Peptides

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Rational Design of Bioorganometallic Foldamers: A Potential Model for Parallel β-Helical Peptides**

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The design of structurally well-defined peptide motifs has been an active area of research aimed at gaining a deeper understanding of biochemical processes[1] to generate molecules with potential biological applications[2] or to develop novel biomaterials.[3] Synthetic foldamers that resemble the structure of helical[4] and sheet conformations,[5] two of the major secondary structural motifs in proteins, can now be designed de novo by using non-proteinogenic amino acids or a molecular scaffold. In some cases, as for gramicidin A, alternating D- and L-amino acid sequences can adopt a β-helical conformation.[6] In 1993, a new kind of β-helix, in which only L-amino acids are present, was discovered in bacterial *Pectate Lyase C*.[7] and was recognized to play various important roles in biological systems.[8] In this structural motif, a series of β strands are linked by a turn region, thus resulting in a virtually perpendicular arrangement of the individual β strands with respect to the axis of the helix. Little effort has been dedicated to designing the model for this important secondary structural domain. In 2001, Nolte and co-workers reported the synthetic analogue, in which peptide strands are attached to a central polyisocyanide backbone and adopt a β sheet conformation, which runs the length of the central helical core.[9] Disubstituted ferrocene compounds, and ferrocene amino acid (Fca) in particular, are known to induce β turns in peptide conjugates and have the appropriate structural rigidity as a result of efficient hydrogen-bonding interactions between the peptide chains attached to the two cyclopentadienyl (Cp) rings.[10] Thus, we hypothesized that it should be possible to design a helical foldamer that exploits the turn-inducing properties of Fca and links them to α-amino acids. It was thought that the hydrogen-bonding interactions between the peptide substituents on opposite Cp rings would favor the formation of a helical foldamer that displays the main features of the natural β-helical motif. Herein, we present the results of a synthetic and structural study of a series of helical foldamers formed from L- and D-alanine and Fca. Our results unequivocally show the utility of our design approach for the formation of a series of unprecedented helical foldamers that display right- and left-handedness.

Starting from the fully protected Fca derivative **1**,[11] the desired "monomers" Fca–L-alanine (**2**) and Fca–D-alanine (**3**), their corresponding oligomers **6**–**11**, and poly(Fca–L-alanine) (**12**) were synthesized in solution by peptide-coupling strategies (Scheme 1) and were characterized spectroscopically (see Supporting Information). The ^{1}H NMR spectra of **2** and **3** in $CDCl_3$ show the presence of an amide NH signal at $\delta = 7.61$ (NH of Fca) and 6.50 ppm (NH of Ala). As the length of the peptide increases, the amide resonances gradually shift downfield, thus indicating an increasingly hydrogen-bonded system. In the case of the polymeric **12**, the two amide NH resonances are observed at $\delta = 10.87$ (for NH attached to Cp) and 9.07 ppm (for NH of Ala), as is expected for strongly hydrogen-bonded systems. ROESY NMR spectroscopic studies of the Fca foldamers **6**, **8**, and **10** in solution show the cross-correlations between Fc–NH_i and β-H of Ala_{i+1}, Ala-NH_{i+1} and α-H of Ala_i, and β-H of Ala_{i+1} and α-H of Ala_i (see Supporting Information), which correspond to the β-helical conformations. Circular dichroism (CD) spectroscopy is a reliable tool to evaluate the conformation of chiral Fc conjugates that display a band (+ or −) at about 450 nm.[12] CD studies of solutions of our foldamers in acetonitrile (Figure 1) show that the oligomers **6**, **8**, and **10**, derived from L-Ala, exhibit a weak positive Cotton effect (*P* helicity) in the ferrocene region at $\lambda = 450$ nm, whereas the D-Ala derivatives

