Macrocycles

DOI: 10.1002/ange.200502788

Discovery of a Pseudo β Barrel: Synthesis and Formation by Tiling of Ferrocene Cyclopeptides**

*Somenath Chowdhury, David A. R. Sanders, Gabriele Schatte, and Heinz-Bernhard Kraatz**

Inspired by channel-forming proteins involved in transmembrane transport and controlling biological functions,[1] the construction of porous organic architectures with well-defined shapes and sizes from simple building blocks has

[*] S. Chowdhury, Prof. D. A. R. Sanders, Prof. H.-B. Kraatz
Department of Chemistry
University of Saskatchewan
110 Science Place, Saskatoon, SK, S7N 5C9 (Canada)
Fax: (+1) 306-966-4730
E-mail: kraatz@skyway.usask.ca
Dr. G. Schatte
Saskatchewan Structural Science Centre
University of Saskatchewan
110 Science Place, Saskatoon, SK, S7N 5C9 (Canada)

[**] We acknowledge support from the NSERC in the form of an operating grant. H.-B.K. is the Canada Research Chair in Biomaterials.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

emerged as an important subject in biological chemistry and materials science.[2–8] In this regard, recently introduced peptide-derived materials are used to mimic biological channels and serve as models for transmembrane pores or as antibacterial pore-forming agents,[9–10] with potential applications in drug delivery or as constrained chiral reaction environments.[2,11–12] These channels are mainly self-assembled from cyclopeptides, which stack on top of each other to give large hollow tubes, with the individual cyclopeptides connected by hydrogen-bonding akin to that found in antiparallel β sheets. The peptide β barrel presents a particular challenge. This ubiquitous structural motif is present in a wide range of proteins, including pore-forming proteins present as membrane proteins of gram-negative bacteria, mitochondria, and chloroplasts, and control many sophisticated biological functions.[13] In a β barrel, the peptide strands are arranged more or less parallel to the axis of the barrel. To date, it has not been possible to synthesize a model for such an ubiquitous biological structural motif.[14] Herein, we report the synthesis and the structure of the first artificial β barrel obtained by self-assembly from smaller bio-organometallic ferrocene–peptide conjugates. In this model, eight β strands are arranged almost parallel to the axis of the channel with an internal-pore diameter of 8 Å. Experimental evidence shows that the pore appears to be filled with water molecules (see below). Our approach offers the potential to exploit such structures for the new design of functional transmembrane channels. Such structures may also find applications in separation science, supramolecular host–guest chemistry, and molecular electronics.

The structure of 1,*n*′-disubstituted ferrocene–peptide conjugates is controlled by a fine balance between inter- and intramolecular hydrogen-bonding, and the attached peptide strands attain a β-sheet conformation.[15–18] In general, *P*- and *M*-helical conjugates with respect to ferrocene are formed if L- and D-amino acids are attached, respectively, to the metallocene and the substituents adopt a flexible *anti* conformation, which is not suitable for the sheetlike intramolecular interactions of the elongated peptide strands. We decided to force the attached strands to align parallel to each other by chemically linking the two pendant peptide chains with cysteamine (CSA), a disulfide linker, thereby restricting the mobility of the strands. We expected this to have a profound consequence in that this chemical modification should foster intermolecular hydrogen bonding, which is akin to that found in antiparallel β sheets.

To optimize intramolecular hydrogen bonding between the two peptide substituents, a small twist of the cyclopentadiene (Cp)/amide planes is required, thus resulting in the peptide strands to turn towards each other.[19] From this observation we predicted that restricting the formation of a "flat" β sheet by tying the two peptide substituents through a cystamine linker will induce additional curving of the peptide strands and thus may allow hollow hydrogen-bonded assemblies based on a β-sheet motif to be created. For this purpose, we designed two ferrocene (Fc) cyclopeptides, Fc(Gly-Val-CSA)$_2$ (**5**) and Fc(Gly-Ile-CSA)$_2$ (**6**; Gly = glycine, Val = valine, Ile = isoleucine), each with a glycine unit as the first amino acid attached to the ferrocene unit, thus giving maximum flexibility to the pendant peptide chains, followed by valine or isoleucine, amino acids with a very high propensity to form β sheets. Compounds **5** and **6** were synthesized by the cyclization of 1,1′-ferrocene dicarboxylic acid and cysteamine peptide conjugates at very high dilution and were characterized spectroscopically (see the Supporting Information). These compounds are poorly soluble in organic solvents, as expected for strongly hydrogen-bonded materials.[21] Compound **5** was readily obtained from solutions of low concentration in a crystalline form suitable for structural analysis by X-ray diffraction studies. The molecular structure of **5**, together with its supramolecular arrangement, is shown in Figure 1.

