Communication

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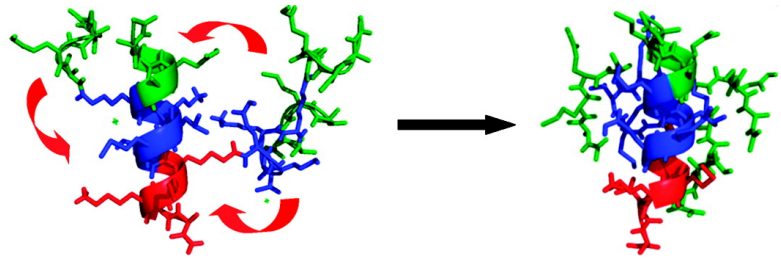

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α-Helix Stabilization within a Peptide Dendrimer

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Dendrimers are branched synthetic macromolecules with promising properties for a variety of applications in technology and medicine.[1] Dendrimers adopt protein-like globular shapes by means of their topology rather than by folding; however, they are usually conformationally labile and lack the stable and dense packing of proteins.[2] The incorporation of designed secondary structure elements provides a strategy toward more protein-like dendrimers, for example using amide hydrogen-bonding networks in 4-aminopyridine-2,4-dicarboxamide-based dendrimers as elegantly demonstrated by Parquette et al.[3,4] While peptide dendrimers[5] containing proline helices have been reported,[6] α-helices have sofar only been reported as long linear sequences forming the dendrimers outermost branches and did not include the branching points.[7] Herein we show that a peptide dendrimer with three very short 4-residue helical segments separated by two successive branching points can adopt an α-helical conformation, and that this dendrimer is more stable than the corresponding linear peptide toward pH-induced unfolding and temperature-induced intermolecular aggregation. Although less pronounced than the stabilization reported for α-helices longer than 11 residues in dendrimers[7] and designed α-helix bundles and TASPs,[8] the effect is unprecedented and opens the way to folded dendritic analogues of proteins using natural amino acids only.

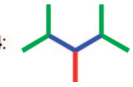
To search for an α-helical peptide dendrimer, we prepared the first and second generation peptide dendrimers **D1**−**D4** containing a branching lysine residue at position 8 or at positions 5 and 10, with branches of 7 or 4 residues in length, respectively. We used helix-forming ALMKE amino acids aligning negatively and positively charged side chains to form ion-pairs in α-helical conformation, with anionic glutamates at the N-terminus to stabilize the α-helix dipole.[9] The linear peptides **L1**−**L4** with alanine replacing the lysine branching points were also prepared.

The α-helix content of **D1**−**D4** and **L1**−**L4**, measured by circular dichroism in aqueous acetate buffer containing 0 → 20% v/v trifluoroethanol (TFE), ranged between 13 and 77%, which is typical for short synthetic peptides, and did not change significantly with concentration, suggesting that it was not affected by aggregation effects (Table 1, Figure S1, Table S1). While most dendrimers were less helical than their linear analogues, the second generation dendrimer **D3** showed a higher α-helix content and a lower *β*-sheet fraction than the linear control **L3**, suggesting a dendritic effect on folding, and was investigated closer.

Both **D3** and **L3** showed fully reversible thermal denaturation curves by circular dichroism (Figure S2).[10] However, the α-helix propensity of **D3** was conserved in the absence of TFE and was pH-independent, while the linear control **L3** interconverted to a *β*-sheet conformation from pH 3.8 to pH 5.8 (Figure 1).

