A role of the Trp–His interaction in the conformational switch between α-helix and β-sheet in short alanine-based peptides

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The interaction between aromatic residues (Trp, Tyr, and Phe) and a histidine residue (His) is often present in proteins and plays an important role in determining the conformation of peptides and the folding of globular proteins. The role of the Trp–His interaction in the conformation of peptides and the folding of globular proteins has been investigated for a series of alanine-based peptides having a pair of Trp–His in different geometrical spacing and positions. A conformational switch between the α-helix and β-sheet due to the Trp–His interaction was found with the result that the pairs of Trp–His with (*i*, *i* + *4*) and (*i*, *i* + *2*) spacing at the C terminus led to α-helix and β-sheet conformations, respectively. The possible factors contributing to the positional effect of the Trp–His interaction are also discussed in the paper. The role of the Trp–His interaction in the conformational switch between the α-helix and β-sheet in peptides is important in the evolution of new protein folding by accumulations of simple mutations in peptides and proteins.

Introduction

Protein sequences in biological systems are evolved by random mutations, including substitutions and *en bloc* changes resulting from frame-shifts or large insertions and deletions, to result in occasional structural alteration to a new or dramatically different three-dimensional fold.[1] However, little is known about how many or what kind of sequence changes might lead to significant structural changes. A few years ago, Rose and Creamer formulated the Paracelsus challenge: transform the conformation of one globular protein into that of another by changing no more than half of the sequence.[2] A large number of biophysical chemists and structural biologists have been working to meet the challenge over the past several years.[3] Recently, Sauer and his colleagues reported that mutations at adjacent positions in the antiparallel β-sheet of the Arc repressor are sufficient to change the local secondary structure to a right-handed helix.[4]

Short alanine-based peptides are useful simple models for studying interactions that contribute to the peptide conformation and the folding of globular proteins.[5,6] They have been used in our previous work to study the conformational transition between an α-helix and β-sheet, and the effect of some metal ions, especially Cu^{2+}, on the conformations of short alanine-based peptides.[6] The interaction between aromatic residues (Trp, Tyr, and Phe) and the histidine residue (His) is often present in proteins and plays an important role in determining the conformation of peptides and the folding of globular proteins.[7–9] Here, we report that Trp–His pairs with (*i*, *i* + *4*) and (*i*, *i* + *2*) spacing at the C terminus in short alanine-based peptides lead to α-helix and β-sheet conformations, respectively. This suggests that the role of the Trp–His interaction in the conformational switch between an α-helix and a β-sheet in peptides is important for the evolution of new protein folding by accumulations of simple mutations.

Experimental

The series of short alanine-based peptides employed in this work (Table 1) was *de novo* designed and synthesized by the Fmoc method on a Pioneer Peptide Synthesis System (Perseptive Biosystems Inc.). The crude peptides were purified on a UV-8020/CCPM-II high performance liquid chromatography system (TOSOH Co.) and the purified peptides (>95% according to HPLC analysis) were characterized using a Voyager-DE mass spectrometer (Perseptive Biosystems Inc.) using a α-cyano-4-hydroxycinnamic acid matrix. Concentrations of the peptide stock solutions were determined from the absorption of the tryptophan (Trp) residue in each peptide at 278 nm (extinction coefficient $\varepsilon_{278} = 5500\ M^{-1}\ cm^{-1}$) with a UV/VIS/NIR Spectrophotometer (JASCO Co. Ltd.).[6] Samples were prepared by diluting the stock solution with the appropriate buffers containing 1 mM each of sodium citrate, sodium phosphate, sodium borate, 10 mM sodium chloride, and adjusted by HCl or NaOH to different pHs.[6–9]

The conformations of the peptides were monitored from the mean residue ellipticity θ (deg cm^2 $dmol^{-1}$) at characteristic peaks, that is, the peaks of 208 nm and 222 nm for the α-helix and that of 218 nm for the β-sheet. Circular dichroism (CD) spectra of the peptides were obtained by using a JASCO J-600 spectropolarimeter (JASCO Co. Ltd.) with 0.1 cm path length quartz cell at 2 °C, and interfaced to a Dell OptiPlex GXi computer. The cell holder was thermostated by a JASCO PTC-348 temperature controller and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid water condensation on the cuvette exterior. All measurements were carried out in 50 μM concentration of peptide unless otherwise noted. The CD spectrum was the average of three scans with data taken at 0.1 nm intervals from 260 nm to 190 nm. The contents of different

