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Bundle-Forming α -Helical Peptide-Dendron Hybrid

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Abstract

Peptides and dendrons are enticing building blocks from which to construct hybrid macromolecules because each can be prepared as monodisperse and sequence defined materials. Folding and assembly properties designed into the amino acid sequence of a peptide-dendron hybrid manifest in the formation of a dendronized bundle of α -helices.

Hybrid materials that harness the properties of peptides constitute a broad class of functional supramolecular materials.^{1,2} α -Helical bundles are especially attractive building blocks for materials because the helix bundle motif is found in the functional domain of diverse proteins (e.g., metalloenzymes, heme proteins, and transmembrane channel proteins).^{3,4} Motivated by the goal of creating tractable models for understanding complex proteins, rules for designing peptides that self-assemble into helix bundle mimetics have been developed and leveraged to create proteins with functions that are not found in nature.⁴ Bundle-forming α -helical peptides can be programmed to assemble into discrete aggregates or into one-dimensional assemblies.^{3,4} The supramolecular and functional properties of these designed helix bundles can be retained when a polymer chain is conjugated to the peptide.² We therefore hypothesized that bundle-forming α -helical peptides could direct the assembly of dendrons. Our long-term goal is to make the helix bundle motif compatible with non-biological environments by varying the structure of the peripheral end groups of the dendrons. While there has been significant interest in peptide-dendron hybrids,^{5–10} there are no examples of dendronized α -helix bundle-forming peptides. Herein, we demonstrate that folding and assembly properties encoded in the amino acid sequence will direct the assembly of dendrons attached to solvent-exposed, exterior residues of an α -helical bundle (Fig. 1a).

Peptide-dendron hybrid **1** (Fig. 1b) was designed by arranging polar and non-polar amino acid residues such that in the folded state an amphipathic α -helix would form. DeGrado and Lear demonstrated that, following this design principle, peptides as short as fourteen residues and composed of only polar lysine and non-polar leucine residues cooperatively fold and self-assemble into a tetrameric bundle of α -helices.¹¹ Because of the simplicity of the design and compact size of the peptides, a family of α -helical “LK” peptides has been

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developed as self-assembling modules for biomaterials.¹² Starting from an amino acid sequence similar to these α -helical “LK” peptides,¹³ we replaced two surface lysine residues with residues bearing water-soluble poly(alkyl ether) dendrons in the side chain.

Hybrid **1** reflects several design elements that are specific to α -helical peptide-dendron conjugates. The spacing between the dendrons ($i, i + 7$) was chosen to match the seven-residue periodicity of the α -helix. The density (one dendron/heptad) at which the dendrons are grafted to the peptide was intentionally kept low to accommodate the large volume that the dendrons occupy. The hydrophilic peripheral groups contribute a great deal of steric bulk to the second-generation dendron. Lee and Fréchet showed that, when grafted to each repeat unit of poly(L-lysine), sufficiently large dendrons induce a conformational transition from an α -helix to a β -sheet.¹⁴ Furthermore, the poly(alkyl ether) dendrons were chosen for their dense branching pattern and non-planar structure. Shao and Parquette encountered an unanticipated transformation of a monomeric α -helical peptide to a self-assembling β -sheet peptide-dendron hybrid due, in part, to π -stacking interactions between dendrons.^{8a} The poly(alkyl ether) dendrons were expected to minimize favorable dendron-dendron interactions that might promote misfolding of the conjugate.

Peptide-dendron hybrid **1** was prepared by conjugating pre-formed second-generation dendrons to the unprotected peptide (a.k.a., graft-to approach). Both the peptide and dendron fragments are prepared through iterative chemical synthesis. Removing impurities that have small structural defects introduced during iterative syntheses can be especially challenging when dealing with large molecules. The graft-to approach employs monodisperse starting materials to avoid structural defects in the product. The graft-to approach has been adopted for the synthesis of most peptide-dendron conjugates,^{5–7,10,14–15} however the chemistries employed in those examples were not amenable to site-specific conjugation of dendrons to unprotected peptides. Several groups have successfully conjugated unprotected peptides to the peripheral groups of dendrons using copper-catalyzed azide-alkyne cycloaddition (CuAAC) reactions.¹⁶ That approach appeared promising for our objective because CuAAC reactions have also proven to be suitable for grafting dendrons¹⁷ or polymers¹⁸ to linear polymers. We recently showed that a high density of dendrons can be grafted to a peptide via CuAAC.¹³

