Chiral N-substituted glycines can form stable helical conformations

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Background: Short sequence-specific heteropolymers of N-substituted glycines (peptoids) have emerged as promising tools for drug discovery. Recent work on medium-length peptoids containing chiral centers in their sidechains has demonstrated the existence of stable chiral conformations in solution. In this report, we explore the conformational properties of these Nα chiral peptoids by molecular mechanics calculations and we propose a model for the solution conformation of an octamer of (*S*)-N-(1-phenylethyl)glycine.

Results: Molecular mechanics calculations indicate that the presence of N-substituents in which the Nα carbons are chiral centers has a dramatic impact on the available backbone conformations. These results are supported by semi-empirical quantum mechanical calculations and coincide qualitatively with simple steric considerations. They suggest that an octamer of (*S*)-N-(1-phenylethyl)glycine should form a right-handed helix with *cis* amide bonds, similar to the polyproline type I helix. This model is consistent with circular dichroism studies of these molecules.

Conclusions: Peptoid oligomers containing chiral centers in their sidechains present a new structural paradigm that has promising implications for the design of stably folded molecules. We expect that their novel structure may provide a scaffold to create heteropolymers with useful functionality.

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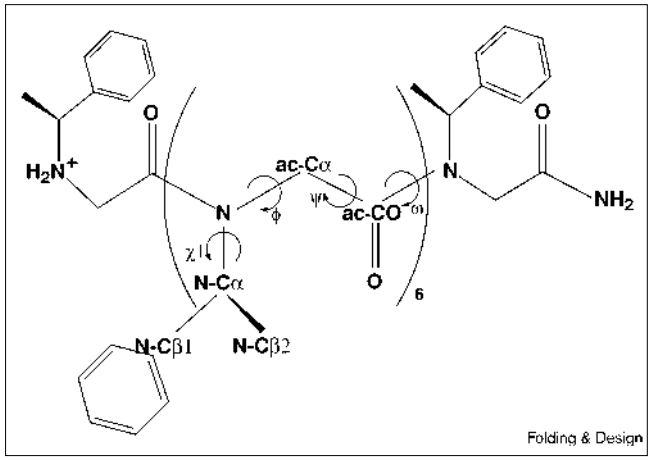
Introduction

Peptoids are polymers of N-substituted glycines; they differ from peptides in that the peptoid sidechains are attached to the backbone nitrogens instead of to the backbone α-carbons. Heteropolymeric peptoids have the potential to become a powerful and flexible system with which to create new molecules able to carry out therapeutic, diagnostic or structural functions [1,2]. Their synthesis is sequence specific, efficient, automatable, and inexpensive, owing to the simplicity of the component synthons [1]. Moreover, a submonomer synthesis protocol allows the incorporation of a large variety of amines as the N-substituent sidechains [3]. But ultimately, the feasibility of rationally designing functional peptoids will depend on the structural properties of this class of molecules, as well as on our ability to understand and use these properties. Therefore, we are currently investigating the nature, stability, and origins of the folded conformations adopted by medium-length peptoids, as well as the effect of the sidechains on the backbone conformation, using both experimental and computational methods.