Fourier transform infrared (FTIR) spectroscopy of dendrimers and their linear analogues in D_2O displayed an intense absorption in the amide I′ region around 1636−1641 cm^{-1}, typical for hydrated α-helices as observed in proteins[14] and peptides.[15] Thermal denaturation (Figure S3) induced a decrease of the α-helical component and a shift toward higher wavenumbers (of about 7 cm^{-1}) indicative of a transition to random coil, and the appearance of an absorption band at 1615−1617 cm^{-1} typical for intermolecular *β*-sheets.[16] While **L3** showed a regular temperature induced aggregation, **D3** was stable up to 40 °C, and then strongly aggregated up to 75 °C (Figure 2). Unexpectedly, aggregation decreased above 80 °C suggesting an additional phase transition into

Table 1. Secondary Structure Content of Peptide Dendrimers and Linear Controls

G1 dendrimers **D1**, **D2**:  G2 dendrimers **D3**, **D4**: 

no.	Sequence[a]	0 % v/v TFE 3.8 α/β	0 % v/v TFE 5.8 α/β	20 % v/v TFE 3.8 α/β	20 % v/v TFE 5.8 α/β
D1	$(AcEALKMAE)_2$*K*AKALEMA	20/24	15/25	48/8	40/13
L1	AcEALKMAEAAKALEMA	36/10	28/16	68/2	58/4
D2	$(AcEAKMAEA)_2$*K*KMEELAA	15/25	12/27	39/15	24/20
L2	AcEAKMAEAAKMEELAA	17/23	13/23	60/3	44/8
D3	$(AcAMEA)_4$(*K*KLME$)_2$*K*MKLA	28/16	25/17	57/3	51/5
L3	AcAMEAAKLMEAMKLA	31/14	13/34	49/11	57/4
D4	$(AcALKM)_4$(*K*EAKM$)_2$*K*ELKA	15/21	14/21	49/5	42/7
L4	AcALKMAEAKMAELKA	20/15	19/14	77/1	65/3

[a] *K* = lysine branching points, both amines are elongated. Ac = acetyl. The C-terminus is $CONH_2$. The products were obtained by Fmoc-synthesis (see Supporting Information). [b] Percentages of α-helix/*β*-sheet as determined by CD spectroscopy at 0.20 mg·mL^{-1} of TFA salt, at 20 °C, in acetate buffer at pH 3.8 and 5.8 with 0% or 20% v/v trifluoroethanol (TFE). The CD spectra were deconvoluted using Dichroweb[11] (Contin-LL regression[12] with data set 4) as recommended for short peptides.[13]

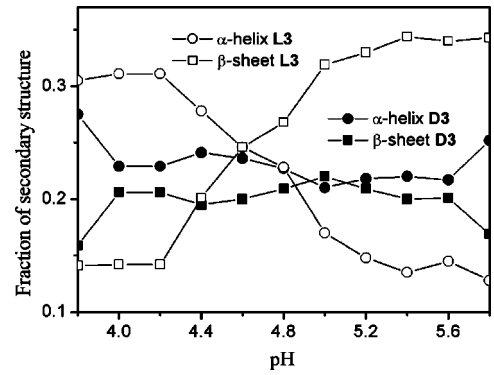


Figure 1. Secondary structure content for **D3** and **L3** as a function of pH at 0.20 mg·mL^{-1} of TFA salt in acetate 8.0 mM buffer pH 3.8→5.8. All solutions were freshly degassed and buffers were prepared from p.a. quality acids and bases in milli-Q water. All CD experiments were performed with a scan rate of 10 nm·min^{-1} using 0.1 cm quartz cuvettes.

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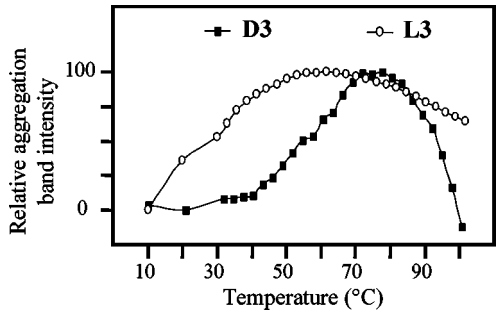

Figure 2. Temperature dependence of **D3** and **L3** aggregation (in D_2O at 0.50 mg·mL^{-1}, pH 3.8) determined by the FTIR band intensity at 1615−1617 cm^{-1}, taken from the inverted second derivative[16b,18] of their measured absorption spectra (see Supporting Information).

