De Novo Left-Handed Synthetic Peptidomimetic Foldamers

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De novo Left-Handed Synthetic Peptidomimetic Foldamers

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Abstract

Development of peptidomimetic helical foldamers with a wide repertoire of functions is of significant interest. Herein, for the first time, we report the X-ray crystal structures of a series of homogeneous L-sulfonyl-γ-AA foldamers, and elucidate their folding conformation at the atomic level. Single crystal X-ray crystallography reveals that this class of oligomers fold into unprecedented dragon-boat-shaped and surprisingly unexpected left-handed helices, which are stabilized by the 14-hydrogen bonding pattern unanimously present in the sequences. With a helical pitch of 5.1 Å and exactly four side chains per turn, these L-sulfonyl-γ-AAPeptides bear side chains which lie perfectly on top of each other along the helical axis. The 2D-NMR, computational simulation and CD studies consistently support the folding conformation in solution. Our results provide a structural basis at the atomic level for the design of novel biomimetics with precise arrangement of functional groups in three dimensions, which is envisaged to lead to the development of foldamers with unique biomimetic scaffold and new functions.

COMMUNICATION

Unprecedented dragon-boat-shaped, surprisingly unexpected left-handed helices comprised of homogeneous L-sulfonyl-γ-AA foldamers were reported for the first time. The results provide a structural basis at the atomic level for the design of novel biomimetics with precise arrangement of functional groups in three dimensions, which is envisaged to lead to the development of foldamers with unique biomimetic scaffold and new functions.

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Keywords

biomimetics; Left-handed foldamer; homogeneous L-sulffono-γ-AApeptides; X-ray crystal; computational studies

Foldamers,[1] which are a class of synthetic unnatural oligomers with defined and predictable structures, are capable of mimicking or complementing the three-dimensional structure and function of natural biopolymers such as proteins, peptides and nucleic acids. [2] In the past two decades, synthetic oligomeric architectures such as β-peptides,[3] peptoids,[4] oligoureas,[5] β-peptoids,[6] aza-peptides,[7] Aib foldamers,[8] aromatic amide foldamers,[9] oligoproline,[10] and others, have been characterized by crystallographic analysis, leading to various applications in molecular self-assembly and recognition.[11] However, as natural macromolecules exhibit an endless set of folding structure and function, continuing exploration of unnatural foldameric architecture with new frameworks and molecular scaffolds is still in an urgent need.[1b]

γ-AApeptides (N-acetylated-N-aminoethyl amino acid oligomers, stemming from chiral PNA backbone[12]) are receiving increasing attention as a new class of peptidomimetics, owing to their enormous chemical diversity imparted by arbitrary side chains and their resistance to proteolytic degradation (Figure 1a).[13] More recently, we have reported the crystal structures of de novo heterogeneous 2:1 α/D-sulffono-γ-AA hybrid oligomers capable of adopting right-handed 4.5–16 helical conformations,[14] as well as heterogeneous 1:1 α/L-sulffono-γ-AA hybrid oligomers that form right-handed 413 helices, [15] demonstrating that peptidomimetics containing γ-AApeptide units can be unique heterogeneous foldamers. However, the crystal structures of homogeneous sulffono-γ-AApeptides, which would be much more significant by elucidating the folding conformation of sulffono-γ-AApeptides, were not yet obtained. It could be an important addition to the foldamer development if homogeneous sulffono-γ-AApeptides, rather than heterogeneous hybrids, are identified to form defined folding structures. Although attempts were made to investigate the folding propensity of sulffono-γ-AApeptides by 2D NMR,[16] the structure generated based on NOE-restrained molecular dynamics remains ambiguous since the helical handedness could not be derived, and the hydrogen-bonding pattern is inconclusive due to dynamic solution structures. Atomic level of structures are highly demanded to precisely elucidate the helicity and hydrogen-bonding pattern of this new helix. Herein, we report the first crystal structures of homogeneous L-sulffono-γ-AApeptide oligomers. High-resolution X-ray crystal structures of these homogeneous foldamers unambiguously delineate their sequence-structure relationships, revealing well-folded, left-handed helical structures of the entire set of oligomers. These results provide a structural basis for designing de novo foldameric structure of this type as ordered biopolymers and potential therapeutic agents in the future.

The homogeneous L-sulffono-γ-AA oligomers were developed with five building units, with or without acetylation on the N-terminus. To exclude the potential impact of side chains on the folding propensity, initially L-methyl-sulffono-γ-AA with chlorobenzene sulfonyl group was chosen (Figure 1b). All together four oligomers (oligomers 1a–2b) were synthesized

She et al. Page 2

Angew Chem Int Ed Engl. Author manuscript; available in PMC 2019 July 26.
and feasibly obtained from solid phase Fmoc chemistry according to protocol reported previously.\textsuperscript{[14]} To test the generality of forming foldamers, the side chains on the oligomeric sequences include both cationic NH\textsubscript{2}, anionic COOH, as well as hydrophobic 4-chlorobenzenesulfonyl residues (oligomers 3a−5a). A sequence containing only methyl side chains (6a) was also synthesized.

