



Effects of charge and hydrophobicity on the oligomerization of peptides derived from IAPP



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ABSTRACT

Changes in pH resulting in modifications of charge can dramatically alter the folding and interaction of proteins. This article probes the effects of charge and hydrophobicity on the oligomerization of macrocyclic β -sheet peptides derived from residues 11–17 of IAPP (RLANFLV). Previous studies have shown that a macrocyclic β -sheet peptide containing this IAPP sequence (peptide **1_{Arg}**) does not form oligomers in aqueous solution at low millimolar concentrations. Replacing arginine with the uncharged isostere citrulline generates a homologue (peptide **1_{Cit}**) that forms a tetramer consisting of a sandwich of hydrogen-bonded dimers. The current study probes the role of charge and hydrophobicity by changing residue 11 to glutamic acid (peptide **1_{Glu}**) and leucine (peptide **1_{Leu}**). Diffusion-ordered spectroscopy (DOSY) studies show that peptides **1_{Glu}** and **1_{Leu}** form tetramers in solution. NOESY studies confirm that both peptides form the same sandwich-like tetramer as peptide **1_{Cit}**. ^1H NMR spectroscopy at various concentrations reveals that peptide **1_{Leu}** has the highest propensity to form tetramers. The effects of pH and charge on oligomerization are further probed by incorporating histidine at position 11 (peptide **1_{His}**). DOSY studies show that peptide **1_{His}** forms a tetramer at high pH. At low pH, peptide **1_{His}** forms a new species that has not been previously observed by our research group—a dimer. These studies demonstrate the importance of charge and hydrophobicity in the oligomerization of IAPP-derived peptides.

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1. Introduction

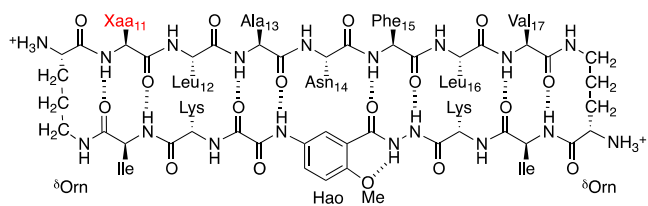
pH provides a critical trigger for changes in protein conformation and structure. Decreases in pH trigger the release of oxygen from hemoglobin in the bloodstream to tissues where it is needed.¹ The cooperative protonation of histidine residues in the hemoglobin tetramer provides the trigger mechanism.^{2,3} Decreases in pH also trigger fusion of the endosomal and viral membranes in the transmission of influenza A viruses. This process occurs through the protonation of histidine residues in influenza hemagglutinin, which causes refolding and binding to receptors on the host cells, which then induce endocytosis of the virus.^{4–6} Researchers have also exploited variations in pH for drug delivery to environments where there are subtle pH changes.^{7–11} Polyhistidine-based micelles were designed to respond to acidic tumour microenvironments, and a copolymer nanoparticle incorporating a polyhistidine sequence was designed to increase uptake of an antibiotic through acidic bacterial cell walls.^{7,10} pH can also provide a conformational switch in the folding of synthetic macromolecules and in peptide self assembly.^{12–15} In a recent study, Andersen and coworkers

designed a pH switch for β -sheet folding in which the hexapeptide sequence HPATGK switches from unstructured to a turn at physiological and higher pH.¹⁴ Conticello and coworkers also designed a reversible pH switch for self assembly where they incorporated three histidine residues into the core of the isoleucine zipper peptide, GCN4-pII, allowing it to toggle between random coil and α -helical assemblies.¹⁵ Histidine residues provide a good trigger for these biological and biomimetic processes because their protonation state changes near physiological pH.

In the current study, we set out to explore the role of charge and hydrophobicity in peptide self-assembly and ask whether pH can modulate assembly. We performed these studies with a well-characterized peptide model system that assembles to form tetramers in both aqueous solution and in the crystal state.^{16–18} The model system consists of macrocyclic β -sheet peptides containing residues 11–17 of the islet amyloid polypeptide (IAPP). Peptides **1_{Arg}** and **1_{Cit}**, which we had previously characterized, are representative of the model system (Chart 1).¹⁹ Peptide **1_{Arg}** consists of a peptide strand and a template strand that are connected by two δ -linked ornithine ($^{\delta}\text{Orn}$) turn units.^{20,21} The peptide strand of **1_{Arg}** contains the IAPP_{11–17} heptapeptide sequence RLANFLV, and the template strand contains isoleucine and lysine residues to reinforce hydrophobicity and increase solubility respectively. The unnatural amino acid Hao is centered within the template strand and mimics

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macrocyclic β -sheet peptides **1**_{Arg}, **1**_{Cit}, **1**_{Glu}, **1**_{Leu}, **1**_{His}

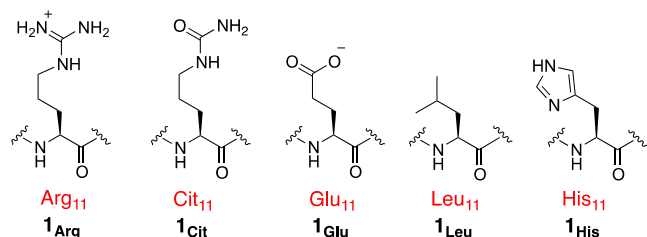


Chart 1. Structures of macrocyclic β -sheet peptides **1**.

