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COMMUNICATION

Intramolecular chiral communication in peptide–dendron hybrids†‡

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The conformational properties of a series of peptide–dendron hybrids progressively incorporating 1–4 dendritic side chains were investigated by circular dichroism. Although the presence of multiple adjacent dendrons along the peptide backbone precluded the formation of α-helical or β-sheet secondary structure, intramolecular packing of the dendrons mediated efficient peptide → dendron chirality transfer in both organic and aqueous media.

The occurrence of coupled motion in biological molecules serves as a mechanism to regulate biological events in response to environmental signals.[1] In such allosteric systems, small structural perturbations are propagated by long-range cooperativity that amplifies the signal. Such correlated equilibria mediate the chiral amplification phenomenon observed in helical polymers[2] and supramolecular assemblies.[3] Synthetic materials that amplify local structural signals have potential to serve as selective catalysts,[4] sensors[5] or smart materials.[6] We have previously observed solvent-dependent chirality amplification and inversion behavior in folded dendrimers.[7] Herein, we demonstrate intramolecular chirality transfer, amplification and switching in a peptide–dendron hybrid.

Peptide–dendron[8] and peptide–polymer[9] hybrids offer the potential to study how the conformational properties of two folded structural elements allosterically communicate structural information. We previously reported a series of 16-mer alanine-rich peptide–dendron hybrids (ARPDH) composed of an intrinsically α-helical, alanine-rich sequence displaying two hydrophobic dendritic side-chains.[10] Intermolecular hydrophobic interactions among the dendritic side-chains induced an α-helix to β-sheet conformational transition in aqueous buffer that ultimately drove further assembly of the sheets into nanotubes or amyloid-type fibrils, depending on environmental conditions. Strong peptide → dendron chirality transfer occurred only for peptide–dendrons that assembled into β-sheet fibrils, indicating the importance of *inter*molecular packing of the dendrons in mediating conformational coupling. In this work, we incorporated dendritic side-chains at every alternate position along the peptide to explore the impact of *intra*molecular dendron packing on β-sheet formation and chirality propagation.

In order to increase the water solubility of the more hydrophobic peptide–dendrons, we installed alternating sequences of the nonpolar dendritic alanine, and either lysine or glutamic acid as charged residues in a manner to maintain a zero net charge. This alternating sequence of polar and nonpolar residues also imparts a greater predisposition toward amphiphilic β-sheet aggregation.[11] Thus, a series of peptide–dendron hybrids were constructed in which the number of dendritic side-chains progresses from 1–4 residues, using standard solid phase Fmoc/*t*-Bu synthesis on Rink amide resin (Fig. 1).

The conformational properties of peptide–dendrons **1–4** were investigated by circular dichroic (CD) and infrared (IR) spectroscopy (Fig. 2). Notably, all three peptide–dendrons (**2–4**) adopt a random coil conformation in water as evidenced by

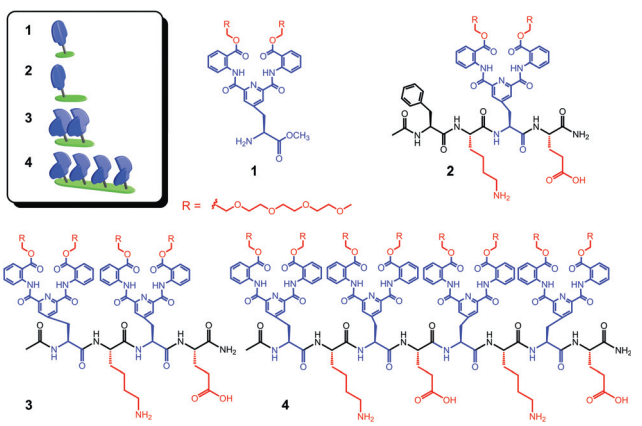

Fig. 1 Structures of peptide–dendrons **1–4** and a notional depiction of potential intramolecular dendron packing interactions.

