ORGANIC LETTERS

2013
Vol. 15, No. 19
5118–5121

Self-Association of Water-Soluble Peptoids Comprising (*S*)-*N*-1-(Naphthylethyl)glycine Residues

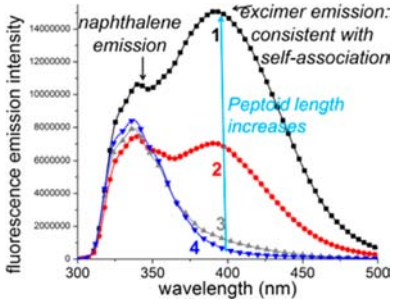
Amelia A. Fuller,* Brett A. Yurash, Erik N. Schaumann, and Frederick J. Seidl

Department of Chemistry & Biochemistry, Santa Clara University, 500 El Camino Real, Santa Clara, California 95053, United States

aafuller@scu.edu

Received September 4, 2013

ABSTRACT



Peptoids (N-substituted glycine oligomers) are widely used peptidomimetics, and an enhanced understanding of their structures is needed to expand their utility, particularly in aqueous applications. We report the synthesis and structural study of four water-soluble peptoids that include strongly helix-promoting (*S*)-*N*-1-(naphthylethyl)glycine residues. Peptoid structure changes with both peptoid length and solvent composition. Multiple data support the self-association of the longest peptoid studied here, 1, via hydrophobic interactions in aqueous solutions.

Peptidomimetic foldamers have served as useful chemical tools to recapitulate protein structures.[1] Among the many available scaffolds, sequence-specific peptoids[1a,2] (N-substituted glycine oligomers) are particularly attractive owing to their ease of synthesis,[3] favorable biostability properties,[4] and capacity to adopt stable secondary structural motifs including helices,[5] turns,[6] sheets,[7] and ribbons.[8] These features have inspired the study and application of peptoids as therapeutics,[9] sensors,[10] and structured nanomaterials,[11] for example. However, the study of water-soluble peptoid structures[12] remains underdeveloped, and examples of peptoid structures that rival the

(1) (a) Sun, J.; Zuckermann, R. N. *ACS Nano* **2013**, *7*, 4715. (b) Martinek, T. A.; Fülöp, F. *Chem. Soc. Rev.* **2012**, *41*, 687. (c) Guichard, G.; Huc, I. *Chem. Commun.* **2011**, *47*, 5933.

(2) Zuckermann, R. N. *Pept. Sci.* **2011**, *96*, 545.

(3) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

(4) (a) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657. (b) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Drug Dev. Res.* **1995**, *35*, 20.

(5) (a) Armand, P.; Kirshenbaum, K.; Goldsmith, R. A.; Farr-Jones, S.; Barron, A. E.; Truong, K. T. V.; Dill, K. A.; Mierke, D. F.; Cohen, F. E.; Zuckermann, R. N.; Bradley, E. K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4309. (b) Wu, C. W.; Kirshenbaum, K.; Sanborn, T. J.; Patch, J. A.; Huang, K.; Dill, K. A.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 13525. (c) Stringer, J. R.; Crapster, J. A.; Guzei, I. A.; Blackwell, H. E. *J. Am. Chem. Soc.* **2011**, *133*, 15559.

(6) (a) Stringer, J. R.; Crapster, J. A.; Guzei, I. A.; Blackwell, H. E. *J. Org. Chem.* **2010**, *75*, 6068. (b) Shin, S. B. Y.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K. *J. Am. Chem. Soc.* **2007**, *129*, 3218.

(7) Crapster, J. A.; Stringer, J. R.; Guzei, I. A.; Blackwell, H. E. *Pept. Sci.* **2011**, *96*, 604.

(8) Crapster, J. A.; Guzei, I. A.; Blackwell, H. E. *Angew. Chem., Int. Ed.* **2013**, *52*, 5079.

(9) (a) Zuckermann, R. N.; Kodadek, T. *Curr. Opin. Mol. Ther.* **2009**, *11*, 299. (b) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.

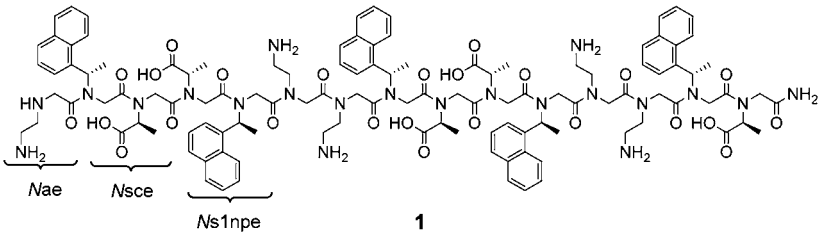
(10) Fuller, A. A.; Holmes, C. A.; Seidl, F. J. *Pept. Sci.* **2013**, *100*, 380.

