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Monovalent cation-promoted ordering of a glycine-rich cyclic peptide

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Abstract—This report describes an accelerated self-assembly of a synthetic cyclic hexapeptide in the presence of alkali metal ions. Time-dependent aggregation of hexapeptide was considerably influenced upon co-incubation with monovalent metal ions, of which K^+ afforded the most significant effect both on the time-scale required for self-assembly and on the morphology of aged structures. Metal ion adducts formation ability of the hexapeptide was confirmed by electrospray ionization mass spectrometry measurements and ^{13}C NMR spectrometry. The effect of metal ion binding on peptide structure was also probed by circular dichroism, optical microscopy, and scanning electron microscopy. K^+ ions not only interacted more efficiently with the hexapeptide enabling it to reach conformational state(s) conducive for self-assembly, but also altered the morphologies of the aged peptide fibers, when compared to the unmetalated peptide.

1. Introduction

Cyclic peptides have attracted considerable focus for the construction of nanotubular supramolecular architectures owing to seminal contributions by Ghadiri and co-workers.[1] Generally speaking, cyclic peptides exhibit a propensity to stack in a β-sheet-like assembly stabilized by backbone hydrogen bonding interactions. In such cases, the peptide rings assume a planar conformation where the backbone is directed almost perpendicular to the plane of the ring structure. Supramolecular assembly of cyclic peptides leads to nanotubular structures in which side chain functionality exerts considerable influence on the self-assembly process and properties of nanotubes.[2]

Naturally occurring ionophoric proteins and peptides,[3] which bind and facilitate transport of ionic species, have further propelled the design and synthesis of functional cyclic peptide architectures. Representative examples of natural cyclic ionophores include valinomycin, bacitracin, gramicidin, and beauvericin.[4] As a typical application, peptide nanotubes derived from cyclic structures display significant ion-transport characteristics through in situ generated hollow channels.[1e]

It is known that cyclic peptides undergo a conformational change upon cation/anion binding where the extent of ion binding can be determined by various biophysical techniques including CD titration experiments, X-ray structure, and NMR spectroscopy.[5–7] Recently, cold electrospray ionization technique has been employed to investigate the formation of cyclic clusters of amino acids and alkali metal ions interaction.[8]

We have already reported on the synthesis and aggregative behavior of a cyclic hexapeptide *cyclo*-(Gly-L-Pro-Gly)$_2$ (**1**) and its linear counterpart.[9] These peptides, despite possessing similar amino acid composition, exhibit remarkably different prefibrillar intermediates leading to aggregate formation: one involving spherulitic structures, while the other invoked 'Maltese-cross' pattern like prefibrillar structures.[9] During the course of our studies with **1**, we became aware of literature reports, which described the interaction of metal ions with *cyclo*-(L-Pro-Gly)$_3$ and other cyclic peptides.[10,11] These reports, coupled with our ongoing interest in determining the aggregative propensity of **1** prompted us to investigate the effect of alkali metal ions on aggregative ordering of **1** as well as change, if any, in fiber morphology in the presence of monovalent cations.

Herein, we report on the ability of **1** to form adducts with alkali metal ions and their observable acceleration of self-assembly perhaps due to a faster approach of aggregating conformational ensembles. Interestingly, K^+ also had curious effects on the morphology of the peptide nanotubes signifying possibilities in morphing peptide nanostructures by cationic species.

Keywords: Peptide; Self-assembly; Monovalent cation; ESI mass spectrometry; Circular dichroism.
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2. Results and discussion

Cyclic peptide **1** was synthesized as previously reported via solution-phase methodology and used for aggregation studies in the presence of monovalent cations. Time-dependent ordering of **1**, which originates from a random-coil-like flexible conformation in the fresh solution to a β-sheet-like pattern formation upon aging for 30 days, is already reported by us.[9] It was suggested that this cyclic peptide approaches intermediary conformations favoring β-sheets, aided by hydrogen bonding interactions, and stackable cyclic rings afford rapid growth of tubular structures, during the aging process. As cyclic peptides show a propensity to bind metal ions, we became interested in determining the possibility of cation-mediated peptide aggregation using **1** as a model cyclic peptide.