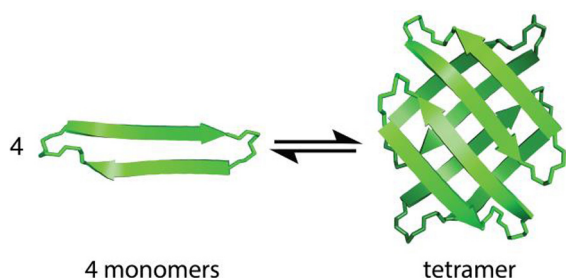


Fig. 1. Tetramer formation of peptide **1**_{Cit}. X-ray crystallographic structure of the tetramer and the monomer subunit of a heavy-atom derivative of peptide **1**_{Cit} (PDB 5UHR). The tetramer consists of a sandwich of hydrogen-bonded dimers laminated through hydrophobic interactions.

a tripeptide β -strand, templates β -sheet formation, and blocks uncontrolled aggregation.²² Peptide **1**_{Cit} is a homologue, in which cationic Arg₁₁ is replaced by the neutral isostere citrulline. Citrulline contains a urea group in place of the guanidinium group. Peptide **1**_{Cit} assembles to form tetramers at low millimolar concentrations in aqueous solution, whereas peptide **1**_{Arg} is monomeric under these conditions (Fig. 1).

Inspired by our previous results and by the role of pH in protein interactions, we further explored the role of charge and hydrophobicity in the assembly of macrocyclic β -sheet peptides **1** derived from IAPP_{11–17}. We incorporated glutamic acid at position 11 to create peptide **1**_{Glu}, and leucine at position 11 to create peptide **1**_{Leu}, and we studied the assembly of the peptides by NMR spectroscopy (Chart 1). Our efforts culminated in peptide **1**_{His}, a macrocyclic β -sheet peptide that changes oligomerization state in response to pH.

2. Results and discussion

2.1. Effects of charge and hydrophobicity on tetramer formation

The ¹H NMR spectrum of macrocyclic β -sheet peptides reflects their oligomerization state.^{17–19,23,24} In the monomeric state, resonances are sharp and are generally at values typical of the corresponding random coil amino acids.²⁵ In the tetrameric state, resonances are often broader, the α -proton resonances are generally shifted downfield, and the aromatic resonances are often shifted upfield.

In aqueous solution at 298 K, the ¹H NMR spectrum of peptide **1**_{Arg} at 4 mM displays a single set of sharp resonances, indicating that the peptide is monomeric (Fig. 2). The resonances associated with the α -protons appear at around 4–5 ppm, and the aromatic resonances of the Phe₁₅ side chain appear at 7 ppm.²⁵ At 8 mM, the ¹H NMR spectrum of peptide **1**_{Arg} shows broadening of the resonances but no additional peaks associated with the onset of self association. The ¹H NMR spectrum of peptide **1**_{Cit} at 1 mM resembles the ¹H NMR spectrum of peptide **1**_{Arg} and displays a single set of sharp resonances, indicating that the peptide is monomeric.¹⁹ At 4 mM, peptide **1**_{Cit} displays two sets of broad resonances associated with the monomer and the tetramer, and at 16 mM only the resonances associated with the tetramer are visible.¹⁹ The α -proton resonances of the tetramer are shifted downfield by up to 1 ppm relative to typical random coil α -protons. The aromatic resonances of the Phe₁₅ side chain are shifted upfield from about 7.0 ppm to about 6.5 ppm. These changes in chemical shift reflect the folding and assembly of the peptide into a tetramer.

The magnetic anisotropy of the diastereotopic δ -protons of the δ Orn turn units reflects β -sheet folding in macrocyclic β -sheet peptides.²¹ Previous studies in our laboratory have shown that complete folding accompanies tetramer formation.^{17,23,24} In a

