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Fe^{III}-Binding Collagen Mimetics

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The synthesis and characterization of hydroxamic acid containing single-chain and TRIS-assembled (where TRIS is tris(carboxyethoxymethyl)aminomethane) collagen mimetics are reported. We have engineered an Fe^{III}-binding domain by placing a hydroxamic acid group at the C termini of collagen mimetic chains composed of the Gly-Pro-NLeu sequence. The circular dichroism spectra and thermal denaturation studies show an enhancement in triple-helical thermal stability upon the addition of Fe^{III} for the TRIS-assembled structure. No triple-helical structure was detected for the single-chain collagen mimetic. From the absorbance shown in the UV–vis spectra, we believe that the thermal stabilization of the triple helix is the direct result of a coordination complex between Fe^{III} and the hydroxamate groups tethered to the C termini of the collagen mimetic peptide chains.

Collagen is a large and vital component of the extracellular protein matrix in mammals.[1,2] The triple-helical structure confers rigidity as well as load-bearing strength to collagen and generates a framework for the attachment and differentiation of cells. Triple helices form by the association of three polypeptide chains, each in a left-handed helix, coiling about a common axis to form a right-handed triple helix.[3,4] This sterically demanding conformation requires every third residue to be glycine (Gly), resulting in a repeating Gly-*Xaa*-*Yaa* sequence where imino acids often populate the *Xaa* and *Yaa* positions.[5]

The field of protein and collagen mimetic de novo design has flourished,[6−11] largely through the use of templates,[9,12] disulfides,[13] and transition metals,[14−16] to create protein-like structures. Koide et al.[16] and Cai et al.[17] have independently published the thermal stabilization of collagen triple helices by the utilization of Fe-binding domains. In both reports, aromatic-based binding groups were used, which resulted in very tight binding complexes.

We report a collagen mimetic structure composed of the Gly-Pro-NLeu sequence (where NLeu denotes *N*-isobutylglycine), which exhibits enhanced triple-helical stability upon Fe^{III} addition. The goal of this research was to prepare a new class of collagen mimetic biomaterials to be used as adhesives. We chose to model our system after the siderophore Desferrioxamine B (DFB), a trihydroxamic acid containing linear molecule with exquisite Fe^{III}-binding capacity.[18−20] DFB has been used to treat Al^{III} overload and is the preferred clinical treatment for iron poisoning.[18,21] The three hydroxamate groups of DFB, as well as other siderophores, bind to Fe^{III} in an octahedral complex. These complexes persist between pH 2 and 10 with a corresponding UV−vis adsorption between 470 and 425 nm, respectively.[20−22] We have included a hydroxamic acid based Fe^{III}-binding domain at the C termini of single-chain and TRIS-derived scaffold-assembled collagen mimetic structures

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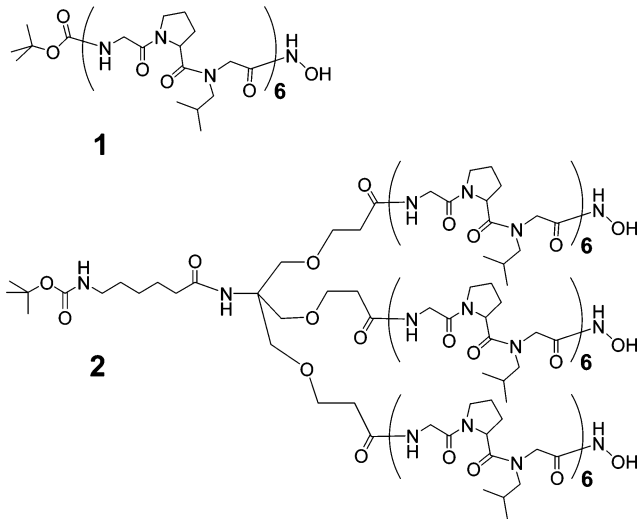

Figure 1. Single-chain (**1**) and TRIS-scaffold-assembled (**2**) Gly-Pro-NLeu structures containing the C-terminal hydroxamic acid based Fe^{III}-binding domain.

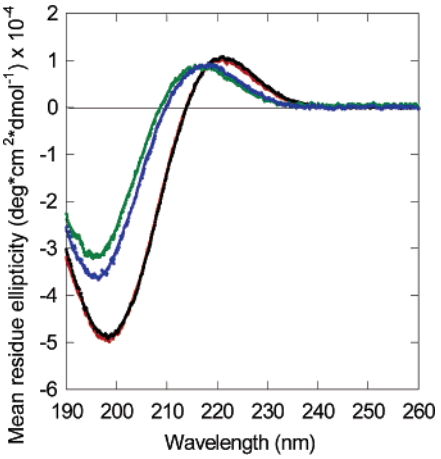

Figure 2. CD spectra of **1** (green), **1**·Fe^{III} (3:1, blue), **2** (black), and **2**·Fe^{III} (1:1, red). Measurements were acquired at 5 °C in H_2O (0.2 mg/mL, pH 4.5).

