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Metalated peptide fibers derived from a natural metal-binding peptide motif

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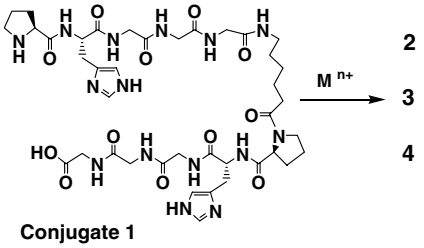
Abstract—Biological molecules serve as convenient scaffolds for the construction of nanoscopic architectures which can effectively interact with small molecules and metal complexes to extend their scope for nano(bio)technological applications. Metalloproteins possess natural metal ion binding motifs and the possibility of using these sequences to generate metalated peptide conjugates with defined metal ion coordination offers a facile entry into metalated supramolecular aggregates. This report describes the formation of metalated fibers from Cu-binding octarepeat motifs of the prion protein. Conjugate **1** effectively binds copper, silver, and manganese, leading to persistent length and thermally stable peptide fibers, which could be applied for molecular bioelectronic applications.

Proteins and peptides possess tremendous potential as useful building blocks for self-assembly owing to their defined secondary and tertiary structures and propensity to interact with the help of various non-covalent interactions. These naturally occurring motifs allow rapid access to an array of three-dimensional structures and various side chain functionalities present in constituent amino acids allow further fine-tuning of properties governing the self-assembly process.[1–6]

Self-assembling proteins and synthetic peptides are convenient systems for nanotechnology and nano(bio)-technology applications due to the availability of reproducible scale-up synthetic methodologies and the ease of functionalisation of fibrous aggregates with groups that can favorably interact with small molecules, metal ions, and inorganics.[7–9] In this context, it is interesting to explore naturally occurring metal binding peptide segments for self-assembly to obtain peptide fibers possessing high affinity for metal ion interactions.

Mammalian and yeast prions are self-propagating proteins rich in β-sheet structure which are held responsible for the etiology of transmissible spongiform encephalopathies such as scrapie in sheeps, bovine spongiform encephalopathy in cattle, and Creutzfeldt-Jakob disease in humans.[10–12] Cellular prion protein (PrP^C) is a cuproglycoprotein whose conformational transition to its infectious, aberrant isoform results in catastrophic cognitive dysfunction in a hallmark of neurological spongiform encephalopathies.[13] Interestingly, PrP^C contains an evolutionary conserved stretch of octapeptide repeats (PHGGGWGQ) that selectively bind Cu^{2+} ions with high affinity. Other metal ions also bind to this octarepeat albeit with lower affinity compared to copper.

We have previously demonstrated aggregation in truncated prion octarepeats.[14,15] The purpose of this study was to gain facile access to peptide fiber formation from prion octapeptide fragments and to extend this strategy to construct metalated fibers from naturally occurring metal binding motifs. Herein, we report the synthesis of a novel bis-peptide conjugate **1**, its interaction with



Scheme 1. Molecular structure of conjugates **1**–**4**.

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copper, silver, and manganese ions, and morphology and thermal stability of metalated peptide fibers formed upon aging (Scheme 1).

The synthesis of conjugate **1** was achieved by solution-phase methodology using a fragment coupling approach and the deprotected peptide was fully characterized.[14,16] Synthetic details will be reported elsewhere. Conjugate **1** was metalated by incubation with various metal ions and the respective metal adducts (**2**–**4**) were characterized by mass spectroscopy. Peaks corresponding to the presence of two metal ions were observed for conjugate **2** [m/z 1003 ($L+Cu^{2+}-2H^{+}$) and 1065 ($L+2Cu^{2+}-4H^{+}$)], **3** [1286 ($L+2Ag^{+}+2NO_3^{-}+4H^{+}$)] and **4** [1047 ($L+2Mn^{2+}-4H^{+}$)], respectively.

Conjugate **2** displayed an absorbance around 625 nm which may be attributed to the Cu^{2+} d–d transition (Fig. 1, inset). This was verified in the CD spectra of conjugate **2** (Fig. 2, inset). A similar absorption maximum ~620 nm has been reported for copper nanocrystal-coated peptide nanotubes.[17] Conjugate **3** revealed two absorbances: a shoulder around 260 nm and a broad peak around 450 nm. The former band could arise from a π–π intraligand transition, while the latter is reflective of a metal–ligand charge transfer phenomenon (MLCT).[18] Incidentally, the UV spectrum of conjugate **4** was devoid of useful features.