(11) Nam, K. T.; Shelby, S. A.; Choi, P. H.; Marciel, A. B.; Chen, R.; Tan, L.; Chu, T. K.; Mesch, R. A.; Lee, B. C.; Connolly, M. D.; Kisielowski, C.; Zuckermann, R. N. *Nat. Mater.* **2010**, *9*, 454.

(12) (a) Fuller, A. A.; Seidl, F. J.; Bruno, P. A.; Plescia, M. A.; Palla, K. S. *Pept. Sci.* **2011**, *96*, 627. (b) Shin, S. B. Y.; Kirshenbaum, K. *Org. Lett.* **2007**, *9*, 5003. (c) Sanborn, T. J.; Wu, C. W.; Zuckermann, R. N.; Barron, A. E. *Biopolymers* **2002**, *63*, 12.

(13) (a) Murnen, H. K.; Rosales, A. M.; Jaworski, J. N.; Segalman, R. A.; Zuckermann, R. N. *J. Am. Chem. Soc.* **2010**, *132*, 16112. (b) Lee, B. C.; Chu, T. K.; Dill, K. A.; Zuckermann, R. N. *J. Am. Chem. Soc.* **2008**, *130*, 8847. (c) Lee, B. C.; Zuckermann, R. N.; Dill, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 10999. (d) Burkoth, T. S.; Beausoleil, E.; Kaur, S.; Tang, D.; Cohen, F. E.; Zuckermann, R. N. *Chem. Biol.* **2002**, *9*, 647.

10.1021/ol4025502
Published on Web 09/19/2013



Peptoid	Sequence
1	H-*N*ae-*N*s1npe-(*N*sce)$_2$-*N*s1npe-(*N*ae)$_2$-*N*s1npe-(*N*sce)$_2$-*N*s1npe-(*N*ae)$_2$-*N*s1npe-*N*sce-NH$_2$
2	H-*N*sce-*N*s1npe-(*N*ae)$_2$-*N*s1npe-(*N*sce)$_2$-*N*s1npe-(*N*ae)$_2$-*N*s1npe-*N*sce-NH$_2$
3	H-*N*ae-*N*s1npe-(*N*sce)$_2$-*N*s1npe-(*N*ae)$_2$-*N*s1npe-*N*sce-NH$_2$
4	H-*N*sce-*N*s1npe-(*N*ae)$_2$-*N*s1npe-*N*sce-NH$_2$

Figure 1. Sequences of peptoids **1**–**4**. The full structure of **1**, shown, is labeled with the peptoid residue abbreviations.

complexity of biomolecular structure (e.g., by intra- or intermolecular self-association in aqueous environments) are few.[13]

Given the absence of H-bond donors in the peptoid backbone, peptoid secondary structure is chiefly controlled by adding side chains that influence the amide bond rotation as well as φ and ψ dihedral angles.[14] The effects of steric and electronic factors on this equilibrium have been used to guide peptoid structural predictions. As an excellent example of this design in practice, the sterically bulky (*S*)-*N*-(1-naphthylethyl)glycine (*N*s1npe) residue was predicted to be strongly helix-promoting because it confers a substantial energetic preference for the *cis* amide bond conformation.[14b] Peptoid helices studied at high resolution comprise *cis* amide bonds.[5] The Blackwell laboratory confirmed this prediction; they detail highly structurally homogeneous poly(*N*s1npe) oligomer helices,[5c] including the crystal structure of a four-residue oligomer. Similar to peptoid helices that comprise other sterically bulky hydrophobic side chains, the poly(*N*s1npe) helix has three residues per turn, *cis* amide bonds, and a pitch of approximately 6 Å.

In this study, our aim was to advance understanding of the influence of *N*s1npe on peptoid structure in neutral aqueous conditions; findings here will facilitate biological application of *N*s1npe-containing peptoids. We have previously studied 15-residue water-soluble peptoids that adopt a putative amphiphilic helical secondary structure,[12a] originally derived from a structure reported by the Zuckermann laboratory.[13b] We adapted this simple design to peptoids **1**–**4** studied here (Figure 1). The peptoid scaffold includes just three residues: (*S*)-*N*-(1-carboxyethyl)glycine (*N*sce, anionic at neutral pH), *N*-(2-aminoethyl)glycine (*N*ae, cationic at neutral pH), and the *N*s1npe aromatic residues. The sequence ordering follows general criteria for the design of helical peptoids: two-thirds of the residues are bulky and chiral (*N*sce and *N*s1npe), and every third residue is aromatic.[15] Assuming that **1**–**4** would maintain the putative amphiphilic helix structure, **1**–**4** were designed to enable us to compare peptoids with 5, 4, 3, or 2 helix turns, respectively.

