Peptidomimetics

Sulfono-$\gamma$-AApeptides as a New Class of Nonnatural Helical Foldamer


Abstract: Foldamers offer an attractive opportunity for the design of novel molecules that mimic the structures and functions of proteins and enzymes including biocatalysis and biomolecular recognition. Herein we report a new class of nonnatural helical sulfono-$\gamma$-AApeptide foldamers of varying lengths. The crystal structure of the sulfono-$\gamma$-AApeptide monomer S6 illustrates the intrinsic folding propensity of sulfono-$\gamma$-AApeptides, which likely originates from the bulkiness of tertiary sulfonamide moiety. The two-dimensional solution NMR spectroscopy data for the longest sequence S1 demonstrates a 10/16 right-handed helical structure. Optical analysis using circular dichroism further supports well-defined helical conformation of sulfono-$\gamma$-AApeptides in solution containing as few as five building blocks. Future development of sulfono-$\gamma$-AApeptides may lead to new foldamers with discrete functions, enabling expanded application in chemical biology and biomedical sciences.

Introduction

Natural biopolymers including proteins and nucleic acids adopt well-defined and compact three-dimensional folding conformations providing a structural basis for their complex biological functions.[1] This paradigm suggests that nonnatural foldamers with new molecular frameworks and folding propensities may also exhibit unique biomimetic properties that can be exploited in pharmaceutical development and advanced biotechnology applications.[2] Specifically, nonnatural foldamers offer opportunities to better understand biomolecular structure-function relationships, facilitate the design of novel nanostructures, and develop targeted diagnostic agents and potential drug candidates.[3] Because nonnatural monomers have an enormous diversity in size, shape, and backbone structure, nonnatural foldamers can theoretically be developed to display a wide variety of three-dimensional conformations and biomimetic function.[4] In addition, many nonnatural foldamers are resistant to proteolytic degradation, augmenting their potential application in biological systems. The potential importance of nonnatural foldamers has led to the development of numerous foldamer systems including $\beta$-peptides,[5] $\alpha$-aminoxy-peptides,[6] peptoids,[7] and oligoureas.[8] However, nonnatural foldamers have just recently begun to find biological applications and thus the continued development of new building blocks, molecular frameworks, and backbones are of key interest.

We recently developed a new class of peptidomimetics-$\gamma$-AApeptides, oligomers of N-acylated-$N$-aminoethyl amino acids (Figure 1).[9] As half of the side chains are introduced through acylation, $\gamma$-AApeptides have virtually limitless potential in functional group diversity. Although $\gamma$-AApeptides are based upon a chiral PNA backbone,[10] they are designed to capture the function of bioactive peptides rather than nucleic acids.[11] For instance, certain $\gamma$-AApeptides display both antimicrobial activity[12] and anti-inflammatory activity[13] by mimicking host-defense peptides, whereas others mimic the Tat peptide by binding to HIV-1 RNA with high affinity[11b] and permeating cell membranes with excellent efficiency.[11a] In addition, $\gamma$-AApeptides have been developed to mimic the RGD peptide[14] and to

Figure 1. The general chemical structures of $\alpha$-peptides, $\gamma$-AApeptides, and sulfono-$\gamma$-AApeptides.
form one-bead-one-compound libraries for the discovery of bioactive protein/peptide based ligands. Furthermore, γ-AApeptides can also form novel nanostructures akin to peptide-based biomaterials. The emerging importance of γ-AApeptides has heightened the interest in the folding propensity regarding biomolecular structure.

Results and Discussion

One of the most attractive features of γ-AApeptides is that half of the side chains do not have to be derived from carboxylic acids. As shown in Figure 1, replacement of carboxylic acids with sulfonyl chlorides leads to the generation of sulfono-γ-AApeptides. As a subclass of γ-AApeptides, sulfono-γ-AApeptides possess essentially unlimited functional diversity, as a wide variety of functionalized sulfonyl chlorides are either available commercially or can be readily synthesized. Moreover, Sulfono-γ-AApeptides contain the same number of side chains as regular peptides of the same length, affording the potential of sulfono-γ-AApeptides to mimic bioactive peptides. The presence of protons in the second amide moieties in sulfono-γ-AApeptides indicate these polymers may exhibit folding propensities through intramolecular hydrogen bonding akin to α-peptides. In addition, the tertiary sulfonamido moieties are sufficiently bulky to induce intrinsic curvature into the sulfono-γ-AApeptide backbone. Furthermore, half of the side chains of sulfono-γ-AApeptides are chiral, which may also impose conformational bias to further promote the formation of specific secondary conformation.