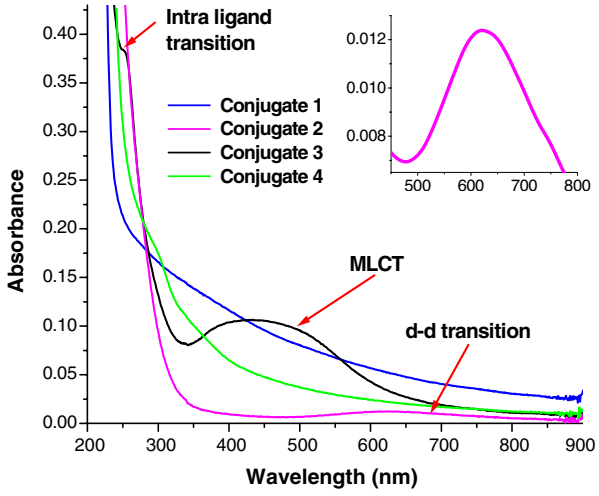

Figure 1. UV–vis spectra of conjugates **1**–**4** in water. Inset shows the d–d transition in **2**.

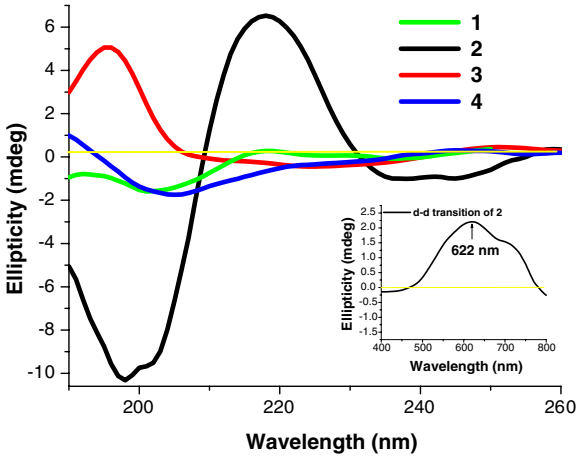

Figure 2. CD spectra of **1**–**4** in aqueous medium. Inset shows the d–d transition in **2**.

We resorted to X-band EPR spectroscopy to investigate possible mode(s) of metal ion coordination to peptide conjugate **1**.[19] The EPR spectrum of **2** in 50% aqueous methanol displayed an isotropic spectrum at room temperature; however, an anisotropic spectrum with hyperfine splitting was observed at liquid nitrogen temperature with a $g_{\|}$ of 2.37 and the hyperfine splitting $A_{\|}$ was 134.33 gauss, thus suggesting a possible [2N, 2O] binding mode.[20–22] At pH 8, $g_{\perp}$ was 2.06, $g_{\|}$ was 2.28 and the hyperfine splitting $A_{\|}$ was 199 gauss which suggested a three-nitrogen and one-oxygen [3N, 1O] binding mode.[23–27] Both spectra indicate a tetragonal coordination environment.[28–31] In the absence of a crystal structure, the existence of $d_{x^2-y^2}$ or d_{xy} ground states in square planar, square pyramidal or tetrahedral elongated geometry may be proposed based on spectral data. The EPR spectrum of **4** displayed a characteristic six-line spectral pattern with g_{iso} of 2.018 and hyperfine splitting A_{iso} of 96 G.

FT-IR spectroscopy offers useful information about metal-peptide interactions and aggregate formation upon prolonged incubation.[19] The following inferences can be drawn from the IR spectra of unmetalated conjugate **1** versus its metalated versions **2**–**4** (Table 1; spectra recorded after 10 days of incubation).

- Amide I and amide II band frequencies were shifted in comparison to **1**. In the case of **2** and **4**, amide I and II bands were shifted to a lower frequency indicating interactions of copper and manganese ions with both carbonyl and NH groups.
- In the case of conjugate **3**, both the amide I and II band frequencies are shifted to slightly higher wavenumbers suggesting that perhaps silver is bound to the imidazole ring of the histidine residue rather than the backbone amides.