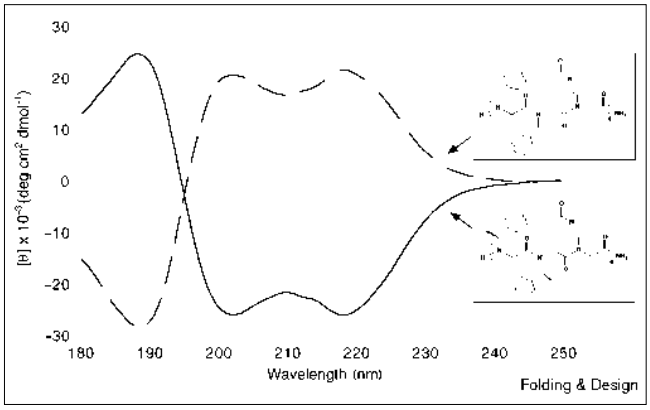
Recent work on peptoids containing chiral centers in their sidechains at the Nα position has demonstrated the existence of stable folded conformations in aqueous and organic solvents for chain lengths of 5–30 residues [4]. The CD spectra of molecules with specific sidechain sequences show a double minimum with the lowest points around 200 nm and 220 nm accompanied by a maximum around 190 nm, which is strongly reminiscent of the α-helical signal in peptides. Our data strongly support the argument that this signal arises from a regular ordering of the backbone amide chromophores, and that the ordering does not occur through aggregation [4]. This suggests that a significant fraction of the molecules in solution are monomeric and adopt a regularly repeating conformation of specific handedness. In order to develop a basic understanding of peptoid structure, to assist in the interpretation of our experimental data, and to guide peptoid design efforts, we have modeled this conformation at the atomic level. While there have been previous reports on the modeling of oligopeptoids [1,5], they have focused on sarcosine (in which the sidechain is simply a methyl group). But the presence of chiral di-substituted Nα carbons drastically affects the spectroscopic characteristics of the molecules [4]. Thus, a re-examination of their conformational properties is warranted. In this report, we study the available helical geometries of Nα–chiral peptoids (a subset of peptoid secondary structure) and the minimum-energy conformation. The molecule that we have chosen for study, based on its spectroscopic characteristics, is an octomer of (*S*)-N-(1-phenylethyl)glycine, which we call $(Nspe)_8$. It is shown in Figure 1, along with the atom and dihedral angle nomenclature used in this report. The CD

Figure 1



An Octamer of (*S*)-N-(1-phenylethyl)glycine, $(Nspe)_8$. The N terminus is free and the C terminus is amidated. This is the molecule that was used in all calculations unless stated otherwise. Also shown are the mainchain dihedral angles: ϕ, ψ and ω are defined as for glycine; $\chi 1$ refers to the $C_{(i-1)}$,$N_{(i)}$,N–C$\alpha_{(i)}$,N–C$\beta 1_{(i)}$ dihedral angle. Atom names follow the nomenclature devised in [17]. Note that as we are dealing here with homopolymers, the sidechain name in subscript has been omitted from the names of sidechain atoms (e.g. N_{xxx}Cα is referred to as N–Cα).

Figure 2



CD spectra of peptoids containing N-(1-phenylethyl)glycines. This shows the characteristic double peak around 200 nm and 220 nm, as well as the peak of opposite sign around 190 nm, for a dodecamer containing two-thirds chiral sidechains Nspe and one-third achiral sidechains (N-(2-acetamidoethyl)glycine). Note how changing the chirality of the Nspe sidechain inverts the sign of the ellipticity.

spectra of Nspe homopolymers display the maximum and double minimum just described. Figure 2 shows that this signal is present even when one-third of the sidechains are achiral, and that reversing the chirality of the Nspe sidechains reverses the sign of the ellipticity.

