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Torsion Angle Based Design of Peptidomimetics: A Dipeptidic Template Adopting β-I Turn (Ac-Aib-AzGly–NH_2)

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Abstract—We have attempted to design a model dipeptide (acetyl dipeptide amide, Ac-CA1-CA2–NH_2) that can adopt specifically typical torsion angles of the β-I turn (ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2}, ψ_{i+2} = −60°, −30°, −90°, 0°). The key of the design is the combination of constrained amino acids that prefer to adopt the desired torsion angles. We chose Aib (aminoisobutyric acid) as the first residue of which ϕ and ψ angles must be −60° and −30°, respectively. Then, we selected an azaamino acid as the second residue since previous studies have indicated that they prefer to adopt ±90° of ϕ angle and 0° or 180° of ψ angle. The conformational preference of the resulting Ac-Aib-AzGly–NH_2 is investigated using ab initio methods. The conformations implying β-I and β-I′ turns are energetically most favorable, as we expected. Thus, we synthesized the designed molecule on the solid phase considering the future generation of combinatorial libraries using an automatic peptide synthesizer. Then, NMR spectroscopy was carried out to confirm their conformational preference in solution was carried out. The results indicated that the Ac-Aib-AzGly–NH_2 adopt β-I or β-I′ turns in solution forming an intramolecular hydrogen bonding between Ac–C(O) and terminal NH_2. We believe that such a small peptidomimetic template is highly useful for the design of drug candidates and molecular devices. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

Recently, two areas of cutting edge science, drug discovery and nanotechnology, share the need of constrained peptidomimetics implicating predictable and controllable secondary structures. In drug discovery, peptidomimetics have been needed to obtain drug candidates to mimic the function of bioactive peptides that can initiate or control the physiological process of the human body. To execute their functions, peptides interact with corresponding proteins, adopting a specific conformation. Thus, the accurate mimic of bioactive conformations using constrained peptidomimetics is one of the most important tasks in modern drug discovery.[1] On the other hand, in nanotechnology, constructing atomically precise molecular machinery is a goal and it can be achieved by the design of non-periodic linear polymers that fold into the desired 3-D structure like proteins. Thus, new constrained peptidomimetic monomers that can display clear secondary structures in solution are eagerly desired to obtain the polymer that can show a strong tendency to adopt a specific tertiary structure.[2]

Usually, secondary structures for peptides and proteins can be described by torsion angles (mostly, backbone torsion angles, ϕ and ψ angles) and there are many constrained amino acids to stabilize specific ϕ and ψ angles.[1] Thus, we believe that small peptidomimetics implicating predictable and controllable secondary structures can be constructed by the combination of such constrained amino acids. Namely, using the defined torsion angles for secondary structures as blue prints and constrained amino acids as building blocks, we can generate the desired secondary structure on the small peptide. In the present work, we describe an example to design small peptidomimetics (dipeptide size) in which a specific secondary structure is embedded.

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The general procedure for the design of such peptidomimetic templates (we call 'torsion angle based design of peptidomimetics') is composed of five steps. The first step is to define torsion angles to mimic. Then, we design a model peptidomimetics in which constrained amino acids are combined on the basis of the defined torsion angles. Since most constrained peptidomimetics are incorporated in extra functional group(s) to restrict ϕ and ψ angles, we carefully choose them to avoid steric bumps. In the third step, we confirmed our design by computational methods with identification of preferred conformations of the model compounds. If the desired secondary structure appears as one of the lowest energy conformations in these calculations, we synthesize the model compound. Finally, we investigated the conformational preference of the model peptidomimetics in solution. Clear conformational preference in solution is highly important in both drug discovery and nanotechnology because all the bioactive peptides are recognized by proteins in solution and a foldameric monomer for molecular machine should have stable 3-D arrangements in solution, respectively. Thus, we use NMR spectroscopy in this step because it is the most powerful tool to identify preferred structures in solution.

Among many peptide secondary structures, β-turns are at the center of interest in drug discovery since they have been identified as bioactive conformations of many peptides.[3] β-turns are formed by four amino acids. They are classified into several types according to the torsion angles of the second and the third resides ($i+1$ and $i+2$, respectively). Additionally, they contain hydrogen bonding between carbonyl oxygen of the residue i and amide proton of the $i+3$ residue. In the present study, we attempt to design a model template (acetyl dipeptide amide: Ac-CA1-CA2–NH$_2$) that can adopt specifically the typical torsion angles of a β-turn. We believe, if the conformation of each residue is fixed by the typical torsion angles for $i+1$ and $i+2$ residues of a β-turn, the resulting molecule can adopt the desired β-turn type.