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conformations (α-helix, β-sheet, and random coil) were estimated from the corresponding CD spectra with a least-squares procedure in an Indigo 2 Silicon Graphics Computer System (Silicon Graphics Inc.).[10] The effect of concentration on the CD spectra was monitored by diluting the solution of peptides with buffer from 100 μM to 25 μM and by measuring their CD spectra. Since an accurate determination of helical content from CD spectra is particularly difficult for peptides containing aromatic residues,[11] we assumed in the calculation of the Trp–His interaction energy (ΔG) that the experimental helicities are correct to plus or minus 3%, as described in the calculation of the Trp–His interaction energy with the helix2 algorithm, the most recent version of SCINT.[12,13] A similar problem has been encountered and analogous method has been used in the study of the Trp–His interaction by other researchers.[9]

The Trp–His interaction was also monitored using the fluorescence emission spectra of the Trp residue, measured in a F-3010 Fluorescence Spectrophotometer (Hitachi Co. Ltd.) with a 1.0 cm path length quartz cell at 2 °C. The temperature of the cell holder was maintained using a Pharmacia LKB MultiTemp II and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid water condensation on the cuvette exterior. A wavelength of 278 nm was used for the excitation of tryptophan and the emission intensity was measured in the range 320 nm to 500 nm, where there was an emission peak near 350 nm. Unless otherwise noted, a 5 μM concentration of peptide was used in the measurements. According to the fluorescence intensity of Trp–His at different pHs, a sigmoidal titration curve with pH dependence was fitted using the MacCurveFit version 1.4 of Kevin Raner Software on a Macintosh computer.

In order to monitor the formation of amyloid fibril, the incubation of all the peptides was performed at a 400 μM concentration for 20 h at pH 7.0 and 37 °C in a B1-515 Block Incubator (ASTEC). The incubation of amyloid β-peptide (1–42), Aβ (42), as a reference was also performed at a 100 μM concentration for 6 h at pH 7.0 and 37 °C in the same incubator. The solution was not agitated during incubation. The formation of amyloid fibril was monitored with the thioflavin T, ThT, fluorescence method in an F-3010 spectrophotometer. ThT binds specifically to amyloid fibril and such a binding results in a fluorescent signal that is proportional to the mass of the fibril formed.[14] After incubation, 25 μL of solution was added to 50 mM glycine buffer at pH 9.0 containing 5 μM ThT to make a final volume of 1.2 mL in an ice–water bath at 2 °C and its fluorescence spectrum from 440 nm to 600 nm was immediately measured with an excitation wavelength of 435 nm.

This paper reports the calculated Trp–His interaction energies (ΔG) at different pHs using the helix2 algorithm.[12] The helical content of the peptides at different pHs were estimated from the corresponding CD spectra using a least-squares procedure as described above. $P_{W/H}$ (pH = 5.0) and $P_{W/J}$ (pH = 9.5), defined as equilibrium constants for the formation of the (*i*, *i* + *4*) Trp–His^+ (pH = 5.0) and Trp–His (pH = 9.5) interactions in the α-helix conformation respectively, were changed until the experimental helicities at the different pHs were predicted by the algorithm. The Trp–His interaction energies at different pHs were calculated from the corresponding $P_{W/H}$ and $P_{W/J}$. Since an accurate determination of the helical content from CD spectra is particularly difficult for peptides in which aromatic residues are present,[11] we allowed the experimental helicities to increase or decrease by 3% and estimated the error in interaction energy (ΔG) from the re-calculation value of $P_{W/H}$ and $P_{W/J}$. The helix2 algorithm is available *via* anonymous ftp at ftp://cmgm.stanford.edu/pub/helix/helix2. It should be mentioned that the helix2 algorithm can only be used in a helix–coil conformational equilibrium because it is based on the Lifson–Roig helix–coil theory.[12,13]

Table 1 Peptide sequence and its notation[a]