There are three different types of hydrogen bonding present in **5**. Intramolecular hydrogen bonding is observed between the C=O group and the adjacent (H)N group (N(21)-O(12): 2.7848(4), N(23)-O(13): 2.848(3), N(11)-O(22): 3.013(4) Å). The Fc(Gly-Val-CSA)$_2$ units are linked by intermolecular hydrogen bonding (2.898(4) and 3.036(4) Å). The intermolecular hydrogen-bonding interactions between head-to-tail connected ferrocene peptides results in the formation of a 14-membered ring, as expected for two peptides that interact in an antiparallel β-sheet fashion (see Figure 1b).[16] The β sheets are parallel to the *b*–*c* plane. Additional hydrogen bonding (O23···N13: 2.824(4) Å) between adjacent sheets results in the formation of β barrels with a diameter of approximately 8 Å (measured between adjacent oxygen atoms of the C=O groups, see Figure 1c). Importantly, the structural rigidity allows the molecules to act as tiles that form what can be described as pseudo barrels (Figure 1d).

Figure 2 gives a stereoview of a pseudo barrel formed by tiling of the Fc conjugates and superimposes the direction of the individual β strands. The barrel is formed by eight β strands arranged in a up-up-down-down-up-up-down-down pattern. The axis of the barrel is parallel to the *a*–*c* plane and perpendicular to the *b*–*c* plane. The ϕ/ψ angles for the four residues are (Gly1: −134/194°, Val1: −56/146°, Gly2: −76/161°, Val2: −115/64°). These residues all fit close to the theoretical region of β-sheet conformations (ϕ: −180–−45°, ψ: 45–225°).[22] As is seen with naturally occurring β sheets, there is distortion, but the angles still fall within the "allowed" Ramachandran region associated with β-sheet structures.[23–27] The third "residue" (the cystamine) of one substituent (chain 1) has a ϕ value of approximately −97°, thus making the one substituent of the molecule a fairly close approximation of a β strand.[22]

There is precedence for naturally occurring eight-stranded β barrels. For example, OmpX,[28] OmpA,[29] and PagP[30] are all eight-stranded barrels with various biological functions, which range from toxin binding to controlling enzymatic activity. Another example is NspA from *N. meningitides*, which is involved in cell adhesion and has potential applications in structure-based vaccine design.[31] In these natural β barrels, the β strands are tilted from the barrel axis, whereas the β strands are almost parallel to the barrel axis in our pseudo β barrel.

Figure 3a provides a view of the interior surface of the pseudo barrel, thus showing hydrophilic and hydrophobic