Design of Model Peptidomimetics

We target β-I turn and its prime type (β-I$'$ turn) as the first case. Typical torsion angles of β-I and β-I$'$ turns (ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2}, ψ_{i+2}) are (−60°, −30°, −90°, 0°) and (60°, 30°, 90°, 0°), respectively.[3] Thus, the ϕ and ψ angles of the first constrained amino acid (CA1) in the model peptidomimetics (Ac-CA1-CA2–NH$_2$) must assume −60° and −30°, respectively, and those of the second one (CA2) must adopt −90° and 0°, respectively.

Extensive studies of Balaram, Toniolo and Benedetti have shown that α-methylated amino acids and α-amino-cycloalkane carboxylic acids prefer to adopt 3_{10}-helical structures (ϕ and $\psi = -60°$ and $-30°$). Thus, one of these amino acids can be the choice for the first residue. Since we wanted to design the simplest model at first, we chose Aib (aminoisobutyric acid) among others. The Aib is formed by the replacement of the α-hydrogen of alanine with a methyl group. In general, the Aib prefers to adopt the conformations in which (ϕ and ψ) are (−60° and −30°) or (60° and 30°) and rarely exhibits fully extended structures (180° and 180°).[4]

As the second residue, we chose an azaamino acid that is formed by replacement of α-carbon of the amino acids with a nitrogen atom allowing retention of the side chains.[5] X-ray crystallographic studies performed by Aubry, Boussard and Marraud and our extensive ab initio studies have indicated that the azaamio acid prefer to adopt ±90° as ϕ angle and 0° or 180° as ψ angle.[5,6] In a typical β-I turn, the ϕ angle is ±90° and the ψ angle is about 0°. Therefore, of the two preferred ψ angles, 0° should be stabilized. For this purpose, we incorporate acetyl group into the N-terminus and amide group at the C-terminus of the dipeptide as hydrogen bonding acceptor and donor, respectively. Namely, if other torsion angles (ϕ_{i+1}, ψ_{i+1} and ϕ_{i+2}) are fixed by the desired values for β-I turn, only in the case that ψ_{i+2} adopt 0°, the hydrogen bond can be formed. Thus, the overall structure becomes more stable in the case that ψ_{i+2} is 0° than in the case that ψ_{i+2} is 180°. In these studies, we incorporated the simplest azaamino acid, azaglycine(AzGly).

Ab Initio Calculation of the Designed Model Peptidomimetic Dipeptide

The resulting Ac-Aib-AzGly–NH$_2$ (Fig. 1) is simple to include only 14 heavy atoms and non-chiral. To confirm our speculation, we carried out ab initio calculations of the designed molecule since appropriate force field for azaamino acids has not been developed. The Aib favorably adopts three conformations (−60°, −30°), (60°, 30°) and (180°, 180°) and AzGly preferentially adopts five conformations (−90°, 0°), (90°, 0°), (−90°, 180°), (90°, 180°) and (180°, 180°). Thus, total 15 conformations of Ac-Aib-AzGly–NH$_2$ were considered as starting structures. They were optimized at the RHF/3-21G* level[7] using the Gaussian 94,[8] and the results are summarized in Table 1. The conformation including β-I and β-I$'$ turns (conformations 1 and 2 of Table 1) are energetically most favorable, as we expected. Thus, we decided to synthesize the designed molecule.

Synthesis of Ac-Aib-AzGly–NH$_2$

The synthesis was carried out on solid phase considering future generation of combinatorial libraries. We used an automatic peptide synthesizer (ABI 431A Peptide Syn-

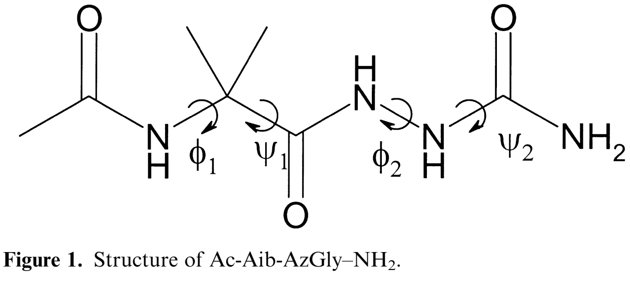


Figure 1. Structure of Ac-Aib-AzGly–NH$_2$.

thesizer, Perkin-Elmer) on which the synthetic procedure was programmed. The programmed synthetic steps include (1) deprotection of Rink amide MBHA resin by piperidine, (2) treatment with CDI, (3) addition of hydrazine, (4) coupling of Fmoc-Aib–OH using HBTU/HOBt, (5) removal of Fmoc protecting group by piperidine, (6) treatment with acetic anhydride and (7) acidolytic cleavage from the resin (Scheme 1). The HPLC purification of the crude product led to the desired Ac-Aib-AzGly–NH_2.