CD spectra for **1**, both fresh and an aged solution, in the presence of monovalent cations such as Li^+, Na^+, K^+, Rb^+, and Cs^+ were recorded to investigate possible interaction and their effect on aggregation (Fig. 1). Cyclic peptide **1** was co-incubated with metal ions and the CD spectra were recorded at various time intervals (data not shown) and the results are qualitatively discussed with the help of three time-points: 0, 15, and 30 days. A plot of CD signal (ellipticity) versus time (in days), at two wavelengths suggestive of β-sheet formation, indicated a gradual change toward sheet-like structure during the initial phase of aggregation (till 15 days) (Fig. 2). However, there was a sudden increase in the ellipticities from 15 to 30 days of aggregation revealing a relatively fast approach toward sheet-like structure during the later part of the aggregation process.

It was interesting to observe that the presence of monovalent cations accelerated aggregation of peptide **1** with an appearance of a β-sheet-like CD pattern exhibiting negative ellipticity at ~222 nm within 15 days in contrast to 30 days taken by **1** in the absence of any added metal ion. This observation suggested a curious role of monovalent cations in facilitating the approach of a structured conformation, which was primed to aggregate in a shorter time period compared to peptide alone.

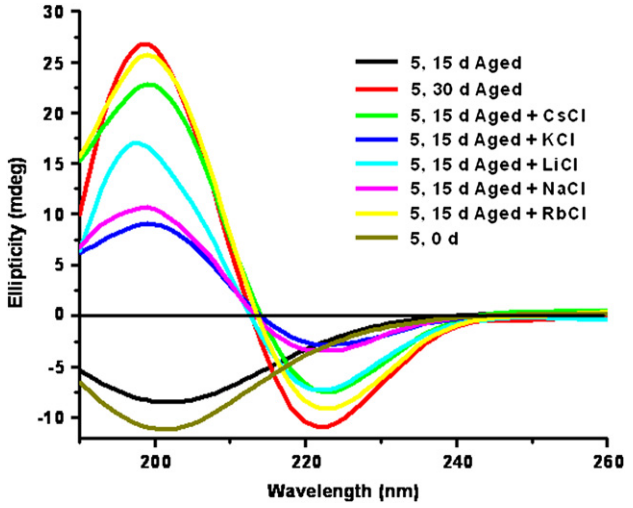


Figure 1. CD spectrum of 15 and 30 days aged sample of **1** (0.3 mmol) and 15 days aged sample with 2 equiv monovalent cations.

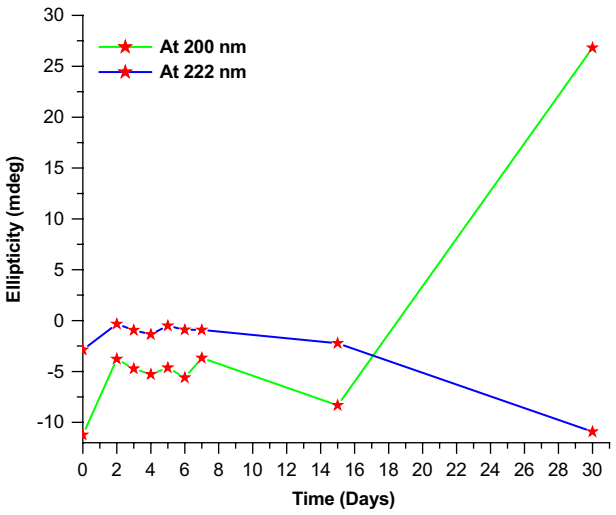


Figure 2. Time course CD spectrum of sample of **1** (0.3 mmol) at 200 and 222 nm.

This prompted us to further probe the cation–peptide interaction with the help of NMR spectroscopy and mass spectrometry in order to confirm the relative affinities of these cations for **1**. Electrospray ionization (ESI) is a soft ionizing-desorption technique, which is effectively used for facile transfer and detection of intact, metalated neutral peptide species.[12] This technique is frequently employed to provide accurate mass assignments for metal ion adducts and complexes by deciphering stoichiometric speciation in the gas phase. An aqueous solution of **1** (1 mmol) containing chloride salts of Li^+, Na^+, K^+, Rb^+, and Cs^+ (2 mmol) was incubated for 15 days at 37 °C. ESI mass spectra were recorded for these incubated samples to evaluate the metal ion binding efficiency of cyclic peptide **1** using water as the mobile phase.