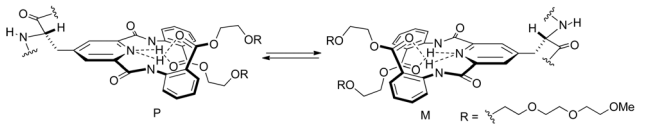

Fig. 2 Helical conformational equilibria of dendritic side-chain of peptide–dendrons **1–4**.

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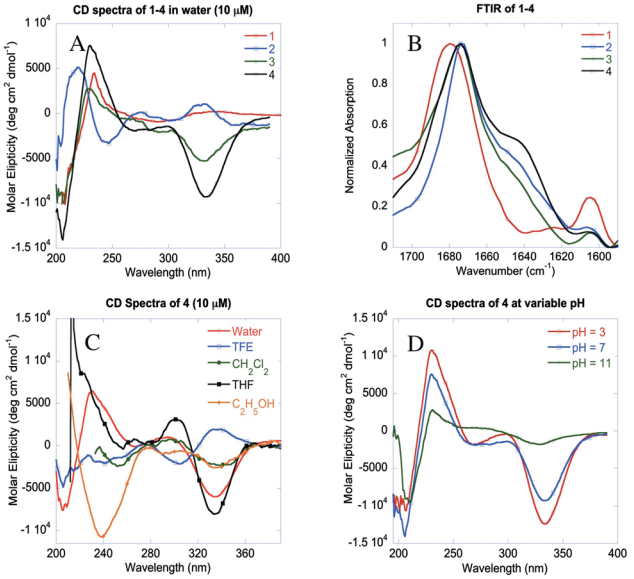

Fig. 3 (a) Molar CD and (b) infrared spectra of **1–4** in water at RT. (c) Solvent- and (d) pH dependence of the CD spectra of **4**.

negative peaks at *ca.* 204 nm in the CD spectra and IR absorptions at ~1640 cm^{-1} (Fig. 3a–b, S5‡). Monte Carlo simulations of protein secondary structure suggest that entropy losses arising from the steric restriction of side chain fluctuations reduces the stability of the α-helical state.[12] Thus, the lack of α-helical structure in the peptide–dendrons, especially **4**, can be attributed to the steric influence of the dendritic side-chains. Similarly, the coplanar orientation of dendritic side chains required for efficient intermolecular packing along the direction of the β-sheet axis[10b] is precluded by their proximal positioning along the peptide backbone. Hence, both α-helical and β-sheet structures are destabilized in the peptide–dendron structures.

The dendritic alanine control, **1**, exhibited a flat line in the region of the anthranilate π–π* absorption at 316 nm in the CD spectrum, indicating the lack of a helical bias relating these chromophores of the dendron (Fig. 3).[7a] This observation reflects prior studies showing that chiral groups at this position have little influence on the helical bias of the dendron.[13] In contrast, peptide–dendrons **2–4** exhibited excitonic couplets centered at 316 nm consistent with the emergence of a chiral bias in the helicity of the dendron side chains. The presence of a phenylalanine residue at the $i + 2$ position adjacent to the dendron in **2** induced a weak, positive couplet reflecting a P-type bias. Peptide–dendrons **3** and **4** having two and four adjacent dendron groups, respectively, on the same face of the extended peptide sequence exhibited M-type helicities. Overall, the absolute magnitude of the excitonic couplets correlated strongly with the number of adjacent dendron substituents. The conformational behavior of these peptide–dendrons diverges significantly from that of the ARPDH sequences.