Results and discussion

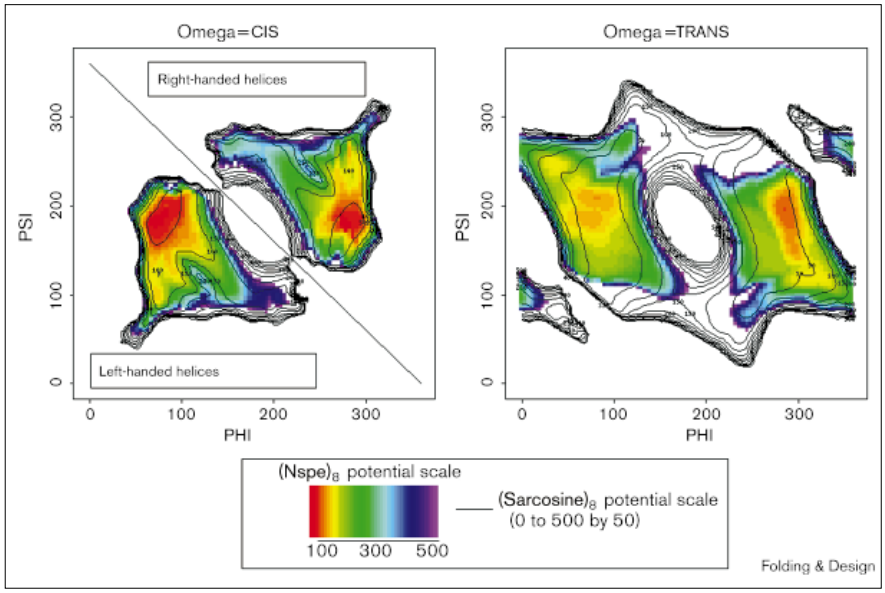
Conformational maps

Following the classic approach of Ramachandran *et al.* [6] for peptides, we obtained maps of the allowed regular conformations of a sarcosine octomer $(sarcosine)_8$, which is unsubstituted at N–Cα, and $(Nspe)_8$, which is N–Cα substituted, with the amide bond (the ω dihedral angle) adopting either a *cis* or a *trans* conformation (Figure 3). In all cases, bond lengths and angles were kept constant, and conformations were evaluated using the all-atom AMBER molecular mechanics force field [7]. We also performed the calculations on an Nspe-containing dipeptoid while allowing all bond lengths and angles to relax; the maps thus obtained were flatter, but the positions of the minima and their relative depths did not change appreciably (data not shown). We draw three conclusions from this analysis. First, the maps for $(Nspe)_8$ are distinctly asymmetric, unlike those for $(sarcosine)_8$. This asymmetry implies that the chiral centers in the sidechains can give rise to a significant preference of backbone handedness. This is consistent with the CD features of Nspe-containing peptoids: the double minimum indicates a chiral backbone conformation, the handedness of which is determined by the chirality of the sidechain (Figure 2). Second, the maps show that the minima for $(Nspe)_8$ in the *cis* conformation are lower in energy (more favorable) than those in the *trans* conformation, while this preference is reversed for $(sarcosine)_8$. This can be understood from simple steric considerations, by assuming that the bulkiest groups on either side of the C–N amide bond will prefer to be *trans* to each other (Figure 4). Of the two groups bonded to the carbonyl carbon, the methylene group of the mainchain Cα is the bulkier. It will prefer to be *trans* to the bulkier group bonded to the nitrogen. In the case of $Nspe_8$, this will be the di-substituted sidechain N–Cα (Figure 4a,b), and in the case of $sarcosine_8$, this will be the mono-substituted mainchain ac–Cα (Figure 4c,d). Third, for each value of ω, there are apparently only two broad regions of backbone conformational space that are easily accessible, and their backbones are mirror images of each other. In those regions, the presence of the sidechain has little impact on the shape of the energy landscape in the *cis* conformation. This holds true for a variety of sidechains (data not shown).

Together, these results argue that the secondary structure of Nα chiral peptoids may be easier to predict than that of peptides. Indeed, chirality at the Nα atoms should be sufficient to impart a chiral preference to the backbone. In addition, any chiral carbon at the Nα position will be bulkier than the mainchain mono-substituted ac–Cα, leading the amide bond to adopt a *cis* geometry (Figure 4). Taken together, these two restraints could then force backbone dihedral angles to lie within a narrow and predictable range (Figure 3): ϕ between –120° and –60° for the (*S*)-N-(1-phenylethyl)glycine isomer (or between 60° and 120° for the *R* isomer), and ψ between 150° and 210°. Here, we consider only regular backbone conformations, and not turns or other irregular structures.

Figure 3

Conformational maps of peptoid octamers. The potential of $(Nspe)_8$ is shown in color and the potential of $(sarcosine)_8$ by black contour lines. 'CIS' denotes octamers with ω (omega) values around 0°, while 'TRANS' denotes octamers with ω values around 180°. The cutoffs are 500 units in all cases. Note that as bond lengths and angles are fixed, the units cannot be interpreted in kcal/mol. A line has been drawn on the *cis* map to separate right-handed from left-handed helices. Points that are symmetrical about the center of a map represent mirror-image backbone structures.



Minimum energy conformation

To model in greater detail the most energetically favorable conformation of $(Nspe)_8$, we sampled the space of simple helical conformations (see the Materials and methods section), again using the AMBER force field [7] and allowing all bond lengths and angles to relax. After minimization, we retained the four conformations of lowest energy: two enantiomeric *cis* helices ($\omega \approx 0°$, $\phi \approx -75°$, $\psi \approx 170°$, right-handed; $\omega \approx 0°$, $\phi \approx 75°$, $\psi \approx -170°$, left-handed), and two enantiomeric *trans* helices ($\omega \approx 170°$, $\phi \approx -75°$, $\psi \approx 180°$, left-handed; $\omega \approx 185°$, $\phi \approx 70°$, $\psi \approx 170°$, right-handed). Of these, the *cis* right-handed helix had the lowest energy (Table 1a).