Peptide	Sequence	Notation
1	Ac-**W**KAAAAAAAAAAAAAK-NH_2	**W**KA_{13}K
2	Ac-KAAAAA**W**AAA**H**AAAAK-NH_2	KA_5**W**A_3**H**A_4K
3	Ac-K**W**AAA**H**AAAAAAAAK-NH_2	K**W**A_3**H**A_9K
4	Ac-KAAAAAAAAA**W**AAA**H**K-NH_2	KA_9**W**A_3**H**K
5	Ac-KAAAAAAA**W**AAAAAAK-NH_2	KA_7**W**A_6K
6	Ac-**W**KAAAAAAAAAAAA**H**K-NH_2	**W**KA_{12}**H**K
7	Ac-KAAAAAAAAAAA**WAH**K-NH_2	KA_{11}**WAH**K
8	Ac-KAAAAAA**WAH**AAAAAK-NH_2	KA_6**WAH**A_5K

[a] Peptides were *de novo* designed and synthesized by the Fmoc method.

Results

Design and characterization of the peptides

The peptides shown in Table 1 are the derivatives of an alanine-based host peptide, where a Trp residue or a pair of Trp–His was introduced in different geometrical positions and spacing to study the role of the Trp–His interaction on the conformations of peptides. Generally, the high helix-forming tendency of the Ala (A) residue is able to provide a helical structure in this simple model system with only 16 residues. Two Lys (K) residues were placed at the two termini to make the peptides water-soluble. One Trp (W) residue was introduced into all the peptides for both the peptide concentration measurement and its interaction with the His (H) residue. The peptides were acetylated (Ac-) at the N termini and amidated (-NH_2) at the C termini to reduce the destabilising interactions of the helix dipoles. Sequence **1** (**W**KA_{13}K) with a Trp residue at the N terminus was designed and used as a host peptide to compare with the guest peptides. Sequence **5** (KA_7**W**A_6K) with a Trp residue in the middle of a peptide was used to study the effect of the Trp residue in different positions on the peptide conformation and also to compare with other guest peptides having a Trp–His pair. We used an (*i*, *i* + 4) Trp–His pair as a possible α-helix geometrical spacing for the Trp–His interaction on the basis that a regular α-helical structure is of 3.6 residues per turn. It was introduced in the middle, the N terminus, and the C terminus of the short alanine-based peptide to form three guest peptides, Sequence **2** (KA_5**W**A_3**H**A_4K), Sequence **3** (K**W**A_3**H**A_9K), and Sequence **4** (KA_9**W**A_3**H**K), respectively. An (*i*, *i* + 2) Trp–His pair was used as a possible β-sheet geometrical spacing for the Trp–His interaction and introduced at the C terminus and middle of the short alanine-based peptides to form two guest peptides, Sequence **7** (KA_{11}**WAH**K) and Sequence **8** (KA_6**WAH**A_5K). A Trp residue was introduced at the N terminus and a His residue at the C terminus of the same peptide to form Sequence **6** (**W**KA_{12}**H**K), where the Trp–His pair is of neither (*i*, *i* + 4) nor (*i*, *i* + 2) geometrical spacing. The amino acid residues of **2**, **3**, **4**, **6**, **7**, and **8** are identical and the only difference within them is the geometrical spacing and position of the Trp–His pair.

Conformation of the peptides

The conformations of the peptides were monitored from the corresponding CD spectra shown in Fig. 1. The content of α-helix (f_α), β-sheet (f_β), and random coil (f_r) conformations were estimated from the corresponding CD spectra using the least-squares procedure and are shown in Table 2. It can be seen that the Trp–His pair has a different effect on the conformation according to its different geometrical spacing and position in the short alanine-based peptides. The guest peptides with an (*i*, *i* + 4) Trp–His pair in the middle and the N terminus (**2** and **3**) are less helical than the host peptide (**1**). In contrast, the guest peptide with an (*i*, *i* + 4) Trp–His pair at the C terminus (**4**) is more helical than the host peptide (**1**). The peptide with only a Trp residue in the middle (**5**) is less helical than that with a Trp residue at the N terminus (**1**). The guest peptide with an

(i, i + 2) Trp–His pair at the C terminus (**7**) is a typical β-sheet, whereas the guest peptide with an (i, i + 2) Trp–His pair in the middle (**8**) shows a random coil form. In addition, the β-sheet is the main conformation in the guest peptide with a Trp residue at the N terminus and a His residue at the C terminus (**6**). It is shown that (i, i + 4) and (i, i + 2) Trp–His pairs at the C terminus resulted in typical α-helix and β-sheet conformations, respectively, in short alanine-based peptides with identical amino acid residues. This suggests that the Trp–His interaction has a role in the conformational switch between the α-helix and β-sheet of peptides, which should be useful in the evolution of new protein folding by accumulations of simple mutations.

