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Collagen Mimetic Dendrimers

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Collagen is the main structural protein in mammals and consists of triple helical peptides composed of Gly-Xaa-Yaa trimer repeats.[1−5] Templates,[6] disulfides,[7] and transition metals[8] have been utilized to create protein-like structures.[9−11] We report here the synthesis and conformational properties of 162-residue collagen mimetic dendrimers which exhibit enhanced triple helical stability compared to equivalent scaffold terminated structures.

Many synthetic collagen mimetics exhibit triple helical conformations.[12−15] In this communication we also report the utilization of a new scaffold to assemble collagen mimetics: *N*-(benzyloxycarbonyl)-tris(carboxyethoxymethyl)aminomethane (Z-TRIS[OH]$_3$), possessing three carboxyl groups for peptide chain attachment and a protected primary amine for further reaction (Figure 1).[16]

The collagen-like dendrimers were built from Gly-Pro-Nleu and Gly-Nleu-Pro sequences (where Nleu denotes *N*-isobutylglycine). The Z-protecting groups on the scaffold-terminated peptides were removed and allowed to react with trimesoyl chloride to create the dendritic structures (Figure 2 and Supporting Information). Figure 2 shows the structure of the dendrimer built from the Gly-Pro-Nleu sequence, TMA[TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$ where TMA denotes the trimesic acid core. We also prepared an analogous dendrimer from the Gly-Nleu-Pro sequence which possess a β-alanine spacer between the TRIS amine and the TMA core, TMA[β-Ala-TRIS[(Gly-Nleu-Pro)$_6$-OMe]$_3$]$_3$ (see Supporting Information Figure S1).

The triple helicity of all structures were determined by thermal denaturation monitored by optical rotation and circular dichroism measurements (Figures 3−5). These spectroscopic studies were carried out in H_2O as well as the triple helicity-enhancing solvent ethylene glycol:H_2O (EG:H_2O, 2:1, v/v).[14]

The optical rotation data shown in Figures 3 and 4 indicate that the molecules studied exhibit a cooperative melting transition with the exception of the single-chain compounds shown in Figure 3A and B which do not exhibit a transition in H_2O. A shallow transition for the linear molecules can be seen in EG:H_2O (2:1) (Figure 4A and B). This indicates that the single-chain structures studied form triple helices to a small extent in EG:H_2O (2:1).

The CD data shown in Figure 5A are indicative of a triple helical conformation for the scaffold-assembled and dendritic Gly-Pro-Nleu-containing molecules at low temperature, whereas the single-chain molecule is not triple helical. In Figure 5B the data were acquired at 22 °C. Only the dendrimer remains triple helical, while the scaffold-terminated structure and the single-chain molecule are not triple helical. This result is consistent with data from the melting curves contained in Figure 3B. The Gly-Nleu-Pro-containing structures also exhibit CD spectra of triple helical collagen-like molecules except for the single-chain compound in H_2O (see Supporting Information Figure S2 and S3).

It can be readily seen from Figures 3 and 4 that mimetics prepared from the Gly-Nleu-Pro sequence form more thermally

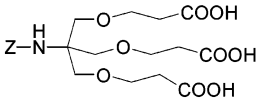

Figure 1. The Z-protected TRIS scaffold (Z-TRIS[OH]$_3$).

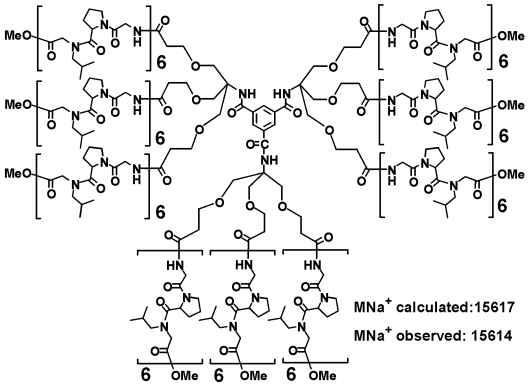

Figure 2. The collagen mimetic dendrimer TMA[TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$.