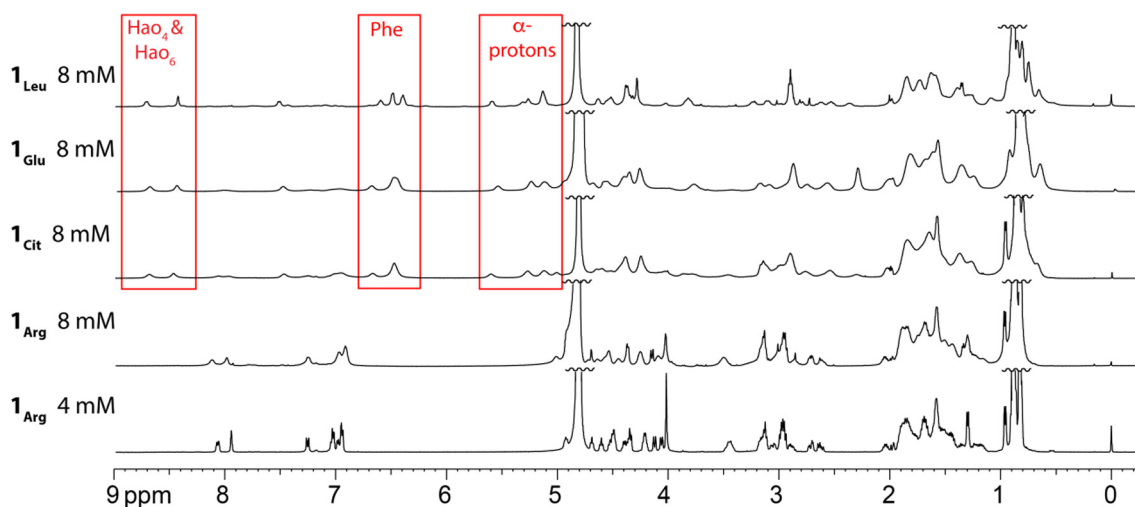


Fig. 2. ¹H NMR spectra of macrocyclic β -sheet peptides **1**_{Arg}, **1**_{Cit}, **1**_{Glu}, and **1**_{Leu} in 50 mM CD₃COOD and 50 mM CD₃COONa buffer in D₂O at 500 MHz and 298 K. The red boxes highlight key resonances in the tetramer.

Table 1
 $\Delta\delta^{\text{Orn}}$ values^a of peptides **1Arg**, **1Cit**, **1Glu**, **1Leu**, and **1His** in D₂O at 298 K.

	pH	Conc (mM)	$\Delta\delta^{\text{Orn}}$ (ppm)	$\Delta\delta^{\text{Orn}}$ (ppm)	Average $\Delta\delta^{\text{Orn}}$ (ppm)
1Arg	4.7 ^b	4	0.31	0.31	0.31
1Cit	4.7 ^b	16	0.70	0.66	0.68
1Glu	4.7 ^b	16	0.69	0.59	0.64
1Leu	4.7 ^b	16	0.71	0.60	0.66
1His	2.5 ^c	1	0.29	0.29	0.29
1His	2.5 ^c	16	0.47	0.47	0.47
1His	4.7 ^b	16	0.51	0.51	0.51
1His	7.2 ^d	16	0.77	0.57	0.67

^a Difference in chemical shift of the diastereotopic δ -protons of the δ^{Orn} turn unit.

^b 50 mM CD₃COOD and 50 mM CD₃COONa buffer in D₂O.

^c Unbuffered peptide in D₂O.

^d 50 mM CD₃COOD and 50 mM CD₃COONa buffer in D₂O with the pH adjusted to 7.2 with NaOD.

well-folded macrocyclic β -sheet peptide, each diastereotopic *pro-S* δ -proton resonance is about 0.6 ppm downfield of the corresponding *pro-R* δ -proton resonance. The ¹H NMR spectrum of monomeric peptide **1Arg** shows that it is partially folded, with an average difference in chemical shifts of 0.31 ppm (Table 1). In contrast, the monomer subunits of the tetramers formed by peptides **1Cit**, **1Glu**, and **1Leu** are well folded, with average differences in chemical shifts of 0.68, 0.64, 0.66 ppm, respectively (Table 1).

To better understand how charge and hydrophobicity affect oligomerization, we compared homologues **1Cit**, **1Glu**, and **1Leu** and evaluated the effects of the respective polar, acidic, and hydrophobic residues at position 11. Peptides **1Cit**, **1Glu**, and **1Leu** are monomeric at submillimolar concentrations in aqueous solution, but form tetramers at millimolar concentrations (Figs. S1 and S2). The ¹H NMR spectra of peptides **1Cit**, **1Glu**, and **1Leu** show only single sets of sharp resonances associated with the monomer at 0.1 mM. The chemical shifts of the α -protons and aromatic resonances are similar to those of peptide **1Arg**, reflecting the monomeric state of the peptides at low concentrations. At 8 mM, each of the three peptides displays two sets of resonances, which correspond to the monomer and the tetramer (Fig. 2). The ¹H NMR spectra of the three peptides are broad, and key resonances appear at similar positions, with the α -proton resonances of the tetramers shifted downfield and the aromatic resonances shifted upfield.

To assess the propensities of peptides **1Cit**, **1Glu**, and **1Leu** to form tetramers, we measured the monomer-tetramer ratios at 0.1, 1, 2, 4, and 8 mM by ¹H NMR spectroscopy. Integration of the Hao₄ and Hao₆ resonances permits the measurement of the percentage of tetrameric peptides (Table S1). Peptide **1Cit** is about 50% tetrameric at 4 mM, peptide **1Glu** is about 50% tetrameric at 2 mM, and peptide **1Leu** is 50% tetrameric at just above 1 mM (Fig. 3). These studies show the following propensities of these peptides to form tetramers: **1Leu** > **1Glu** > **1Cit** >> **1Arg**.