ESI mass spectra were recorded for free peptide **1** and its Na^+ and K^+ adducts. It is important to notice that ESI-MS of free peptide show minimal adventitious metal ion adduct formation usually associated with this methodology and a 100% abundant molecular ion peak corresponding to $[M^+{+}1]$ (m/z=423) was identified in the spectra (see Supplementary data). Scrutiny of MS data indicated stable adduct formation between **1** and Na^+ (m/z=445) and K^+ ions (m/z=461), where 1:1 peptide to metal stoichiometry afforded 100% abundant molecular ion peaks. In addition, peaks at m/z values of 446 and 447, corresponding to $[M+Na+1]^+$ and $[M+Na+2]^+$, respectively, were observed (see Supplementary data). Similarly, peaks at m/z values of 462 and 463, corresponding to $[M+K+1]^+$ and $[M+K+2]^+$, respectively, were also detected (see Supplementary data). The mass spectrometry data suggest a 1:1 adduct formation between **1** and Na^+/K^+ ions and it is worth mentioning that we did not observe 1:2 or higher stoichiometries in the ESI-MS data.

Yamaguchi and co-workers have recently reported the use of cold-spray ionization mass spectrometry, a variant of electrospray ionization, to ascertain the formation of cyclic clusters from L-proline in the presence of alkali metal ions.[8c] A combined application of both ESI and CSI-MS for the characterization of self-assembled Pt cages has also been documented.[13] Similarly, Cooks and co-workers have studied

solution-phase periodic aggregates of L-serine with alkali metal ions by ESI-MS technique.[14]

In contrast to Na^+ and K^+ ions, Li^+ interacted weakly with **1**, while Rb^+/Cs^+ and Cl^- did not interact with **1**. This suggested that preferential interaction of Na^+ and K^+ ions is dictated by cationic size. Such selectivity is commonly encountered in natural transmembrane ion channels where the passage of certain ions is permissible, while inhibiting transport of other ions. The deciding factors may include interatomic interactions, electrostatic considerations, and the overall pore size of the ion channel.[15] These observations also suggest that despite an observable change in ellipticity at ~225 nm, Rb^+ and Cs^+ ions did not produce stable adducts in ESI-MS analysis. In light of these observations, we decided to pursue detailed studies with Na^+ and K^+ ions to further understand their role in the ordered aggregation of cyclic peptide **1**.

It is important to note that amino acid constituents of **1** are devoid of any cation interacting side chains, thus interaction of **1** with Na^+/K^+ occurs via backbone amide carbonyl oxygens. It has been proposed that the identification of carbonyl carbons, involved in complexation of diamagnetic cations, can be achieved by determining the difference in the downfield shifts of these carbons (from 0.4 to 3.4 ppm) in ^{13}C NMR spectra.[5e,10b] Cyclic peptide **1** was incubated with Na^+/K^+ ions in D_2O and ^{13}C NMR spectra were recorded after 15 days incubation. The glycine residue attached to the imino group of proline is labeled as glycine-1, while the one attached to the proline carboxyl group is labeled as glycine-2 according to a reported nomenclature.[16]

An appreciable downfield shift in the ^{13}C chemical shifts of both the glycine-1 and -2 and proline carbonyl carbons was observed for the two cations (Table 1). This downfield shift may be attributed to the change in diamagnetic anisotropy following complexation of the carbonyl carbon to the monovalent ions, as the π electron density of oxygen gets attracted toward monovalent ion thus making the carbonyl carbons more deshielded.[17]

Table 1. ^{13}C chemical shifts of **1** and its 1:1 equiv Na^+/K^+ complexes (solvent D_2O at 25 °C, *c* 0.12 M)

Resonance	Fresh **1**, ppm	**1** (30 days aged), ppm	**1**/NaCl (15 days aged), ppm	**1**/KCl (15 days aged), ppm
Pro C=O	174.02	176.43	177.58	177.51
Gly_1 C=O	172.22	171.97	173.16	173.09
Gly_2 C=O	166.18	168.45	169.79	169.67

Compared to the fresh sample, the proline carbonyls' shift was observed 3.56 ppm downfield in the presence of Na^+ ions, while glycine-1 and glycine-2 carbonyls were shifted 0.94 and 3.61 ppm downfield, respectively. The corresponding ^1H chemical shifts were also observed for **1** in the presence of K^+ ions (Table 1). With the 30 days aged sample, the corresponding δ values were downfield shifted ~1.15 ppm for the proline carbonyls, while shifted 1.19 and 1.34 ppm for glycine-1 and -2 carbonyls, for Na^+ and K^+ ions, respectively. These downfield shifts provide additional proof for metal ion complexation with the carbonyl groups.