pH dependence of the Trp–His interaction

The pH dependence of the CD spectra of some of the peptides is illustrated in Fig. 2. It can be observed that the pH dependence of the conformations was different for peptides with different Trp–His geometrical spacing. The α-helix conformation was promoted in an acidic solution for **4** with an (i, i + 4) Trp–His interaction at the C terminus, whereas some of the α-helix conformation was converted to random coil in a basic solution. Although the β-sheet conformation of **7** with an (i, i + 2) Trp–His interaction at the C terminus was similarly promoted in an acidic solution, there was some aggregation in a basic solution. It is interesting that there was a partial conformational transition from α-helix to β-sheet for **6** when the pH of its solution was changed from 5.0 to 9.0. The pH dependence of the Trp–His interaction was also monitored from the fluorescence emission spectra of **4** with an (i, i + 4) Trp–His interaction at the C terminus. Fig. 3 shows that the fluorescence intensity at 350 nm for **4** is considerably enhanced from 11.10 to 51.33 with the titration of pH from 5.0 to 9.0. The titration curve was sigmoidal and was fitted to a theoretical curve for the ionization of a single residue with a pK_a of 7.32, using MacCurveFit version 1.4. The His residue may be responsible for the ionization because it is the only residue which dissociates in this pH range, and the pK_a was the apparent dissociation constant of

Table 2 Conformational contents of peptides used in this paper[a]

Sequence	f_α (%)	f_β (%)	f_r (%)
1	39.05	28.18	32.77
2	31.38	33.73	34.89
3	28.21	27.02	44.78
4	72.39	16.69	10.92
5	29.24	24.78	45.98
6	1.74	59.62	38.64
7	0.00	62.23	37.77
8	13.49	30.36	56.15

[a] The conformational contents were estimated using a least-squares procedure from the CD spectra of peptides which were measured at pH 7.0. The detailed method and conditions are described in the Experimental section.

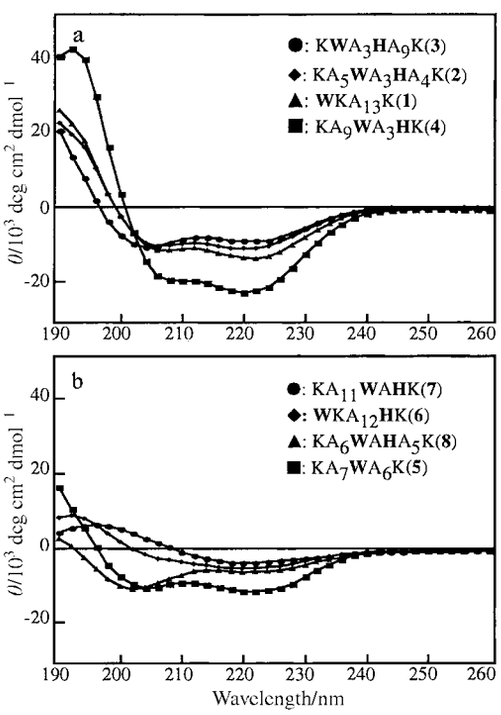

Fig. 1 CD spectra of a series of short alanine-based peptides. CD spectra were measured using a 1 mm cell at 50 μM, 275 K, and pH 7.0.

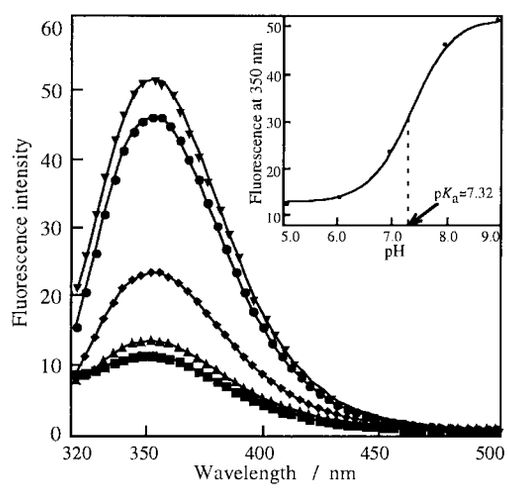

Fig. 3 Fluorescence spectra and pH dependence of the intensity at 350 nm of KA_9WA_3HK. Fluorescence spectra were measured using 10 mm cell at 5 μM, 275 K, and pH 5.0 (■), 6.0 (▲), 7.0 (◆), 8.0 (●), 9.0 (▼), respectively. Fluorescence intensities are shown in arbitrary units.