After a series of attempts, we first obtained single crystals of 1a suitable for X-ray diffraction analysis at resolution of 1.1 Å from a mixture of acetonitrile and H\textsubscript{2}O. Surprisingly, unlike classic α-helices or recently developed 4.5\textsubscript{16}−14 and 4\textsubscript{13} helices based on heterogeneous backbones,\textsuperscript{[14−15]} this homogeneous L-sulfono-γ-AA oligomer adopts an unprecedented left-handed helical structure with unanomous 4-helical fold, with radius of 2.8 Å and helical pitch of 5.1 Å (Figure 2a). The helix possesses four distinct helical faces, with side chains aligned at 90º intervals to form a rectangular shape when viewed down the helix axis. The side chains on the four helical faces perfectly line on top of each other, somewhat resembling dragon boat paddling. In addition to unusual folding parameters, the handedness of the crystal structure was surprising, since L-peptides are well known to generally adopt right-handed helical conformations.\textsuperscript{[17]} This finding is critical as it demonstrates that our previously hypothesized structure of this class of foldamer\textsuperscript{[16]} to be the right-handed helix is wrong. Moreover, oligomer 1a shows highly consistent 14-hydrogen bonding pattern (Figure 2b), formed between the N-H group of the L-sulfono-γ-AA residue and the C=O group of the L-sulfono-γ-AA three residues later, namely $i \rightarrow i + 3$ hydrogen bonding with a distance of 2.2 Å (N−H···O=C distance). This lead to the formation of a macrodipole with partial positive charge at the C-terminus and partial negative charge at the N-terminus, which is opposite to the macrodipole formed in canonical α-helix. Due to its unique hydrogen bonding pattern and arrangement of side chains, the name 4\textsubscript{14}-helix is designated hereafter, indicating that four side chains (three residues) are included in the pseudo helical loop, and 14 atoms are involved in the ring formed by the intramolecular hydrogen bonds. The 4\textsubscript{14}-helix is less tightly packed compared with 3\textsubscript{10}-helix, α-helix, and even π-helix, however possessing an unprecedented ordered C\textsubscript{2} symmetric helix. In crystal packing, the individual helical segments are arranged in a hydrogen-bonding driven head-to-tail manner to give regularly elongated helical treads (Figure 2c).

Direct comparison of the 4\textsubscript{14}-helix with other types of natural helical peptides (Table 1) unveils that it resembles the left-handed counterpart of the right-handed π-helix, with similar helical pitch and diameter. However, the projection of side chains are completely distinct from that of π-helix, demonstrating a de novo scaffold. The side chains of 4\textsubscript{14}-helix project away from the helical axis, and it is of particular interest that both chiral side chains at γ-position and sulfono side chains on the L-sulfono-γ-AA residues form a C\textsubscript{2} symmetric rectangle structure when viewed along the peptide axis. Both type of side chains point toward the C-terminus due to the (S)-configuration of the sulfono-γ-AA residues in the scaffold.

Notably, we were able to obtain more crystalline structures when incorporating both cationic NH\textsubscript{2} and anionic COOH groups on the chiral side chains. From solvent CHCl\textsubscript{3}/MeOH and acetonitrile, oligomers 3a and 4b (Figure 3), which bear with both amino and carboxylic groups, with or without acetyl capping group at N-terminus respectively, were crystalized.
and resolved by high-resolution single-crystal X-ray crystallography with resolution of 1.0 Å. 4b shows slightly different packing mode as the adjacent parallel helices are packing in different way (Figure 3b), however, the crystal structure of 4b reveals exactly the same left-handed 414-helix. Compared with 1a, oligomer 4b bears one more L-sulfono-γ-AA residue in length and one Lys and two Glu chiral side chains, which confer it with much better solubility in the majority of solvents, even in the presence of 10% H2O. Further attempts furnished the crystal structure of 3a, which has one more L-sulfono-γ-AA residues in length with a Lys chiral side chain compared to 4b. Consistent with the helical conformation of 4b, 3a (Figure 3c) also adopts the same left-handed 414-helix configuration.

Finally, oligomer 6a, bearing just methyl side chains, also crystallized from CH3CN/MeOH/CH2Cl2 with suitable quality for X-ray crystallography analysis. As anticipated, the crystal analysis revealed a left-handed helical structure, with the same helical pitch and diameter as those of the other oligomers (Figure 4). The ability of L-sulfono-γ-AApeptides to form ubiquitous left-handed helices regardless of side chain identity demonstrates their unanimous backbone folding propensity and augments their potential for applications in biological functional materials or self-assembly architectures.