To test the hypothesis that sulfono-γ-AApeptide foldamers can form discrete secondary structures, we have synthesized a series of sulfono-γ-AApeptides of differing lengths. The longest sulfono-γ-AApeptide (S1) contains eight building blocks, comparable in length to a 16-mer peptide. The shortest sequences S5 and S6 are sulfono-γ-AApeptide monomers, which are equivalent to dipeptides (Figure 2).

The sulfono-γ-AApeptides S1–S5 were obtained through solid-phase synthesis following our previously published protocol (Scheme 1). In brief, the desired N-alloc γ-AApeptide residues were attached sequentially on the solid support. After each N-alloc γ-AApeptide residue was added, the alloc protecting group was removed with Pd(PPh3)4 (10 mol% equiv) and Me2NH·BH3 (6 equiv) in DCM. Subsequently, the sulfonyl side group was introduced by reacting a sulfonyl chloride with the secondary nitrogen on the γ-AApeptide backbone. The synthetic cycle was repeated until the desired sequence was assembled, followed by cleavage and purifications by HPLC (Figure S1). The monomer S6 was prepared as previously reported.

The crystal structure of the monomer S6 was successfully obtained and shown in Figure 3 a. The crystal structure indicates that the S6 adopts a right-handed turn conformation. Consistent with our hypothesis, the bulky tertiary sulfonamido group appears to force the formation of the backbone curvature. In addition, there is a hydrogen bond formation between NH of N-terminus and CO of C-termini. An overlay of S6 with a canonical α-helical scaffold reveals that this turn curvature matches that of the α-helical sense (Figure 3 b). The demonstration that such a short sulfono-γ-AApeptide has a defined pre-organized structure due to the intrinsic folding propensity.
leads to the possibility that longer sulfono-γ-AAA peptides can be formed with more defined and stable secondary structures.

We then carried out NMR studies of the longest sequence, S1. In order to solve the structure unambiguously, different hydrophobic and hydrophilic groups are included in the S1 sequence. The NMR spectra were collected on an Agilent dd600 with a triple resonance cold probe. The 1D 1H NMR spectra were first obtained with differing concentrations (0.05–1 mM) and the chemical shifts of the backbone protons were compared. There were no obvious changes in the chemical shifts, suggesting that S1 does not aggregate under the experimental conditions (Figure S4). Next, 2D NMR spectroscopy was employed to investigate the solution structure of S1 in methanol (2 mM in CD3OH, 10°C). Two-dimensional spectra (zTOCSY, NOESY) were collected using standard pulse sequences with the number of acquisitions typically set to 200 for the NOESY and 6 for the zTOCSY spectra. The Water suppression through Enhanced T1 Effects (WET) method was used to suppress the proton peak in the CD3OH solvent. In general, a 2 s delay was applied before each scan. Experiments were collected with 2 K complex data points in F2 for each of 300 t1 increments with a sweep width of 6009 Hz in each dimension. Residue-specific assignments were made based on a combination of DQFCOSY, zTOCSY, ROESY, and NOESY spectra. The presence of different side chains eliminates potential overlaps between proton signals and is helpful for the unambiguous assignment of different building blocks. The CaHs were successfully assigned based on the short-range and/or sequential NOEs with neighbor side chains or amide proton (Figure 4).

Medium/long range NOEs revealed clear i–i+3 correlations between related side chains, that is, 1bHPA–3aHB, 2aHB–3bHPA, 3bHPA–5aHB, 4aHG–5bHPA, 5bHPE–7aHB, 6aHY–7bHPA (Figure 5). The i–i+3 correlation pattern implies that there is a defined folding pattern in S1, which displays proximity between every first and third building block.