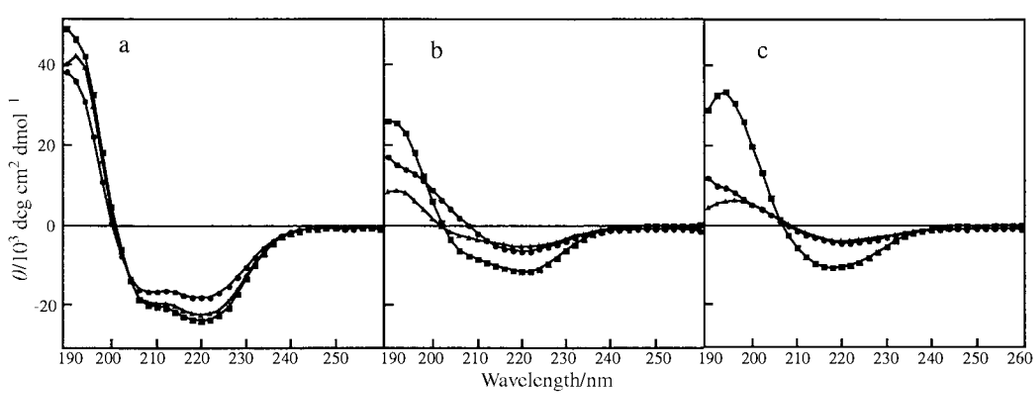

Fig. 2 pH dependence of CD spectra of KA_9WA_3HK (a), $WKA_{12}HK$ (b), and $KA_{11}WAHK$ (c). CD spectra were measured using a 1 mm cell at 50 μM, 275 K, and pH 5.0 (■), 7.0 (▲), 9.0 (●), respectively.

Table 3 Concentration-dependence of CD spectra of peptides used in this paper[a]

Sequence	$\Delta\theta$ (%)		
	66.7 μM	50 μM	25 μM
1	−3.32	4.48	6.85
2	−0.58	−0.85	3.61
3	−1.53	−1.48	6.53
4	−0.56	0.55	−5.83
5	0.95	2.65	6.00
6	−9.00	−0.38	9.17
7	−3.33	24.98	50.29
8	−2.01	−2.24	0.82

[a] The mean residue ellipticity θ at 222 nm was used as the characteristic θ for **1**, **2**, **3**, **4**, **5**, and **8**. θ at 218 nm was used for **6** and **7**. θ of the peptide at 100 μM was used as the reference and the relative changes, $\Delta\theta$ (%), at different concentrations were calculated from the following: $\Delta\theta$ (%) = $(\theta_1 - \theta_2) \times 100/\theta_1$, in which θ_1 was θ at 100 μM, and θ_2 was that at 66.7 μM, 50 μM, and 25 μM, respectively.

the His residue in the peptide. The Trp–His interaction is the main factor which results in the increase of pK_a of the His residue because the Trp residue is suitable for binding protonated His residue and stabilizing its H^+. The hydrogen bond between the protonated His residue and the exposed backbone carbonyl oxygen in the last turn of the the C terminus of the α-helix and the interaction between the protonated His residue and the α-helix macrodipole are other possible factors which result in the increase of pK_a of the His residue because of their stabilization of H^+ in the protonated His residue. This is consistent with the results of other investigators.[7–9]

Aggregation assay

Aggregation assays of the peptides, especially the peptides with a β-sheet conformation, were carried from the concentrational dependent CD spectra of all the peptides used in this work. The concentration dependence was monitored by the relative change ($\Delta\theta$ (%)) of the characteristic ellipiticity at 222 nm or 218 nm and comparing it with that at 100 μM. As shown in Table 3, the mean residue ellipticity of most of the peptides, including **6** which was shown to be in the β-sheet conformation as described above, was not dependent on the concentration in the range of 25 μM to 100 μM. This indicates that these peptides existed in a monomeric form and that their conformation was related to the intramolecular interaction under the experimental conditions. In constrast, the concentration dependence of **7** with a typical β-sheet conformation was evident in the range of 25 μM to 100 μM. This indicates that there is a certain intermolecular interaction, which usually diminishes upon dilution. This was in agreement with the result that there was some aggregation in basic solutions as shown in Fig. 2c.