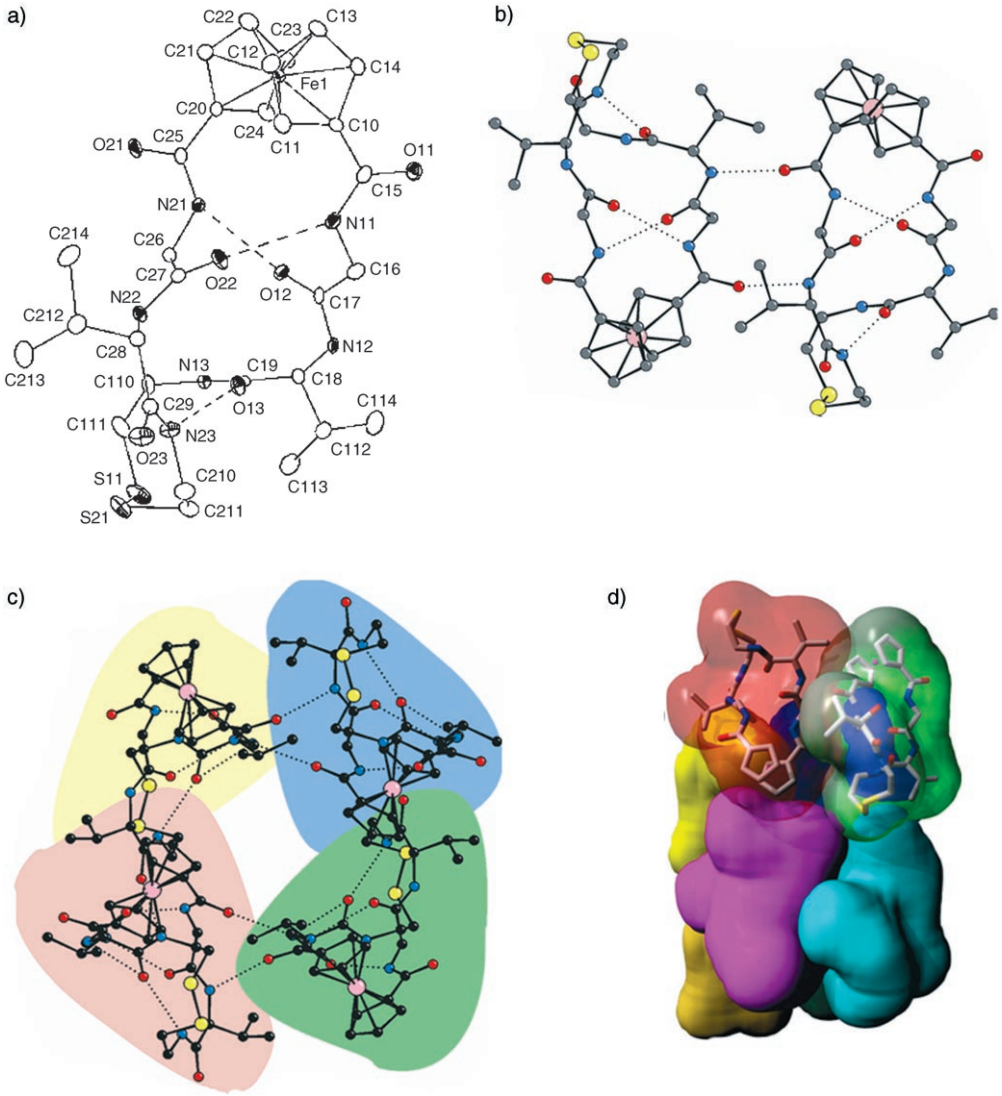

Figure 1. The crystal structure of Fc(Gly-Val-CSA)$_2$ (**5**) and a schematic view of the formation of the pseudo β barrel. a) Molecular structure of **5** showing the *P*-helicity of the ferrocene unit and the intramolecular hydrogen bonding between the two pendant peptide strands. The geometry of the Fc group, the amide substituents, and the bond lengths and angles are well within established parameters for Fc–peptide conjugates.[20] b) Formation of β sheets through intermolecular N(H)···O=C hydrogen bonding. The head-to-tail interaction of the molecules results in an unusual up-up-down-down arrangement of the peptide strands. The arrangement between the two peptide strands on the two Fc conjugates is antiparallel. c) Four molecules interact through hydrogen bonding to form a β barrel; a view down the *c* axis is shown, and the disordered water molecules inside the cavity of the barrel are omitted for clarity. d) Molecular-surface representation showing a side view of the half barrel viewed along the *c* axis formed by tiling of cyclic ferrocene peptides.[35]

characteristics. Some of the peptide carbonyl groups point towards the inside of the pseudo barrel. We assume that this imparts sufficient hydrophilic character on the interior of the barrel to enable water to be inside the pseudo barrels. X-ray analysis shows a significant amount of residual electron density inside the pore, which is best modeled as approximately 12 disordered water molecules, with adjacent water molecules separated from each other by approximately 2.8 Å, which corresponds to strong hydrogen bonding. The closest contact between the water and the interior walls of the pseudo barrel is 3.5 Å, thus indicating weak hydrogen-bonding interactions with the walls. ^{1}H NMR spectroscopic analysis of **5** in solution clearly indicates the presence of water as part of the crystalline solid. The ^{1}H NMR spectra indicate the presence of approximately 1.2–1.5 molecules of water per molecule of Fc–peptide for a total of approximately 9–12 molecules of water per 8-mer or pseudo barrel. As the space within the barrel is 386 Å^3, theoretically 10 water molecules, each occupying 40 Å^3, could fit the pore. Therefore, a model including only water molecules inside the pore best fits all the experimental evidence.

