendrons fully cover the backbone, even at conjugation density of 80%.
(9) α-Helical conformations of the DPP backbones were confirmed using circular dichroism (CD) and infrared (FTIR) spectroscopies. These data are presented in the Supporting Information.
(10) The volume available for each cylindrical molecule in a concentrated suspension is maximized when the DPP molecules separate into well-defined layers with efficient hexagonal packed order in each layer. See: Herzfeld, J. *Acc. Chem. Res.* **1996**, *29*, 31−37.

JA046965Q