Communication

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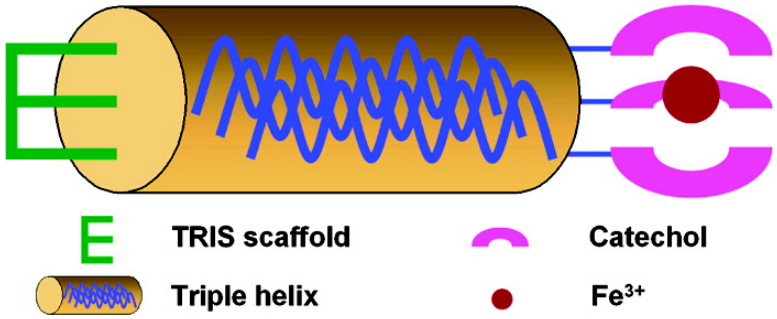

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# Metal-assisted Assembly and Stabilization of Collagen-like Triple Helices

Weibo Cai, Sen Wai Kwok, Joseph P. Taulane,* and Murray Goodman†

*Department of Chemistry and Biochemistry, University of California-San Diego, La Jolla, California 92093*

Received September 23, 2004; E-mail: jpt@chem.ucsd.edu

Collagen is the most abundant extracellular protein in vertebrates. It has a unique triple helical motif in which three polypeptide chains form left-handed helices and are supercoiled into a right-handed triple helix.[1,2] The primary sequence of triple helical collagen is composed of Gly-Xaa-Yaa trimer repeats.[3,4] A variety of scaffolds have been utilized to facilitate the assembly of collagen-like triple helices.[5−10] In particular, Koide and co-workers reported the assembly and stabilization of a collagen-like triple helix by an N-terminal Fe(II)−bipyridine complex.[10] Here, we report the incorporation of an $Fe^{3+}$−catechol complex as a scaffold to assemble peptide chains into triple helices.

Enterobactin, a naturally occurring triscatechol, is the most powerful $Fe^{3+}$ chelator ever reported with an overall stability constant of $10^{49}$.[11,12] Therefore, we have chosen to use the $Fe^{3+}$−catechol complex as a scaffold because of the robust nature of the complex. The $Fe^{3+}$−catechol complex has been well studied. A 1:3 $Fe^{3+}$−catechol complex is formed at a pH above 9.5, which has a characteristic absorption peak at around 500 nm (for the octahedrally coordinated $Fe^{3+}$) in the UV−vis spectra.[13,14]

Two molecules incorporating the catechol moiety have been synthesized (Figure 1). In the single-chain compound (**A**), a dopamine residue was attached to the N-terminus of the peptide chain composed of the Gly-Nleu-Pro sequence (where Nleu denotes N-isobutylglycine) via a flexible succinic acid linker. In the TRIS-assembled peptide (**B**), 2,3-dihydroxybenzoic acid was attached to the C-terminus via an ethylenediamine linker. The structure of the TRIS scaffold is noted in Figure 1.[15] The linkers were incorporated to accommodate the one amino acid residue register between the three strands of a triple helix.[16] Molecules **A** and **B** have the same peptide sequence and chain length.

The UV−vis spectra of the molecules with and without $Fe^{3+}$ in 50 mM (pH 10) CAPS buffer are shown in Figures 2 and 3 (where CAPS denotes 3-cyclohexylamino-1-propane sulfonic acid). Fresh, but nondegassed, solutions were stored under $N_2$, and no oxidation of the catechol was observed. In the case of compound **A**, $^1/_3$ equiv of $Fe^{3+}$ was added. For compound **B**, 1 equiv of $Fe^{3+}$ was added since one molecule of **B** contains three catechol groups. Both spectra exhibit the broad absorbance band at around 500 nm, which clearly suggests octahedral coordination of $Fe^{3+}$ (red wine color).