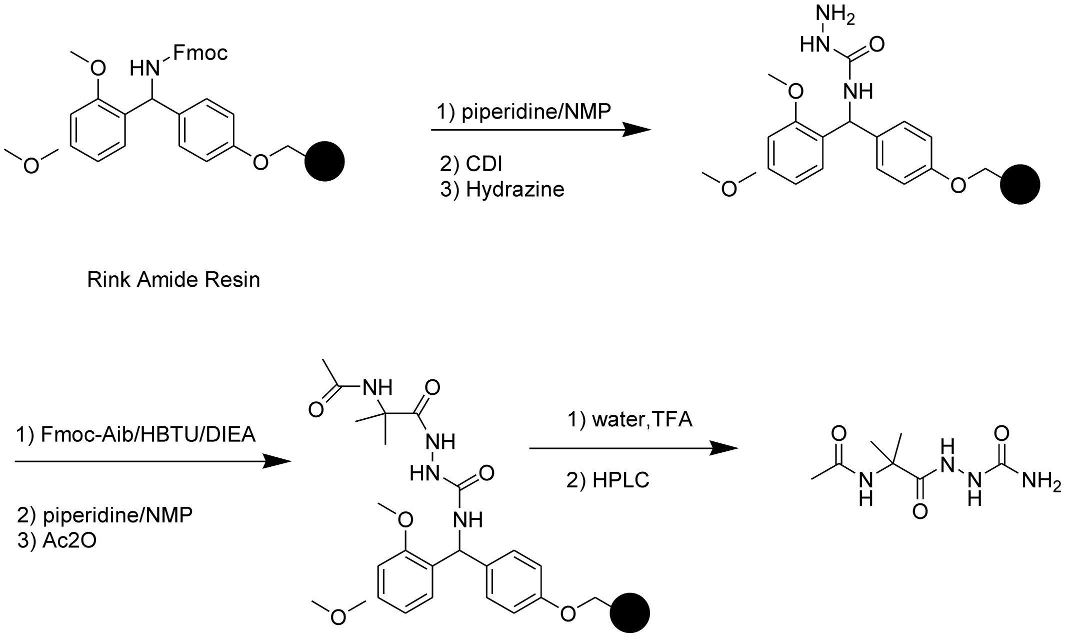
NMR Studies of Ac-Aib-AzGly–NH_2

To prove that the Ac-Aib-AzGly–NH_2 form β-I or β-I′ turns in solution, we carried out NMR studies. The proton resonance of Ac-Aib-AzGly–NH_2 was assigned employing 2-D Hartmann–Hahn type experiments.[9] The NOEs were obtained from the Nuclear Overhauser Effect Spectroscopy (NOESY).[10] Variation of mixing time from 400 to 800 ms resulted in linearity of cross-peak intensities to 800 ms. The NOESY experiments with this mixing time were used to measure the intensities of NOEs. The volumes of cross peaks were measured and are summarized in Table 2. Temperature coefficients were also measured for all NH protons (Table 3).

The NOEs of Ac-Aib-AzGly–NH_2 demonstrate typical pattern for β-I or β-I′ turn formation of peptide [i.e., NOE between Aib NH and AzGly NH and NOE between AzGly NH and C(O)NH_2]. The inter-proton distances corresponding to each NOE were calculated from the NOE intensities using the formula $d_{ij}=d_{\mathrm{ref}}(I_{\mathrm{ref}}/I_{ij})1/6$, where I is intensity and d is distance between protons.[11] As the standard distance for the calculation, the inter-proton distance between Ac-Me and Aib–NH was employed because the amide bond conformation is