These results compare well with pore-forming cyclopeptides, described before by Ghadiri, who interpreted residual electron density present within the pores of the cylindrical β-sheet peptide as that from water molecules.[32]

FT-IR spectroscopy is also a powerful technique for the examination of the secondary structure of peptides.[33] In particular, the amide-I region offers a characteristic footprint that allows us to distinguish between an α helix, a β sheet, and a random-coil conformation. The FT-IR spectrum of **5** in the solid state exhibits amide-I bands at 1640 and 1682 cm^{-1} and an amide-II band at 1536 cm^{-1}, which are the characteristic peaks for a β sheet.[33,34] Importantly, the IR absorptions are virtually identical for the Ile derivative **6** (1640, 1680, and 1535 cm^{-1}, respectively; see the Supporting Information), thus indicating that the structures of **5** and **6** are similar in the solid state. As expected, the amide N–H bonds are involved in hydrogen bonding, and absorptions are observed in the amide-A region at 3280 and 3282 cm^{-1} for **5** and **6**, respectively. Our observations are closely related to those made by Ghadiri and co-workers who showed that cyclopeptides with alternating D- and L-amino acid sequences, assemble by intermolecular hydrogen bonding into peptide nanotubes, in which the individual building blocks have an antiparallel β-sheet conformation.[5]

In summary, we have outlined a potential strategy for the design of porous peptide materials, in which the barrellike

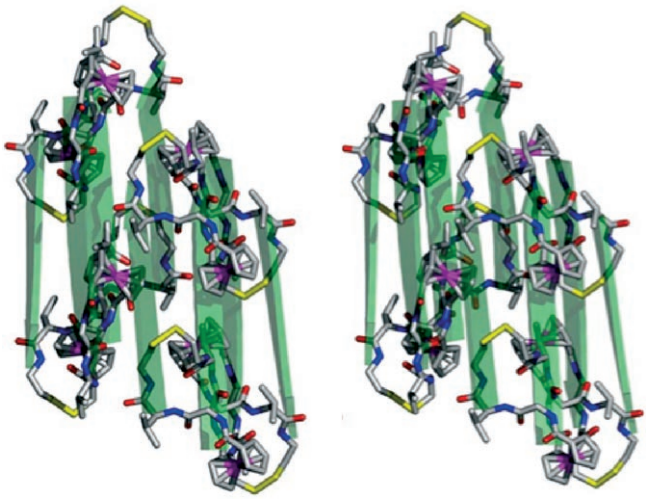

Figure 2. Stereoview of the pseudo barrel formed by tiling of **5**. The individual molecules are indicated as well as the direction of the individual β strands.[22]

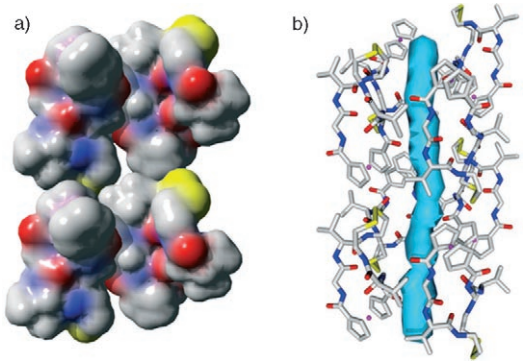

Figure 3. a) Van der Waals surface representation of a cut-away side view of the interior of the pseudo barrel that shows the hydrophilic and hydrophobic characteristics (red: oxygen, blue: nitrogen, yellow: sulfur, gray: carbon). b) The same side view of the pseudo barrel in (a) generated by tiling of **5**. This channel has an interior diameter of approximately 8 Å. The residual electron density within this channel is interpreted as water molecules that are disordered over several positions and only the approximate envelope of the channel content is shown.

pores are formed by the self-assembly of bio-organometallic peptide conjugates. Although solubility of these systems is a challenge at present, the use of hydroxy-containing amino acids, such as serine and threonine, should allow us to study self-assembly in solution. Our approach is promising for the use of readily available starting materials in the assembly of large, porous peptide materials, which may find applications in biological chemistry and materials science. If successful, the strategy described herein should lend itself to the preparation of novel proteins that incorporate the β-barrel motif.[36]

Received: August 6, 2005
Revised: October 19, 2005
Published online: December 27, 2005

