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Low pH-triggering Changes in Peptide Secondary Structures

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We developed a novel methodology using cyclic α,α-disubstituted α-amino acids (dAAs) with an acetal-side chain to control peptide secondary structures. The introduction of cyclic dAAs into peptides contributed to the stabilization of peptide secondary structures as a helix, while an acidic treatment of peptides resulted in a marked conformational change.

Foldamers, which were defined by S. H. Gellman as any polymers with a strong tendency to adopt a specific compact conformation,[1] have been the focus of studies for the past decade due to their unique properties[2–4] and are expected to become drug candidates.[5] In order for the functions of synthetic foldamers to become similar to those of natural proteins, foldamer need to make distinct changes in their conformation in response to an external stimulus or environmental change.[6] Proteins often start to function after an appropriate structural change in a suitable environment. α,α-Disubstituted α-amino acids (dAAs) are promising tools for the design of foldamers.[7,8] Peptides with cyclic dAAs are more likely to adopt a helical structure, even those with short sequences.[9–13] These helical peptide foldamers have been reported to function as antimicrobial peptides,[14,15] peptide catalysts,[16,17] and drug delivery peptides.[18–20] In contrast, peptides composed of acyclic dAAs with two bulky substituents equal to or larger than ethyl groups are more likely to form extended planar C5 conformations.[4,21–25] Therefore, the side chains of dAAs in peptides change from a cyclic to an acyclic structure with two bulky substituents, as a consequence, peptide secondary structures may change from a helical to a planar or random structure.

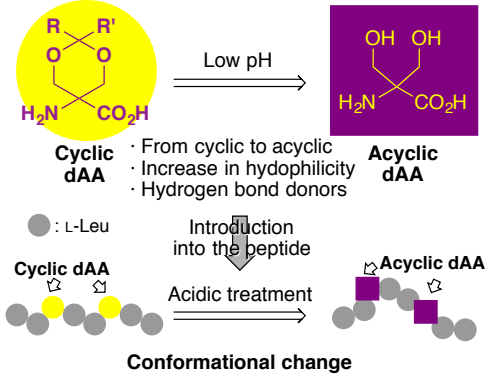
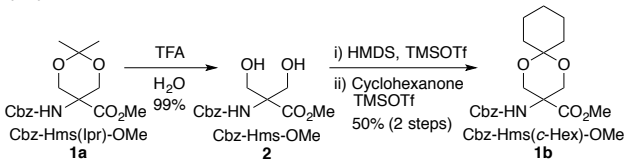


Fig. 1. Conformational changes in peptides containing cyclic dAAs by an acidic treatment.

In the present study, we designed dAAs with a cyclic acetal-side chain. A cyclic acetal is hydrolyzed under acidic conditions and generates an acyclic diol. Besides structural changes from a cyclic to an acyclic structure, the hydrolysis of an acetal to a diol increases its hydrophilicity and produces two hydrogen bond donors, which may break intra- and intermolecular peptide interactions. We assumed that the synergistic effects of structural changes (from cyclic to acyclic), hydrophilic changes (from an acetal to a diol), and the production of hydrogen bond donors contribute to conformational changes in peptides with dAAs (Fig. 1). We prepared two types of dAAs with a cyclic acetal and evaluated their low pH-triggering structural changes. We also introduced dAAs into L-leucine (Leu) sequences and examined their peptide secondary structures before and after an acidic treatment in order to clarify conformational changes in peptides.



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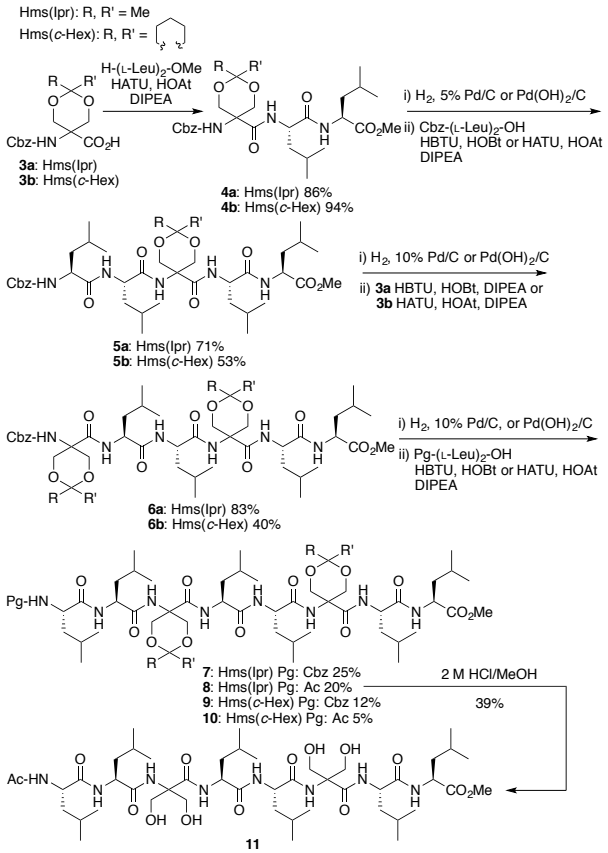
Scheme 1. Synthesis of a cyclic dAA with an acetal side chain. Hms: α-hydroxymethylserine; Hms(Ipr): *O,O*-isopropylidine-α-hydroxymethylserine; Hms(*c*-Hex): *O,O*-cyclohexylidine-α-hydroxymethylserine.