Figure 4

Interactions around the amide bond: a representative residue from $(Nspe)_8$ with **(a)** *cis* or **(b)** *trans* ω (ω is the amide bond dihedral angle defined by the green mainchain carbons) and poly-sarcosine with **(c)** *cis* or **(d)** *trans* ω. The bulkiest groups on either side of the amide bond are highlighted with orange (conformations where the two orange groups are *trans* to each other avoid the steric repulsion (double line) that occurs when they are *cis*). Mainchain carbons are in green, nitrogens in blue, oxygens in red, and sidechain carbons in yellow.

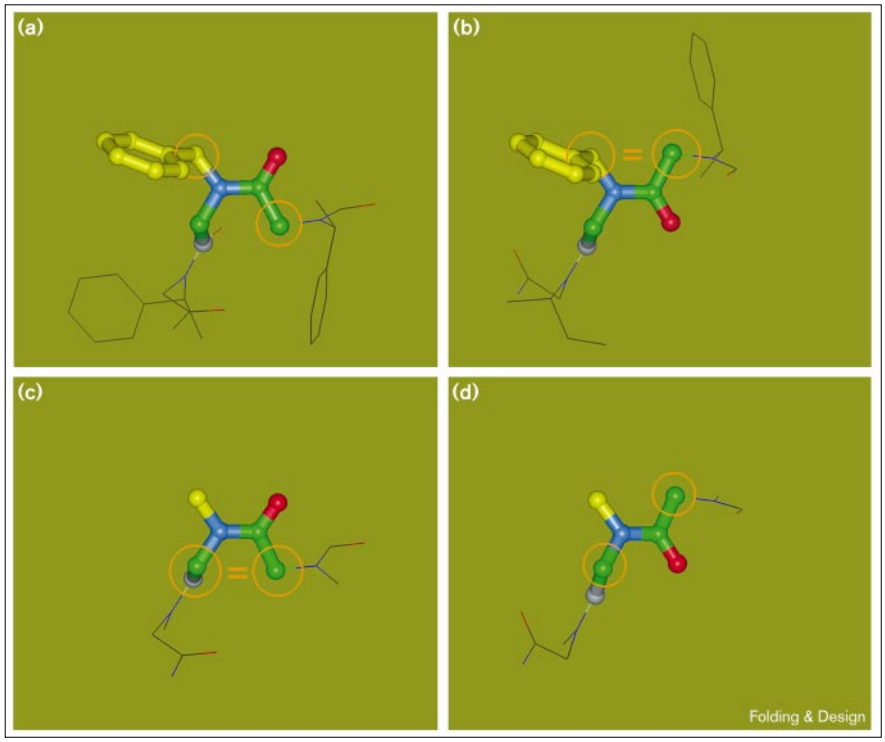


Table 1

Conformational comparisons.

(a) AMBER octapeptoid calculations.

Helix type	ϕ^*	ψ^*	ω^*	AMBER energy†
Cis RH	−70	165	*cis*	0.0‡
Cis LH	70	185	*cis*	5.6
Trans RH	−75	185	*trans*	34.5
Trans LH	70	180	*trans*	23.8‡

(b) AMSOL dipeptoid calculations.

Helix type	ϕ	ψ	ω	$\chi1$§	AMSOL energy† Gas phase	AMSOL energy† Solvated
Cis RH	−76.5	−167.2	*cis*	−113.2	0.0	0.0
Cis LH	+72.0	165.6	*cis*	−130.8	1.12	0.58
Cis LH	+85.1	165.1	*cis*	86.2	1.46	2.01
Cis RH	−82.1	−113.9	*cis*	89.3	2.01	2.50

RH, right-handed; LH, left-handed. *Values for dihedral angles are average for the helix. †Values are in kcal/mol, relative to the lowest-energy conformation. ‡These two values are for a $\chi1$ of +60°, which for these two structures gives a lower AMBER energy. §$\chi1$ refers to the $C_{(i-1)}$,$N_{(i)}$,N–$C\alpha_{(i)}$,N–$C\beta1_{(i)}$ dihedral angle.