Azide **3** was conjugated to peptide **2** under ligand-free CuAAC reaction conditions¹⁹ in DMSO/H₂O (Fig. 2). Peptide **2** is soluble in the reaction solvent at concentrations >1 mM, and does not contain residues that are prone to side reactions.²⁰ We were therefore able to execute the CuAAC reaction in the presence of substoichiometric amounts of CuSO₄·5H₂O and sodium ascorbate. The reaction was performed under an N₂ atmosphere, but no further effort was made to deoxygenate the reaction mixture. An excess of azide (1.3 eq./alkyne) was used to help drive the reaction to completion. The reaction was judged complete when the target conjugate was the only observable species in MALDI-TOF mass spectra of the reaction mixture. The conjugate was purified by preparative reversed-phase HPLC. The identity and purity of the conjugate were confirmed by MALDI-TOF mass spectrometry (Fig. 3) and analytical reversed-phase HPLC, respectively. Peptide-dendron hybrid **1** was also characterized by NMR spectroscopy.

Amides in the peptide backbone give rise to circular dichroism (CD) spectra that are characteristic for different secondary structures. In particular, negative bands are observed at 208 and 222 nm in the CD spectrum when the peptide adopts an α -helical conformation.²² The poly(alkyl ether) dendrons are optically transparent in this region of the UV spectrum, so CD spectroscopy reports the conformation of **1**. The measured CD spectrum of **1** in tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.3) is shown in Fig. 4. The spectrum confirms that the peptide is α -helical and validates the design considerations described above.

The CD spectrum of a bundle-forming peptide without the dendrons (i.e., Ac-YLKLLKLLKLLK-NH₂) is shown in Fig. 4 for comparison with peptide-dendron hybrid **1**. The peptide composed entirely of natural amino acids produces a more intense CD spectrum, which suggests that some fraying of the helix is caused by the introduction of the dendrons. The mean residue ellipticity at 222 nm ($[\theta_{222}]$) is proportional to the amount of folded, α -helical peptide for both **1** and Ac-YLKLLKLLKLLK-NH₂. We can compare $[\theta_{222}]$ for the dendronized and conventional peptides to estimate the extent to which dendronization disrupts folding of the peptides. From this analysis we estimate that **1** is approximately 64% as helical as Ac-YLKLLKLLKLLK-NH₂.

In the α -helical conformation, peptide-dendron hybrid **1** has a globally amphipathic structure that is the driving force for assembly of **1** into bundles of α -helices. Hydrophobic leucine residues lie on one face of the α -helix, and the lysine residues and hydrophilic dendrons reside on the opposite face. To investigate whether the α -helical peptide-dendron hybrid self-associates to form a dendronized helix bundle, we measured CD spectra at various concentrations of **1**. The mean residue ellipticity at 222 nm ($[\theta_{222}]$) for each concentration reports the amount of folded, α -helical peptide in the solution. If folding into an α -helical conformation and association of the conjugates into bundles are coupled equilibria, then $[\theta_{222}]$ will depend on the concentration of **1**. Fig. 5a plots $[\theta_{222}]$ for various concentrations of **1**, and shows that the α -helical peptide-dendron conjugate self-assembles as a bundle. Additionally, the sharp change of $[\theta_{222}]$ at concentrations below 125 μ M suggests that the assembly process is cooperative. DeGrado and Lear demonstrated similar behavior for their α -helical “LK” peptide.¹¹ The folding and assembly behavior that is apparent from Fig. 5a distinguishes **1** from previous examples of α -helical peptide-dendron hybrids,^{8a,14–15} which were monomeric, and from hybrids that rely upon an extended peptide conformation for self-assembly.^{5–8,10}

Combining rationally designed peptides with dendrons provides a modular platform for creating peptide-dendron hybrid materials with predictable structure and function. The “LK” peptide upon which hybrid **1** is based is sensitive to the concentration of chloride anions.¹¹ Increasing chloride concentration shields electrostatic repulsions between the positively charged lysine residues on the polar face of the amphipathic helix. Fig. 5b plots $[\theta_{222}]$ at various concentrations of chloride anion ($[\text{Cl}^-]$). The Tris buffer is a mixture of Tris and Tris-HCl, which contains 15 mM chloride, and the concentration of chloride was increased by adding KCl. The data plotted in Fig. 5b indicate a transition from an unfolded state at low chloride concentration to a dendronized bundle of α -helices at higher concentrations of chloride. While other self-assembling peptide-dendron hybrids were found to be responsive

to environmental cues (e.g., solvent, temperature, ionic strength, and pH),^{5–8,10} the response of peptide-dendron hybrid **1** to chloride anions exemplifies how rationally designed properties in helix bundle-forming peptides can be transplanted into hybrid materials.