Electrostatic and hydrophobic interactions explain the relative propensities of peptides **1Cit**, **1Glu**, **1Leu**, and **1Arg** to form tetramers in aqueous solution. We have previously observed that a derivative of peptide **1Cit** containing a heavy atom forms sandwich-like tetramers (Fig. 1). The crystallographic structure (PDB 5UHR) shows that the side chain of Cit₁₁ is proximal to the protonated α -amine of Orn in the dimer subunits of the tetramer. Peptide **1Leu** has the highest propensity to oligomerize, as it has a non-polar residue at position 11, which can further contribute to the hydrophobic core. Peptide **1Glu** has the second highest propensity to oligomerize, as it has a negatively charged residue at position 11, which may allow electrostatic attraction or the formation of a salt bridge with the protonated α -amine of Orn. Peptide **1Cit** has the lowest propensity to oligomerize, as it has an uncharged polar side chain. In contrast, peptide **1Arg** does not oligomerize, as the electrostatic

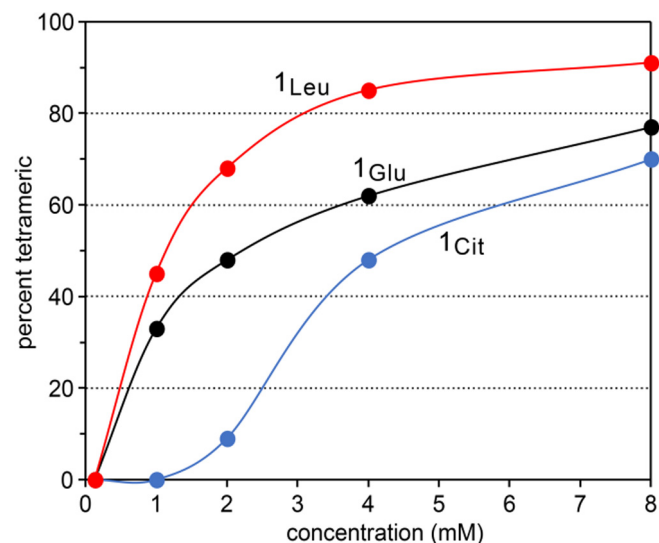


Fig. 3. Percentage of tetrameric peptides **1Cit**, **1Glu**, and **1Leu** at 0.1, 1, 2, 4, and 8 mM. The values are derived from integrations of the Hao₄ and Hao₆ resonances of the tetramer and the monomer.

repulsion of the positively charged side chain of Arg₁₁ and the protonated α -amine of Orn inhibits the formation of the dimer.

2.2. Assembly of tetramers **1Glu** and **1Leu**

Diffusion-ordered spectroscopy (DOSY) provides a convenient method to assess the relative oligomerization state of macrocyclic β -sheet peptides **1**. Dimers typically have a diffusion coefficient of about 0.75–0.79 times that of monomers, and tetramers have a diffusion coefficient of about 0.58–0.63 times that of monomers.^{17,18,23,26,27} We have previously observed that similarly sized monomers exhibit a diffusion coefficient of about 20×10^{-7} cm²/s, while tetramers exhibit a diffusion coefficient of about 12×10^{-7} cm²/s. At 16 mM and pH 4.7 or 7.2, the diffusion coefficients of peptides **1Glu** and **1Leu** are consistent with tetramers (Table 2).

NOESY NMR spectroscopy establishes that the structures of the tetramers formed by peptides **1Leu** and **1Glu** in solution are similar to the tetramer previously observed for peptide **1Cit**.¹⁹ The NOESY spectrum of peptide **1Leu** exhibits NOE crosspeaks that reflect intramolecular contacts within the monomer subunits and intermolecular contacts within the dimers and tetramers. Three strong crosspeaks in the NOESY spectrum of peptide **1Leu** indicate that the macrocyclic β -sheet monomers are well-folded—one between the α -protons of Leu₁₂ and Lys, one between the α -protons of Leu₁₆ and Lys, and one between the α -proton of Asn₁₄ and the proton at the 6-position of the tripeptide mimic Hao (Fig. 4B). A strong

Table 2
Diffusion coefficients (*D*) of peptides **1Glu**, **1Leu**, and **1His** in D₂O at 298 K.

	Conc (mM)	pH	<i>D</i> (10 ^{−7} cm ² /s)	Oligomer state
1Glu	16	4.7 ^a	12.1 ± 0.6	Tetramer
1Glu	16	7.2 ^c	12.4 ± 0.3	Tetramer
1Leu	16	4.7 ^a	11.4 ± 0.5	Tetramer
1Leu	16	7.2 ^c	12.3 ± 1.3	Tetramer
1His	1	2.5 ^b	19.8 ± 0.8	Monomer
1His	4	2.5 ^b	16.8 ± 0.6	Dimer
1His	8	2.5 ^b	16.4 ± 0.9	Dimer
1His	16	2.5 ^b	16.4 ± 0.3	Dimer
1His	16	4.7 ^a	16.4 ± 0.3	Dimer
1His	16	7.2 ^c	12.3 ± 1.2	Tetramer

^a 50 mM CD₃COOD and 50 mM CD₃COONa buffer in D₂O.

^b Unbuffered peptide in D₂O.

^c 50 mM CD₃COOD and 50 mM CD₃COONa buffer in D₂O with the pH adjusted to 7.2 with NaOD.

