Helical Supramolecules and Fibers Utilizing Leucine Zipper-Displaying Dendrimers

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The programmed assembly of biomolecules into higher-order self-organized systems is central to innumerable biological processes and biomaterial construction.[1–4] Noncovalent interactions guide the organization of functionally and architecturally impressive assemblies spanning the 10 nm to 10 μm regime that includes enzymatic complexes such as the ribosome and proteosome, along with structural constructs, such as viral capsids. Recent design efforts have utilized a bottom-up approach toward both understanding and engineering supramolecular peptide and protein assemblies such as peptide dendrimers,[5,6] cyclic peptide nanotubes,[7] peptide and peptide–lipid micelles,[8,9] hydrogels,[10] and fibers.[11–13] Here, we demonstrate for the first time that discrete molecules comprising leucine zippers tethered to a core dendrimer provide the necessary molecular framework for constructing both monodisperse and fibrillar supramolecular assemblies that span the nanometer to micrometer regime (Figure 1). The ability to integrate protein–protein self-assembly principles with dendrimer architecture will allow for the facile self-assembly of hybrid dendrimer–protein complexes for protein, DNA, or RNA recognition. Furthermore, the novel leucine-zipper dendrimers provide a rich platform for constructing and utilizing a new class of programmable biomaterials with helical secondary structure.

Our design entailed choosing a dendrimer scaffold that did not sterically occlude peptide assembly and a series of peptides capable of noncovalent assembly. For our dendrimer, we utilized the zero generation PAMAM[14] core-functionalized with maleimides (pink ball in Figure 1), thus allowing for chemoselective covalent tethering to cysteine-containing peptides. Our peptides consisted of the pH-sensitive leucine zippers, EZ and KZ, with hydrophobic cores and polar exteriors that can form tetramers,[15,16] which were further modified with a Cys linker at the C-terminus. In our first generation design, we did not tune the specificity or orientation of the leucine zippers[17] because we wanted to maintain very high affinity for driving both multivalent and polyvalent assemblies.

Two macromolecules, D-EZ$_4$ and D-KZ$_4$, were synthesized (Supporting Information) for validating our design (Figure 1). We predicted that the acidic D-EZ$_4$ dendrimer would be able to assemble four copies of the cognate basic KZ-peptide and that the basic D-KZ$_4$ would be able to assemble four copies of the acidic EZ peptide. We utilized circular dichroism (CD) spectroscopy to monitor the complexation of D-EZ$_4$ (5 μM) with 4 equiv of KZ peptide (20 μM) at pH 8.4 (Figure 2a) and the complexation of D-KZ$_4$ (5 μM) with 4 equiv of the EZ peptide (20 μM) at pH 5.6. The pH 8.4 and pH 5.6 buffers were chosen so the CD spectra of the folded peptides, KZ at pH 8.4 and EZ at pH 5.6, could be subtracted from that of the complexes, allowing us to selectively monitor the change in secondary structure of the D-EZ$_4$ and D-KZ$_4$ as a function of increasing peptide concentration.

The titration results (Figure 2a,b) clearly showed that both D-EZ$_4$ and D-KZ$_4$ supported the assembly of the cognate peptides with concomitant increase in helicity. The helical content of D-KZ$_4$ in
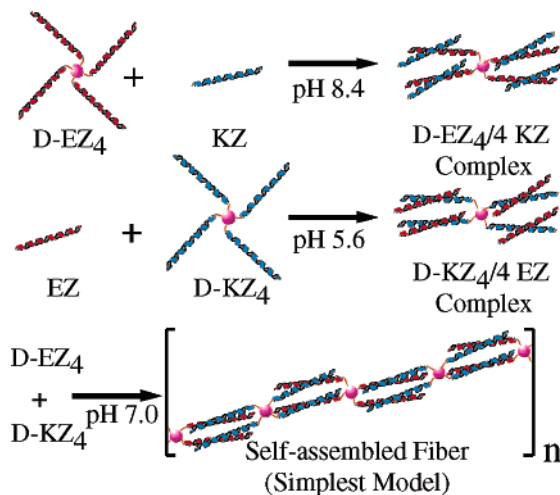