The empirical AMBER force field was not parameterized for peptoids, however; we therefore used semi-empirical quantum mechanical calculations, performed with the AMSOL force field [8], to compare the *cis* conformations more accurately. We calculated the helical handedness and sidechain rotamer preferences on a *cis* Nspe-containing dipeptoid with full relaxation (Table 1b). The dipeptoid gas-phase calculations revealed a 0.5 kcal/mol per residue preference for the right-handed helix (negative value of ϕ) when the sidechain adopts the most favorable rotamer (in which the sidechain N–Hα hydrogen of residue *i* points toward the carbonyl oxygen of residue *i*–1). These energetic differences may change in magnitude or direction as the helix grows and residues begin to interact with each other. But our molecular mechanics results for the octamer agree qualitatively with the dipeptoid calculations in favoring the *cis* right-handed helix backbone for the *S* isomer of N-(1-phenylethyl)glycine.

These preferences can be rationalized as follows (Figure 5):

1. The preferred rotamer (Figure 5a) avoids a weak steric interaction between the two N–Cβ carbons of sidechain *i* and the carbonyl oxygen of *i–1*. The other possible rotamer (Figure 5b) would have these N–Cβ carbons straddling the oxygen, which would introduce some steric strain.

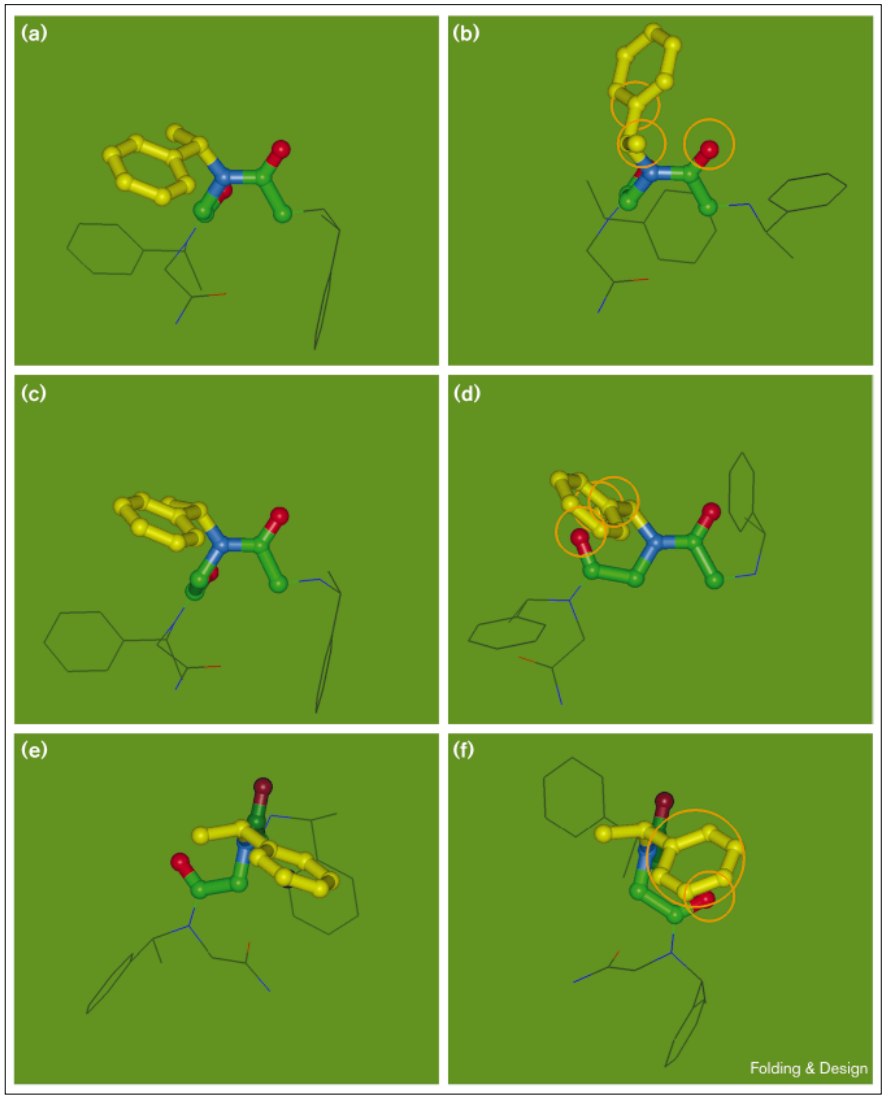
2. This rotamer prevents ϕ from adopting *trans* values (~180°), because then the N–$C\beta_i$ carbons would have an unfavorable steric interaction with the ac–CO_i carbonyl carbon (Figure 5d). Therefore, ϕ is limited to values around +90° or –90° (Figure 5c).