Table 1. The optimized conformations of Ac-Aib-AzGly–NH_2

No.	Starting conformation				Optimized conformation				ΔE (kcal/mol)
	ϕ_1 (°)	ψ_1 (°)	ϕ_2 (°)	ψ_2 (°)	ϕ_1 (°)	ψ_1 (°)	ϕ_2 (°)	ψ_2 (°)	
1	−60	−30	−90	0	−59.2	−31.3	−77.8	−10.0	0.0
2	60	30	90	0	59.2	31.3	77.8	9.9	0.0
3	180	180	−90	0	179.9	−179.9	−86.6	−14.2	3.9
4	180	180	90	0	−179.9	178.9	86.6	14.1	3.9
5	180	180	−90	180	179.5	180.0	−70.4	171.4	4.2
6	180	180	90	180	−179.5	−180.0	70.4	−171.4	4.2
7	−60	−30	90	180	−64.4	−30.1	77.2	−170.4	5.6
8	60	30	−90	180	64.4	30.1	77.1	170.1	5.6
9	180	180	180	180	180.0	180.0	−180.0	180.0	5.8
10	−60	−30	90	0	−58.1	−39.4	92.7	13.0	6.0
11	60	30	−90	0	58.1	39.4	−92.7	−13.0	6.0
12	−60	−30	−90	180	−53.6	−45.0	−109.7	170.6	7.0
13	60	30	90	180	53.6	45.0	109.8	−170.6	7.0
14	−60	−30	180	180	−62.7	−33.3	−165.3	176.0	8.3
15	60	30	180	180	62.7	33.3	165.3	175.9	8.3



Scheme 1. Synthesis of Ac-Aib-AzGly–NH_2 using automatic peptide synthesizer.

Table 2. NOE data and calculated interproton distances of Ac-Aib-AzGly-NH_2

NOE	NOE intensity (volume)	Distance from NOE (Å)	Distance from calculations (Å)
Ac CH_3–Aib NH	-2.77×10^5	2.575	2.575
Aib NH–Aib CH_3	-4.9×10^5	2.332	2.425
Aib NH–AzGly NH	-4.4×10^5	2.901	2.898
Aib CH_3–AzGly NH	-2.6×10^4	2.591	2.770
AzGly NH–AzGly $N^{\alpha}H$	-7.9×10^4	2.632	2.693
AzGly NH–C(O)NH_2	-5.1×10^4	2.831	2.732
AzGly $N^{\alpha}H$–C(O)NH_2	-2.6×10^4	3.167	3.523

always about 180° in solution. The distance of 2.575 Å is determined using the optimized conformations in Table 1. These results are summarized in the third column of the Table 2.

To obtain conformations complying with NOE data, the calculated inter-proton distance based on the NOE data were compared with the inter-proton distances measured from the optimized conformations of Table 1. All the inter-proton distances obtained from the conformations 1 and 2 are essentially the same as those of NOE based distances while the inter-proton distances measured from other conformations show big differences. The inter-proton distances of the conformations 1 and 2 summarized in the fourth column of Table 2. These results indicate that the Ac-Aib-AzGly-NH_2 adopts β-I or β-I′ turn conformations. In addition, the differentiation between β-I or β-I′ turn conformations is not possible because this dipeptide is non-chiral.

When a peptide adopts a β-I or β-I′ turn, the carbonyl group of the residue in the *i* position [AcC(O)] and the amide proton of the residue in the $i+3$ position [C(O)NH_2] form an intramolecular hydrogen bonding. Usually, temperature coefficient provides evidence for presence of such intramolecular hydrogen bonding. Since the terminal NH_2 is contained in a urea structure, the indicative temperature coefficient for intramolecular hydrogen bonding in DMSO and water is about −4 ppb/K or below.[12] In cases of amides, it is about −3 ppb/K or below. As shown in Table 3, the temperature coefficient of the terminal NH_2 is about −4 ppb/K while those of the other amide protons are much bigger than −3 ppb/K. These results indicate that only the C-terminal NH_2 is involved in an intramolecular hydrogen bonding and the other amide protons are exposed to the solvent.[13] The resulting solution conformation of the Ac-Aib-AzGly–NH_2 is depicted in Figure 2.

Table 3. Temperature coefficient (ppb/K) of NH protons in Ac-Aib-AzGly–NH_2

	Aib NH	AzGly NH	AzGly $N^{\alpha}H$	C(O)NH_2
DMSO	−7.0	−4.7	−5.0	−3.9
H_2O	−9.1	−9.8	−6.8	−4.3

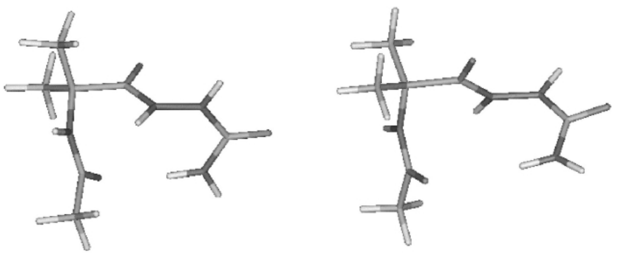

Conformation 1 **Conformation 2**

Figure 2. Preferred conformation of Ac-Aib-AzGly–NH_2 in solution.