The inclusion of *N*s1npe residues offers several advantages. Because they are strongly helix-promoting, we anticipated that we would observe robust peptoid structures, and we could compare our spectroscopic data to those of the poly(*N*s1npe) oligomers.[5c] Moreover, the fluorescence of the naphthalene chromophore provides a chemical tool that has not yet been used to derive additional structural information about the peptoids.

Peptoids are efficiently prepared on solid support by the "submonomer" synthesis method wherein bromoacetylation and amine displacement reactions are iterated to generate a sequence-specific oligomer (Supporting Information (SI) Scheme S1).[3] For the synthesis of **1**–**4**, an amine-functionalized Rink amide resin was bromoacetylated. The terminal bromide was then displaced by the appropriate primary amine to install the *N*s1npe, *N*sce, or the *N*ae residues (see SI for details). Peptoids **1**–**4** were then simultaneously deprotected and cleaved from the resin. Finally, peptoids were purified by reverse-phase HPLC (Figure S1), and their identities were confirmed by mass spectrometry (Table S1).

To assess the effects of peptoid length on structure, we acquired circular dichroism (CD) spectra for **1**–**4** (Figure 2a). Correlations between CD spectral features and the existing high-resolution structures are commonly used to infer structural information.[5] In buffered aqueous solution, peak wavelengths and intensities both vary with peptoid length, indicating very different conformations for these peptoids. The CD spectrum of the shortest

(14) (a) Roy, O.; Caumes, C.; Esvan, Y.; Didierjean, C.; Faure, S.; Taillefumier, C. *Org. Lett.* **2013**, *15*, 2246. (b) Gorske, B. C.; Stringer, J. R.; Bastian, B. L.; Fowler, S. A.; Blackwell, H. E. *J. Am. Chem. Soc.* **2009**, *131*, 16555. (c) Shah, N. H.; Butterfoss, G. L.; Nguyen, K.; Yoo, B.; Bonneau, R.; Rabenstein, D. L.; Kirshenbaum, K. *J. Am. Chem. Soc.* **2008**, *130*, 16622.

(15) (a) Wu, C. W.; Sanborn, T. J.; Huang, K.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 6778. (b) Armand, P.; Kirshenbaum, K.; Falicov, A.; Dunbrack, R. L., Jr.; Dill, K. A.; Zuckermann, R. N.; Cohen, F. E. *Folding Des.* **1997**, *2*, 369.

peptoid, **4**, closely resembles spectra reported for poly(*N*s1npe) oligomers of at least four residues,[5c] suggesting **4** adopts a similar helical conformation; it has an intense minimum at 231 nm and a broad maximum near 215 nm. The minimum at 231 nm was correlated with the overlap of naphthalene units in the side chains of the i and $i + 3$ residues in the peptoid helix.[5c] While the CD spectrum of **3** maintains the same general shape as the spectrum of **4**, CD spectra for **1** and **2** are substantially different. Most notably, **1** and **2** exhibit spectral *maxima* at 227 and 231 nm, respectively. The dramatic length-dependent spectral differences for **1**–**4** diverge substantially from the CD studies of poly(*N*s1npe) oligomers, wherein spectral features change minimally with increased peptoid length.[5c]

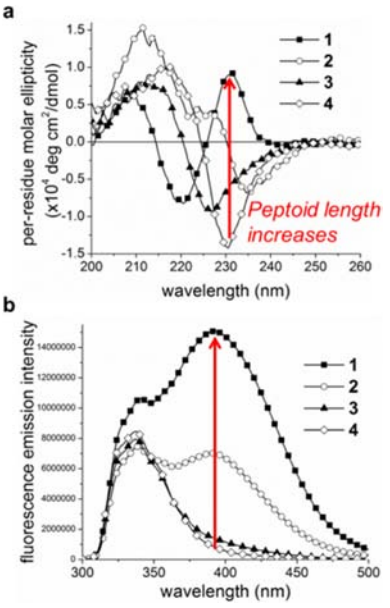


Figure 2. Spectroscopic features of peptoids **1**–**4** change with peptoid length. (a) CD spectra and (b) fluorescence emission spectra of 40 μM **1**–**4** in aqueous buffer (5 mM Tris, pH 7.5).