3. There are then two important interactions to consider between the sidechain and the backbone atoms of *i* which determine the sign of ϕ, and thus the handedness of the helix (Figure 5e,f). First, there is a steric interaction between the carbonyl atoms and either the bulkier ring (if ϕ is positive) or the smaller Nβ methyl group (if ϕ is negative). This should favor a negative ϕ. Second, an electrostatic repulsion between the π orbitals of the aromatic ring and the carbonyl oxygen can reinforce this preference.

We therefore predict that $(Nspe)_8$ will form a helix with the following dihedral angles: $\omega \approx 0°$, $\phi \approx -75°$, $\psi \approx 170°$, $\chi1 \approx -120°$ (where $\chi1$ refers to the C_{i-1}, N_i, N–$C\alpha_i$, N–$C\beta1_i$ dihedral angle, as shown in Figure 1). This helix has *cis* amide bonds, a right-handed twist, and a periodicity of about three residues per turn (Figure 6). Its backbone dihedral angles are essentially the same as those of a polyproline type I helix. Of note is the fact that the C=O dipoles in this model are nearly parallel to the long axis of the helix, which is consistent with the strength of the molecule's CD signal. This alignment should also generate a helix macrodipole much like that of the α helix [9], equivalent to the presence of a partial negative charge at the N terminus and a positive one at the C terminus. But the direction of this dipole is opposite to that of the α helix in peptides because, in our model, the carbonyls point towards the N terminus (Figure 5). Again by analogy to peptides [10–12], we expect this conformation to be stabilized by positively charged sidechains near the N terminus and by negatively charged sidechains near the C terminus. Similarly, a molecule with a free positively charged amine at the N terminus and a free negatively charged carboxylic acid group at the C terminus should more readily adopt the proposed conformation and hence display a stronger CD double minimum signal than a molecule acetylated at the N terminus and amidated at the C terminus.

Although we have focused on the minimum energy conformation, it appears that the energetic differences associated with changes in ω, in handedness, or in $\chi1$ are small (Table 1). In specific situations, therefore, polypeptoid conformations may depart from the proposed helix, if in so doing they can be stabilized by solvation effects, sidechain interactions, etc. The most conformationally significant changes would be a change of the amide bond conformation (from *cis* to *trans*) or a reversal of the sign of ϕ. The barrier between the *cis* and *trans* conformations of the amide bond is probably close to the 20 kcal/mol barrier for proline isomerization [13]. To estimate the barrier to handedness reversal, we considered a dipeptoid in the minimum energy conformation (negative ϕ). Based on the conformational map obtained with AMBER, we determined the lowest-energy maximum separating this conformation from the minimum energy conformation of opposite handedness (positive ϕ). On this basis, we estimate the barrier to be around 10 kcal/mol. Finally, the sidechains could

