Anti-Bacterial Self-Assembled Nanotubes of Cyclic *D*, *L*-α-Peptides

M. Al-Sayah[a] and M. R. Ghadiri[b]

[a]*Department of Biology and Chemistry, American University of Sharjah, P.O. Box: 26666, Sharjah, UAE*
[b]*Departments of Chemistry and Molecular Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California, 92037, USA*
malsayah@aus.edu

Abstract. Cyclic *D*, *L*-α-peptides can be designed to spontaneously partition into lipid and cell membranes where they self-assemble into membrane-permeating nanotubes and result in disruption of membrane potentials leading to rapid cell death. The self-assembly of the flat ring-shaped cyclic *D*, *L*-α-peptides in lipid membranes is based on the formation of intermolecular hydrogen-bonds through the perpendicular backbone amide groups. Single channel conductance measurements and proton-transfer assays have demonstrated the ability of such constructs to act as trans-membrane channels while dye-release assays have been employed to determine the pore size created by the self-assembled structures. The dynamics of the self-assembly of pyrene-labeled cyclic peptides into nanotubes in lipid membranes were monitored through the change in the fluorescence of pyrenes and the bio-activity is studied through MIC assays.

Keywords: Cyclic peptides, nanotubes, antibacterial, self-asssembly.
PACS: 87.83.+a

INTRODUCTION

Cyclic peptides made up of an even number of alternating *D*- and *L*-α-amino acid residues adopt flat ring-shaped conformations in which all amide backbone functionalities reside approximately perpendicular to the plane of the ring structure. In this conformation, the spatial disposition and juxtaposition of amide hydrogen-bond donor and acceptor sites on either side of the ring structure allows the peptide subunits to stack under conditions that favor hydrogen bonding to form uniformly-shaped and contiguously hydrogen-bonded β-sheet-like nanotube ensembles (Figure 1). [1-14] All peptide side chains lie on the outside of the tube due to steric considerations and the alternating amino acid configuration. By appropriate choice of amino acid sequence, cyclic *D, L*-α-peptides can be designed to spontaneously partition into lipid membranes and self-assemble into functional pores that enable ion and small molecule transport across the membrane. [2,3,14].

CHRACTERIZAION OF NANOTUBES

Solid-State Peptide Nanotubes

The internal diameter of cyclic *D, L*-α-peptide nanotubes can be controlled by simply varying the size of the peptide ring. The aggregates formed by octapeptide cyclo[-(l-Gln-d-Ala-l-Glu-d-Ala)$_2$-] has a van der Waal internal diameter of 7 Å while that of cyclo[-(l-Gln-d-Ala-l-Glu-d-Ala)$_3$-] is 13 Å. [14, 15] The microcrystalline aggregates formed by

CP929, *Nanotechnology and Its Applications, First Sharjah International Conference*
edited by Y. I. Salamin, N. M. Hamdan, H. Al-Awadhi, N. M. Jisrawi, and N. Tabet

controlled acidification of the former were thoroughly examined by electron microscopy, electron diffraction, FT-IR spectroscopy, and crystal structure modeling. These analyses strongly support the formation of the expected structure in which the ring-shaped subunits stack to form hollow tubes. The unit cell parameters of solid-state nanotubular assemblies, obtained from cryoelectron microscopy and electron diffraction analyses, are in full agreement with the expected tubular structures of cyclic octapeptides. [6] Finally, the high stability of the aggregates formed from the cyclic *D, L*-α-peptide subunits and their low solubility suggests significant cooperative self-assembly process of the monomeric units[14].

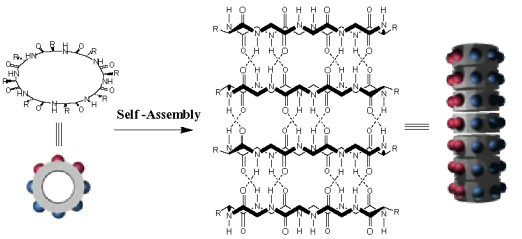

FIGURE 1. Schematic representation of the self-assembly of cyclic *D, L*-□-peptides. (Side chains were removed for clarity)

Self-Assembling Peptide Nanochannels

Cyclic *D, L*-α-peptides bearing appropriate hydrophobic side chains partition into nonpolar lipid bilayers and self-assemble to form nanochannels. Single-channel conductance measurements, glucose transport studies and fluorescence proton-transport assays have demonstrated the ability of such constructs to act as transmembrane channels.[2,3,14] The self-assembling ion channels of cyclic octapeptides display transport activities also for H^+, K^+ and Na^+.

