Helical Foldamers

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The Canonical Helix of Urea Oligomers at Atomic Resolution: Insights Into Folding-Induced Axial Organization**

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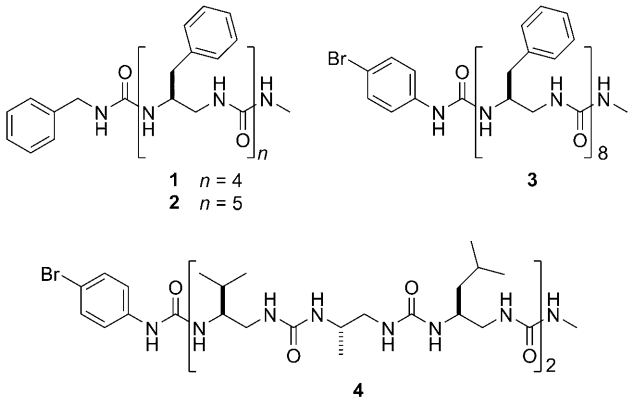
Foldamers are discrete artificial oligomers with defined and predictable folding patterns akin to naturally occurring helices, turns, and linear strands.[1–3] Because of their diversity in size, shape, and side chain appendages, and also their resistance to enzymatic degradation, peptidomimetic helical foldamers are unique scaffolds for use in a range of biological and biomedical applications.[4] Characterizing such helical folds at atomic resolution is of prime importance if molecules are to be designed that can target biological surfaces and for reliable structure–function analysis. To date, extensive crystallographic data sets have been gathered on aliphatic (β- and α/β-peptides) and aromatic oligoamides,[3] thus providing a detailed picture of the structural diversity within these foldamer families. Few other helical peptidomimetic backbones have been characterized by crystallographic analysis.[5–10] Crystal structures are also central to gain precise insight into axial[11] and lateral[12,13] self-assembling properties of helical foldamers, en route to new tertiary and quaternary structural motifs and more sophisticated self-assembled nanostructures. Notable achievements include the atomic structure determination of large (> 8 kDa) aromatic oligoamide foldamers[14] and helix-bundle quaternary structures formed by designed β- and α/β-peptides.[12,13]

Oligomers consisting of *N*,*N*′-linked urea bridging units are receiving increasing attention as folding backbones.[9,15–20] Peptidomimetic oligoureas belonging to the γ-peptide lineage,[21] (-NH-CH(R^i)-CH_2N′H-CO)$_n$-, have a remarkable propensity to fold into helical secondary structures in solution[22–26] and show promise for interaction with biologically relevant targets.[27,28] Compared to γ-peptides,[5,29,30] helix stabilization in oligoureas is promoted by the presence of additional backbone conformational restriction and H-bond donor sites. Although three-centered H-bonding between C=O(*i*) and urea HN(*i*−3) and HN′(*i*−2) to form 12- and 14-membered pseudo rings is likely to occur, detailed NMR investigations provided some evidence for H-bond dissymmetry with d(O(*i*)–N(*i*−3)) < d(O(*i*)–N′(*i*−2)). Notwithstanding the high quality of structure calculations recently achieved using NMR spectroscopy at ^{13}C natural abundance,[31] structural data at atomic resolution are clearly needed to precisely describe the H-bonding pattern of the 2.5 helix and facilitate the design of functionally active oligoureas and more complex structures. Herein, we report the first crystal structures of enantiopure *N*,*N*′-linked oligoureas with from 5 to 9 urea groups and containing exclusively acyclic residues with proteinogenic side chains (that is, Me, *i*Pr, *i*Bu, and Bn).



1 *n* = 4
2 *n* = 5
3
4

Oligoureas **1**–**4** have been prepared in solution by stepwise assembly of *N*-Boc protected monomers.[32] The nature of the capping groups at both ends of oligomers was critical to grow high-quality crystals. The 4-bromophenyl moiety in **3** and **4** was introduced to facilitate phasing. Crystal structures of **1**–**4** were solved in the *C*2, *C*2, $P4_1$, and $P2_12_12$ space groups, respectively.[32]

All four oligoureas, including short tetramer **1**, are fully helical in the solid state (Figure 1 and 2; Supporting Information, Figure S1).[33] They adopt a right-handed 2.5-helical fold that matches the conformation initially proposed from