Figure 5



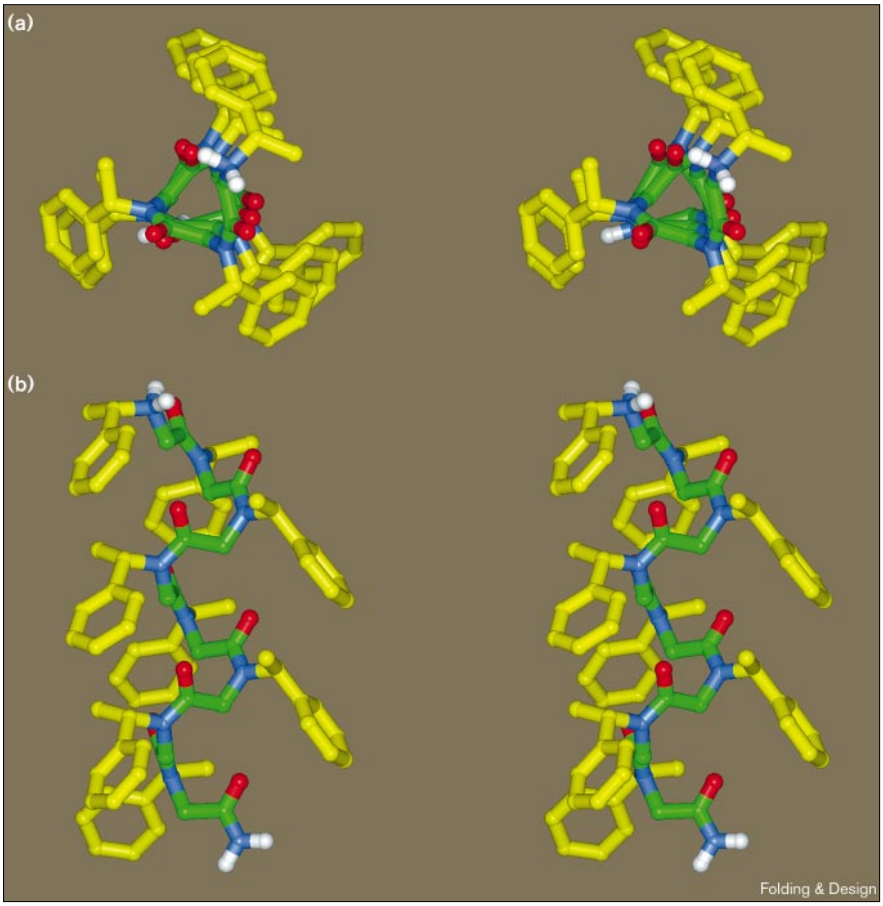
Rotamer handedness preferences for a representative residue of $(Nspe)_8$. Steric repulsion is denoted by orange circles drawn around the clashing atoms or groups. All relevant atoms are rendered in ball-and-stick model and colored as described in the legend to Figure 4. Rotamer preferences: **(a)** a value for $\chi 1$ of −120° avoids the steric repulsion between the carbonyl oxygen and the Nβ groups; **(b)** this repulsion occurs if $\chi 1$ is +60°. Exclusion of *trans* ϕ dihedral angle: **(c)** a value for ϕ of ± 90° avoids the steric repulsion between the carbonyl oxygen and the Nβ groups; **(d)** this repulsion occurs if ϕ is 180°. Handedness preference: **(e)** a value for ϕ of −90° avoids the steric and electrostatic repulsion between the carbonyl oxygen and the ring; **(f)** this repulsion occurs if ϕ is +90° (this preference would be reversed for the *R* isomer).

easily adopt the other possible rotamer ($\chi 1 = +60°$ instead of the proposed $\chi 1 = -120°$), because this would incur only a minimal steric cost and may permit favorable interactions of sidechain or backbone atoms with other sidechains or with solvent molecules. Using the strategy just described, we estimate the barrier to this change to be ~5 kcal/mol.

All of the calculations described above were done in the gas phase and largely ignore the effects of solvation (except for the distance-dependence of the AMBER dielectric constant). But the solvent probably does not have a major impact on the conformation responsible for the double-minimum CD signal; indeed, we have observed this signal in a variety of both aqueous and organic solvents [4]. Furthermore, when we repeated the AMSOL dipeptoid calculations taking into account solvation energies, the conclusions were qualitatively unchanged (Table 1b). This argues for the robustness of our calculations, and the primacy of the steric term in determining the conformation of these molecules.

Peptoids whose sidechains lack the Nα chiral center should behave differently. Changing the degree of substitution at the Nα atom would probably affect both the ω and the ϕ preference, as well as the number of allowed rotamers. This should be reflected in a change in the spectroscopic properties of the molecules. The decreased energy of *trans* conformations and the lack of an enantiomeric preference would increase the number of accessible conformations at a given temperature, which would be entropically favorable. Therefore, as the number of achiral sidechains increases in an oligopeptoid, we expect a decrease in the strength of the double minimum CD signal, as more molecules depart

Figure 6



Stereo diagrams of the predicted structure of the $(Nspe)_8$. All heavy atoms are shown colored as described in the legend to Figure 4. The model is viewed **(a)** parallel and **(b)** perpendicular to the long axis of the helix.

from the regular helix conformation. This decrease in the magnitude of the signal may be accompanied by an increase in its heat stability, reflecting the entropic benefit of the greater number of accessible conformations.