The analysis of spectroscopic results of polarized attenuated total reflectance (ATR) and transmission Fourier transform infrared (FT-IR) of the complexes formed from multiple lipid bilayers and peptides supports this model of the peptide nanotubes as the active channel species.[9] The pore size created by the self-assembled structures has also been studied using dye-release assays.[13] All these studies have indicated that the mode of membrane permeation depends upon the amino acid sequence comprising the cyclic *D, L*-α-peptide units.

Fluorescence Studies of the Self-Assembly of Pyrene-Peptides

Pyrene units are utilized as the spectroscopic handle to monitor the self-assembly of cyclic *D, L*-α-peptide rings. Monomer pyrenes exhibit characteristic fluorescence emission (λ_{em} = 397 nm) upon excitation with UV light (λ_{ex} = 340 nm). However, cofacial interaction between one pyrene ring in its excited state with a second ring in its ground state at a 3.5 Å distance leads to quenching of the monomer fluorescence and the formation of excimers (λ_{em} = 470 nm). [16] Since the inter-unit separation distance of cyclic *D,L*-α-peptides assembled via β-sheet interactions is 4.8 Å, the assembly of pyrene-modified cyclic peptides places the pyrene rings in close proximity and enhances the formation of the excimer (Figure 2).

Solutions of pyrene-labeled cyclic peptides exhibit fluorescence spectra characteristic of monomer pyrene. Upon the introduction of a lipid bilayer, initial enhancement in the pyrene monomer fluorescence is observed due to the partition of the peptides into the hydrophobic membrane. As time progresses, a decrease in the monomer emission at 397 nm concomitant with an increase of the excimer emissions at 470 nm is noticed (Figure 3). This increase in the excimer emission provides evidence that the peptides self-assemble slowly upon partitioning into the membrane. The rate and the extent of the self-assembly of these peptides increase as the peptide-to-lipid ratio increases. This suggests that the

self-assembly of the pyrene-labeled cyclic peptides is a corporative process in which the partition and the assembly are concurrent processes at high peptide-to-lipid ratios.

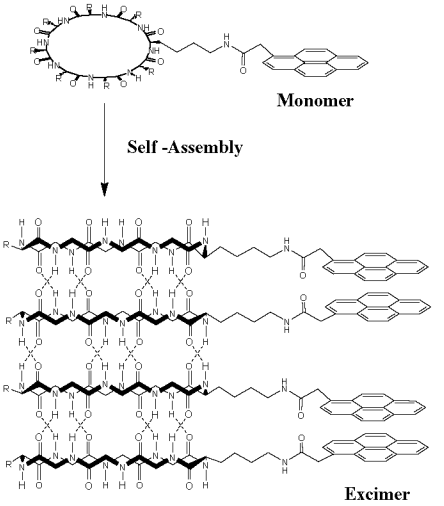

FIGURE 2. The self-assembly of pyrene-labeled cyclic *D, L*-α-peptides.

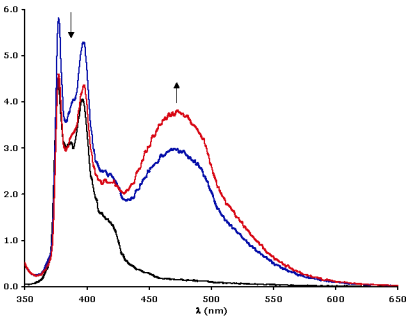

FIGURE 3. Fluorescence spectra of c[QXHRWLWK] (5μM) before (black) and after the addition of lipid bilayer (20 molar equivalence of 9:1 DMPC/POPS) by 1 min (blue) and 20 mins (red).