Helix bundle motifs in natural proteins provide the framework to support diverse functions by precisely positioning functional groups from the side-chains of different amino acids. Motivated by the rich functionality embedded in helix bundles, peptides that emulate the structure and function of helix bundle motifs have been developed as simpler models of biological macromolecules. These studies have also led to the development of principles for designing helix bundles de novo and the creation of proteins with non-biological (or non-natural functions). Utilizing these design principles to program the assembly of dendrons into larger supramolecular structures provides a pathway to create supramolecular dendrimers with a folded, protein-like core. In addition to the structural precision gained through this strategy, it is intriguing to consider whether dendron-peptide hybrids can support additional functions (e.g., catalytic activity) that have been successfully designed into model helix-bundle proteins. Several groups have explored how designed helix bundle-forming peptides conjugated to polymers program assembly and impart functionality to peptide-polymer conjugates.² Dendron-peptide conjugates offer additional insight by virtue of being monodisperse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Aida T, Meijer EW, Stupp SI. *Science*. 2012; 335:813. [PubMed: 22344437]
2. (a) Shu JY, Panganiban B, Xu T. *Annu Rev Phys Chem*. 2013; 64:631. [PubMed: 23331303] (b) Klok HA. *Macromolecules*. 2009; 42:7990.
3. (a) Marsden HR, Kros A. *Angew Chem, Int Ed*. 2010; 49:2988.(b) Cavalli S, Albericio F, Kros A. *Chem Soc Rev*. 2010; 39:241. [PubMed: 20023851]
4. Apostolovic B, Danial M, Klok HA. *Chem Soc Rev*. 2010; 39:3541. [PubMed: 20676430]
5. (a) Jang WD, Jiang DL, Aida T. *J Am Chem Soc*. 2000; 122:3232.(b) Jang WD, Aida T. *Macromolecules*. 2003; 36:8461.
6. (a) Percec V, Dulcey AE, Balagurusamy VSK, Miura Y, Smidrkal J, Peterca M, Nummelin S, Edlund U, Hudson SD, Heiney PA, Duan H, Magonov SN, Vinogradov SA. *Nature*. 2004; 430:764. [PubMed: 15306805] (b) Percec V, Dulcey A, Peterca M, Ilies M, Miura Y, Edlund U, Heiney PA. *Aust J Chem*. 2005; 58:472.(c) Percec V, Dulcey AE, Peterca M, Ilies M, Ladislav J, Rosen BM, Edlund U, Heiney PA. *Angew Chem, Int Ed*. 2005; 44:6516.(d) Percec V, Dulcey AE, Peterca M, Ilies M, Sienkowska MJ, Heiney PA. *J Am Chem Soc*. 2005; 127:17902. [PubMed: 16351122] (e) Percec V, Dulcey AE, Peterca M, Ilies M, Nummelin S, Sienkowska MJ, Heiney PA. *Proc Natl Acad Sci USA*. 2006; 103:2518.(f) Peterca M, Percec V, Dulcey AE, Nummelin S, Korey S, Ilies M, Heiney PA. *J Am Chem Soc*. 2006; 128:6713. [PubMed: 16704274] (g) Kaucher MS, Peterca