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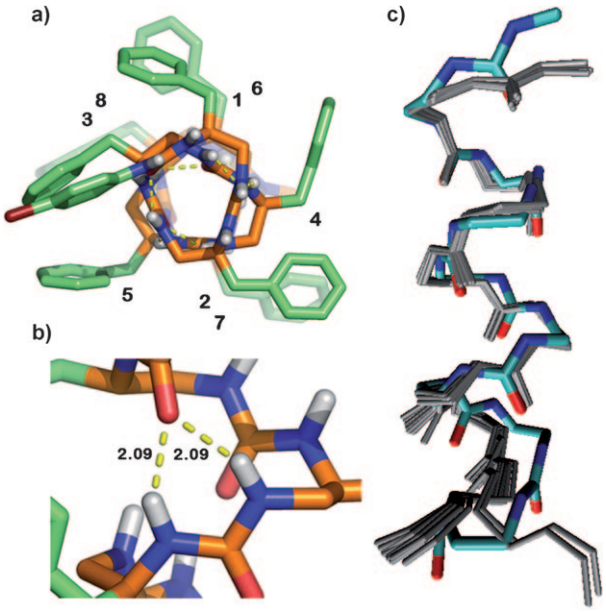

Figure 1. Crystal structure of **3**. a) View along helical axis. b) Detail of the three-centered H-bonding. O(7)···HN(4) and O(7)···HN′(5) distances in Å. c) Overlay with the 20 best NMR structures (in gray) of a related nona-urea.[31] Side chains are omitted for clarity.

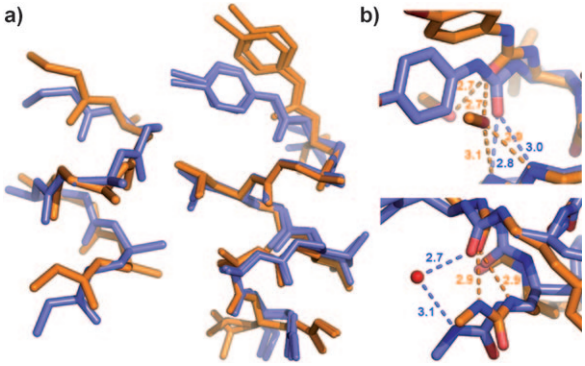

Figure 2. X-ray crystal structures of **1** and **4**. a) Overlay of independent molecules (blue and orange) in **1** (left) and **4** (right). Side chains in **1** have been removed for clarity. b) H-bond network at upper and lower extremities of the helix in **4** (top) and **1** (bottom), showing conformational differences between independent molecules (carbon atoms in blue and orange). D···A distances in Å.

NMR spectroscopy studies of oligoureas in solution.[23–25] Octamer **3**, which has one single conformation in the crystal, has a nearly perfect repeat of 2.5 residues per turn (Figure 1a) and a rise per turn of 5.1 Å. The average backbone torsion angles ϕ, θ_1, and θ_2[34] are $-103°$, $+57°$, and $+80°$, and are characterized by relatively low RMSD values (11°, 5°, 7°). Such a regular fold is allowed, as all possible intramolecular 12- and 14-membered H-bonded rings (i.e. 14) are present in the crystal structure of **3** (Figure 1). Distances between O(i) and H-bonded N′($i-2$) and N($i-3$) are very similar, and range from 2.78 to 2.99 Å and 2.82 to 3.00 Å, respectively. The O(i)-H-N′($i-2$) and O(i)-H-N($i-3$) angles are comparable with an average value of 139° (RMSD of 9° and 11° respectively).

