Short Azapeptides of Folded Structures in Aqueous Solutions

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ABSTRACT: Building folded short peptides that are driven by the intramolecular hydrogen bonding in aqueous solutions remains challenging because of their highly competitive intermolecular hydrogen-bonding interactions with water solvent molecules that would have favored the extended conformations. Here, we show folded β-turn structures in acyl amino acid-based N-amidothioureas, the nonclassic azapeptides, in aqueous solutions, as well as in solid-state and organic solvents, by X-ray crystal structures, DFT calculations, 1D and 2D NMR spectra, and absorption and CD spectra. The achiral phenylthiourea chromophore acts as a CD reporter for the β-turn structure that communicates the chirality of the amino acid residue to the achiral thiourea moiety and the relative intensity of the intramolecular hydrogen bond that stabilizes the turn structure. The amidothiourea moiety represents a versatile structural framework for folded short peptides in aqueous environments.

INTRODUCTION

Folded short peptides have exhibited potential applications in organocatalysis, molecular sensing, and therapeutics.1−3 However, short peptides prefer to adopt extended conformations in aqueous solutions to maximize the intermolecular hydrogen-bonding interactions with water molecules. Therefore, building folded short peptides brought about by intramolecular hydrogen bonding in aqueous environments remains challenging. Several strategies have been explored, such as by forming cyclic peptides that fix the folded structures via covalent bonds4,5 or incorporating proline or the D-amino acid residue that promotes restricted conformation,6,7 yet they are limited by their complicated molecular design and syntheses. We set to develop a facile structural framework for folded short peptides in aqueous solutions, based on our recent observation of β-turn structures in a series of short nonclassic azapeptides, the peptide-based N-amidothioureas (1a, 1b, and 1c, Scheme 1) in solid-state and organic solvents.8 We have shown that these folded structures can be employed to build molecular beacons9 and supramolecular helical structures.10 The conformations of such azapeptides in aqueous conditions have yet to be clarified that if the folded structures would remain. We envisaged that such azapeptides may be structural candidates for the short folded peptides in aqueous solutions. This is because (i) the extended backbone of such azapeptides is actually not linear but folded, which is driven by lone pair–lone pair repulsion of the N−N bond and manifested by the distinct Ramachandran φ and ψ dihedral angles,11 (ii) the intramolecular hydrogen bond that stabilizes the β-turn structure is enhanced by the higher acidity of the thioureido −NH proton compared to that of the amido −NH protons in peptides and in classic azapeptides (2a, 2b, and 2c, Scheme 1)12,13 and (iii) the S atom is a weaker hydrogen bonding acceptor than the O atom14 that reduces the intermolecular hydrogen bonding of the azapeptides with water molecules.
As a proof-of-principle, we chose acyl-alanine-, acyl-leucine-, and acyl-phenylalanine-based N-amidothioureas (1a, 1b, and 1c, Scheme 1) as molecular targets for folded structures in aqueous solutions. The conformational properties of these azapeptides were characterized by X-ray crystal structures, DFT calculations, absorption and CD spectra, and 1D and 2D NMR spectra. Folded \( \beta \)-turn structures were shown in aqueous solutions. The phenylthiourea chromophore showing absorption at longer wavelength than those of many amino acid residues acts as a CD reporter for the \( \beta \)-turn structure. Our results suggest that the peptide-based N-amidothioureas could be a structural motif for folded short peptides in aqueous solutions.

## RESULTS AND DISCUSSION

Acy1 amino acid-based N-amidothioureas (1a, 1b, and 1c, Scheme 1) were synthesized through the procedures outlined in Scheme S1 (Supporting Information). Crystals of 1a, 1b, and 1c were obtained by slow evaporation of the respective solutions in the CH\(_2\)OH/CH\(_2\)CN mixture, and the structures were elucidated by X-ray crystallography (for crystallographic data, see Table S1). For 1a, a ten-member intramolecular ring hydrogen bond (N\(^+4\)–H\(^\delta\)…O\(^1\)) is defined, which is indicative of the \( \beta \)-turn structure (Figure 1). The distance from C\(\alpha\) to C\(\alpha_{i+3}\) is 5.342 Å, within the distance criterion of a \( \beta \)-turn structure (C\(\alpha\) – C\(\alpha_{i+3}\) ≤ 7 Å) for peptides and proteins. The \( \beta \)-turn structure in 1a is of type II, according to the Ramachandran \( \varphi \) and \( \psi \) dihedral angles (Table S3). 1b and 1c show similar type II \( \beta \)-turn structures (Figure 1). DFT calculations revealed the \( \beta \)-turn structures in these azapeptides in the solid state too (Figure S1).