Fluorescence emission spectra of **1**–**4** also exhibit peptoid length-dependent trends that provide some insight into the origin of the observed CD spectral differences (Figure 2b). In the emission spectra of both **1** and **2**, the longest peptoids studied, there are two emission peaks at λ_{max} = 340 and 392 nm. In the spectrum of **1**, the peak at 392 nm is 52% more intense than the 340 nm peak. The two peaks are approximately equal in intensity in the spectrum of **2**. The spectra of **3** and **4**, however, exhibit just one maximum at 337 nm.

The broad, red-shifted emission peak at 392 nm is attributed to a naphthalene excited state dimer (excimer).[16] Excimers are observed when chromophores are positioned ~3 Å from one another due to molecular conformation or intermolecular association, making them useful photophysical probes.[17] The appearance of this peak in the fluorescence spectra of **1** and **2** prompted us to hypothesize that **1** and **2** undergo hydrophobically driven self-association in water. Given that peptoid helices have a helix pitch of ~6 Å in all high-resolution structures,[5] an intermolecular assembly is the most likely arrangement that would position the chromophores at a close distance.

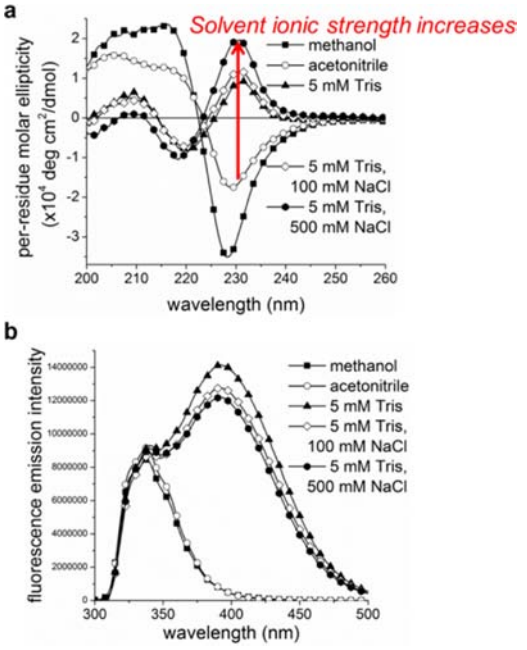


Figure 3. Spectral features of **1** are solvent-dependent. (a) CD spectra and (b) fluorescence spectra of 40 μM **1** in varied solvents. All aqueous buffers are pH 7.5.

To evaluate our hypothesis of peptoid self-association, we first compared solvent effects on the CD spectra of **1** (Figure 3a). We focused our studies on **1** because it showed the most dramatic CD spectral differences from the poly(*N*s1npe) oligomers and the most intense excimer fluorescence emission. CD spectra of **1** were evaluated in two organic solvents, methanol (protic) and acetonitrile (aprotic), and in aqueous buffers with increasing ionic strength. In the organic solvents, CD spectral features of **1** match those of poly(*N*s1npe) oligomers: there is a broad maximum near 210 nm and an intense minimum at 227 nm. Notably, CD spectra of **1**–**4** are similar in organic solvents (Figure S2), suggesting that all have a helical conformation similar to the poly(*N*s1npe) oligomers. With increasing ionic strength, the spectrum of **1** changes; the intensity of the signal at 231 nm is increased. CD spectra of peptoids **2**–**4** exhibit more modest changes in response to changes in solvent (Figure S3).

In the fluorescence spectra of **1**, there are also significant differences between organic and aqueous solvents (Figure 3b). In the organic solvents, there is no emission attributed to excimer fluorescence. The single fluorescence maximum at 340 nm is common to **1**–**4** in organic solvents

(16) Förster, T. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 333.
(17) (a) Duhamel, J. *Langmuir* **2012**, *28*, 6527. (b) De Schryver, F. C.; Collart, P.; Vandendriessche, J.; Goedeweeck, R.; Swinnen, A.; Van der Auweraer, M. *Acc. Chem. Res.* **1987**, *20*, 159.

(Figure S4). While added NaCl impacts the ratio of peak intensities for **2**–**4** (Figure S5), increased solvent ionic strength has minimal effect on the fluorescence spectra of **1**. At this peptoid concentration (40 μM), we propose that fluorescence efficiency of the excimer is maximized. Solvent-dependent changes in both the CD and fluorescence spectra of **1** can be attributed to a hydrophobically driven process wherein aqueous solvents promote peptoid self-association or a conformational rearrangement induced by altering electrostatic interactions of the side chains or some combination of these two effects.