The triple helicity of all of the structures were determined by thermal denaturation monitored by optical rotation and circular dichroism (CD) measurements (Figures 4 and 5). All of the measurements for compound **A** were carried out at 0.2 mg/mL, while all of the measurements for compound **B** were carried out at 0.1 mg/mL due to a solubility problem. Since catechol, in the absence of $Fe^{3+}$, is easily oxidized at elevated temperatures, the thermal denaturation measurements for both compounds without $Fe^{3+}$ were carried out in 1 mM HCl to prevent oxidation. Melting curves for model compound Boc-$\beta$-Ala-TRIS-[(Gly-Nleu-Pro)$_6$-OMe]$_3$ were obtained in 1 mM HCl (pH 3), in $H_2O$ (pH 7) and 50 mM CAPS buffer (pH 10), and they are superimposable with

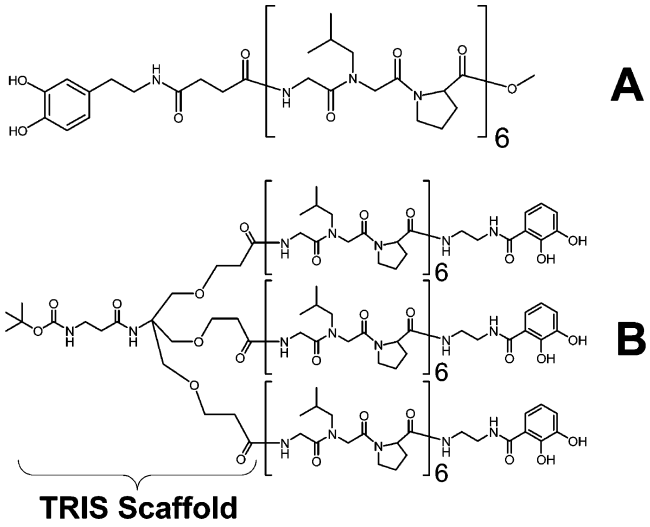


***Figure 1.*** Single-chain (A) and TRIS-assembled (B) peptides incorporating catechol groups.

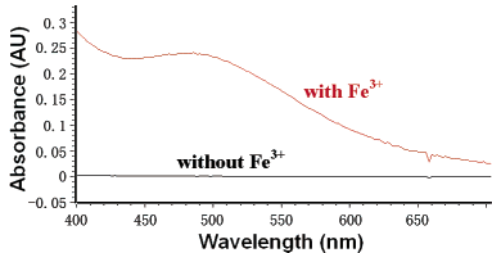


***Figure 2.*** The UV−vis spectrum of compound **A** (0.2 mg/mL) with and without $Fe^{3+}$ in 50 mM CAPS buffer.

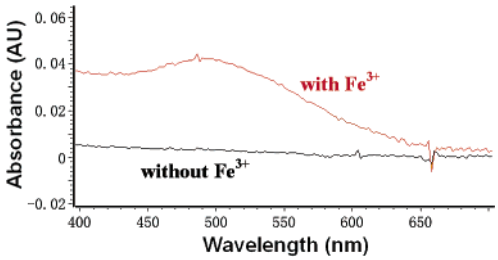


***Figure 3.*** The UV−vis spectrum of compound **B** (0.1 mg/mL) with and without $Fe^{3+}$ in 50 mM CAPS buffer.

the same melting temperature ($T_m$) (see Supporting Information). Therefore, the difference in triple helical stability between the peptide without $Fe^{3+}$ in 1 mM HCl and the peptide with $Fe^{3+}$ in 50 mM CAPS buffer would only come from the $Fe^{3+}$−catechol complex.

For peptides composed of the Gly-Nleu-Pro sequence, the positive peak in the CD spectrum is only present when it is triple helical.[17,18] The CD data shown in Figure 4 indicate that compound **A** does not form a triple helix when $Fe^{3+}$ is absent. This was further

† Professor Murray Goodman died on June 1, 2004.



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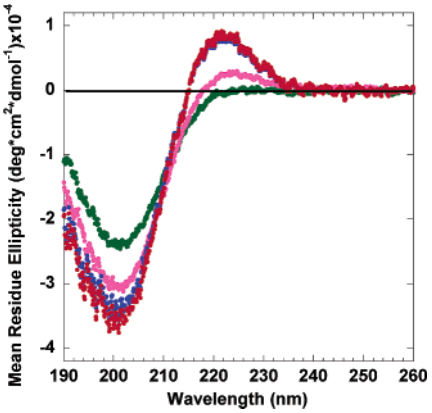


***Figure 4.*** Circular dichroism spectra of **A** (dark green), **A** + $Fe^{3+}$ (magenta), **B** (blue), and **B** + $Fe^{3+}$ (red). All measurements were carried out at 6 °C in 50 mM CAPS buffer (pH 10).