(where TRIS is tris(carboxyethoxymethyl)aminomethane). The single-chain (**1**) and TRIS-assembled (**2**) structures are shown in Figure 1.[23]

The single-chain (**1**) and scaffold-assembled (**2**) peptidomimetics were analyzed by circular dichroism (CD) spectroscopy (Figure 2), thermal denaturation observed by optical rotation (Figure 3), and UV−vis spectroscopy (Figure 4) in the absence and presence of Fe^{III} (a 20 mM $Fe(NO_3)_3$ solution was used as the source of Fe^{III}). The solution containing compound **1** and Fe^{III} was prepared in a 3:1 ratio, respectively, while the scaffold-assembled structure **2** and Fe^{III} solution was prepared in a 1:1 ratio. All solutions containing collagen mimetics were adjusted to pH 4.5 using Na_2HPO_4 and incubated at 4 °C for 7 days before analysis to allow for proper equilibration of triple-helix formation.[24] Scaffold-assembled formation of triple helices is rapid because of intramolecular strand association. However, single-chain

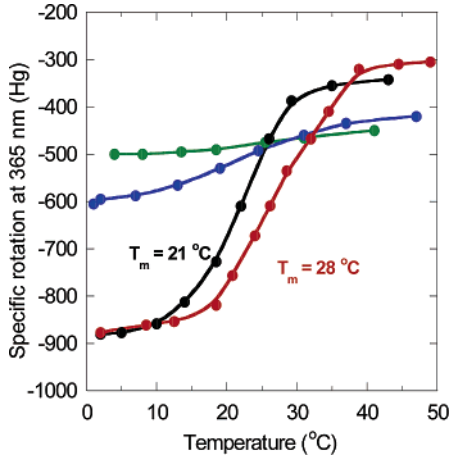

Figure 3. Thermal denaturation spectra of **1** (green), **1**·Fe^{III} (3:1, blue), **2** (black), and **2**·Fe^{III} (1:1, red). Measurements were acquired in H_2O (0.2 mg/mL, pH 4.5).

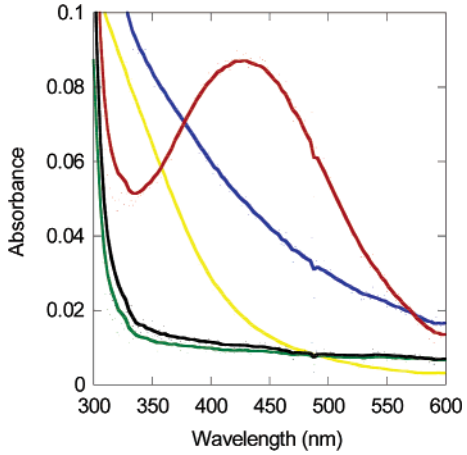

Figure 4. UV−vis spectra of solutions **1** (green), **1**·Fe^{III} (3:1, blue), **2** (black), **2**·Fe^{III} (1:1, red), and Fe^{III} in the absence of collagen mimetic (yellow). Measurements were carried out in H_2O at 22 °C (0.2 mg/mL, pH 4.5).

collagen mimetics strand association is intermolecular and, therefore, time- as well as concentration-dependent. For consistency, all collagen mimetics were incubated under identical experimental conditions. The CD spectra (Figure 2) were acquired in water (0.2 mg/mL with respect to the peptidomimetic) at 5 °C. A collagen-like triple helix possesses a characteristic CD profile with maximum, crossover, and minimum spectral positions near 220, 214, and 198 nm, respectively.[24−27] A blue shift is observed in the above wavelengths when triple-helical structures composed of the Gly-Pro-NLeu sequence are denatured. The single-chain collagen mimetic Boc-(Gly-Pro-NLeu)$_6$-NH-OH is not triple helical with or without the presence of Fe^{III}, determined by the positive peak between 216 and 7 nm observed by CD spectroscopy (Figure 2). The TRIS-assembled collagen mimetic Boc-Ahx-TRIS[(Gly-Pro-NLeu)$_6$-NH-OH]$_3$ (where Ahx is 6-aminohexanoic acid) clearly is triple-helical in the

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absence and presence of Fe^{III}, as observed by the collagen-like CD spectra with a positive peak at 221 nm.[25,28]

Little to no melting transition is observed for the single-chain molecule Boc-(Gly-Pro-NLeu)$_6$-NH-OH in the absence or presence of Fe^{III} (Figure 3). Previous studies of the single-chain analogue Boc-(Gly-Pro-NLeu)$_6$-OMe in H_2O did not show triple-helical propensity in our laboratory. The TRIS-assembled structure **2**, which contains C-terminal hydroxamic acids, exhibits a cooperative melting transition with a melting temperature (T_m) of 21 °C in the absence of Fe^{III}. In the presence of Fe^{III}, the T_m of the melting transition is raised to 28 °C.

Further evidence of the complex between Fe^{III} and the collagen-based hydroxamate-binding domain is provided by the UV−vis spectra shown in Figure 4. The trace representing the TRIS-assembled collagen-like structure in the presence of Fe^{III} possesses a spectral maximum around 430 nm. The absorbance at 430 nm is consistent with the spectra obtained from hydroxamate siderophore complexes with Fe^{III}.[21,22,29,30] No similar absorbance is observed for the single-chain analogue. It seems clear that Fe^{III} binding is favored when the C-terminal hydroxamate groups are brought into close proximity by triple-helix formation. It also appears that the iron(III) hydroxamate interaction is not sufficient to bring the three single chains together and act as a covalent scaffold.

Among the molecules studied, only compound **2** in the presence of Fe^{III} shows an enhancement in triple-helical thermal stability and an Fe^{III}-binding complex, as observed by UV−vis spectroscopy. We therefore believe the enhancement in thermal stability shown is the direct result of a specific binding interaction between the Fe^{III} metal and the three staggered hydroxamic acid groups positioned at the C termini of the collagen triple helix. Here we report a model system of metal-stabilized triple helices. This tunable system could be advanced to form adhesive collagen-like gels or matrices by the incorporation of selected metal-binding domains and the addition of corresponding metal ions.

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

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