Aggregation assays of the peptides were also carried out by incubating all the peptides used in this paper and Aβ (42), a reference used to monitor the formation of amyloid fibril, using the ThT fluorescence method. It is known that there is a strong tendency for the formation of amyloid fibril in Aβ (42).[15] A characteristic ThT fluorescence spectrum of amyloid fibril of Aβ (42) with an emission peak at 484 nm was observed using an excitation wavelength of 435 nm. In contrast, no characteristic ThT fluorescence spectrum of the amyloid fibril was observed for any of the peptides including **7** (data not shown).

Calculation of the Trp–His interaction energy using the helix2 algorithm

Since it is very difficult to describe a three-state conformational equilibrium, the helix–coil transition theory is generally used to calculate the approximate intrahelical interaction energy between side-chain residues by comparing the helical contents of the peptide in the absence and presence of such an interaction. Two such theories have been developed and implemented recently in useful computer algorithms such as AGADIR[16] and SCINT.[12,13] In this paper we have quantitatively analyzed the Trp–His interaction energies (ΔG) at different pHs using the helix2 algorithm, the most recent version of SCINT. Because of its high α-helical and poor β-sheet contents as shown in Fig. 1 and Table 2, **4** with an (i, i + 4) Trp–His interaction at the C terminus of an alanine-based peptide was selected for the calculation and the results are shown in Table 4. Trp–His interaction energies (ΔG) in different pHs were calculated with acceptable accuracy in Table 4. At pH 5.0 where the His residue is protonated, the (i, i + 4) Trp–His interaction was worth -1.4 ± 0.3 kcal mol^{-1}. An aromatic interaction of (i, i + 4) Trp–His at high pH (pH 9.5), in which the His residue is not protonated, was indicated by the interaction energy of -0.4 ± 0.2 kcal mol^{-1} although this was small. The Trp–His interaction energies calculated using the helix2 algorithm indicated the stabilization of the (i, i + 4) Trp–His interaction in the α-helix conformation.

Table 4 Interaction energies (ΔG) of (i, i + 4) Trp–His pair **4** at different pHs

	pH 5.0	pH 9.5
Helix2[a]	-1.4 ± 0.3	-0.4 ± 0.2
SCINT[9]	-1.2 ± 0.2	0.1 ± 0.5
AGADIR[9]	-0.8 ± 0.1	-0.2 ± 0.2

[a] Experimental helicities at different pHs were estimated from the corresponding CD spectra using the least-squares procedure and multiplied by 1.04 because the helicities were determined at 2 °C and the helix2 algorithm predicts helical contents at 0 °C. The 1.04 factor was derived from the ratio of helical contents predicted by the AGADIR algorithm at 0 °C and 2 °C. Interaction energies (ΔG) were calculated as following: $\Delta G = -RT\ln P$, where P was estimated until the experimental helicities were predicted using the helix2 algorithm.

Discussion

Effect of the Trp–His interaction on peptide conformation

It was observed in this paper that the (i, i + 4) Trp–His interaction at the C terminus led to the characteristic α-helix conformation of **4**. In contrast, the (i, i + 2) Trp–His interaction at the C terminus led to the typical β-sheet conformation of **7** which was dependent on its concentration although a characteristic ThT fluorescence spectrum of the amyloid fibril was not observed. The results indicate that there is an apparent effect of the Trp–His interaction on the conformations of the short alanine-based peptides studied in this paper. The conformation of the peptide depends not only on the type of Trp–His interaction, that is (i, i + 4) for the α-helix or (i, i + 2) for the β-sheet, but also on its position in the peptide. There may be several factors that lead to the result that both the (i, i + 4) Trp–His and (i, i + 2) Trp–His interactions at the C terminus are useful in improving and stabilizing the formation of α-helix and β-sheet conformations, respectively.