- M, Dulcey AE, Kim AJ, Vinogradov SA, Hammer DA, Heiney PA, Percec V. *J Am Chem Soc.* 2007; 129:11698. [PubMed: 17784763] (h) Percec V, Dulcey AE, Peterca M, Adelman P, Samant R, Balagurusamy VSK, Heiney PA. *J Am Chem Soc.* 2007; 129:5992. [PubMed: 17429976] (i) Kim AJ, Kaucher MS, Davis KP, Peterca M, Imam MR, Christian NA, Levine DH, Bates FS, Percec V, Hammer DA. *Adv Funct Mater.* 2009; 19:2930. (j) Rosen BM, Peterca M, Morimitsu K, Dulcey AE, Leowanawat P, Resmerita AM, Imam MR, Percec V. *J Am Chem Soc.* 2011; 133:5135. [PubMed: 21391688]
7. Lee J, Kim JM, Yun M, Park C, Park J, Lee KH, Kim C. *Soft Matter.* 2011; 7:9021.
8. (a) Shao H, Lockman JW, Parquette JR. *J Am Chem Soc.* 2007; 129:1884. [PubMed: 17256863] (b) Shao H, Parquette JR. *Angew Chem, Int Ed.* 2009; 48:2525.
9. Shao H, Bewick NA, Parquette JR. *Org Biomol Chem.* 2012; 10:2377. [PubMed: 22334247]
10. (a) Sato K, Itoh Y, Aida T. *J Am Chem Soc.* 2011; 133:13767. [PubMed: 21627140] (b) *Chem Sci.* 2014; 5:136. (c) Amorín M, Pérez A, Barberá J, Ozoires HL, Serrano JL, Granja JR, Sierra T. *Chem Commun.* 2013; 50:688.
11. DeGrado WF, Lear JD. *J Am Chem Soc.* 1985; 107:7684.
12. (a) Jain A, Buchko GW, Reback ML, O'Hagan M, Ginovska-Pangovska B, Linehan JC, Shaw WJ. *ACS Catal.* 2012; 2:2114. (b) Fu L, Liu J, Yan ECY. *J Am Chem Soc.* 2011; 133:8094. [PubMed: 21534603] (c) Mermut O, Phillips DC, York RL, McCrea KR, Ward RS, Somorjai GA. *J Am Chem Soc.* 2006; 128:3598. [PubMed: 16536533] (d) Long JR, Oyler N, Drobny GP, Stayton PS. *J Am Chem Soc.* 2002; 124:6297. [PubMed: 12033857] (e) Kiyota T, Lee S, Sugihara G. *Biochemistry.* 1996; 35:13196. [PubMed: 8855958]
13. Marine JE, Liang X, Song S, Rudick JG. *Biopolymers.* 2015 Accepted. 10.1002/bip.22634
14. Lee CC, Fréchet MJM. *Macromolecules.* 2006; 39:476. [PubMed: 18833337]
15. Zhuravel MA, Davis NE, Nguyen ST, Koltover I. *J Am Chem Soc.* 2004; 126:9882. [PubMed: 15303837]
16. (a) Wan J, Huang JX, Vetter I, Mobli M, Lawson J, Tae HS, Abraham N, Paul B, Cooper MA, Adams DJ, Lewis RJ, Alewood PF. *J Am Chem Soc.* 2015; 137:3209. [PubMed: 25710197] (b) Tang W, Ma Y, Xie S, Guo K, Katzenmeyer B, Wesdemiotis C, Becker ML. *Biomacromolecules.* 2013; 14:3304. [PubMed: 23931528] (c) Schellinger JG, Danan-Leon LM, Hoch JA, Kassa A, Srivastava I, Davis D, Gervay-Hague J. *J Am Chem Soc.* 2011; 133:3230. [PubMed: 21341746] (d) Rijkers DT, van Esse GW, Merckx R, Brouwer AJ, Jacobs HJF, Pieters RJ, Liskamp RMJ. *Chem Commun.* 2005:4581.
17. Helms B, Mynar JL, Hawker CJ, Fréchet MJM. *J Am Chem Soc.* 2004; 126:15020. [PubMed: 15547986]
18. Engler AC, Lee H-i, Hammond PT. *Angew Chem, Int Ed.* 2009; 48:9334.
19. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. *Angew Chem, Int Ed.* 2002; 41:2596.
20. (a) Hong V, Presolski SI, Ma C, Finn MG. *Angew Chem, Int Ed.* 2009; 48:9879. (b) Kumar A, Li K, Cai C. *Chem Commun.* 2011; 47:3186.
21. Strohalm, M. mMass 5.5.0. Available at: <http://www.mmass.org/>
22. Greenfield NJ. *Nat Protoc.* 2006; 1:2876. [PubMed: 17406547]

Fig. 1. (a) The folding and assembly of a peptide (green ribbon) with two dendritic side-chains (spheres) to form a dendronized bundle of α -helices, and (b) the structure of peptide-dendron hybrid **1**.

Fig. 2.
Graft-to synthesis of peptide-dendron hybrid **1**.

Fig. 3. MALDI-TOF Mass spectrum of **1**. The inset compares the simulated isotope pattern²⁰ (upper) with the observed isotope envelope (lower) for the $[1 + H]^+$ ion ($C_{187}H_{352}N_{25}O_{60}$).

Fig. 4. Circular dichroism (CD) spectra of peptide-dendron hybrid 1 (500 μM , ●) and Ac-YLKKLLKKLLK-NH₂ (500 μM , +) in 20 mM Tris buffer (pH 7.3) with 285 mM KCl (total $[\text{Cl}^-] = 300 \text{ mM}$) at 25 °C.

Fig. 5.

Plots of the mean residue ellipticity at 222 nm versus (a) the concentration of peptide-dendron hybrid **1** (285 mM KCl; total $[Cl^-] = 300$ mM), and (b) the total concentration of chloride ion ($[1] = 500$ μM). All spectra were recorded in 20 mM Tris buffer (pH 7.3) at 25 °C.