Metal ion-induced conformational changes in conjugate **1** were studied by CD spectroscopy.[32] Conjugate **1** did not reveal any definite structure (Fig. 2). However, **2** displayed a structure similar to a poly-L-proline II helix upon interaction with copper, while **3** exhibited a switch from the random-coil like structure present in **1** to a β-sheet-like orientation upon interaction with silver ions (Fig. 2). In contrast, **4** lacked a definite structure in solution.

Table 1. IR (KBr pellet) amide absorptions of conjugates **1**–**4**

Conjugate	Amide I (cm^{-1})	Amide II (cm^{-1})
1	1656	1594
2	1635	1575
3	1660	1605
4	1646	1585

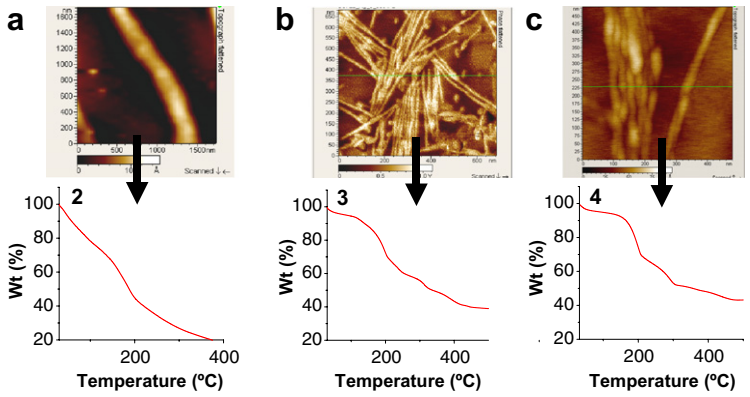

Figure 3. AFM micrograph and TGA traces of metalated peptide fibers after 15 days incubation in water at 37 °C. (a) Conjugate **2** shows a fiber cross-section of 220–230 nm and stability 185 °C; (b) **3** (15–40 nm; ~200 °C); (c) **4** (20–30 nm; ~200 °C).

It can be reasoned that Cu(II) with an intermediary acidic character is likely to interact with histidine and backbone nitrogens and carbonyl oxygens, while Ag(I) primarily coordinates to histidine nitrogens possibly in a linear Ag–N–Ag fashion, without interacting with the peptide backbone.[33–35] Although speculative in the absence of crystal data, it can be proposed that intramolecular coordination in the case of Cu(II) and intermolecular coordination for Ag(I), may contribute to the two geometries observed in the CD spectra. Interestingly, the existence of a poly-L-proline II helix secondary structure has previously been demonstrated for prion octarepeat peptides.[36–38]

Aging of metalated peptides **2**–**4** for 15 days lead to extensive growth of nanofibers (incubation in water at 37 °C). The cross-sectional diameter of fiber adducts **2**–**4** were ~220–230, 15–40, and 20–30 nm, respectively.[39] Gazit and co-workers have recently reported thermogravimetric analyses (TGA) to describe the thermal stability of the diphenylalanine nanotubes.[40] We also performed TGA to evaluate the thermal stability of these fibers.[41] It was found that metalated peptide fibers derived from **2** were stable up to 185 °C, while fibers from **3** and **4** exhibited stability up to 200 °C (Fig. 3), compared to unmetalated **1** which is stable up to 160 °C (data not shown). These results suggest appreciable thermal stability of the metalated fibers.

In conclusion this report describes an expeditious entry for the generation of thermally stable metalated peptide fibers from a naturally occurring metal binding motif derived from the prion octarepeat sequence. It is proposed that conjugation of peptide sub-segments, from metal binding regions, may provide opportunities for the design and growth of metalated fibers with potential applications in molecular bioelectronics and nano(bio)-technological research.