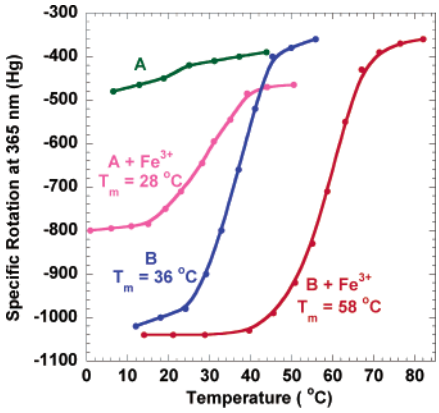


***Figure 5.*** Thermal melting transitions of **A** (dark green), **A** + $Fe^{3+}$ (magenta), **B** (blue), and **B** + $Fe^{3+}$ (red). Measurements without $Fe^{3+}$ were carried out in 1 mM HCl to prevent oxidation of the catechol groups.

confirmed by the absence of a melting transition in the thermal melting measurement (Figure 5). However, when $^1/_3$ equiv of $Fe^{3+}$ was added, the positive peak appears in the CD spectra. Thermal melting measurement revealed that the $T_m$ of the triple helix is 28 °C. Since the UV−vis spectrum indicates the presence of a 1:3 $Fe^{3+}$−catechol complex, it is clear that this complex acts as a scaffold to assemble the collagen-like triple helix. The triple helix folding rate of compound **A** when $Fe^{3+}$ is present is comparable to the rate of scaffold-assembled peptides of the same sequence (data not shown).

For TRIS-assembled peptide **B**, both CD and thermal melting experiments indicate that the molecule is triple helical in solution even when $Fe^{3+}$ is not present. The melting temperature is 36 °C. When 1 equiv of $Fe^{3+}$ is added, the molecule exhibits an extraordinary $T_m$ of 58 °C. The formation of an $Fe^{3+}$−catechol complex increased the $T_m$ of **B** by a remarkable 22 °C!

Two possibilities exist. If the $Fe^{3+}$−catechol complex is intermolecular (where $Fe^{3+}$ is coordinated by catechol groups from three different TRIS-assembled molecules), the resulting structure, as well as the stabilization effect, would be similar to that of the collagen mimetic dendrimer reported earlier (where three TRIS-assembled structures were attached to a trimesic acid core structure via the $\beta$-Ala linker).[19,20] An increase of 3−4 °C in the $T_m$ value over the corresponding TRIS-assembled structure would be observed. However, an increase of 22 °C in the $T_m$ value can only be explained

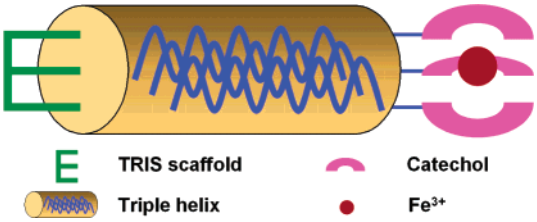


***Figure 6.*** Schematic representation of the triple helix formed by compound **B** when $Fe^{3+}$ is added.

by an intramolecular complex where $Fe^{3+}$ is coordinated by three catechol groups from the same molecule **B**.

On the basis of the above data, we believe that the $Fe^{3+}$−catechol complex formed in each case acts as an extra scaffold to facilitate the folding of the peptide into triple helices. For compound **A**, the complex acts as an N-terminal scaffold. For compound **B**, the complex acts as a C-terminal scaffold, which confers extra stability to the TRIS-assembled triple helix. A schematic of this discaffold-assembled triple helical collagen mimetic structure is shown in Figure 6.

In conclusion, we report here the assembly of a triple helix which is tethered at both the N- and the C-terminus by a TRIS scaffold and an $Fe^{3+}$−catechol complex, respectively. The $Fe^{3+}$−catechol complex raised the $T_m$ of the TRIS-assembled triple helix by a remarkable 22 °C. Further characterization of the discaffold-assembled structures is currently underway.

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

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