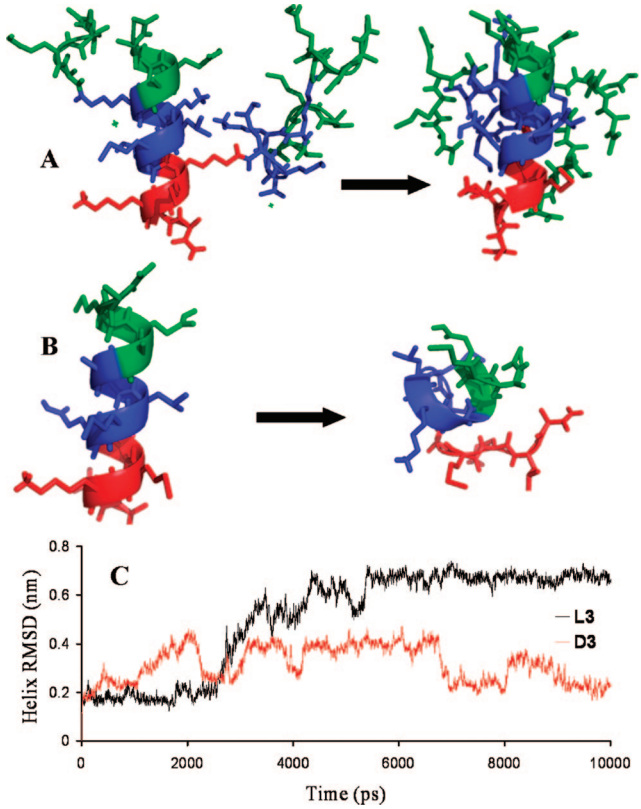

Figure 3. MD simulation using GROMACS 3.3.[19] and the ffG43a1 force field in explicit (SPC) water at 300 K: (A) **D3** $t = 0 \rightarrow 10$ ns; (B) **L3** $t = 0 \rightarrow 10$ ns; (C) root mean square deviation from the starting structure of the α-helix in **D3** and **L3**. The models are depicted in stick representation and secondary structures are highlighted in cartoon representation. The different residues are colored according to Table 1. Picture generated using PyMOL software.[20]

an unordered structure.[17] Overall, both CD and FTIR data indicated that the α-helical content of **D3** was more stable than that of the linear control **L3**.

A molecular dynamics (MD) study was carried out to gain a structural insight into the α-helical conformation of dendrimer **D3** and its linear analogue **L3**. Structural models were assembled in a prefolded α-helical conformation and simulated over 10 ns at 300 K. **L3** unfolded after 3 ns to produce a partially folded structure with only 1.5 turns of the helix corresponding to approximately 50% α-helical content, somewhat above that observed by CD and FTIR. MD of dendrimer **D3** by contrast showed that the core α-helix along the α-amino groups of the branching lysines remained completely folded, exhibiting 3 of the 3.5 turns possible by design, while the other branches were unfolded to wrap around the helical core, which corresponds well to the 30% helical content in pure water observed by CD. Because the 4-residue branches in **D3** are much shorter than the 11−13 residues known to be necessary to form a stable α-helix,[21] the MD model of **D3** with an α-helix spanning successive generations provides the best explanation for the observed pH and thermal stabilization effects (Figure 3).

In summary, dendritic branching of helix-forming peptide sequences was found to be compatible with folding. The second generation dendrimer **D3** exhibits a higher α-helical content that its linear analogue **L3** and is more stable toward thermal aggregation. MD suggests that the dendrimer branches wrap around a central α-helix including two successive branching points of the dendrimer leading to a more stable α-helical conformation than the linear peptide. The use of α-helical sequences spanning several generations might provide a general strategy toward stably folded peptide dendrimer mimics of proteins.

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Supporting Information Available: Synthetic procedures, HPLC and MS (ES+) analysis of all synthesized dendrimers and peptides, procedures for CD and FTIR measurements, MD parameters, and procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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