The acyclic dAA with a diol-side chain, Cbz-Hms-OMe **2**, was synthesized via Cbz-Hms(Ipr)-OMe **1a** (Scheme 1). Acetalization using cyclohexanone was achieved according to previously reported methods,[26] giving Cbz-Hms(*c*-Hex)-OMe **1b** in a moderate yield. We then prepared octapeptides with Hms(Ipr), Hms(*c*-Hex), or Hms in the L-Leu sequences (Scheme 2), in order to evaluate the effects of side chain structures on peptide secondary structures and low pH sensitivity. The synthesis of peptides was achieved using fragment condensation solution-phase methods with HATU/HOAt or HBTU/HOBt as coupling reagents. The coupling yields to obtain octapeptides **7**-**10** were relatively low, which was probably due to a steric hindrance of cyclic dAA at N-terminus of hexapeptides. The Ac-protected Hms(Ipr) octapeptide **8** was converted into the Hms octapeptide **11** not by TFA/H_2O but 2 M HCl/MeOH in moderate yield.



Scheme 2. Synthesis of peptides having Hms(Ipr), Hms(*c*-Hex), or Hms in L-Leu sequences.

Low pH-triggering changes in the side chain structures of cyclic dAAs **1a** and **1b** were evaluated by ^{1}H NMR measurements (Fig. S1). The cyclic dAA **1** was dissolved in pH 2 or pH 3 DCl-D_2O/CD_3OD (50/50). After an incubation at 25˚C for the indicated times, ^{1}H NMR were measured and the conversion (%) of cyclic dAA **1** into the acyclic dAA **2** was calculated based on the peak intensity of methylene protons in the side chain. Fig. S1 reveals that side chain structures have a significant impact on low pH-triggering changes from a cyclic acetal to an acyclic diol. Fig. S2 showed the time-dependent MALDI-TOF-MS spectra of the Hms(Ipr) octapeptide **7** and Hms(*c*-Hex) octapeptide **9** in pH 7 and pH 2 TFE/H_2O at room temperature in order to demonstrate low pH-triggering side chain structural changes in dAAs in peptides. The Hms(Ipr) octapeptide **7** was completely transformed into the Hms octapeptide **11** at pH 2 after 1 day (Fig. S2b), but remained intact at pH 7 (Fig. S2a). In the case of the Hms(*c*-Hex) octapeptide **9** at pH 2 (Fig. S2d), two acetal moieties in the peptides were gradually changed into diols and completely deprotected after 4 days. On the other hand, no change was observed in the spectrum at pH 7, even after 4 days (Fig. S2c). These results were consistent with those shown in Fig. S1, in which the Hms(Ipr) dAA **1a** was more sensitive to low pH than the Hms(*c*-Hex) dAA **1b**. Peptides having dAAs with a cyclic acetal designed herein were stable at neutral pH, but reactive at acidic pH.

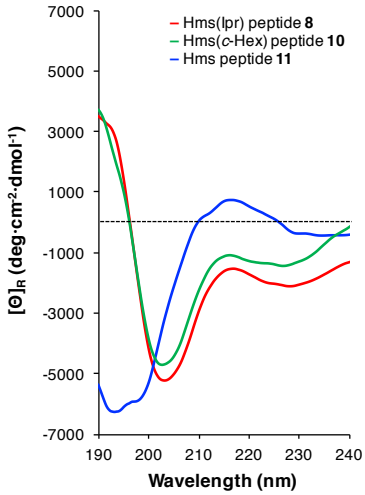


Fig. 2. CD spectra of the Hms(Ipr) octapeptide **8**, Hms(*c*-Hex) octapeptide **10**, and Hms octapeptide **11**. The peptide concentration was 0.1 mM in TFE.