Overlay of the X-ray structure of octamer **3** and the optimized structure of a related nona-urea determined by NMR spectroscopy[31] provides precise insight into the behavior of this helical fold in solution (Figure 1c). First, the helix in solution is clearly distorted at both ends, which is a direct consequence of an incomplete H-bond network. Indeed, the carbonyl group of the residue 9 in the nonamer only establishes an H-bond with the amide proton of the residue 6. Compared to the crystal structure, the absence of H-bonding between O(9) and HN′(7) modifies the orientation of the residue 9 urea plane and thus slightly distorts the helix axis. The same feature is found for the residue 4, which is not H-bonded to the HN' amide of residue 2 but only to HN of residue 1. As no H-bond occurs for the preceding residues, the corresponding tail appears much more flexible than in the solid state structure. The detailed conformations of residues 4 and 9 highlight the weaker H-bond character of the HN' amide compared to the HN amide. Such an observation is also made for central residues of the helix, for which slightly greater distances accompanied with smaller O-H-N angles are found between O(i) and N′($i-2$) than for O(i) and N($i-3$). This structural feature is in very good agreement with experimental data recorded on oligoureas in pyridine, for which temperature coefficients of well-structured regions were all found to be greater for the HN amide (between -2.0 and -3.5 ppb K^{-1}) than for HN′ (between -4.4 and -5.6 ppb K^{-1}),[25] but differs from the crystal structure of **3**, in which both H-bonds are equivalent. Torsion angles ϕ, θ_1, and θ_2 obtained over the 20 best NMR spectroscopically determined structures for the well-defined segments are $-100°$, 43°, and 96°, with RMSD values of 1°, 6°, and 7°; these values are comparable to those measured in the crystal structure of **3**. The difference of about 15° for θ_1 and θ_2 could be related to the significant planarity deviations found for the urea motifs in the crystal structure. Indeed, C-*N*′-C′-O dihedral angles range from 5° to 18° and C-*N*-C′-O angles from $-23°$ to $-6°$. As a consequence, whereas C-*N*′-*N*-C dihedral angles are between 0 and 5° for all the calculated structures determined from NMR spectroscopy, they range between $-23°$ and 18° in the X-ray structure. This result shows that the double-bond character of the amide bond is significantly reduced within urea motifs. Overall, slight differences in H-bonding in NMR and X-ray structures and in the distortion of urea planes observed in the crystal structure account for the 2.5 Å root-mean-square deviation obtained when aligning solution and crystal structures (Figure 1c).

Shorter oligoureas **1**, **2**, and **4** crystallized with two (**1** and **2**) or four (**4**) independent molecules in the asymmetric unit (Figure 2a; Supporting Information, Figure S1). In all three cases, the conformations of independent molecules differ perceptibly, with the largest differences being localized at both ends (Figure 2). These distortions, which are presumably caused by packing effects and/or H-bonded interaction with solvent molecules (H_2O, MeOH), reflect conformational plasticity of shorter helices. For example, in the crystal of tetra-urea **1**, the O–N distance between O(3) and NH(Me) increases from 2.91 Å (molecule A) to 3.67 Å (molecule B) owing to the insertion of a bridging water molecule (Fig-

ure 2b, lower). Differences between **1**(A) and **1**(B) propagate along the backbone, with a shift of O(4) from an equidistant position between N(1) and N′(2) in **1**(A) to a N′(2) distal position with loss of the corresponding 12-membered H-bonded ring in **1**(B). This situation parallels that observed in solution (see below), thus reflecting a hierarchy in the formation of H-bonds. Distortions imposed by solvation at the helix termini are even more pronounced in the structure of hepta-urea **4**. Whereas HN′(5) and HN(4) are directly H-bonded to O(7) in **4**(A) and **4**(B), a bridging methanol molecule is intercalated in **4**(C) and **4**(D), thus the O(7)–N′(5) and O(7)–N(4) distances increase to about 4.7 Å and 4.3 Å, respectively (Figure 2c). At the other end of the molecule, residue 1 in **4**(B) adopts a non-canonical geometry, with a θ_2 angle value of +156° (compare with +86° in **4**(A); Supporting Information, Figure S2).

Oligourea helices possess four free donor and two acceptor groups that are capable of H-bonding at both ends of the molecule. Head-to-tail arrangements of helices are frequently observed in the crystal structures of DNA oligonucleotides,[35] hydrophobic helical α-peptides,[36,37] and β-peptide foldamers.[11] Generally these molecules form long cylindrical helical columns.