Having established a strong interaction between **1** and Na^+/K^+ ions, we became interested in determining the effect of these cations on the aggregation of the cyclic peptide and the possibility of altering ultrastructural morphologies due to metal ion interaction(s). Time-dependent aggregative propensities of **1** and the metalated peptide were evaluated by staining aged peptide samples with the Congo red dye, followed by optical microscopy. Interestingly, dissimilar morphologies were observed in the aged solutions of **1** when co-incubated with Na^+ and K^+ ions. Co-incubation with Na^+ ions afforded short, ill-defined fibrils (Fig. 3a), while 15 days aged solution of K^+-metalated peptide exhibited longer and thicker fibrillar patterns (Fig. 3b). This suggests that K^+ ions not only bind to cyclic peptide **1** and cause a shift in the CD pattern, but is also able to accelerate its aggregation to persistent length fibers, when compared to Na^+ ions. Fresh solutions of metalated peptide did not reveal any definite structure (data not shown). A remarkable difference in the solution-phase assembly of **1** and the metalated peptides, suggests for different structural ensembles in the presence of two metal ions leading to altered self-assembled structures.

Encouraged by the optical microscopic evidence for the K^+ ion interaction with **1**, we decided to further analyze the ultrastructural morphology of 15 days aged potassium-metalated peptide fibers with scanning electron microscopy (SEM). Interestingly, we observed fibrous bundles, stacked one over the other, having barbed-wire-like multidirectional growth from the main fiber length (Fig. 4a). Upon further

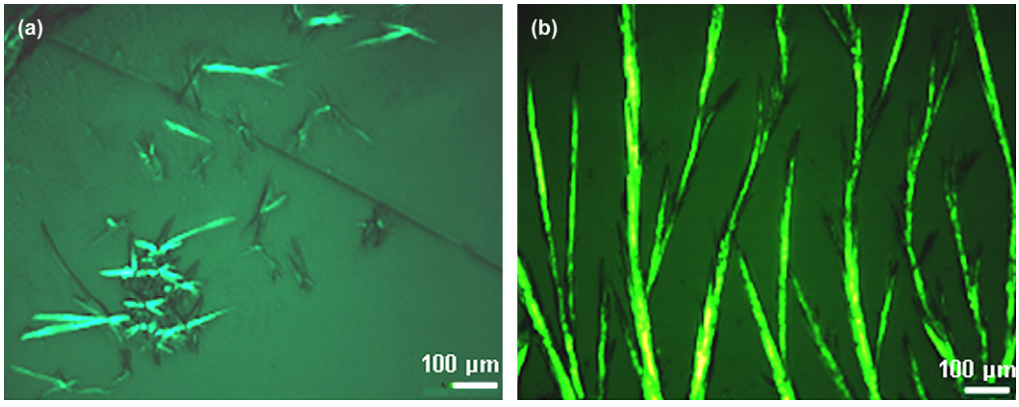


Figure 3. Optical micrographs of metalated peptide **1** self-assembly: (a) 15 days aged solution with NaCl; (b) 15 days aged solution with KCl.