We then investigated the secondary structures of octapeptides in TFE solution using CD spectral measurements (Figs. 2 and S3). Negative maxima at 205–209 nm (π→π*) and 222–225 nm (n→π*) are diagnostic of right-handed (*P*) helical structures.[27–29] The ratio of *R* ($\theta_{n\to\pi^*}/\theta_{\pi\to\pi^*}$) has been used as a parameter to distinguish α-helical from 3_{10}-helical structures (*R* ≈ 1: α-helix; *R* ≤ 0.4: 3_{10}-helix).[30,31] A typical random structure shows a negative maximum at 195–200 nm (π→π*) and a positive maximum at 217 nm (n→π*). Cbz- and Ac-protected Hms(Ipr) and Hms(*c*-Hex) octapeptides showed similar results, in which negative maxima at approximately 205 nm and 225 nm were observed (Fig. S3). Based on the *R* values (**7**: 0.44; **8**: 0.41; **9**: 0.23; **10**: 0.31), they adopted a right-handed (*P*) 3_{10}-helical structure. The FT-IR absorption spectra (the 3250–3500 cm^{-1} region in $CDCl_3$) of Hms(Ipr) peptides **5a**/**6a**/**7** and Hms(*c*-Hex) peptides **5b**/**6b**/**9** supported these results (Fig. 3). The low

frequency band at 3350 cm^{-1} in pentapeptides **5a**/**5b** shifted to a lower wavenumber of 3330 cm^{-1} in octapeptides **7**/**9**, and its intensity increased with the elongation of peptide lengths, suggesting that the dominant secondary structure was a helix. Hexapeptides **6a**/**6b** showed bands assigned to peptide NH groups intramolecularly hydrogen bonded to acetal oxygens, which is consistent with a previous study.[26,32] On the other hand, the Hms octapeptide **11**[33] showed the typical CD spectrum of a random structure with a negative maximum at 195 nm and positive maximum at 215 nm (Fig. 2). Furthermore, the conformations of octapeptides **7** and **11** were analyzed via CD in MeOH/H_2O (50/50) (Fig. S4). The preferred conformation of Hms octapeptide **11** was a random structure, while that of Hms(Ipr) octapeptide **7** was an α-helical structure (*R* = 0.86). Changes in the side chain structures from cyclic acetals to acyclic diols led to marked changes in peptide secondary structures from a helical to a random structure. We previously reported that the preferred conformation of L-Leu-based octapeptides containing two cyclic dAAs with a seven-membered ring was similar to that having two dipropylglycines as acyclic dAAs, a right-handed (*P*) 3_{10}-helical structure.[34] These findings implied that a structural change in the side chain of dAAs from cyclic to acyclic is not sufficient to induce conformational changes in dAA-containing L-Leu peptides. However, in the present study, a marked conformational change was observed in peptides with Hms(Ipr/*c*-Hex) and Hms, suggesting that no change in the side chain from cyclic to acylic, but a change in the hydrophilicity and/or generation of hydrogen bond acceptors is important for changes in peptide secondary structures.

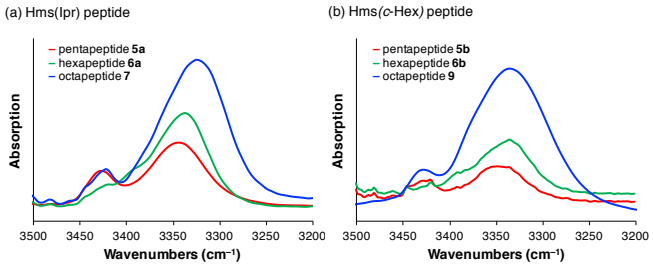


Fig. 3. FTIR absorption spectra of a) Hms(Ipr) pentapeptide **5a**, hexapeptide **6a**, and octapeptide **7**, and b) Hms(*c*-Hex) pentapeptide **5b**, hexapeptide **6b**, and octapeptide **9**. The peptide concentration was 5 mM in $CDCl_3$.