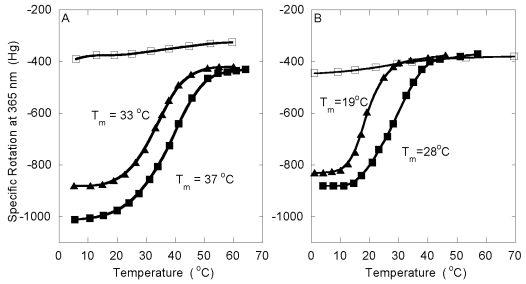

Figure 3. Thermal denaturations measured by changes in optical rotations carried out in H_2O (0.2 mg/mL): (A**)** Boc-(Gly-Nleu-Pro)$_6$-OMe (□), Boc-β-Ala-TRIS[(Gly-Nleu-Pro)$_6$-OMe]$_3$ (▲) and TMA[β-Ala-TRIS[(Gly-Nleu-Pro)$_6$-OMe]$_3$]$_3$ (■); (B**)** Boc-(Gly-Pro-Nleu)$_6$-OMe (□), Boc-β-Ala-TRIS-[(Gly-Pro-Nleu)$_6$-OMe]$_3$ (▲) and TMA[TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$* (■, *0.1 mg/mL).

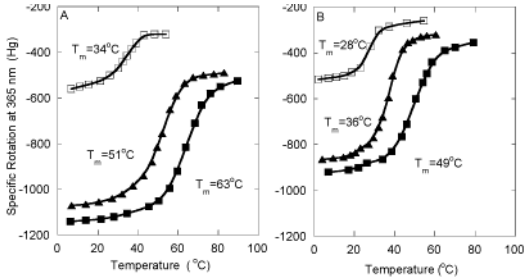

Figure 4. Thermal denaturations measured by changes in optical rotations carried out in EG:H_2O (2:1, 0.2 mg/mL): (A**)** Boc-(Gly-Nleu-Pro)$_6$-OMe (□), Boc-β-Ala-TRIS[(Gly-Nleu-Pro)$_6$-OMe]$_3$ (▲) and TMA[β-Ala-TRIS-[(Gly-Nleu-Pro)$_6$-OMe]$_3$]$_3$ (■); (B**)** Boc-(Gly-Pro-Nleu)$_6$-OMe (□), Boc-β-Ala-TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$ (▲) and TMA[TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$* (■,*0.1 mg/mL).

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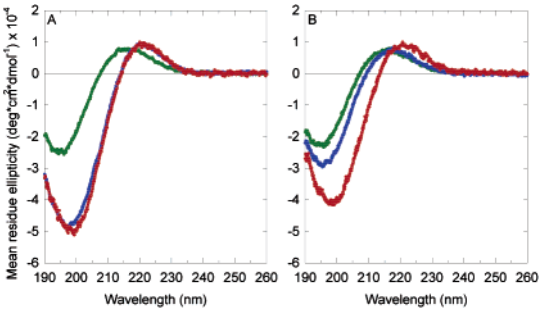

Figure 5. CD spectra in H_2O of Boc-(Gly-Pro-Nleu)$_6$-OMe (green, 0.2 mg/mL), Boc-β-Ala-TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$ (blue, 0.2 mg/mL) and TMA[TRIS[(Gly-Pro-Nleu)$_6$-OMe]$_3$]$_3$ (red, 0.1 mg/mL) at (A**)** 8 °C and (B**)** 22 °C.

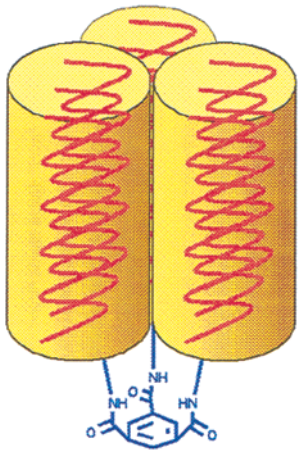

Figure 6. A schematic representation of the clustering of triple helices about the trimesic acid core.

stable triple helices in comparison to equivalent structures from the Gly-Pro-Nleu sequence for all of the molecules prepared. These results are consistent with previous findings from our laboratory.[14] It is intriguing that both dendritic structures precipitate from solution at critical elevated temperatures. The origin of this effect is currently under investigation.[17]

To determine whether the stabilizing effect of the dendrimer is inter- or intramolecular, we measured the concentration dependence for the melting transition and found no effect between 0.05 and 2.0 mg/mL in H_2O (see Supporting Information Figure S4). We therefore believe that the stabilizing effects arise from an intramolecular clustering of the triple helical arrays about the core structure. Figure 6 shows a schematic representation for such a clustered structure. This ensemble excludes solvent from the interior portion of the array which stabilizes the triple helical bundle.

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

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