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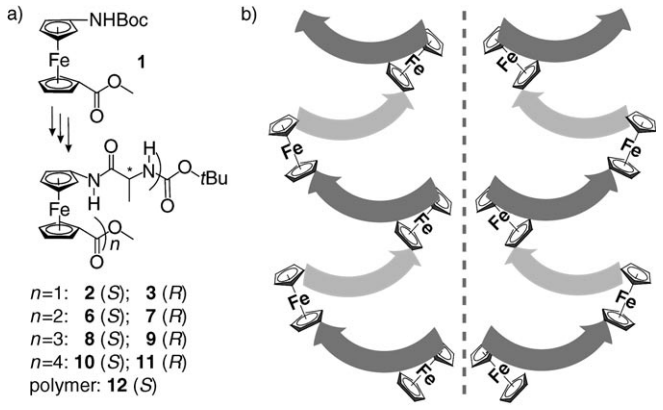

Scheme 1. a) Preparation of *P*- and *M*-helical foldamers from the conjugates of Fca with L- and D-alanine, respectively (see Supporting Information for a detailed description of the synthesis and characterization). b) Schematic representation of the formation of right- and left-handed β-helical structures from the alternating conjugates of Fca and L- and D-amino acids, respectively, which are mirror images of each other.

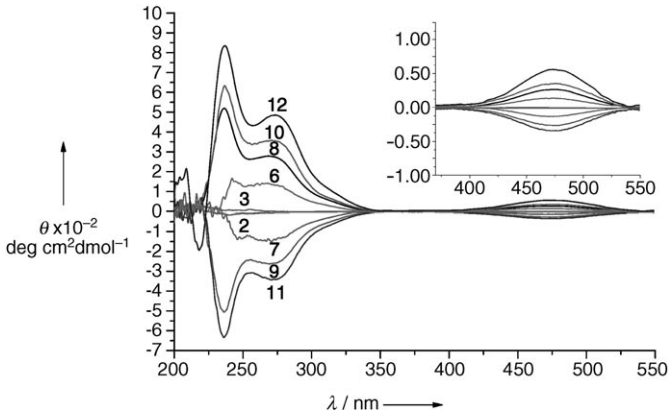

Figure 1. CD spectra of the Fca–Ala "monomers" **2** and **3** and the right- and left-handed parallel β-helical Fca–Ala oligomers (**6**–**11**) and polymer (**12**) in acetonitrile at 25 °C. The intensity is normalized for one repeating unit.

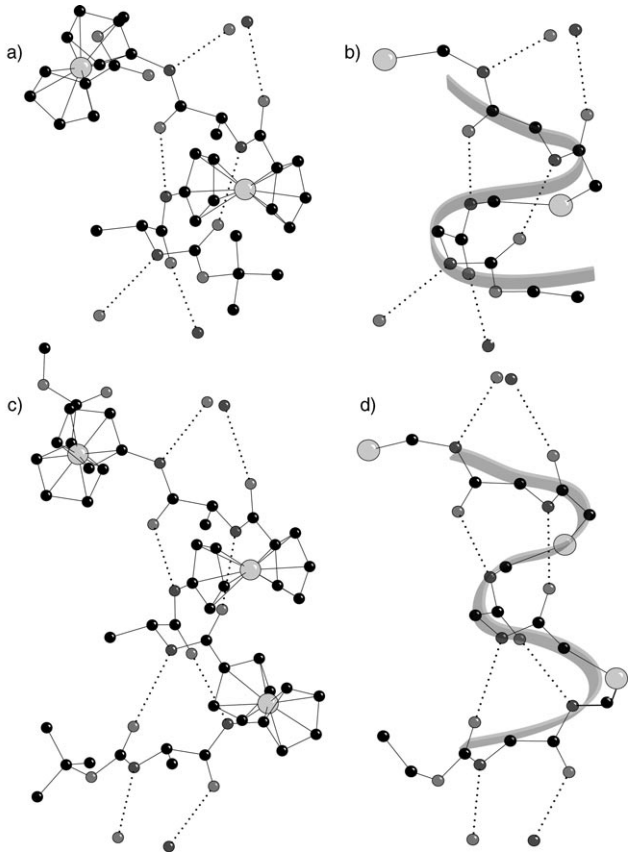

Figure 2. a) Crystal structure of **6**, b) the helical backbone of **6**, c) crystal structure of **8**, and d) the helical backbone of **8**. C black; N dark gray; O mid-gray; Fe pale gray (large spheres). Hydrogen atoms are omitted for clarity, and the dashed lines represent hydrogen bonds. The intramolecular and intermolecular hydrogen-bonding patterns are identical (12-membered rings including the hydrogen atoms, parallel β-sheet-like). The crystal structure of **7** is the mirror image of **6**, and that of **9** is the mirror image of **8** (see Supporting Information).