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References and notes

1. Reches, M.; Gazit, E. *Curr. Nanosci.* **2006**, *2*, 105.
2. Zanuy, D.; Nussinov, R.; Aleman, C. *Phys. Biol.* **2006**, *3*, S80.
3. Scheibel, T. *Curr. Opin. Biotechnol.* **2005**, *16*, 427.
4. Gao, X.; Matsui, H. *Adv. Mater.* **2005**, *17*, 2037.
5. Zhao, X.; Zhang, S. *Trends Biotechnol.* **2004**, *22*, 470.
6. Rajagopal, K.; Schneider, J. P. *Curr. Opin. Struct. Biol.* **2004**, *14*, 480.
7. Carny, O.; Shalev, D.; Gazit, E. *Nano Lett.* **2006**, *6*, 1594.
8. Sarikaya, M.; Tamerler, C.; Jen, A. K.-Y.; Schulten, K.; Baneyx, F. *Nature Mat.* **2003**, *2*, 577.
9. Patolsky, F.; Weizmann, Y.; Willner, I. *Nature Mat.* **2004**, *3*, 692.
10. Harris, D. A.; True, H. L. *Neuron* **2006**, *50*, 353.
11. Ross, E. D.; Minton, A.; Wickner, R. B. *Nature Cell Biol.* **2005**, *7*, 1039.
12. Weissmann, C. *Nature Rev. Microbiol.* **2004**, *2*, 861.
13. Millhauser, G. L. *Acc. Chem. Res.* **2004**, *37*, 79.
14. Madhavaiah, C.; Verma, S. *Chem. Commun.* **2004**, 638.
15. Madhavaiah, C.; Krishna Prasad, K.; Verma, S. *Tetrahedron Lett.* **2005**, *46*, 3745.
16. Synthesis of metalated peptide conjugates (**2**–**4**). PHGGG pentapeptide was prepared using a fragment condensation approach following standard solution-phase synthetic protocols.[14] Short peptide conjugate **1** was prepared by tethering two such pentapeptide sequences with 6-aminocaproic acid, followed by purification by silica gel column chromatography. Detailed synthesis will be reported elsewhere. The purity of conjugate **1** was determined by FPLC (>98%) and was further confirmed by FAB mass spectral and CHN analysis. Conjugate **1** was treated with 2 equiv of $CuCl_2{\cdot}2H_2O$, 2 equiv of $AgNO_3$ and 2 equiv of $MnCl_2{\cdot}4H_2O$ for 10 h in 50% aqueous methanol at room temperature and then the solvent was evaporated under reduced pressure. The residues were washed with methanol and dried under vacuum. Metalated peptide conjugates **2**–**4** were satisfactorily characterized through MALDI ToF and ESI mass spectral analyses.
17. Banerjee, A. I.; Yu, L.; Matsui, H. *Proc. Natl. Acad. Sci.* **2003**, *100*, 14678.
18. Mayer, C. R.; Dumas, E.; Secheresse, F. *Chem. Commun.* **2005**, 345.
19. EPR spectra were recorded on a Varian spectrometer at X-band frequency and the magnetic field strength was calibrated using DPPH ($g = 2.0036$). The preformed 1:2 (conjugate/copper and manganese) copper and manganese conjugates **2** and **4** were dissolved in 50% aqueous

methanol and EPR spectra were recorded at liquid nitrogen temperature. For pH dependent EPR studies of conjugate **2** (1 mM), the pH was maintained by addition of 10 mM NaOH or HCl. Methanol was added to the aqueous solution to increase the resolution and to avoid aggregation.