First, the overall conformational content of peptides are generally affected by the conformational propensities of the substituted residues and their positions. It is known that the intrinsic propensities of Trp and His residues to form the α-helix conformation are generally less than that of the Ala residue.[13,17] This means that Trp and His residues act as helix-destabilizing residues in alanine-based peptides when compared to the Ala residue. The helix-destabilization of Trp and His residues and the sheet-destabilization of the positively charged His residue usually have less effect near either end than in the middle of a peptide because of the frayed termini.[18] This may be seen by comparing the CD spectra and α-helix conformational contents between **1** and **5** and the CD spectra and

β-sheet conformational contents between **7** and **8** as shown in Fig. 1 and Table 2, respectively. A similar effect was also observed recently in alanine-based peptides with an (i, i + 4) Phe–Met pair, where helical contents are higher with the Phe–Met at the termini than in the middle of the peptides.[12] Second, the introduction of a pair of (i, i + 4) Trp–His or (i, i + 2) Trp–His at the C terminus contributes to regulating the frayed terminus and to promoting the formation of hydrogen bonds on the main-chain near the C terminus *via* the Trp–His interaction. This effect is less pronounced at the N terminus because the C terminus usually appears to be more frayed than the N terminus of the peptides.[19] Third, the Trp–His interaction at the N terminus is weaker than that at the C terminus, because it is possibly decreased by the interaction between Trp and Lys residues where Lys is positively charged at the N terminus of the peptides used here. Therefore, the Trp–His interaction at the C terminus plays an important role as a conformational switch to control and induce the formation of either an α-helix or a β-sheet for the short alanine-based peptides. The important role of the Trp–His interaction at the C terminus is also indicated by the changeable conformation of **6** in which there is a Trp–His pair with neither (i, i + 4) nor (i, i + 2) geometrical spacing at the C terminus as shown in Fig. 2b.

Our results on the role of the Trp–His interaction in peptide conformations are also consistent with the distribution of amino acids in natural proteins. Statistical study indicated that basic residues such as His occur preferentially near the C terminus rather than the N terminus,[20] which may be important in stabilizing the structures of proteins in nature.

Relationship between the Trp–His interaction and the α-helix macrodipole

The interaction of the partially positively charged His with the α-helix macrodipole is another possible factor contributing to the helicity enhancement of the peptide with a pair of (i, i + 4) Trp–His at the C terminus. This is because the His residue is placed near the C terminus to maximize the effect due to the charged-helix macrodipole interaction. Studies of the helix dipole model show that the peptide bond has a substantial dipole moment and the peptide dipole moments add end-to-end across H-bonds to generate a macrodipole in the α-helix.[21] The positive pole is near the N terminus and the negative pole is near the C terminus in the macrodipole. The interaction of charged side-chains with the α-helix macrodipole should be helix-stabilizing when the charged side-chain and the nearby pole of α-helix macrodipole are of the opposite sign (attractive) and helix-destabilizing when they are of the same sign (repulsive). This phenomenon has been termed as a "charged group and helix dipole" interaction. Introducing a pair of Trp–His at the C terminus is useful in reducing the helix macrodipole at pH 7.0 ($<pK_a$), because the His is a partial positively charged residue under this condition. The interaction between positively the charged His and the α-helix macrodipole may be also observed with the pH dependence of the conformation of **6** in which there is a His residue at the C terminus and a Trp residue at the N terminus. At pH 7.0, the main conformation of **6** was the β-sheet conformation as shown in Fig. 1 and Table 2. Also, there was a partial conformational transition of β-sheet to α-helix in **6** when its solution pH changed from 9.0 to 5.0 as shown in Fig. 2b.

Comparison with other works

It is known that the interaction between aromatic residues and histidine residues is often present in proteins and plays an important role in determining the conformation of peptides and the folding of globular proteins. Recently, the conformational effect of the interaction between aromatic residues (Trp, Tyr, and Phe) and the His residue has also been studied by other researchers.