The sensitivity of the ratio of the two fluorescence emission peaks' intensities to the concentration of **1** offers more compelling support that intermolecular association of **1** influences excimer peak intensity. At concentrations above 10 μM, the ratio of peak intensity at 392 nm (excimer) to 340 nm does not change substantially. Below 10 μM, however, the excimer fluorescence intensity decreases relative to the emission peak at 340 nm (Figure S6).

Monitoring the interaction of 1-analinonaphthalene-8-sulfonate (1,8-ANS) with **1** (Figure S7) provided more evidence of peptoid self-association. As has been previously studied, the fluorescence emission of 1,8-ANS is minimal in aqueous solution but is enhanced when the dye binds to a hydrophobic environment created by peptoid self-association.[13d] Increasing the concentration of **1** from 5 to 100 μM results in a dramatic 39 nm blue shift of the λ_{max} and a 36-fold enhancement of 1,8-ANS fluorescence intensity at 471 nm (λ_{max} for 1,8-ANS when [**1**] = 100 μM).

The temperature dependence of CD spectral features of **1** is also consistent with its self-association in aqueous buffer (Figure 4). As temperature increases, the intensity of the maximum at 231 nm decreases. At 98 °C, the spectrum of **1** exhibits a minimum at 227 nm, similar to the spectrum of **1** in organic solvent. There is an isodichroic point at 222 nm which is characteristic of a transition between two conformational states. This temperature sensitivity is common for processes driven by hydrophobic interactions and was not observed for poly(*N*s1npe) oligomers. Temperature effects on the CD spectra of **2** are less prominent (Figure S8), and the CD spectrum of **3** does not change in response to temperature.

To estimate the size of the peptoid aggregates, we subjected **1** to size exclusion chromatography. The retention time of **1** was compared to retention times of standards eluted from the column with 50 mM Tris-HCl, 150 mM NaCl, pH 7.3 (TBS buffer) to approximate the degree of assembly of **1**. Peptoid **1** elutes from the column as a broad peak (Figure S9), and the calculated molecular weight of the peptoid assembly is approximately twice the molecular weight of **1**. The fraction eluted at the apex of the broad peak exhibits strong excimer fluorescence emission, as expected given the likely fast equilibration of peptoid monomer and multimer species (Figure S10).

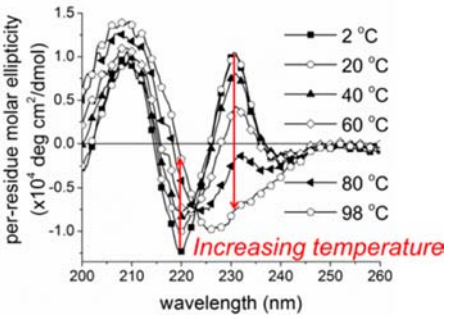


Figure 4. CD spectral features of **1** vary with temperature. All spectra were acquired for 50 μM **1** in 5 mM Tris, pH 7.5.

In conclusion, we have prepared new water-soluble peptoids whose self-association has been characterized by complementary spectroscopic techniques. This is the first time the fluorescence of *N*s1npe peptoid residues has been measured directly, establishing the utility of this analytical tool for peptoid structural studies. We detail specific fluorescence (excimer emission) and CD spectroscopic characteristics (maximum at 231 nm) that correlate with enhanced self-association promoted by increased peptoid length, increased solvent ionic strength, or decreased temperature. Peptoid **1** is among a small number of peptoids reported to self-associate at very low concentrations in aqueous solution, providing a template for the design of molecules that can ultimately rival the structural complexity of proteins. Ongoing studies in our laboratory will seek to characterize the specific roles of peptoid length, helicity, and hydrophobicity on the dynamics of their self-association.

Acknowledgment. This work was supported by a CAREER award (NSF CHE1056520), a Cottrell College Science Award from the Research Corporation for Science Advancement, and a Dreyfus Foundation Faculty Start Up Award. B.A.Y. was supported by a DeNardo Science Scholar Award from Santa Clara University. Routine mass spectra were obtained using an instrument funded by an NSF MRI grant (1126895). The authors are grateful to Prof. Brandon White for assistance with high-resolution mass spectrometry at the PROTEIN laboratory at San Jose State University (NSF MRI 0923573).

Supporting Information Available. Full experimental details and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.