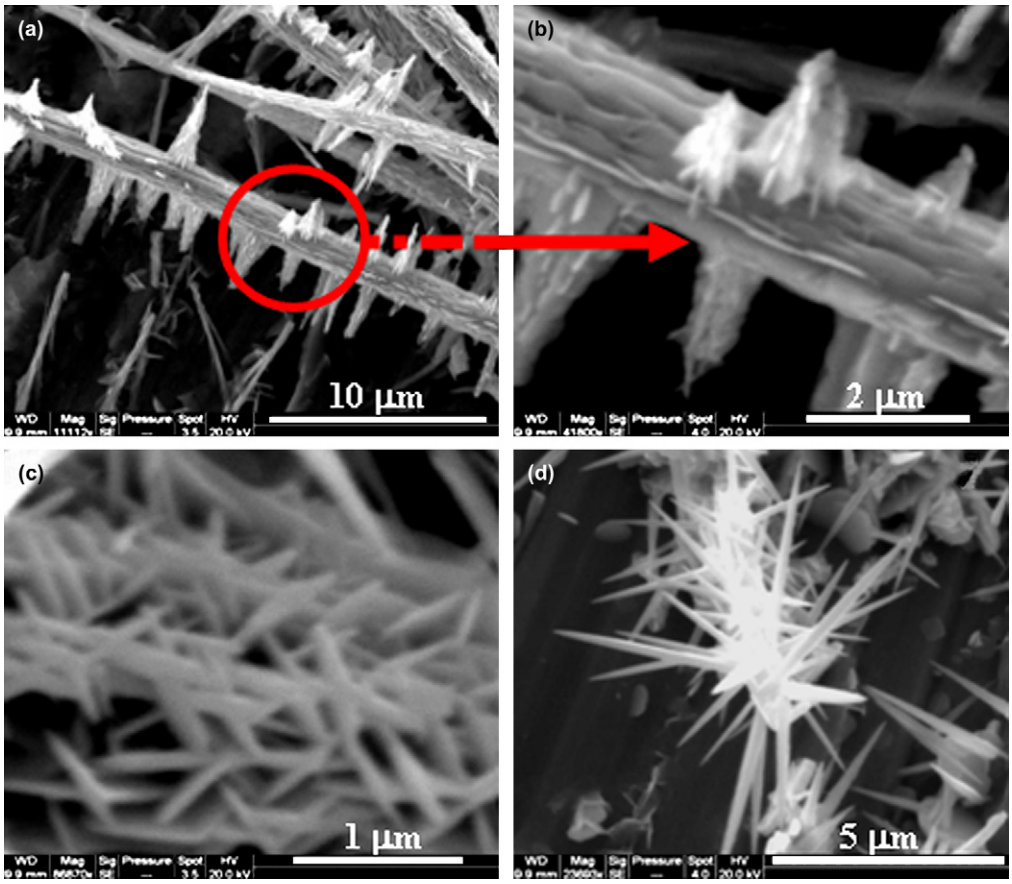
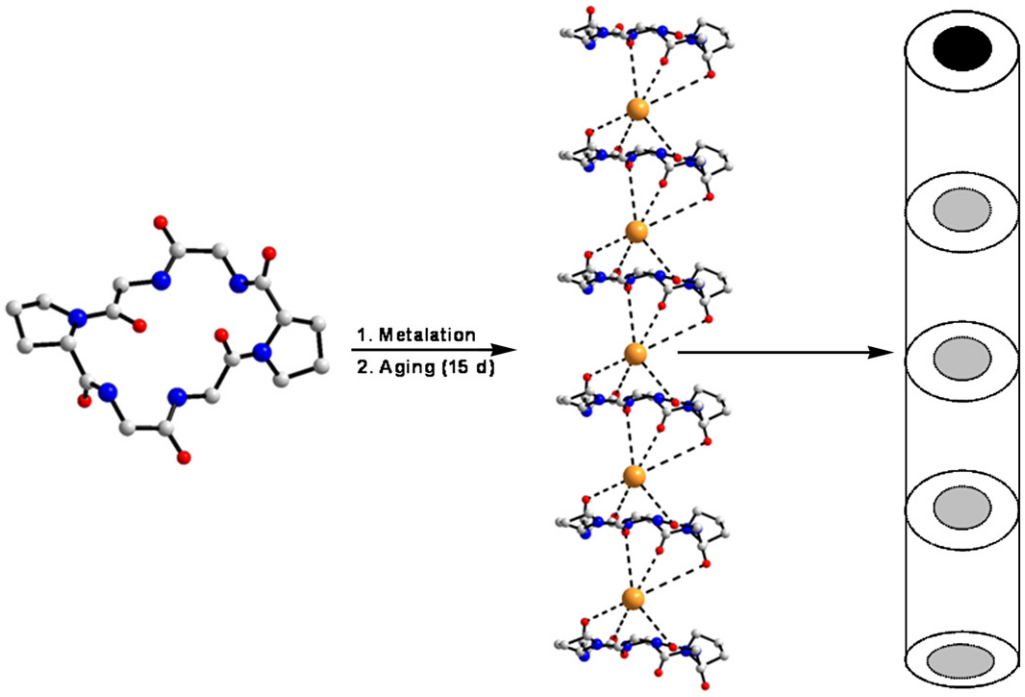


Figure 4. SEM images of 15 days aged solution **1** with K^+ ions.

magnification, these barbed-wire-like fiber revealed a conical shape with an average length of 0.5 μm growing from the main fiber axis (Fig. 4b and c). SEM morphologies look very different to the fibers obtained for the unmetalated cyclic peptide[9] **1** thus suggesting an inextricable role of K^+ ions in peptide adduct formation and on fiber morphology.