To gain insight into the conformational properties of these azapeptides in aqueous solutions, NMR studies were carried out. Temperature coefficients of chemical shifts of the –NH protons have been proposed to predict intramolecular hydrogen bonding in water. We thus recorded variable-temperature \(^1\)H NMR spectra in 95:5 H\(_2\)O–CD\(_3\)OD (v/v, Figure 2). With all of the three azapeptides 1a–1c, the chemical shift of the thioiureido \( –\text{NH}^4 \) proton (Figure 1) showed a more positive temperature coefficient than –4.5 ppb/°C (Table 1), the criterion value for intramolecular hydrogen bonding in proteins/peptides. In contrast, those of \( –\text{NH}^4 \), \( –\text{NH}^4 \), and \( –\text{NH}^4 \) protons exhibited more negative values, suggesting that they do not take part in the intramolecular hydrogen bonding. The temperature coefficients of the chemical shift thus suggested intramolecular hydrogen bonding of the thioiureido \( –\text{NH}^4 \) in 1a, 1b, and 1c in aqueous conditions, as shown in the crystal structures. Moreover, the proton \( –\text{NH}^4 \) in 1a and 1b exhibited substantially more positive temperature coefficients (–2.5 and –2.6 ppb/°C, respectively) than 1c (–4.0 ppb/°C), implying that the hydrogen bonding of the \( \beta \)-turn in 1a and 1b is stronger than that in 1c (Table 1). This difference can be explained by the lower acidity of the thioiureido \( –\text{NH}^4 \) proton in 1c than that in 1a and 1b inferred from their chemical shifts (Table S4). The urea counterparts of 1a, 1b, and 1c (2a, 2b, and 2c, Scheme 1), the classic azapeptides, showed more negative temperature coefficients of the chemical shift of the ureido \( –\text{NH}^4 \) proton (–6.9, –6.7, and –6.5 ppb/°C, respectively, Figure S2), suggesting much less or absence of the intramolecular hydrogen bonding and the turn conformation in aqueous solutions.

Therefore, the amidothiourea moiety exhibited a significantly enhanced promotion of the \( \beta \)-turn structure in short azapeptides in aqueous solutions, compared to that of the urea counterpart in the classic azapeptides. This is likely due to the lower acidity of the ureido \( –\text{NH}^4 \) proton in ureas than that of the thioiureido \( –\text{NH}^4 \) proton in thioureas (Table S4). The acidity of the thioiureido \( –\text{NH}^4 \) proton thus appears to play a key role in promoting the turn conformation of the short azapeptides to remain in aqueous solutions as well. Moreover, the chemical shift of the thioureido \( –\text{NH}^4 \) proton in amidothiourea 1a is less responsive to change in the H\(_2\)O content in H\(_2\)O–CD\(_3\)CN binary solvent than that of ureido \( –\text{NH}^4 \) proton in amidothiourea 2a (Figure S3). Similar observations were made with 1b over 2b and 1c over 2c (Figure S4). These again suggest a higher intramolecular hydrogen bonding capacity of the thioiureido \( –\text{NH}^4 \) proton in the amidothiourea under aqueous conditions.

Two-dimensional NOESY spectra were also recorded for 1a in aqueous solutions (Figure S5). The –CH\(_3\) proton H\(_8\) showed cross-peaks with phenyl –CH\(^2\) and thioureido \( –\text{NH}^4 \) (Figure 2d), despite being located at the two distant terminuses of the molecule. This directly supports the folded conformation of 1a in aqueous solution, as shown in the crystal (the H\(^8\)--H\(^4\) distance is 3.253 Å, whereas the H\(^6\)--H\(^4\) distance is 4.046 Å). The observed NOE signals for –CH\(^2\) and –NH\(^3\) again supported the folded \( \beta \)-turn structure of 1a in aqueous solutions (3.613 Å for H\(^6\)--H\(^4\) in the crystal). The –NH\(^4\) and –NH\(^4\) protons are active for proton exchange and not protected by the intramolecular hydrogen bonding, and their NOE signals were not observed. The NMR studies in aqueous solutions therefore confirmed the folded conformation.

CD spectra in H\(_2\)O were next investigated. CD signals at 215 and 250 nm were observed for 1a, 1b, and 1c. The signal at 250 nm is assigned to the \( \pi–\pi^* \) transition of the achiral phenylthiourea chromophore at the C terminal of the molecule, according to the absorption spectra (Figure 3). This observation confirmed that the chirality of the amino acid residue is transferred to the distant achiral phenylthiourea chromophore. \( \beta \)-Turn structures were thus proposed in these azapeptides in water that bring the achiral chromophore into the hydrogen bonding network containing the chiral center, allowing the long-distance communication of the asymmetric
conformational information. The phenylthiourea chromophore thus operates as a CD reporter for the β-turn structure.8

As the CD response of the phenylthiourea chromophore was induced by the β-turn conformation afforded by the intramolecular hydrogen bonding, the intensity of the CD signal was expected to reflect the strength of the hydrogen bonding.25 Indeed, 1a and 1b in H2O showed stronger induced CD signals at 250 nm than 1c (Figure 3). The g factor values23 calculated from the absorption and CD spectra (Figure S6) exhibited a similar tendency that the g factor values at 250 nm for 1a and 1b (−10.6 × 10−5) are larger than those of 1c (−7.1 × 10−5).