Figure 1. Strategy for the noncovalent assembly of supramolecules and fiber of leucine-zipper dendrimers. The peptide sequences of EZ and KZ are shown below: EZ: NH$_2$–AQALEKELQALEKELQALEWELQALE-KELSGSGC–COOH, KZ: NH$_2$–AQALKKKLQALKKKLQALK-WKLQALKKKLSGSGC–COOH.
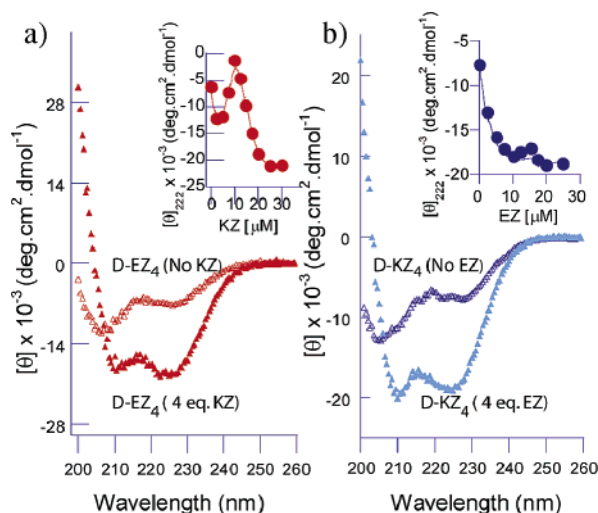

Figure 2. CD spectra of D-EZ$_4$/4KZ and D-KZ$_4$/4EZ assembled at pH 8.4 and pH 5.6, respectively. (a) Subtraction CD spectra of 5 μM D-EZ$_4$ in the presence (red ▲) and absence (red △) of KZ (20 μM). (b) Subtraction CD spectra of 5 μM D-KZ$_4$ in the presence (blue ▲) and absence (blue △) of EZ (20 μM). The insets show the average (±10% error) of three titrations of 5 μM D-EZ$_4$ (red ●) and 5 μM D-KZ$_4$ (blue ●) with increasing concentrations of KZ and EZ, respectively.

the complex was 95% of the fully folded KZ, whereas the overall helical content of D-EZ$_4$ in the complex was 76% of the fully folded EZ. We also noted the presence of interesting higher-ordered complexes when only 2 equiv of KZ were added to the D-EZ$_4$ that is currently under investigation. Sedimentation equilibrium experi-
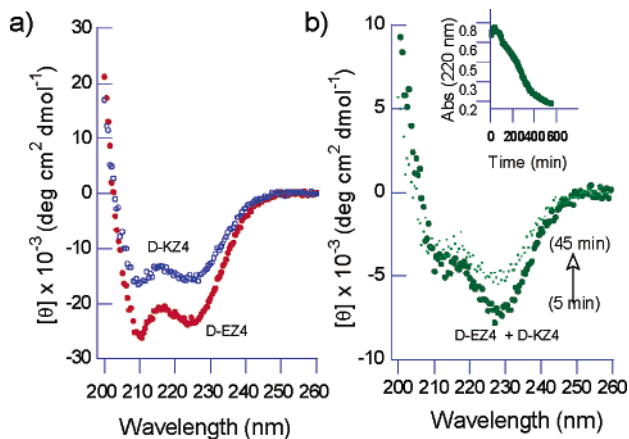
Figure 3. CD spectra of D-EZ$_4$, D-KZ$_4$, and D-EZ$_4$/D-KZ$_4$ at neutral pH. (a) CD spectra of 5 μM D-EZ$_4$ (red ●) and 5 μM D-KZ$_4$ (blue ○) at pH 7 in 10 mM phosphate. (b) CD spectra of an equimolar mixture (5 μM) of D-EZ$_4$ + D-KZ$_4$ after 5 min (green ●) and 45 min (green ⋯) in buffer A. Inset shows the time-dependent loss of UV signal at 224 nm due to precipitation of the complex.

ments unambiguously established that D-EZ$_4$/4KZ at pH 8.4 had a molecular weight of 30.7 kD (31.4 kD theoretical), whereas D-KZ$_4$/4EZ at pH 5.6 had a molecular weight of 30.5 kD (31.4 kD theoretical).