Scheme 1. A proposed model for metal-aided ordering of cyclic peptide **1** (counteranions and hydrogens are omitted for the sake of clarity). Color code: blue, nitrogen; red, oxygen; gray, carbon; orange dummy atom as potassium.

We further decided to probe the role of potassium ions by crystal structure studies. A suitable crystal of **1** was grown from 50% aqueous methanol by slow evaporation, having monoclinic symmetry in the space group $P2_1$, and it matched in all respects to the reported structure of (Gly-L-Pro-Gly)$_2\cdot 4H_2O$.[6b] Therefore, we chose to use the solid-state structure to propose a model of K^+ ion binding to **1** and its possible role in fiber growth (Scheme 1, counteranions and hydrogens are omitted for the sake of clarity).[18] It could be proposed that the binding of **1** with monovalent cations affords a rapid approach toward an organized structure, which is suitably predisposed toward stacking interactions thus affording tubular structures upon aging. Preferential adduct formation between **1** and K^+ ions is perhaps dictated by the size constraints.

3. Conclusions

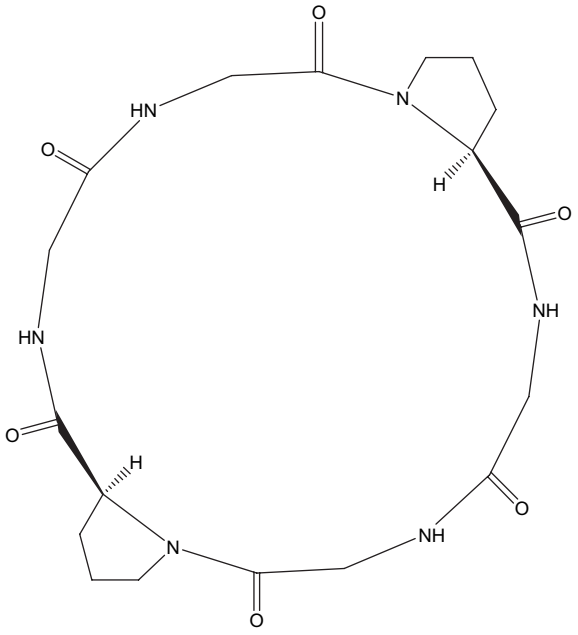
The sizes of solvated alkali metal ions possibly dictate preferential adduct formation with peptide **1** and a concomitant effect on morphology. Although all alkali metal ions displayed changes in the CD spectral pattern, this interaction was not validated by ESI mass spectral analysis. This suggests that stable peptide–metal ion adduct formation, relevant to changes in CD spectral pattern and morphology was limited to Na^+ and K^+ ions in this particular case. In addition, we would also like to eventually study the possible role of anions in aiding the formation of ordered peptide structures.

Thus, this study illustrates the particular role of alkali metal ions in aggregative ordering of cyclic hexapeptide **1**. Spectroscopic evidence provided support for cation–peptide interaction, while microscopic data reveal a pronounced effect of K^+ ions on peptide aging and concomitant fiber formation. In the presence of potassium ions, this glycine-rich cyclic peptide quickly reaches toward conformation(s) suitable for aggregation, aided by hydrogen bonding interactions, and stackable cyclic rings, to afford accelerated growth of tubular structures, when compared to the unmetalated peptide. This study may offer new opportunities in understanding accelerated protein aggregation in the presence of biologically relevant cations.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were recorded on a JEOL-JNM LAMBDA 400 model operating at 400 and 100 MHz, respectively. The sample concentration was 25 mg/0.5 mL (0.12 M) D_2O with 2 equiv monovalent metal salt. The ESI Mass spectra were recorded at Sophisticated Analytical Instrumentation Facility, Lucknow, on a MICROMASS QUATTRO II mass spectrometer. The samples (dissolved in suitable solvents such as methanol/acetonitrile/water) were introduced into the ESI source through a syringe pump at the rate of 5 μL/min. The ESI capillary was set at 3.5 kV and the cone voltage was 40 V. The spectra were collected in 6 scans. The synthesis and characterization of *cyclo*-(Gly-L-Pro-Gly)$_2$ following a modified literature procedure have been reported by us (Scheme 2).[9] Synthetic methodology involved standard solution-phase coupling methods via Boc-chemistry, active ester approach, and fragment condensation. Fully deprotected cyclic hexapeptide **1** and its metal ion adducts were characterized and used for structural investigations and aging experiments. Peptide homogeneity was established by analytical HPLC, followed by ESI and FAB mass spectroscopies as well as by CHN analysis.