Conclusions

In conclusion, we designed a non-chiral dipeptide adopting a typical β-I or β-I′ turn using a combination of constrained amino acids which can stabilize the torsion angles for $i+1$ and $i+2$ positions of the β-turn. After synthesis of the designed dipeptide using automatic solid-phase peptide synthesizer, the β-I or β-I′ turn formation of the compound was confirmed by NMR spectroscopy. We believe that the method described can be a general procedure to generate small peptidomimetics implicating specific secondary structures.

In addition, our recent studies of model peptides containing various azaamino acids using ab initio calculations and NMR spectroscopy indicate that preferred backbone conformations of azaamino acids in peptides are highly similar regardless of side-chain functional groups.[6,14,15] These results indicate that various side chains may be incorporated into the AzGly without major conformational changes. Moreover, in most cases, α-methylated amino acids prefer to adopt the conformation in which ϕ and $\psi = -60°$ and $-30°$.[4] Thus, the β-turn template obtained from these studies will be highly useful for the design of drug candidates or molecular machinery that require a β-I or β-I′ turn.

Experimental

Synthesis of Ac-Aib-AzGly–NH_2

The designed peptidomimetic template was synthesized using an ABI 431A Peptide Synthesizer on which the following procedures were programmed. Fmoc Rink Amide MBHA resin (0.52 mmol/g, 2.09 g) was deprotected with 20% piperidine/NMP for 1 min and 20% piperidine for 20 min. Then, resin was activated with CDI (648 mg, 4 mmol). After 5 h, hydrazine (249 mg, 7.8 mmol) was added. Fmoc Aib (976 mg, 3 mmol) was mixed in 6 g of 0.45 M HBTU/HOBT/DMF and 2 g of NMP, and then, 3 mL of 2 M DIEA was added to initiate the activation of the amino acid. The Fmoc group was removed and then, the N-terminal amino group was acetylated with 4 mL of Ac_2O. The peptide cleaved from the resin by the treatment with 2.5% water in TFA for 1 h. Then, the desired product in a white solid form was obtained by purification with RP-HPLC and freeze-dried (60 mg, overall 27%). ^1H NMR (600 MHz, DMSO, 25 °C): δ 1.32 (s, 6H, Aib $2\times CH_3$), δ 1.86 (s, 3H, Ac), δ 5.90 (s, 2H, $C(O)NH_2$), δ 7.48 (s, 1H, AzGly $N^{\alpha}H$), δ 8.19 (s, 1H, Aib NH), δ 9.50 (s, 1H, AzGly NH) ppm. FAB-MS calcd for $C_7H_{14}O_3N_4$ $[M+H]^+$ 203, found 203.

NMR spectroscopy

^1H NMR spectra were recorded on a Varian Unity plus 600 spectrometer with a 5 mm indirect probe for ^1H at 599.8 MHz. Concentration of the sample 5 mM in dimethylsulfoxyl-d_6 (DMSO-d_6). The chemical shifts are given in ppm downfield from the signal of internal or external DSS. Peak assignment was done by peak integral, TOCSY and NOESY experiment.

The ^1H NMR experiments was collected with 16K data points. For variable temperature experiments, the sample was allowed to equilibrate in the probe for 20–30 min before data collection over temperature range from 5 to 45 °C. The detailed experimental parameters were as follows; spectral width: 20,000 Hz; line broadening: 3 Hz; data size: 8K; number of transients 128.

TOCSY data sets were recorded using the MLEV-17 sequence to generate spin locking time of 100 and 2 ms trim pulse. NOESY spectra were recorded and displayed in the phase-sensitive mode. Mixing time of 400–800 ms was used. All 2-D experiments were obtained with spectral widths of 4950–6600 Hz in both dimensions. 2048 data points in the time domain and 256 blocks in the evolution domain were obtained. For each of the 256 blocks, 64 transients were acquired and the relaxation delay was set to about 1.5 s.

The 1-D and 2-D data sets were processed using the VNMR program. For the 2-D spectra, the data were apodized with a Gaussian function before the Fourier transformation, and then zero-filled 2048×2048 in both dimensions. Volume integration of the cross and diagonal peaks performed using TRIAD NMR software implemented in SYBYL software on IRIX system.

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