7, **9**, and **11** exhibit a negative Cotton effect (*M* helicity). The Fca–Ala "monomers" **2** and **3** do not exhibit any Fca-based band but display a weak signal in the peptide region, which are mirror images of each other. The absence of the band in the Fca region is not surprising, as the intramolecular hydrogen-bonding required to establish a stable secondary structure is not possible in these "monomers". For the dimers **6** and **7**, in which two Fca–Ala units are connected by hed-to-tail strong intramolecular hydrogen bonding (see Figure 2 and below) exists between the two Ala groups. The resulting effect on the CD spectrum normalized to a single Fca–Ala unit is significant enhancement of the bands.

Additional Fca–Ala units increase the signal intensity of the normalized CD spectrum in both the Fca and peptide regions. Peptides of equal length have CD signals of equal magnitude but of opposite sign, thus indicating that the helicity pattern is identical but of opposite handedness. Upon elongation, the conjugates are able to establish stronger intramolecular hydrogen bonding between adjacent Ala units, thus resulting in a more rigid helical arrangement. Importantly, the polymer displays a characteristic band at 215 nm indicative of β-sheet peptides, which is absent in the smaller oligomers. The helices are stable at elevated temperatures and do not show a distinct melting behavior at temperatures up to 70 °C (see Supporting Information).

The dimers (**6** and **7** in $P2_12_12_1$ space group) and trimers (**8** in $P4_12_12$ and **9** in $P4_32_12$ space group) were readily crystallized either by slow diffusion of nonsolvent into the sample solution or by slow evaporation of solvent from the solution (see Supporting Information). The single-crystal structural analyses of those show the expected axial chirality for each of the Fca residues. All systems derived from L-Ala adopt a *P*-helical conformation, whereas D-Ala gives rise to *M* helicity, thus corroborating the CD results. The amino acid residues are involved in intramolecular hydrogen bonding, thereby forming a parallel β-sheet-like structure with 12-membered hydrogen-bonded rings. As the length of the helix increases, the number of 12-membered hydrogen-bonded rings increases. The intramolecular hydrogen-bonding distances (N···O) are 2.95–2.92 Å between the NH of Fca and C=O of

Ala, and 2.99–2.97 Å between the NH of Ala and C=O of Fca. The dihedral angles of all peptide segments for **6** and **8** are $-79 > \varphi > -67$ and $155 < \psi < 138°$, respectively.[13] The dihedral angles for the foldamers **7** and **9** derived from D-Ala are similar in magnitude but opposite in sign. Importantly, on the supramolecular level, the same hydrogen-bonding pattern as intramolecular (a 12-membered parallel β-sheet-like ring formation and similar N–O distances) is propagated and forms a continuous helical structure. The individual supramolecular helices arrange parallel to each other to generate solvent-filled hydrophobic pockets (CH_2Cl_2 for **6** and **7**, hexane for **8**, $CHCl_3$ for **9**). FTIR spectroscopy of the foldamers in KBr show the amide A absorption in the range 3266–3272 cm^{-1}, which clearly indicates the involvement of the amide NH group in the hydrogen bonding. The amide I and II regions of the conjugates are complex and show a series of bands (1627–1639 and 1680–1690 cm^{-1}) that may be attributed to a β-sheet conformation.[14]

In conclusion, we presented the results of a study into the preparation and structural properties of organometallic peptide conjugate foldamers that adopt a structure that shows some structural similarities to the β-helical motif found in naturally occurring proteins, thus exploiting the turn-inducing ability of Fca. This novel redox-active β-helical system may have potential for the electrochemical screening of "β-breakers". We are now exploring the electronic and electrochemical properties of these and related systems in solution and on a surface using surface-supported helical foldamers.[15]

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[15] The Supporting Information includes experimental and spectroscopic details; IR spectra for all compounds; variable-temperature (VT) CD spectra and NMR stack plots for the L-series; ROESY spectra of **6**, **8**, and **10**; and single-crystal data and intermolecular arrangements of **6**–**9**.