Scheme 2. Molecular structure of cyclic peptide **1**.

4.2. Optical microscopy

Congo red (3 μM dye in 100 mM NaCl) was added to aged solution of peptide conjugates and the mixture was left for 6 h at room temperature. This solution of 50 μL was transferred on to glass slides and dried to make a thin film, then viewed under optical microscope (Zeiss) with cross-polarized light (magnification 50×). Images were obtained by using Image-Pro Plus software.

4.3. Scanning electron microscopy (SEM)

SEM analysis was performed on FEI QUANTA 200 microscope with a tungsten filament gun. Image was recorded for unmetalated and metalated peptides at WD 10.6 mm, magnification 9000×–40,000×, HV 20 kV, Spot 5.0, Sig SE. Unmetalated and metalated peptides were dissolved in distilled and double deionized water and aged for 0–30 days. A few drops of fresh and incubated solutions were taken and placed on copper stubs inside the microscope chamber of an FEI QUANTA 200 microscope. The samples were left to equilibrate at 25 °C. The chamber was sealed and evacuated to ~1.00 Torr.

4.4. CD spectroscopy

CD spectra were recorded at 25 °C under a constant flow of nitrogen on a JASCO-810 spectropolarimeter, which was calibrated with an aqueous solution of (+)-ammonium

D-camphor sulfate. Experimental measurements were carried out in water and with different alkali metal ion solutions by using a 1 mm path length cuvette. The CD spectra were recorded in the UV region (190–300 nm). The spectrum represents an average of 5–8 scans and the CD intensities are expressed in mdeg.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.04.017.