Having demonstrated that we could design and assemble non-covalent monodisperse architectures, we turned our attention to building higher-order self-organized systems by the alternate assembly of D-EZ$_4$ and D-KZ$_4$ dendrimers at neutral pH (Figure 1). To this end, we incubated D-EZ$_4$ and D-KZ$_4$ in buffer A (10 mM phosphate, pH 7). The CD spectra of the rapidly precipitating complex (Figure 3b) retained the 222-nm minima indicative of helical structure but with a relative decrease in intensity at 208 nm in comparison to the individual dendrimers (Figure 3a). The decrease at 208 nm was possibly due to aggregation-induced light scattering that has also been observed in linear leucine-zipper fibers.[11]

Transmission electron microscopy (TEM) was used to characterize the structures of the helical assemblies of the D-EZ$_4$/D-KZ$_4$ complexes (5 μM each). Only the two-component D-EZ$_4$/D-KZ$_4$ complex supported the assembly of protofibrils ($\sim$50 nm in diameter) at pH 7 that subsequently assembled into fibers of $\sim$150–200 nm diameter (Figure 4a) and eventually formed even larger assemblies of over 10 μm in length (Figure 4b). FT-IR analysis of the fibers (Supporting Information) further confirmed helical structure, with no change in the amide I′ band of the D-EZ$_4$/D-KZ$_4$ fiber relative to that of the soluble helical complexes of D-EZ$_4$/4KZ and D-KZ$_4$/4EZ.[18] A repeating tetrameric helical bundle subunit structure (Figure 1) is a possible initial step toward fibrillization based upon our solution studies with the D-KZ$_4$/4EZ and D-EZ$_4$/4KZ complexes. The further lateral and longitudinal association of such an initial complex can result in concomitant increases in fiber widths and lengths, respectively. Thus, our work provides the requisite methodology for constructing novel two-component-driven fibrillar assembly at neutral pH with helical secondary structure.

We believe that our ability to control the discrete supramolecular assembly of multiple proteins on a dendrimer scaffold will facilitate
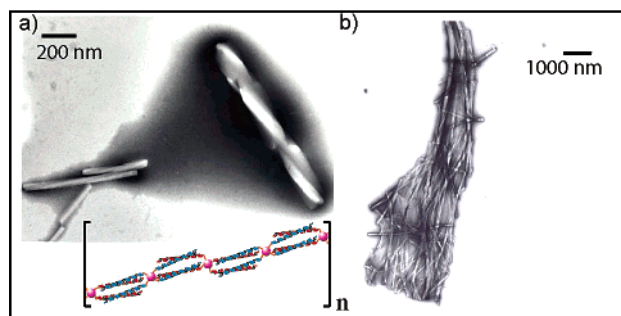

Figure 4. Electron micrograph images of two-component (D-EZ$_4$/D-KZ$_4$) self-assembled fibers formed after 8 h. (a) Protofibrils ($\sim$50 nm diameter) and a larger helical fibril ($\sim$150 nm diameter). (b) Larger fibrillar macrostructure spanning 12 μm in length.

multivalent approaches for protein inhibition[2] as well as allow for the assembly of functional enzymatic complexes, such as polyketide synthetases, that have been recently shown to possess leucine zipper-like motifs.[19] Furthermore, our approach toward the assembly of dendrimer supported fibers with helical secondary structure opens multiple avenues for engineering novel self-organizing biomaterials by judicious manipulation of both the peptide and dendrimer fragments.[4]

Supporting Information Available: Details of syntheses, characterization, CD, sedimentation equilibrium, FT-IR, and TEM data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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