These observations suggest that the hydrogen bonding in 1a and 1b are similar, while being stronger than that in 1c, reminiscent of the conclusion made with the temperature coefficients of the chemical shifts (Table 1). To keep the same solvent conditions for the variable-temperature NMR experiments, absorption and CD spectra were also recorded in 95:5 H2O−MeOH (v/v, Figure S7). We observed CD profiles and g factors (Figure S8) similar to those in H2O. The concentration-independent CD and 1H NMR spectra of 1c exclude the possible contribution of the intermolecular interactions in aqueous solutions (Figures S9 and S10).7

The folded β-turn structures of the three nonclassic azapeptides were also confirmed in the organic solvent CD3CN. CD signals at 235 and 270 nm were observed and assigned to the chiral amino acid residue and the achiral phenylthiourea chromophore, respectively (Figure 4),8 which points to their β-turn conformation in this organic solvent. The chemical shift of the thioureido −NHd proton was found to be reluctant to the change of the temperature (Figure S11), with a more positive temperature coefficient than those of the −NHa, −NHb, and −NHc protons (Table 2). The 2D NOESY spectra of 1a in CD3CN exhibited cross-peaks of the −CHf proton with −NHf and −NHg and with −NHf and −NHd as well (Figure S12), indicative of the folded conformation. Moreover,  

Table 1. Temperature Coefficientsa of −NH Protons’ Chemical Shifts and g Factors at 250 nm of 1a, 1b, and 1c in Aqueous Solutions

| compound | 1a | 1b | 1c |
|----------|----|----|----|
| temperature coefficient (ppb/°C) | | | |
| −NHa | −9.3 | −9.1 | −9.5 |
| −NHb | −10.6 | −10.7 | −8.0 |
| −NHc | −9.7 | −9.4 | −7.5 |
| −NHd | −2.5 | −2.6 | −4.0 |
| g factor at 250 nm (10−5) | | | |
| −10.6 | −10.6 | −7.1 |

aCalculated from variable-temperature 1H NMR spectra in 95:5 H2O−CD3OD (v/v). bObtained from absorption and CD spectra in H2O.
the obvious NOE signals for CH=–NH and NH–NH are consistent with the type II β-turn structure and agree with those shown in the crystal structure of 1a.22 The temperature coefficients of -NHprotons and g factors at 270 nm (Figure S13) indicated a similar strength of the intramolecular hydrogen bonding in 1a and 1b, but which is stronger than that in 1c (Table 2).

Table 2. Temperature Coefficients a of –NH Protons’ Chemical Shifts and g Factors at 270 nm b of 1a, 1b, and 1c in Organic Solvents

| compound | temperature coefficient (ppb°C) | 1a | 1b | 1c |
|----------|---------------------------------|----|----|----|
| –NH | –9.0 | –9.4 | –4.7 |
| –NH | –8.5 | –9.6 | –8.6 |
| –NH | –8.4 | –7.9 | –7.9 |
| –NH | –2.8 | –2.8 | –3.3 |

\( g \) factor at 270 nm (10^{-3})

\( a \)Calculated from variable-temperature 1H NMR spectra in 9:5 CD3CN–DMSO-d6 (v/v). \( b \)Calculated from absorption and CD spectra in CH3CN.

CONCLUSIONS

In summary, β-turn structures were shown in aqueous solutions in a series of acyl amino acid-based N-amidothioareas (the nonclassic azapeptides) and in the solid-state and organic solvent. Our findings afford a facile structural framework for folded short peptides in aqueous solutions that could be readily probed by a simple spectral reporter. The amidothioareo moiety operates like a proline residue in the peptide backbone to promote the folded conformation. This is of significance because the classic short azapeptides, or the urea counterparts of the current peptide-based N-amidothioareas, did not exhibit turn structures in aqueous solutions.19-22 Work is now under way to validate the generality of the present strategy for establishing the folded β-turn conformation in aqueous solutions and to explore the potential impact of such amidothioareo moiety when inserted into peptides or proteins.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00041.

Detailed synthesis procedures, characterization, crystal data, spectral analysis, and others (PDF)

Crystallographic information for 1a (CCDC 1820148) (CIF)

Crystallographic information for 1b (CCDC 1820149) (CIF)

Crystallographic information for 1c (CCDC 1820150) (CIF)

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Notes

The authors declare no competing financial interest.

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