References and notes

1. (a) Horne, W. S.; Ashkenasy, N.; Ghadiri, M. R. *Chem.—Eur. J.* **2005**, *11*, 1137–1144; (b) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988–1011; (c) Hartgerink, J. D.; Clark, T. D.; Ghadiri, M. R. *Chem.—Eur. J.* **1998**, *4*, 1367–1372; (d) Hartgerink, J. D.; Granja, J. R.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996**, *118*, 43–50; (e) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Nature* **1994**, *369*, 301–304; (f) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324–327.
2. (a) Yoshida, K.; Kawamura, S.-I.; Morita, T.; Kimura, S. *J. Am. Chem. Soc.* **2006**, *128*, 8034–8041; (b) Amorin, M.; Brea, R. J.; Castedo, L.; Granja, J. R. *Heterocycles* **2006**, *67*, 575–583; (c) Brea, R. J.; Amorin, M.; Castedo, L.; Granja, J. R. *Angew. Chem., Int. Ed.* **2005**, *44*, 5710–5713; (d) Perlman, Z. E.; Bock, J. E.; Peterson, J. R.; Lokey, R. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5329–5334; (e) Amorin, M.; Castedo, L.; Granja, J. R. *J. Am. Chem. Soc.* **2003**, *125*, 2844–2845; (f) Ozeki, E.; Miyazu, T.; Kimura, S.; Imanishi, Y. *Int. J. Pept. Protein Res.* **1989**, *34*, 97–103; (g) Ishizu, T.; Hirayama, J.; Noguchi, S. *Chem. Pharm. Bull.* **1994**, *42*, 1146–1148.
3. Duax, W. L.; Griffin, J. F.; Langs, D. A.; Smith, G. D.; Grochulski, P.; Pletnev, V.; Ivanov, V. *Biopolymers* **1996**, *40*, 141–155.
4. (a) Easwaran, K. R. K. *Met. Ions Biol. Syst.* **1985**, *19*, 109–137; (b) Ming, L. J.; Epperson, J. D. *J. Inorg. Biochem.* **2002**, *91*, 46–58; (c) Wallace, B. A. *BioEssays* **2000**, *22*, 227–234; (d) Logrieco, A.; Moretti, A.; Ritieni, A.; Caiaffa, M. F.; Macchia, L. *Advances in Microbial Toxin Research and Biotechnological Exploitation*; Upadhyay, R. K., Ed.; Kluwer Academic/Plenum: New York, NY, 2002; pp 23–30.
5. (a) Kuenzel, S.; Strehlow, D.; Poppitz, W.; Willbold, S.; Seyfarth, L.; Keutel, H.; Reissmann, S. *Lett. Pept. Sci.* **2003**, *9*, 261–272; (b) Jois, S. D.; Tibbetts, S. A.; Chan, M. A.; Benedict, S. H.; Siahaan, T. J. *J. Pept. Res.* **1999**, *53*, 18–29; (c) Garcia-Echeverria, C.; Albericio, F.; Giralt, E.; Pons, M. *J. Am. Chem. Soc.* **1993**, *115*, 11663–11670; (d) Shimizu, T.; Fujishige, S. *Biopolymers* **1980**, *19*, 2247–2265; (e) Niu, C.-H.; Medison, V.; Pease, L. G.; Blout, E. R. *Biopolymers* **1978**, *17*, 2747–2751.
6. (a) Karle, I. L.; Flippen-Anderson, J.; Agarwalla, S.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5307–5311; (b) Kostansek, E. C.; Thiessen, W. E.; Schomburg, D.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1979**, *101*, 5811–5815; (c) Dabler, M. *Biochem. Soc. Trans.* **1973**, *1*, 828–832; (d) Sugihara, T.; Imanishi, Y.; Higashimura, T. *Biopolymers* **1973**, *12*, 2823–2830.
7. (a) Kubik, S.; Goddard, R. *Eur. J. Org. Chem.* **2001**, *2*, 311–322; (b) Kubik, S. *J. Am. Chem. Soc.* **1999**, *121*, 5846–5855.
8. (a) Emmert, J.; Pfluger, M.; Wahl, F. *Spectroscopy* **2006**, *21*, 64–67; (b) Huang, H.; Chaudhary, S.; Van Horn, J. D. *Inorg. Chem.* **2005**, *44*, 813–815; (c) Kunimura, M.; Sakamoto, S.; Yamaguchi, K. *Org. Lett.* **2002**, *4*, 347–350.
9. Joshi, K. B.; Verma, S. *Supramol. Chem.* **2006**, *18*, 405–414.
10. (a) Xie, P.; Diem, M. *J. Am. Chem. Soc.* **1995**, *117*, 429–437; (b) Madison, V.; Atreyi, M.; Deber, C. M.; Blout, E. R. *J. Am. Chem. Soc.* **1974**, *96*, 6725–6734; (c) Deber, C. M.; Torchia, D. A.; Wong, S. C. K.; Blout, E. R. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1825–1829.
11. Kubik, S. *Cyclopeptides as Macrocyclic Host Molecules for Charged Guests, Highlights in Bioorganic Chemistry*; Schmuck, C., Wennemers, H., Eds.; Wiley-VCH GmbH: Weinheim, Germany, 2004; pp 124–137.
12. Shudha, R.; Panda, M.; Chandrasekhar, J.; Balaram, P. *Chem.—Eur. J.* **2002**, *8*, 4980–4991.
13. Sakamoto, S.; Yoshijawa, M.; Kusukawa, T.; Fujita, M.; Yamaguchi, K. *Org. Lett.* **2001**, *3*, 1601–1604.
14. Cooks, R. G.; Zhang, D.; Koch, K. J.; Gozzo, F. C.; Eberlin, M. N. *Anal. Chem.* **2001**, *73*, 3646–3655.
15. Corry, B.; Chung, S.-H. *Cell. Mol. Life Sci.* **2006**, *63*, 301–315.
16. Pease, L. G.; Deber, C. M.; Blout, E. R. *J. Am. Chem. Soc.* **1973**, *95*, 258–260.
17. Grell, E.; Funk, T. *Eur. J. Biochem.* **1973**, *34*, 415–424.
18. (a) Clark, T. D.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651–656; (b) Kim, H. S.; Hartgerink, J. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 4417–4424; (c) Simizu, T.; Tanaka, Y.; Tsuda, K. *J. Inclusion Phenom.* **1987**, *5*, 103–108.