The average backbone torsion angles ϕ, θ, η, ξ, ψ, φ′, θ′, η′, ξ′, and ψ′ in each helical loop are quite unanimous across all structures (Table S7). The torsion angles of adjacent L-sulfono-γ-AA residues in each oligomer are also very close. Specifically, the homogeneous L-sulfono-γ-AA endow unique backbone torsion angles ϕ (−138+/−2°), θ (66+/−5°), η (−120+/−5°), ξ (92+/−5°), and ψ (−141+/−5°), which are apparently distinct from that of heterogeneous α/D-sulfono-γ-AA (2:1 pattern) foldamer (ϕ, θ, η, ξ, ψ = 117+/−10°, −77+/−4°, 79+/−2°, 60+/−4°, −169+/−2°) with the incorporation of α-residues.[14] The torsion angles of these residues also reasonably differ from α-helices, β-sheets and the previously reported natural or synthetic peptides.[4b, 6, 18] These unique torsion angles could pave the way to the creation of finite helical bundles in materials or the rational design of unnatural helical structures.

To further investigate the solution conformation for these types of homogeneous foldamers, oligomer 4b was selected as a representative example for 2D NMR experiments. gDQFCOSY, zTOCSY, and NOESY spectra were recorded at a concentration of 5 mM in CD3OH at 10°C in order to assign the backbone protons. As shown in Figure 5, two types of long-range NOEs among protons on the scaffold were detected: (a) i, i+2 NOEs, correlations between amide protons of L-sulfono-γ-AA and methylene/γ-CH protons two residues down either direction of the oligomer; (b) chimeric i, i+3 NOEs, correlations between α protons of the L-sulfono-γ-AA and amide protons of the L-sulfono-γ-AA three residues earlier. These detected NOEs are consistent with the i → i + 3 hydrogen bonding pattern found in crystal structures and suggest that this homogeneous L-sulfono-γ-AApeptide foldamer is helical in methanol.

To further evaluate the helical propensity in solution, circular dichroism (CD) spectroscopy of sequences was conducted. Five oligomers, including a homogeneous sequence with the same side chains (1a), sequences with both cationic and anionic side chains (3a, 4b, and 5a), and an oligomer without any aromatic side chains (6a), revealed a positive cotton effect at

*Angew Chem Int Ed Engl.* Author manuscript; available in PMC 2019 July 26.
215–218 nm (Figure 6a), the intensity of which is both length and side chain dependent. The hexamer 1a displayed a maximum at 218 nm, while the ellipticity of oligomers possessing NH₂/COOH sidechains (5a, heptamer 4b, and octamer 3a) also consistently exhibit the same maximum. The CD signal of 6a was considerably weaker than other oligomers due to its lack of phenyl substituents on the sulfonyl residues, nonetheless, similar pattern of cotton effect was observed. The stability of secondary structures could be slightly affected by solvents as trifluoroethanol (TFE) and acetonitrile, methanol or H₂O (Figure 6b and 6c), but overall the helical structures were very stable in various solvents. It should be noted that the peaks at 240 nm are not indicative of secondary structures, similar to what we have demonstrated for hybrid oligomers before.[14]

The helical stability of oligomer 1a and 4b at various concentrations was also investigated in solution. The CD spectra of oligomer 1a and 4b revealed consistently helical conformation from 6.25 to 100 μM (Figure 7a and 7b). Furthermore, Figure 7c and 7d indicate that only a slight decrease of signal intensity took place over the 5–55 °C temperature range, where 2 nm of red shifts were witnessed when temperature increased over 50 °C. These results indicated that this type of oligomer is highly stable in both diluted solution and at elevated temperatures, possibly due to the strong intramolecular hydrogen bonding and the curvature of sulfonamide moieties.

To investigate the preference of homogeneous L-sulfono-γ-AA foldamers to adopt left-handed helical configurations in solution, molecular dynamics (MD) simulations were performed for 4b in the left-handed helical configuration of the X-ray structure as well as a modelled right-handed helical conformation (Figure S8). These configurations were solvated in methanol, and multiple simulations were run for each system. The left-handed systems retained their helical integrity throughout the 1 μs production runs, whereas the helical structure was quickly lost in the right-handed system (Figure S8a, S8b). The average heavy atom backbone root mean squared deviations (rmsd) of the left-handed 4b systems were significantly lower than that of the right-handed systems (~1 versus ~5 Å).