Notably, the emergence of a helical bias in the dendron side-chains of the latter was strongly coupled to β-sheet formation in water.[10] In contrast, strong helical biases were observed in **2–4** although the peptide backbones lacked any apparent β-sheet secondary structure. Whereas peptide–dendrons **2–3** exhibited significantly weaker excitonic couplets in TFE (Fig. S1–S2‡), indicating lower helical biases, than in water, peptide–dendron **4** exhibited strong helical biases in both aqueous and organic solvents (Fig. 3c, S1‡). Compared with **2–3**, which exhibited M-type helicity in all solvents studied, the helical bias of **4** transitioned to a P-helix exclusively in TFE. Titration experiments indicated that the M → P transition occurs in the range of 20–30% TFE–H_2O (Fig. S3‡).

The increasing efficiency of peptide → dendron chirality transfer going from **1** to **4** can be rationalized by *intramolecular* conformational coupling among the dendron side-chains. This conformational behavior resembles the generation-dependent helical bias and solvent-induced chirality switching observed in folded dendrimers, which was attributed to enhanced packing at the periphery.[7a] Dynamic light scattering experiments were consistent with minimal aggregation in CH_2Cl_2, THF, C_2H_5OH and TFE; whereas particles with a hydrodynamic diameter of 116.9 nm were detected in water (Fig. S6‡). However, the excitonic couplet at 316 nm in the CD spectra in these solvents, including water, showed little concentration dependence in the range of 10–100 μM (Fig. S1–S2‡). 2D-NOESY experiments in [d_8]THF did not reveal any close contacts between adjacent dendrons in **4**, indicating a highly dynamic conformational state. However, the decreased emission intensity of **4** in water and TFE at ~440 nm (exc. ~316 nm), compared to that of **1**, is consistent with an increase in dendritic packing interactions as the number of adjacent dendrons increases going from **1** to **4** (Fig. S4‡). A decrease of emission intensity is often observed in systems with strongly interacting chromophores.[14] These observations along with the progressive trend going from **1–4** support a process in which chirality transfer is mediated by the intramolecular packing of the dendron side chains.

The source of the M → P inversion in TFE must emerge from a change in the conformation of the main peptide chain given the lack of any apparent aggregation in this solvent. It is noteworthy that in contrast to **2–3**, the helical bias of the dendron side chain of **4** was quite sensitive to pH in pure water, progressively diminishing going from pH 3 to 11 (Fig. 3d, S2‡). The impact of changes in the protonation state of the lysine/glutamate side chains may be amplified by the increased packing in **4** compared with **2–3**. Such amplified responses to external stimuli are characteristic properties of folded proteins, which typically exhibit long-range structural cooperativity.[15] Cooperative conformational behavior has been also observed in non-natural systems such as helical oligomers and dendrimers.[2,7a] The addition of 10–50% (v/v%) of TFE in water is known to increase the α-helicity of intrinsically helical peptides and to induce helicity in disordered peptides.[16] It is thought that TFE penetrates the peptide in a manner that dehydrates the backbone and enhances the intramolecular hydrogen-bonding within the α-helical state. In this case, the stability of the α-helical state is not sufficiently improved by TFE to overcome steric destabilization by the dendrons. However, TFE has been shown to form hydrophobic clusters in water that bind to and increase the hydrophobic association of apolar side chains.[17] The clustering of the dendrons in TFE–water could be expected to alter the conformation of the backbone, which is manifested as a helical inversion in the dendrons. The greater importance of TFE-induced local clustering of the dendrons, compared with changes in hydrogen-bonding interactions of the backbone, is suggested by the

observation that ethanol does not induce this inversion although the effect of ethanol and TFE on protein α-helical stability is often qualitatively similar.

Conclusions

In summary, the occurrence of intramolecular packing of the dendritic side chains produces efficient peptide → dendron chirality transfer in both organic and aqueous solvents. Steric congestion produced by the large dendritic groups decreases the stability of both α-helical and β-sheet secondary structures resulting in predominant random coil conformations. An M → P helical transition occurs in **4** upon going from pure water to greater than 20–30% TFE (v/v%), which may be a consequence of increased hydrophobic association of the dendrons in the presence of TFE. Application of these observations to the design of stimuli-responsive materials is an ongoing objective in our laboratory.

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