The Hms(Ipr) octapeptide **7** formed good crystals for an X-ray crystallographic analysis due to slow evaporation of the solvent (EtOH/H_2O) at room temperature.[35] Crystal and diffraction parameters are summarized in Table S1. Relevant backbone and side-chain torsion angles as well as intra- and intermolecular hydrogen bond parameters are listed in Tables S2 and S3, respectively. The molecular structures of **7** are shown in Figs. 4a and 4b. Two right-handed (*P*) α-helices were present in the asymmetric unit along with three ethanol molecules (Fig. 4a). The conformations of the two molecules (molecules A and B) were generally similar. The mean ϕ and ψ torsion angles of the amino acid residues (1–6) were –60.0˚ and –43.4˚ for molecule A and –58.1˚ and –44.3˚ for molecule B, respectively, which are close to those for an ideal right-handed (*P*) α-helix (–60˚ and –45˚, respectively). Five intramolecular hydrogen bonds of the *i* ⟵ *i*+4 type, which corresponded to the α-helical conformation, are shown in molecules A and B (Table S3). Besides the hydrogen bond of the *i* ⟵ *i*+4 type, one hydrogen bond of the *i* ⟵ *i*+3 type (3_{10}-helical conformation) was observed in molecule B [N(7)···O(4)], indicating that this specific hydrogen bond was of the bifurcated type. In the packing mode, two molecules, A and B, were alternatively connected by intermolecular hydrogen bonds along the backbone, forming head-to-tail aligned chains; i.e., ···A···B···A···B···. These results slightly differed from the CD spectral data in TFE (Fig. S3), but were similar to that in MeOH/H_2O (50/50) (Fig. S4). This may have been because the single crystal for the X-ray analysis was obtained by recrystallization from EtOH/H_2O and the CD spectra were measured in TFE or MeOH/H_2O, or, alternatively, a minor (*P*) α-helical structure existing in solution may have been preferentially induced in the crystal state as a consequence of nucleation events. It is important to note that no hydrogen bond from the acetal oxygens was observed in the side chain of Hms(Ipr). Acetal oxygens did not suppress the formation of hydrogen bonds in the peptide backbone. Unfortunately, good crystals of the Hms octapeptide **11** have not yet been obtained for an X-ray analysis. Conformational calculations for α-helical octapeptides **8** and **10** were performed using the statistical software MacroModel 10.0 (MCMM method; AMBER* (H_2O); 20,000 calculations) (Fig. S5). The global minimum energy conformation of the calculated α-helical structure of the Hms(Ipr) octapeptide **8** closely matched the X-ray conformation of **7** (Fig. 4c), although these peptides had a different N-terminal-protecting group (**7**: Ac; **8**: Cbz).

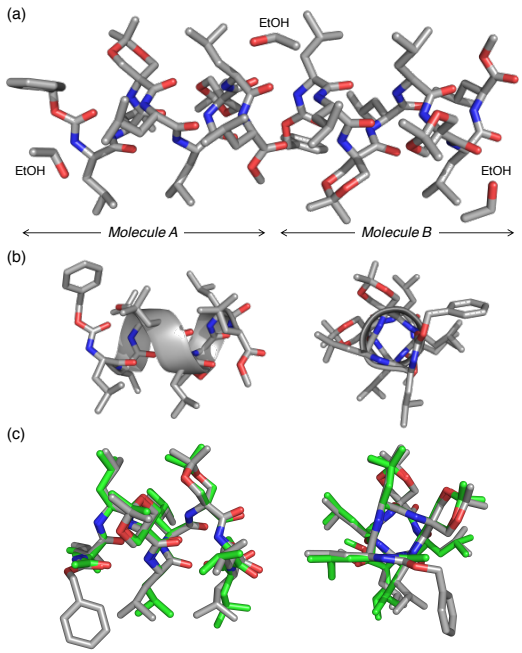


Fig. 4. a) Crystal-state structures of the Hms(Ipr) octapeptide **7**. b) Side and top views of molecule A. c) Superimposition of **7**

(molecule A) assessed by an X-ray analysis (gray) and the calculated α-helical structure of **8** (green).

Conclusions

In summary, we herein developed a strategy using cyclic dAAs for conformational changes in peptides from a helical to a random structure by an acidic treatment. The cyclic acetal-side chain was converted into the acyclic diol-side chain in response to low pH. The acidic treatment of the cyclic dAA-containing peptide afforded an acyclic dAA-containing peptide, and, consequently, peptide secondary structures markedly changed from a helical to a random structure. These results will contribute to the design of functional foldamers in response to an external stimulus or environmental change.

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33 Hms octapeptide **11** was dissolved in MeOH and TFE, but not in $CHCl_3$ and CH_3CN. Therefore, conformational analysis of it by FTIR spectra was impossible.
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35 CCDC-1543113 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via: Available at: www.ccdc.cam.ac.uk/data_request/cif.