The number of backbone hydrogen bonds as a function of rmsd is shown in Figure S8a–S8b. For the left-handed helix 4b, all 7 hydrogen bonds were made for configurations with a rmsd below 1.0 Å. These were the dominant structures in the simulations (Figure S8c). A loss of one hydrogen bond occurred for rmsd values between 1.0 and 2.0 Å; these corresponded to configurations with a frayed N or C terminus. While fraying of the N terminus was more frequently observed than fraying of the C terminus, both were minor species (Figure S8c). A hydrogen bond at each terminus was lost when the rmsd surpassed 2.0 Å, which rarely occurred in the left-handed helical systems. The loss of hydrogen bonding and helical structure of the termini was reversible, reflected by a quick return of all rmsd peaks back to sub 1.0 Å values. In contrast, almost no hydrogen bonds were made in the right-handed system due to its inability to retain a helical structure (Figure S8b, S8d).

The preference of 4b to form a left-handed rather than right-handed helix could also be traced by energy decompositions of the minimized structures. It was found that the dihedral energy (~26 kcal/mol) contributed greatest of all bonded and nonbonded energy terms to the total potential energy difference (~84 kcal/mol). The torsion angles about the C7-N bond
possessed the greatest total differential stability (~19 kcal/mol). The single largest contributor was the Cβ-Cγ-N-C dihedral term, which contributed 1.8 kcal/mol per dihedral or ~14 kcal/mol in total to the potential energy difference (Figure S8e). This dihedral angle was on average −17° in the minimized left-handed helix, and 105° in the minimized right-handed structure. As a result, the carbonyl oxygen and the methyl were staggered in the minimized left-handed helix, while eclipsed in the minimized right-handed helix.

In summary, we report an unprecedented left-handed helical secondary structure of homogeneous L-sulfono-γ-AA foldamers. A series of crystals adopted well-defined left-handed helical conformations with a 4,14-helix pattern in the solid state.[19] The presence of this secondary structure in solution was supported by CD spectroscopic data in various solvents, NMR, and MD simulations. The preference of left-handed helix formation was rationalized by MD simulations in methanol. By showing that sulfono-γ-AA peptides form well-defined left-handed helices, our study greatly expands the repertoire of AApptides for the design of biopolymers, materials and self-assembly architectures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References


[19]. CCDC database 1841091–1841094 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.
Figure 1.
(a) General structures of α-peptides, L-γ-AApeptides, L-sulfono-γ-AApeptides. (b), (c), (d), (e) Homogeneous L-sulfono-γ-AA peptidic oligomers prepared for structural and spectroscopic evaluation in this study.
Figure 2. Single crystal structure of 1a.
(a) Side and top views of crystal structure of 1a. Hydrogen bonding is shown in red. (b) The intramolecular 14-hydrogen-bonding pattern of 1a detected in the crystal structure. (c) Crystal packing of 1a viewed perpendicular and down to the helix axis.
Figure 3. Single crystal structure of 4b.
(a) Side and top views of crystal structure of 4b. (b) Crystal packing or 4b viewed perpendicular and then down to the helix axis. c) Sequence structure of oligomer 3a. (d) Sequence structure of 4b.
Figure 4. Comparison between crystal structures of 1a (a), 3a (b), 4b (c), and 6a (d).
(e) Sequence structure of oligomer 6a.
Figure 5. Summary of detected NOESY crosspeaks of 5 mM oligomer 4b between protons on nonadjacent residues in CD$_3$OH (10 °C). Three types of NOEs are displayed in different color. Each L-sulfono-$\gamma$-AA unit is considered as two residues, since the L-sulfono-$\gamma$-AA building block is equal to two $\alpha$ amino acid in length.
Figure 6.
(a) CD spectra of 1a, 3a, 4b, 5a, and 6a (80 μM) measured at room temperature in TFE. (b) CD spectra of 1a (80 μM) in various solvents at room temperature. (c) CD spectra of 4b (80 μM) in various solvents at room temperature.
Figure 7.
(a) CD spectra of 1a in TFE at various concentrations (6.25–100 μM) at room temperature.
(b) CD spectra of 4b in TFE at various concentrations (6.25–100 μM) at room temperature.
(c) CD spectra of oligomer 1a (80 μM) in TFE at various temperatures. (b) CD spectra of 4b (80 μM) in TFE at various temperatures.
**Table 1.**
Parameters of helical structures found in proteins, $\alpha_{13}$-helix, and $\alpha_{14}$-helix

<table>
<thead>
<tr>
<th>Secondary Structures</th>
<th>Handedness</th>
<th>Helical Pitch $p$ (Å)</th>
<th>Radius of Helix $r$ (Å)</th>
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<td>$\alpha$-helix</td>
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<td>5.4</td>
<td>2.3</td>
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<tr>
<td>$3_{10}$-helix</td>
<td>Right-handed</td>
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<td>1.9</td>
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<tr>
<td>$\pi$-helix</td>
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<td>5.0</td>
<td>2.8</td>
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<td>3.0</td>
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<tr>
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