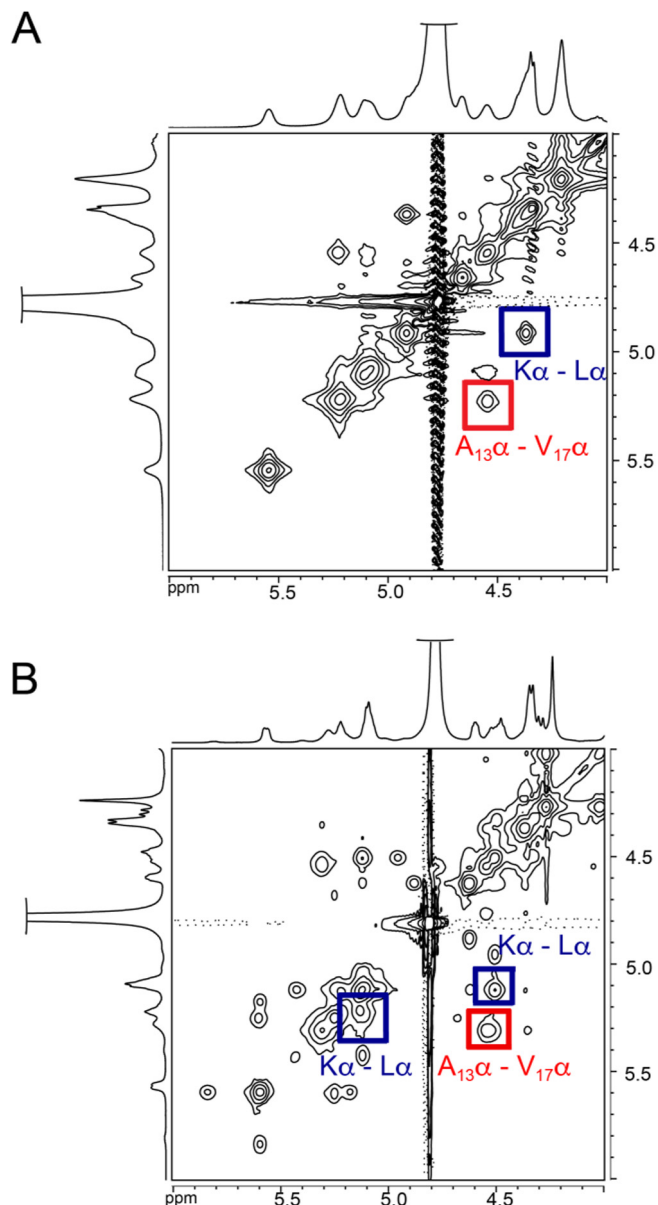


Fig. 4. NOEs associated with the dimer subunits of peptides **1_{Glu}** and **1_{Leu}**. (A) Expansion of the NOESY spectrum of peptide **1_{Glu}** showing selected intramolecular (blue) and intermolecular (red) NOE crosspeaks. (B) Expansion of the NOESY spectrum of peptide **1_{Leu}** showing selected intramolecular (blue) and intermolecular (red) NOE crosspeaks.

crosspeak between the α -protons of Ala₁₃ and Val₁₇ reflect formation of a shifted, antiparallel dimer, in which the two heptapeptide β -strands are shifted by two residues towards the C-termini (Fig. 5). A characteristic NOE between the methoxy group of Hao and the Ile side chain reflects packing of the dimers to form a sandwich-like tetramer. The NOESY spectrum of peptide **1_{Glu}** exhibits the same NOE crosspeaks with the exception of one Leu-Lys crosspeak that cannot be discerned due to overlap (Fig. 4A). These NOEs associated with the folded monomer, dimer, and tetramer indicate that peptides **1_{Leu}** and **1_{Glu}** form the same tetramer structure as peptide **1_{Cit}**.

2.3. A histidine-containing homologue that forms either a dimer or a tetramer

Inspired by these results and the role of pH in triggering changes in protein structure and interactions, we incorporated a

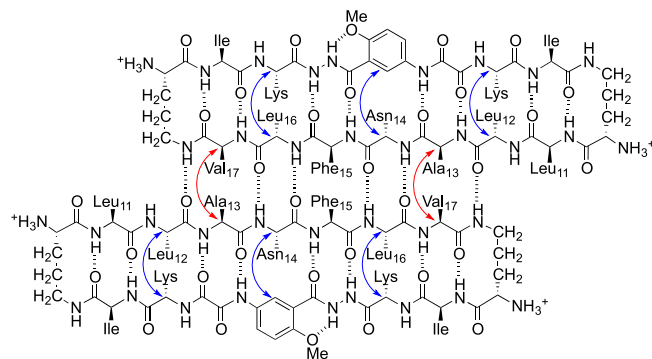


Fig. 5. Shifted, antiparallel hydrogen-bonded dimer of peptide **1_{Leu}**. Blue and red arrows indicate key intra- and intermolecular NOEs observed in the NOESY spectrum. Peptide **1_{Glu}** forms an analogous hydrogen-bonded dimer. For peptide **1_{Glu}**, one Leu-Lys crosspeak cannot be observed due to overlap.

histidine residue at position 11, to create peptide **1_{His}** (Chart 1). We envisioned that peptide **1_{His}** would change oligomerization state upon protonation of histidine ($pK_a \sim 6$). At low pH, the histidine should be protonated, like peptide **1_{Arg}**. At higher pH, the histidine should be deprotonated, like peptide **1_{Leu}** and peptide **1_{Cit}**.