The NMR solution structure was solved and is displayed in Figure 6. Schrödinger Macromodel[20] was used to perform molecular dynamics calculations based upon the NOE constraints, which resulted in the ten best structures. As shown in Figure 6b, the structures display very good overlap among backbone atoms (rmsd = (0.72 ± 0.29), Figure 6b, and Table S3 in the Supporting Information). The average of the ten helical structures for S1 is also displayed in Figure 6c and d. The data demonstrate that S1 adopts a well-defined right-handed helical conformation in methanol, with the side chains pointing away from the helical scaffold. Further analysis of the structure of S1 reveals a helical radius (2.3 Å) that is the equivalent to the canonical α-helix. In addition, the average of helical pitches is 5.7 Å, which is also very close to that of the peptide-based α-helix (5.4 Å). Furthermore, the structure indicates that each turn contains four side chains (Figure 6e), relative to 3.6 residues per turn found in α-helical peptides. This assignment is also consistent with the observation of i–i+3 NOE patterns. These features suggest that the sulfono-γ-AAA peptide could be developed to mimic the structure and function of α-helices.

The NMR structure further suggests that S1 has a 10/16 helix hydrogen-bonding pattern (Figure 7). It is known that the

Figure 3. a) Crystal structure of the sulfono-γ-AAA peptide monomer S6; b) overlay of S6 on an α-helical polyalanine scaffold.

Figure 4. a) Typical sequential NOEs observed for S1. Residue numbers are labeled; b) corresponding peaks in NOE spectrum.
α-helix is the 13-helix; however, the same hydrogen pattern cannot be formed in sulfono-γ-Apeptides due to an alternative secondary amide and tertiary sulfonamide functionalities. Nonetheless, each 10/16 helix cycle in a sulfono-γ-Apeptide is equivalent to two successive 13-helices in the α-helix. This feature may partially explain why the helical pitch and the radius of S1 are similar to those of the α-helix. Additionally, as expected, sulfonyl groups also contribute to the stability of the helical structure by directly participating in hydrogen bonding. SO2 groups (except the first and last one) point away from the helical scaffold and do not participate in hydrogen bonding.

Circular Dichroism (CD) spectroscopy can also provide an assessment of the folding propensity of oligomers including proteins and peptides, as well as nonnatural molecules such as β-peptides and peptoids. The CD spectra of S1 under different solvent conditions are displayed in Figure 8a. The spectra exhibit a maximum at ~220 nm. Interestingly, it appears that the sequence adopts a more stable helical conformation in PBS buffer relative to TFE, suggesting the potential of sulfono-γ-Apeptide for the mimicry of the α-helix and modulation of protein interactions in biological systems. The CD data further indicate that the sequence is not aggregated under the concentration range examined here, as a ten-fold dilution in TFE has little or no effect on the spectrum. The stability of the helix was further examined by temperature-dependent CD analysis (Figure 8b). As expected, S1 forms more defined helical structures at low temperatures. However, the secondary folding structure is still discernible even up to 55°C. To assess the general folding propensity of sulfono-
The shortest sequence S5 displayed some degree of helicity, which is consistent with the crystal structure of S6 foldamer. The clear trend is that longer sequences form better helical structures. The S4 is a trimer sequence that displays increased helicity relative to S5. This is similar to the helical propensity of α-peptides. Additionally, the bulky group appears to stabilize the helical conformation, as observed with another trimer sequence S3, which contains an aromatic group and exhibits much more discernable helicity than S4. Surprisingly, the pentamer sequence S2, which is comparable to a decamer peptide, displays almost identical helicity to the longest sequence S1. This indicates that the general helical propensity of sulfono-γ-Apeptides is quite high. However, as our peptidomimetics do not have canonical peptide backbone, CD data is just used as the supporting data for NMR structures, and should not be overly interpreted. For example, the dichroic bands from the arylsulfonamido chromophores may have a potentially overlapping and disrupting role. In addition, the Cotton effect near 220 nm may not be the accurate indication of molecular helicity.