By analogy, independent helices of pentaurea **1** and hexaurea **2** in the asymmetric unit are aligned end-to-end with formation of all possible three-centered H-bonds in the head-to-tail regions (Figure 3a, top; 3b, left; Supporting Information, Figure S3). In heptamer **4**, the H-bonded network at the boundary between neighboring helices differs substantially: whereas complementary H-bonding sites between helices B and C (A and D) are satisfied, assembly at the boundary between helices C and A (D and B) is mediated in part by a relay of methanol molecules (Figure 3a, bottom). Packing of neighboring helices in **4** curves slightly (16° between A and C, and 31° between C and B) (Figure 3b right) and forms a wavy column. The bend is even more striking in nona-urea **3**, in which helices self-assemble by using a non-canonical water-mediated intermolecular H-bonding pattern (6 H-bonds in the head-to-tail region). In contrast to what is observed in **1**, **2**, and **4**, O(1) and O(2) are not H-bounded to the penultimate and ultimate urea moieties of a neighboring helix, but rather to the ultimate and penultimate ones, respectively (Figure 3c, top). As a result, two neighboring helices are oriented at an angle of ca 65° (Figure 3c, bottom), and crystal formation generates an H-bonded right-handed superhelix (Figure 4).

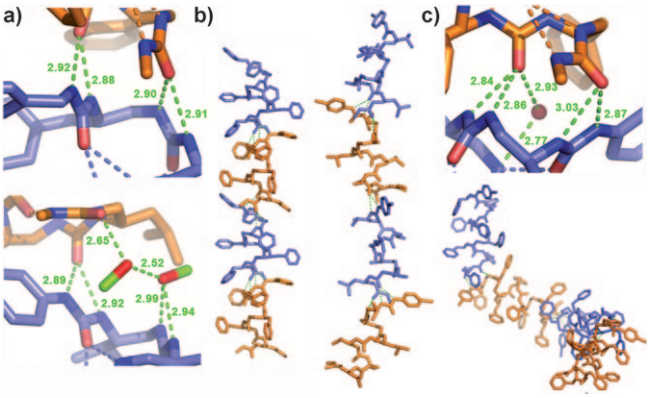

Figure 3. Assembly of urea helices. a) H-bonding network between pairs of helices in **2** (top) and **4** (bottom). b) Packing of helices in **2** (left) and **4** (right). c) Non-canonical intermolecular H-bonding pattern (top) in **3** and orientation of neighboring helices at an angle (bottom). Intermolecular H-bonds in green. D···A distances in Å.

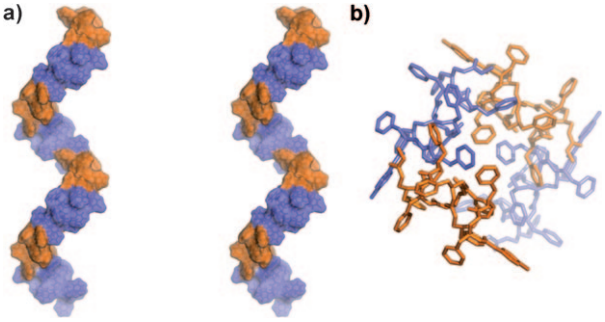

Figure 4. Supramolecular helix formed by H-bonded assembly of helices **3**. a) Side view in stereo. b) View along helical axis. Pitch: ca. 48.0 Å, internal diameter: ca. 7 Å, external diameter (with side chains): ca. 28.0 Å, groove: ca. 29.6 Å.

The canonical 2.5 helical structure of oligoureas has been characterized at atomic resolution. The similarity between the structures reported herein and those deduced earlier from NMR studies in solution is striking and underlines the excellent complementarities of the two techniques to analyze urea-based foldamers.[22–25,31,38] Our study also demonstrates the robustness of the folding process: four acyclic residues are sufficient to drive complete helix formation with all complementary H-bonding sites being satisfied. Overall, this crystallographic data set provides the ground for the structure-guided development of urea foldamers with function, such as recognition of biomolecules and catalysis,[20,39,40] and also for the elaboration of new tertiary and quaternary structural motifs.

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[32] See the Supporting Information.
[33] In solution, as shown by NMR and circular dichroism, oligoureas **1**, **2**, **4**, and **5**, an analogue of 3 with the bromophenyl cap substituted by a benzyl group, have the hallmarks of the 2.5-helical structure. See the Supporting Information.
[34] The torsion angles have been designated by analogy to the torsion angles of α- and β-amino acid residues. See the Supporting Information for details.
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