At pH 2.5 and 1 mM, peptide **1_{His}** is monomeric. The ^1H NMR spectrum of peptide **1_{His}** exhibits a single set of sharp resonances, associated with the monomer (Fig. 6). The resonances associated with the α -protons appear at 4–5 ppm, and the aromatic resonances of the Phe₁₅ side chain appear at approximately 7 ppm. The diffusion coefficient is $19.8 \times 10^{-7} \text{ cm}^2/\text{s}$.

At higher concentrations at pH 2.5, peptide **1_{His}** forms a dimer—an oligomerization state that we have not previously observed in Hao-containing macrocyclic β -sheet peptides. The ^1H NMR resonances are somewhat broadened at 4 mM and 8 mM, but are sharper at 16 mM (Fig. 6). The diffusion coefficients at 4 mM, 8 mM, and 16 mM are 16.8, 16.4, and $16.4 \times 10^{-7} \text{ cm}^2/\text{s}$, respectively (Table 2). These values are 0.84–0.82 times that of the monomer, best matching a dimer and not matching a tetramer. The lack of substantial change in diffusion coefficient from 4 mM to 16 mM indicates that the observed spectrum does not correspond to a rapid monomer-tetramer equilibrium, but instead represents a unique species—a dimer.

The spectrum of the dimer differs from that of the monomer and those of the other tetramers. The aromatic resonances of Phe₁₅ are shifted upfield by about 0.2 ppm, to 6.8 ppm, and the Hao₄ and Hao₆ resonances are shifted downfield by 0.2 ppm, to 8.3 and 8.1 ppm, respectively. The monomer subunit of the dimer is better folded than the free monomer, with the average difference in chemical shifts of the diastereotopic δ -protons of the ^6Orn turn units increasing from 0.29 ppm at 1 mM to 0.47 ppm at 16 mM.

NOESY spectroscopy reveals relatively little about the structure of the dimer. The NOESY spectrum of peptide **1_{His}** at pH 2.5 and 16 mM shows negative NOEs, as would be expected for an oligomer ($\geq 3.5 \text{ kDa}$) but not a monomer (1.7 kDa). A crosspeak between the α -protons of Leu and Lys and a crosspeak between the α -proton of Asn₁₄ and the proton at the 6-position of the Hao residue shows that the monomer subunit is folded into a β -sheet. No additional NOE crosspeaks between α -protons are observed, which may reflect overlap of resonances or the lack of edge-to-edge contacts between the folded monomer subunits. NOE crosspeaks between the side chain of Phe₁₅ and one of the isoleucine residues suggest hydrophobic packing, as do weaker NOE crosspeaks between the isoleucine side chain and the methoxy group of Hao.

Upon increasing the pH to 7.2, the ^1H NMR spectrum changes dramatically. The spectrum broadens slightly at pH 4.7 and 16

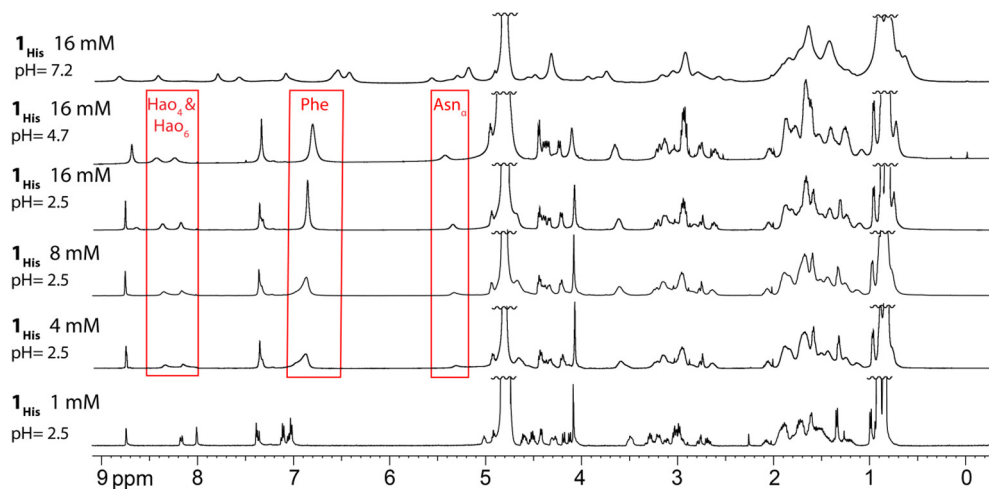


Fig. 6. ^1H NMR spectra of macrocyclic β -sheet peptide **1His** at 500 MHz and 298 K. Spectra at pH 2.5 are in D_2O . Spectrum at pH 4.7 is in 50 mM CD_3COOD and 50 mM CD_3COONa buffer in D_2O . Spectrum at pH 7.2 is in 50 mM CD_3COOD and 50 mM CD_3COONa buffer adjusted to pH 7.2 with NaOD in D_2O . The red boxes highlight key resonances of the dimer.

mM, and resonances begin to shift suggesting the onset of self-association (Fig. 6). At pH 7.2, the diffusion coefficient drops to $12.3 \times 10^{-7} \text{ cm}^2/\text{s}$ and the spectrum resembles those of tetramers **1Cit**, **1Glu**, and **1Leu** (Fig. 6). New resonances appear in the α -region and aromatic region, indicating the formation of a new species. The monomer subunits are fully folded, with 0.67 ppm average difference in chemical shifts of the diastereotopic δ -protons of the $^{\circ}\text{Orn}$ turn units. Several resonances associated with the α -protons of the tetramer are shifted above 5 ppm, reflecting the folding and assembly of the peptide. The aromatic resonances of the Phe₁₅ side chain are shifted upfield, reflecting packing of the aromatic side chains to form a hydrophobic core. The dimer resonances are no longer present at higher pH, reflecting a pH-dependent transition from dimer to tetramer.