20. Hou, L.; Zagorski, M. G. *J. Am. Chem. Soc.* **2006**, *128*, 9260.
21. Karr, J. W.; Lauren, J. K.; Szalai, V. A. *J. Am. Chem. Soc.* **2004**, *126*, 13534.
22. Sanna, D.; Micera, G.; Kallay, C.; Rigo, V.; Sovago, I. *Dalton Trans.* **2004**, *17*, 2702.
23. Zong, X.-H.; Zhou, P.; Shao, Z.-Z.; Chen, S.-M.; Chen, X.; Hu, B.-W.; Deng, F.; Yao, W.-H. *Biochemistry* **2004**, *43*, 11932.
24. Malandrinos, G.; Louloudi, M.; Deligiannakis, Y.; Hadjiliadis, N. *Inorg. Chem.* **2001**, *40*, 4588.
25. Brown, D. R.; Guantieri, V.; Grasso, G.; Impellizzeri, G.; Pappalardo, G.; Rizzarelli, E. J. *Inorg. Biochem.* **2004**, *98*, 133.
26. Aronoff-Spencer, E.; Burns, C. S.; Avdievich, N. I.; Gerfen, G. J.; Peisach, J.; Antholine, W. E.; Ball, H. L.; Cohen, F. E.; Prusiner, S. B.; Millhauser, G. L. *Biochemistry* **2000**, *39*, 13760.
27. Valensin, D.; Luczkowski, M.; Mancini, F. M.; Legowska, A.; Gaggelli, E.; Valensin, G.; Rolka, K.; Kozlowski, H. *Dalton. Trans.* **2004**, *9*, 1284.
28. Pogni, R.; Baratto, M. C.; Busi, E.; Basosi, R. J. *Inorg. Biochem.* **1999**, *73*, 157.
29. Madhavaiah, C.; Verma, S. *Bioorg. Med. Chem.* **2005**, *13*, 3241.
30. Chattopadhyay, M.; Walter, E. D.; Newell, D. J.; Jackson, P. J.; Aronoff-Spencer, E.; Peisach, J.; Gerfen, G. J.; Bennett, B.; Antholine, W. E.; Millhauser, G. L. *J. Am. Chem. Soc.* **2005**, *127*, 12647.
31. Gaggelli, E.; Bernardi, F.; Molteni, E.; Pogni, R.; Valensin, D.; Valensin, G.; Remelli, M.; Luczkowski, M.; Kozlowski, H. *J. Am. Chem. Soc.* **2005**, *127*, 996.
32. 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-10-sulfonate. Experimental measurements were carried out in water (sample concentration was fixed at 0.33 mM, pH 7.5), using a 1 mm path length cuvette between 190 and 400 nm. The spectrum represents an average of 5–8 scans and the CD intensities are expressed in mdeg.
33. Burns, C. S.; Aronoff-Spencer, E.; Dunham, C. M.; Lario, P.; Avdievich, N. I.; Antholine, W. E.; Olmstead, M. M.; Vrielink, A.; Gerfen, G. J.; Peisach, J.; Scott, W. G.; Millhauser, G. L. *Biochemistry* **2002**, *41*, 3991.
34. Aakeröy, C. B.; Beatty, A. M. *Chem. Commun.* **1998**, 1067.
35. Sigel, H.; Martin, R. B. . *Chem. Rev.* **1982**, *82*, 385.
36. Blanch, E. W.; Gill, A. C.; Rhie, A. G. O.; Hope, J.; Hecht, L.; Nielsen, K.; Barron, L. D. *J. Mol. Biol.* **2004**, *343*, 467.
37. Gill, A. C.; Ritchie, M. A.; Hunt, L. G.; Steane, S. E.; Davies, K. G.; Bocking, S. P.; Rhie, A. G.; Bennett, A. D.; Hope, J. *EMBO J.* **2000**, *19*, 5324.
38. Smith, C. J.; Drake, A. F.; Banfield, B. A.; Bloomberg, G. B.; Palmer, M. S.; Clarke, A. R.; Collinge, J. *FEBS Lett.* **1997**, *405*, 378.
39. Atomic force microscopy (AFM). Fresh and aged metalated peptide samples were imaged with an atomic force microscope (Molecular Imaging, USA) operating under Acoustic AC mode (AAC), with the aid of cantilever (NSC 12(c) from MikroMasch). The force constant was 0.6 N/m, while the resonant frequency was 150 kHz. The images were recorded in air at room temperature, with a scan speed of 1.5–2.2 lines/s. Data acquisition was performed using PicoScan 5® software, while the data analysis was done with the aid of visual scanning probe microscopy. Conjugates **2**–**4** (0.33 mM) were incubated in pure water and micrographs were recorded for selected incubation periods. 10 μL of an aqueous solution of each conjugate was transferred onto a freshly cleaved mica surface and uniformly spread with the aid of a spin coater operating at 200–500 rpm (PRS-4000). The sample-coated mica was dried for 30 min at room temperature, followed by AFM imaging.
40. Adler-Abramovich, L.; Reches, M.; Sedman, V. L.; Allen, S.; Tendler, S. J. B.; Gazit, E. *Langmuir* **2006**, *22*, 1313.
41. TGA analysis. Conjugates **1**–**4** were incubated at 37 °C for 15 days after which the thermal stability of the metallic peptide fibers was determined by thermogravimetric analysis. Thermogravimetric analysis curves were obtained on a Perkin–Elmer Pyris6 thermogravimetry analyzer under zero-grade dry nitrogen atmosphere.