Keywords: β barrel · ferrocene · macrocycles · peptides · self-assembly

[1] M. H. Saier, *J. Membr. Biol.* **2000**, 175, 165 – 180.
[2] D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 1016 – 1041; *Angew. Chem. Int. Edn.* **2001**, 40, 988 – 1011.
[3] D. Venkataraman, S. Lee, J. S. Zhang, J. S. Moore, *Nature* **1994**, *371*, 591 – 593.
[4] A. Harada, J. Li, M. Kamachi, *Nature* **1993**, *364*, 516 – 518.
[5] M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, *366*, 324 – 327.
[6] M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature* **1994**, *369*, 301 – 304.
[7] J. C. Nelson, J. G. Saven, J. S. Moore, P. G. Wolynes, *Science* **1997**, *277*, 1793 – 1796.
[8] D. Ranganathan, C. Lakshmi, I. L. Karle, *J. Am. Chem. Soc.* **1999**, *121*, 6103 – 6107.
[9] J. R. Granja, M. R. Ghadiri, *J. Am. Chem. Soc.* **1994**, *116*, 10785 – 10786.
[10] S. Fernandez-Lopez, H. S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen, M. R. Ghadiri, *Nature* **2001**, *414*, 329 – 329.
[11] J. Sanchez-Quesada, H. S. Kim, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 2571 – 2574; *Angew. Chem. Int. Ed.* **2001**, *40*, 2503 – 2506.
[12] B. Eisenberg, *Acc. Chem. Res.* **1998**, *31*, 117 – 123.
[13] W. C. Wimley, *Curr. Opin. Struct. Biol.* **2003** 13, 404 – 411.
[14] N. Sakai, J. Mareda, S. Matile, *Acc. Chem. Res.* **2005**, *38*, 79 – 87.
[15] D. R. van Staveren, N. Metzler-Nolte, *Chem. Rev.* **2004**, *104*, 5931 – 5985.
[16] T. Moriuchi, A. Nomoto, K. Yoshida, A. Ogawa, T. Hirao, *J. Am. Chem. Soc.* **2001**, *123*, 68 – 75.
[17] T. Moriuchi, K. Yoshida, T. Hirao, *Org. Lett.* **2003**, *5*, 4285 – 4288.
[18] A. Nomoto, T. Moriuchi, S. Yamazaki, A. Ogawa, T. Hirao, *Chem. Commun.* **1998**, 1963 – 1964.
[19] S. Chowdhury, G. Schatte, H. B. Kraatz, *Dalton Trans.* **2004**, 1726.
[20] L. Lin, A. Berces, H. B. Kraatz, *J. Organomet. Chem.* **1998**, *556*, 11 – 20.
[21] D. Gauthier, P. Baillargeon, M. Drouin, Y. L. Dory, *Angew. Chem.* **2001**, *113*, 4771 – 4774; *Angew. Chem. Int. Ed.* **2001**, *40*, 4635 – 4638.
[22] G. N. Ramachandran, V. Sasisekharan, *Adv. Protein Chem.* **1968**, 23, 283 – 437.
[23] M. R. Betancourt, J. Skolnick, *J. Mol. Biol.* **2004**, *343*, 635 – 649.
[24] S. Hovmoller, T. Zhou, T. Ohlson, *Acta Crystallogr. Sect. D* **2002**, *58*, 768 – 776.
[25] P. A. Karplus, *Protein Sci.* **1996**, *5*, 1406 – 1420.
[26] A. D. Solis, S. Rackovsky, *Proteins Struct. Funct. Genet.* **2002**, *48*, 463 – 486.
[27] W. Kabsch, C. Sander, *Biopolymers* **1983**, *22*, 2577 – 2637.
[28] J. Vogt, G. E. Schulz, *Struct. Folding Design* **1999**, *7*, 1301 – 1309.
[29] A. Pautsch, G. E. Schulz, *Nat. Struct. Biol.* **1998**, *5*, 1013 – 1017.
[30] P. M. Hwang, R. E. Bishop, L. E. Kay, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9618 – 9623.
[31] L. Vandeputte-Rutten, M. P. Bos, J. Tommassen, P. Gros, *J. Biol. Chem.* **2003**, *278*, 24825 – 24830.
[32] T. D. Clark, J. M. Buriak, K. Kobayashi, M. P. Isler, D. E. McRee, M. R. Ghadiri, *J. Am. Chem. Soc.* **1998**, *120*, 8949 – 8962.
[33] S. Krimm, J. Bandekar, *Adv. Protein Chem.* **1986**, *39*, 181 – 364.
[34] A. Barth, C. Zscherp, *Q. Rev. Biophys.* **2002**, *35*, 369 – 430.
[35] Crystal data for **5**: $C_{30}H_{42}Fe_1N_6O_6S_2$, $M_r = 702.67$, orthorhombic, $P2_12_12_1$, $a = 14.4808(4)$, $b = 16.2650(5)$, $c = 16.4770(4)$ Å, $\alpha = \beta = \gamma = 90°$, $Z = 4$; $\mu = 0.543\ mm^{-1}$, $\rho_{calcd} = 1.209\ Mg\,m^{-3}$.
[36] The synthesis and characterization of **5**, **6**, and intermediate species; IR spectra of **5** and **6**; and X-ray structural data for **5** are given in the Supporting Information.