The observation of a stable dimer of peptide **1His** under acidic conditions is notable, because we do not observe dimer formation for peptides **1Cit**, **1Glu**, or **1Leu**. In all conditions studied, we observe only monomer or tetramer for these peptides. The absence of observed dimer for these peptides does not mean that absolutely no dimer forms, but rather that the monomer-tetramer equilibrium predominates and that tetramer formation is cooperative.

The formation of a dimer by peptide **1His** under acidic conditions suggests that some feature or features of the protonated imidazole group stabilize the dimer, while destabilizing the tetramer. The extra charge of the protonated peptide **1His** likely disrupts tetramer formation. The protonated imidazole group of peptide **1His** is less hydrophilic than the guanidinium group of peptide **1Arg**, which likely facilitates hydrophobic packing of the dimer. It is also notable that the ^1H NMR spectrum of peptide **1Arg** broadens slightly at 8 mM, suggesting the onset of self association. It is likely that this hint of self association reflects the onset of dimerization, rather than tetramerization.

3. Conclusion

These ^1H NMR studies of macrocyclic β -sheet peptides **1Arg**, **1Cit**, **1Glu**, **1Leu**, and **1His** demonstrate how charge and hydrophobicity can affect oligomerization in a peptide model system. In this model system, hydrophobic surfaces and hydrogen-bonding edges are critical in tetramer formation. Increasing positive charge on the hydrophobic surface through mutation or protonation disrupts tetramer formation. A histidine residue provides a convenient pH trigger for modulating the charge of the hydrophobic surface.

Although peptide **1His** is far smaller and simpler than hemoglobin or influenza hemagglutinin, it exhibits pH-triggered behavior like that of large proteins.

4. Experimental section

4.1. Synthesis of macrocyclic β -Sheet peptides

Synthesis of macrocyclic β -sheet peptides was performed on a 0.1 mmol scale as previously published.¹⁷ The peptides were purified by reverse-phase HPLC, and the pure fractions were lyophilized to give 19 mg of peptide **1Glu**, 22 mg of peptide **1Leu**, and 8 mg of **1His**. Based on resin loading, the yields are 7%, 9%, and 4%, respectively. All macrocyclic β -sheet peptides were prepared as the trifluoroacetate salts.

Macrocyclic β -sheet peptide **1Glu**: HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{82}\text{H}_{134}\text{N}_{21}\text{O}_{20}$ 1733.0114; Found 1733.0093.

Macrocyclic β -sheet peptide **1Leu**: HRMS (ESI-TOF) m/z : $[\text{M}+\text{Na}]^+$ Calcd for $\text{C}_{83}\text{H}_{137}\text{N}_{21}\text{O}_{18}\text{Na}$ 1739.0348; Found 1739.0363.

Macrocyclic β -sheet peptide **1His**: HRMS (ESI-TOF) m/z : $[\text{M}+\text{H}]^+$ Calcd for $\text{C}_{83}\text{H}_{134}\text{N}_{23}\text{O}_{18}$ 1741.0277; Found 1741.0278.

4.2. NMR spectroscopy of macrocyclic β -sheet peptides

Sample preparation. NMR spectroscopy of macrocyclic β -sheet peptides at pH 2.5 was performed in D_2O (D, 99.96%; Cambridge Isotope Laboratories, Inc.). NMR spectroscopy of macrocyclic β -sheet peptides at pH 4.7 was performed in D_2O with 50 mM CD_3COOD (D, 99.5%; Cambridge Isotope Laboratories, Inc.) and 50 mM CD_3COONa (D, 99%; Cambridge Isotope Laboratories, Inc.). NMR spectroscopy of macrocyclic β -sheet peptides at pH 7.2 was performed in D_2O with 50 mM CD_3COOD and 50 mM CD_3COONa with the pH adjusted using NaOD (D, 99.5%; Cambridge Isotope Laboratories, Inc.). The solutions were prepared by dissolving a weighed portion of the peptide in the appropriate volume of D_2O or buffer. The molecular weights of the peptides were calculated as the trifluoroacetate salts with all amino groups assumed to be protonated (**1Glu**, MW 2188.87 g/mol; **1Leu**, MW 2172.93 g/mol; and **1His**, MW 2310.90 g/mol). The solutions at pH 4.7 and 7.2 contained 4,4-dimethyl-4-silapentane-1-ammonium trifluoroacetate (DSA) as an internal standard for referencing chemical shifts.²⁸ The solutions were prepared and left for 24 h to allow complete hydrogen-to-deuterium exchange of the amide NH protons.

4.2.1. ^1H NMR, TOCSY, ROESY, and NOESY data collection

NMR spectra were recorded on a Bruker 500 MHz spectrometer with a TCI cryoprobe. TOCSY spectra were recorded with 2048 points in the f_2 dimension and 512 increments in the f_1 dimension with a 150-ms spin-lock mixing time. NOESY spectra were recorded with 2048 points in the f_2 dimension and 512 increments in the f_1 dimension, with a 150-ms mixing time.