**Conclusion**

In summary, we have identified a new class of nonnatural helical foldamer-sulfono-γ-Apeptides. The crystal structure indicates that even the shortest sulfono-γ-Apeptide (monomer) possesses a pre-organized folding structure. NMR studies further suggest that sulfono-γ-Apeptides adopt well-defined right-handed helical conformations in solution, similar to peptide-based α-helices. Similar to α-peptides, the sulfono-γ-Apeptide S1 is also stabilized by intramolecular hydrogen bonding. CD studies suggest that the similar folding propensity is generally observed throughout the sulfono-γ-Apeptide library examined here, and longer sequences exhibit more pronounced helicity in their secondary structures. Because a virtually endless set of functional groups can be incorporated into sulfono-γ-Apeptides, the folding propensity can be further programmed by a number of chemical approaches, such as inclusion of constrained residues and hydrocarbon stapling. We envision that sulfono-γ-Apeptide foldamers can be readily developed to address a variety of challenges in chemical biology.

**Experimental Section**

**Synthesis of sulfono-γ-Apeptides**

Solid-phase synthesis of S1 was carried out on 100 mg Rink-amide resin (0.7 mmol g⁻¹) at room temperature. The resin was swelled in DMF for 1 h before use. The Fmoc protecting group was removed by treating the resin with piperidine/DMF solution (3 mL 20%) for...
15 min (x2). The resin was washed with dichloromethane (3 x 3 mL) and DMF (3 x 3 mL). A premixed solution of N-allo γ-Apeptide building block[30](3 equiv), HOBT (6 equiv, HOBT = 1-hydroxybenzotriazole), and DIC (6 equiv) in DMF (2 mL) was added to the resin. The mixture was shaken for 4 h. After being washed with DCM and DMF, the resin was treated with Pd(PPh 3) 4 (8 mg, 0.007 mmol) and DIPEA (6 equiv) in CH 2Cl 2/isopropanol containing 1-hydroxybenzotriazole (95/2.5/2.5; TFA = trifluoroacetic acid, TIS = triisopropylsilanilic) for 2 h. The solvent was evaporated and the crude was analyzed on a preparative (1 mL/min) and preparative (16 mL/min) Waters HPLC systems, respectively. 5 % to 100 % linear gradient of 0.5 mL) in a 5 mm NMR tube. The 1H shift assignment was contained in APEX2, WinGX v1.70.01[30], and OLEX2[31]. All non- H atom positional parameters were derived using a multiscan method implemented in SADABS[29]. Absorption correction was performed using a mixing time of 200 ms. Vnmrj was used to process the data acquired with the wet solvent suppression. All these experiments were performed by collecting 6009 points in f2 and 300 points in f1. A DIPS2 spin-lock sequence with a spin-lock field of 6 kHz and a mixing time of 80 ms were used in zTOCSY. NOESY experiments used a mixing time of 200 ms. Vnmrj was used to process the data and 2D NMR spectra were analyzed by using the SPARKY program.[28]

X-ray crystallography

The crystal of S6 was obtained by slow solvent evaporation of 1:1 CH 2Cl 2/isopropanol containing S6 (5 mg mL −1 ). The X-ray diffraction data for S6 were collected on a Bruker D8 Venture PHOTON 100 CMOS system equipped with a Cu Kα, INCOATEC Imus microfocus source (λ = 1.54 Å). Indexing was performed using APEX2 (difference vectors method).[27] Data integration and reduction were performed using SAINTPlus 6.01.[30] Absorption correction was performed using a multiscan method implemented in SAADABS[28] Space groups were determined using XPREP implemented in APEX2. The structure was solved using SHELXS-97 (direct methods) and refined using SHELXL-2013 (full-matrix least-squares on F 2 ) contained in APEX2, WinGX v1.70.01[30], and OLEX2[31]. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms of –CH 3, –CH 2–, –CH = O and –NH groups were placed in geometrically calculated positions and included in the refinement process using a riding model with isotropic thermal parameters: U eq (H) = 1.2U eq (–CH 3), –CH 2–, –NH and U eq (H) = 1.5U eq (–CH = O). Disordered benzyl group has been refined using constraints (AFIX66 for phenyl group) and restraints RIGU and SADI. The atomic displacement parameter (ADP) values for disordered C21A and C21B atoms have been set to be equal (EADP). CCDC–1081941 (S6) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data request/cif.

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