ANTIBACTERIAL ACTIVITY OF CYCLIC *D, L*-α-PEPTIDES

In the presence of various microbial, bacterial, and other cellular systems, self-assembling cyclic *D, L*-α-peptides have shown the ability to disrupt membrane potentials, leading rapidly to cell death.[13] The activity and selectivity of this behavior is strongly dependent upon the amino acid sequence of the cyclic *D, L*-α-peptide units (Table 1). Minimum inhibitory concentration assays have determined the relative activity of respective derivatives, and

fluorescence depolarization studies have indicated that such activity coincides with disruption of the transmembrane potential gradient.

TABLE 1. Minimum Inhibitory concentration (μg/mL) vs the indicated bacteria strain.

Peptide*	MRSA	E. coli
c[KQRWLWLW]	6	80
c[KSKWLWLW]	5	40
c[KKKWLWLW]	7	80
c[K**X**KRWLWR]	25	> 40
c[Q**X**HRWLWK]	12.5	> 40

*Single letter codes for amino acids and shorthand representation of cyclic peptide sequences are used. The brackets indicate cyclic structure and underlining represents *D* amino acid residues; **X** = pyrene-CH_2CO—Lys.

ACKNOWLEDGMENTS

The authors thank the Skaggs Institute for Chemical Biology and the National Institutes of Health (USA) for financial support.

REFERENCES

1. Ghadiri, M. R., Granja, J. R., Milligan, R. A., Mcree, D. E., and Khazanovich, N., *Nature* **366** (1993) 324-327.
2. Ghadiri, M. R.; Granja, J. R., and Buehler, L. K., *Nature* **369** (1994) 301-304.
3. Granja, J. R. and Ghadiri, M. R., *J. Am. Chem. Soc.* **116** (1994) 10785-10786.
4. Ghadiri, M. R.; Kobayashi, K., Granja, J. R., Chadha, R. K., and Mcree, D. E., *Angew. Chem., Int. Ed. Engl* **34** (1995) 93-95.
5. Kobayashi, K., Granja, J. R., and Ghadiri, M. R., *Angew. Chem., Int. Ed. Engl.* **34** (1995) 95-98.
6. Hartgerink, J. D., Granja, J. R., Milligan, R. A., and Ghadiri, M. R., *J. Am. Chem. Soc.* **118** (1996) 43-50.
7. Motesharei, K. and Ghadiri, M. R., *J. Am. Chem. Soc.* **119** (1997) 11306-11312.
8. Clark, T. D., Buriak, J. M., Kobayashi, K., Isler, M. P., Mcree, D. E., Ghadiri, and M. R., *J. Am. Chem. Soc.* 102 (1998) 8949-8962.
9. Kim, H. S., Hartgerink, J. D., andGhadiri, M. R., *J. Am. Chem. Soc.* **120** (1998) 4417-4424.
10. Hartgerink, J. D.; Clark, T. D.; Ghadiri, M. R., *Chem. Eur. J.* **4** (1998) 1367-1372.
11. Rapaport, H., Kim, H. S., Kjaer, K., Howes, P. B., Cohen, S., Als-Nielsen, J., Ghadiri, M. R., Leiserowitz, L., and Lahav, M., *J. Am. Chem. Soc.* **121** (1999) 1186-1191.
12. Bong, D. T., Clark, T. D., Granja, J. R., and Ghadiri, M. R., *Angew. Chem., Int. Ed. Engl.* **40** (2001) 988-1011.
13. Fernandez-Lopez, S., Kim, H. S., Choi, E. C., Delgado, M., Granja, J. R., Khasanov, A., Kraehenbuehl, K., Long, G., Weinberger, D. A., Wilcoxen, K., and Ghadiri, M. R., *Nature* **412** (2001) 452-455.
14. Sanchez-Quesada, J., Kim, H. S., and Ghadiri, M. R., *Angew. Chem., Int. Ed. Engl.* **40** (2001) 2503-2506.
15. Khazanovich, N., Granja, J. R., McRee, D. E., Milligan, R. A., and Ghadiri, M. R., *J. Am. Chem. Soc.* **116** (1994) 6011 - 6012.
16. Forster, T. *Angew. Chem., Int. Ed. Engl.* **8** (1969) 333-343.