4.2.2. ^1H NMR, TOCSY, and NOESY data processing

NMR spectra were processed with Bruker XwinNMR software. Automatic baseline correction was applied in both dimensions after phasing the spectra. TOCSY spectra were Fourier transformed to a final matrix size of 4096×1024 real points using a Qsine weighting function and forward linear prediction. NOESY and ROESY spectra were Fourier transformed to a final matrix size of 4096×2048 real points using a Qsine weighting function and forward linear prediction.

4.2.3. Diffusion-ordered spectroscopy (DOSY) experiments

DOSY experiments were performed on a Bruker 500 MHz spectrometer equipped with a TCI cryoprobe, with a diffusion delay (Δ) of 75 ms and a diffusion gradient length (δ) of 2.5 ms. Sixteen sets of FIDs were recorded with the gradient strength incremented from 5% to 95% using a linear ramp. The combined FIDs were Fourier transformed in Bruker's TopSpin™ software to give a pseudo-2D spectrum. After phasing and performing baseline correction, each pseudo-2D spectrum was processed with logarithmic scaling on the Y-axis. The Y-axis was calibrated to the diffusion coefficient of the residual HOD peak in D_2O ($1.9 \times 10^{-9} \text{ m}^2/\text{s}$ at 298 K).²⁹ The diffusion coefficients of the peptides were read and converted from logarithmic values to linear values.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bmc.2017.10.001>.

References

1. Kilmartin JV, Breen JJ, Roberts GCK, Ho C. *Proc Natl Acad Sci USA*. 1973;70:1246–1249.
2. Edelstein SJ. *Annu Rev Biochem*. 1975;44:209–232.
3. Eaton WA, Henry ER, Hofrichter J, Mozzarelli A. *Nat Struct Mol Biol*. 1999;6:351–358.
4. Kampmann T, Mueller DS, Mark AE, Young PR, Kobe B. *Structure*. 2006;14:1481–1487.
5. Fritz R, Stiasny K, Heinz FX. *J Cell Biol*. 2008;183:353–361.
6. Kalani MR, Moradi A, Moradi M, Tajkhorshid E. *Biophys J*. 2013;105:993–1003.
7. Lee ES, Gao ZG, Kim D, Park K, Kwon IC, Bae YH. *J Control Release*. 2008;129:228–236.
8. Griset AP, Walpole J, Liu R, Gaffey A, Colson YL, Grinstaff MW. *J Am Chem Soc*. 2009;131:2469–2471.
9. Yang YQ, Zheng LS, Guo XD, Qian Y, Zhang LJ. *Biomacromolecules*. 2011;12:116–122.
10. Radovic-Moreno AF, Lu TK, Puscasu VA, Yoon CJ, Langer R, Farokhzad OC. *ACS Nano*. 2012;6:4279–4287.
11. Qu W, Li Y, Hovgaard L, et al. *Int J Nanomed*. 2012;7:4983–4994.
12. Wada K, Mizuno T, Oku J, Tanaka T. *Protein Peptide Lett*. 2003;10:27–33.
13. Jones IM, Lingard H, Hamilton AD. *Angew Chem Int Ed*. 2011;50:12569–12571.
14. Anderson JM, Andersen NH. *Angew Chem Int Ed*. 2017;56:7074–7077.
15. Zimenkov Y, Dublin SN, Ni R, et al. *J Am Chem Soc*. 2006;128:6770–6771.
16. Cheng PN, Liu C, Zhao ML, Eisenberg D, Nowick JS. *Nat. Chem.*. 2012;4:927–933.
17. Truex NL, Wang YL, Nowick JS. *J Am Chem Soc*. 2016;138:13882–13890.
18. Truex NL, Nowick JS. *J Am Chem Soc*. 2016;138:13891–13900.
19. Wang Y, Kreutzer AG, Truex NL, Nowick JS. *J Org Chem*. 2017;82:7905–7912.
20. Nowick JS, Lam KS, Khasanova TV, et al. *J Am Chem Soc*. 2002;124:4972–4973.
21. Nowick JS, Brower JO. *J Am Chem Soc*. 2003;125:876–877.
22. Nowick JS, Chung DM, Maitra K, Maitra S, Stigers KD, Sun Y. *J Am Chem Soc*. 2000;122:7654–7661.
23. Khakshoor O, Demeler B, Nowick JS. *J Am Chem Soc*. 2007;129:5558–5569.
24. Pham JD, Demeler B, Nowick JS. *J Am Chem Soc*. 2014;136:5432–5442.
25. Wishart DS, Sykes BD. *Methods Enzymol*. 1994;239:363–392.
26. Polson A. *J Phys Colloid Chem*. 1950;54:649–652.
27. Pham JD, Chim N, Goulding CW, Nowick JS. *J Am Chem Soc*. 2013;135:12460–12467.
28. Nowick JS, Khakshoor O, Hashemzadeh M, Brower JO. *Org Lett*. 2003;5:3511–3513.
29. Longworth LG. *J Phys Chem*. 1960;64:1914–1917.