Conclusions

The results presented here lend some insight into the conformational properties of Nα chiral oligopeptoids, and into the differences between the protein folding and peptoid folding problems. Indeed, the constraints that determine the allowed values of the backbone dihedral angles for peptoids seem subtly different from those that operate in polypeptides. Polypeptide backbones can adopt very distinct regular conformations (most notably α helix and β sheet), but almost always with negative values of ϕ [13]. This handedness of the backbone is driven by the chirality of the Cα, which is identical for all 19 naturally occurring amino acids with sidechains. Thus, backbone conformations in natural proteins derive not from local chiral choices, but from the balance between local conformational propensities of the different amino acids and nonlocal interactions [14]. This renders rational design of proteins very difficult, due to the delicate balance between local and nonlocal interactions. By contrast, peptoid backbones may be forced by the presence of Nα chiral sidechains to adopt conformations close to the helix described above. The handedness of this conformation can be changed by the choice of the sidechains' chirality. Thus, it may ultimately prove easier to design peptoids than proteins by using Nα chiral sidechains to force the backbone dihedral angles into a predictable region of conformational space and different chemical groups distal to the Nα atom to direct the combination of the backbone elements and to introduce functionality.

Peptoids present a new and promising structural paradigm, which could translate into new functional possibilities. We are involved at present in determining the solution structure of Nα chiral oligopeptoids, and in exploring the ramifications of their conformational properties for molecular design.

Materials and methods

Synthesis and circular dichroism

Synthesis and spectroscopy were performed as described in [4]. For the CD spectra shown in Figure 2, the molecules were dissolved in

96% H_2O/4% methanol to a concentration of 0.1 mM, and the spectra collected at 25°C.

Conformational maps

The values of the ϕ and ψ dihedral angles of the octamers were independently changed from 0° to 360° in increments of 4°. At each point, the sidechain dihedral angles and the ω dihedral angle were allowed to relax in a force field that consisted of the nonbonded (van der Waals' and electrostatics) and torsional components of the all-atom AMBER potential [7], with (1,4) interactions scaled as described in [7]. To improve computational efficiency, we kept bond lengths and angles fixed to the monomer equilibrium values. Missing partial charges and torsional parameters were determined simply by similarity to peptides. Because of the lack of accurate parameters for peptoids, we have repeated these calculations without the torsional potentials (data not shown). The maps were essentially unchanged, implying that their salient features are sterically determined. The minimization was performed with a downhill simplex algorithm [15]. For the dimers, bond lengths and angles were allowed to relax; ϕ and ω were scanned in increments of 10°, using an internal coordinate conjugate gradient minimizer. All calculations reported here were done on SGI IndigoII workstations, and all software was written for this work, unless stated otherwise. Graphical displays were created and printed out using the *Insight® II* 95.0 molecular modeling system (Biosym/MSI, San Diego, USA).

Minimum energy conformation

Conformations were generated in two steps. In the first step, octamers were minimized in the force field described above, with all bond lengths and angles fixed and all dihedral angles constrained to lie within 40° of each other (this excluded irregular conformations, but was necessary to keep the problem computationally tractable). The ω torsion was constrained to lie between −15° and 15° (*cis*), or between 165° and 195° (*trans*). The minimization was performed using a simulated annealing protocol [16]. In the second step, low-energy structures were further minimized in the full AMBER force field, allowing all dihedral angles, bond angles, and bond lengths to relax, using an internal coordinate conjugate gradient minimizer. Again missing parameters were inferred from similarity to peptides.

Semi-empirical quantum mechanical calculations were performed with AMSOL version 4.5 [8], using the AM1 parameter set. The calculations were also done in solvent by using the AM1-SM2.1 parameter set. The dipeptoid was N-acetyl-(*S*)-N-(1-phenylethyl)glycine with an N-dimethyl amide cap.

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