The effect of the Phe–His interaction on the conformations of peptides has been described with a model peptide by Baldwin and his colleagues.[7] The helix stability of the Phe–His interaction is present in an alanine-based peptide and depends only on the correct spacing of Phe and His residues, that is (i, i + 4) spacing. Helix stabilization by the (i, i + 4) Phe–His interaction with a positively charged His residue, His^+, is about two times stronger than with a neutral His residue, His. The (i, i + 4) Phe–His interaction causes a change of helix conformation shown in $[\theta]_{222}$ which is twice as large when the Phe–His pair is at the C terminus as when it is in the middle of a peptide. The effect of the Phe–His interaction on the conformation of alanine-based peptides is similar to that of the Trp–His interaction described in this paper. Unfortunately, the effect of the (i, i + 2) Phe–His interaction on the conformation of peptides was not shown in the paper.[7] Our research indicated that the (i, i + 2) Trp–His interaction at the C terminus also has an apparent effect on the conformations of peptides, which resulted in a typical β-sheet conformation in the short alanine-based peptide as described above. The reasons are similar to that of the (i, i + 4) Trp–His interaction. One of the reasons is that the sheet-destabilization of the positively charged His residue usually has less effect near either end than in the middle of the peptide because of the frayed termini. Another is that the (i, i + 2) Trp–His interaction at the C terminus contributes to regulating the frayed terminus and promoting the formation of hydrogen bonds in the β-sheet conformation near the C terminus.

A similar Trp–His interaction as described here was also discovered in barnase where His18 stabilizes the protein with a tertiary interaction with Trp94 by Fersht's group.[8] The Trp–His interaction stabilizes the protein more at low pH than at high pH and the interaction energy between a charged His (at low pH) and a Trp residue is in the range of −1.2 to −1.4 kcal mol^{-1}. The pH dependence of the Trp–His interaction was observed with the fluorescence intensity which varied with pH according to an ionization of a pK_a of 7.75. The mutational experiments indicated that the order of the effect is Trp > Tyr > Phe in barnase.

A study on the stability of isolated α-helices has also been made concerning the effect of Trp–His located in the middle of a peptide by Sancho and his colleagues.[9] The (i, i + 4) Trp–His spacing is found to be the most stabilizing and gives rise to the highest helical content when the His residue is protonated. As shown in Table 4, the energy (ΔG) of the (i, i + 4) Trp–His(+) interaction at low pH (pH 5.0) was calculated as -1.2 ± 0.2 kcal mol^{-1} using the SCINT algorithm. A similar energy value of -1.4 ± 0.3 kcal mol^{-1} at pH 5.0 was also calculated in this paper. Also, the values calculated using the SCINT algorithm[9] and obtained in this paper are not far from the value found in barnase for a tertiary interaction between a charged His and a Trp residue (−1.2 to −1.4 kcal mol^{-1}).[8] But the energy of the same interaction was calculated as -0.8 ± 0.1 kcal mol^{-1} using the AGADIR algorithm shown in Table 4.[9] The difference between the SCINT (including helix2) and AGADIR algorithms is one of the possible factors which results in the difference in Trp–His interaction energies shown in Table 4. The pK_a of 7.0 for the His residue was obtained by modifying the pK_a to optimize the agreement between the predicted helical content as a function of pH and the observed one.[9] A similar value ($K_a = 7.32$) was obtained in this paper from the pH-dependent sigmoidal fluorescence titration curve of Trp, as shown in Fig. 3. The similar interaction energy (ΔG) of the (i, i + 4) Trp–His(+) and pK_a of the His residue, which is present in different peptides and calculated with different algorithms, suggests that the conformational stability of a peptide could be increased by means of a Trp–His interaction. It also indicates that both AGADIR and SCINT algorithms are useful in the conformational study of peptides.

The helical contents of peptides used in this paper were also predicted by both the AGADIR and helix2 algorithms. A similar tendency on the effect of the Trp–His interaction was observed in most of the peptides (**1, 2**, **3**, **4**, **5**, and **8**) between experimental results and the predictive value with AGADIR and helix2 algorithms, although the algorithms are based on helix–coil transition theory. However, apparent differences between the experimental and the predictive values were observed in **6** and **7** because of their high content of β-sheet conformation (data not shown).

The positional dependence of non-polar and non-charged polar amino acids on intrinsic helical propensities has also been noticed and reported by Serrano's group recently.[22] It was found that the amino acid residues including non-polar and non-charged polar residues at the first helical turn (N terminus) and at a central helical position had different intrinsic helix-forming propensities because of the differences in van der Waals' interactions, entropy of side-chain, and solvation. Considering the positional effects of intrinsic helix-forming propensity should significantly improve the predictive power of AGADIR. As shown in this paper and the results of Serrano's group,[22] the positional effect is a comprehensive expression of several factors. It is known that the termini of a peptide are, in general, not equivalent to the rest of the peptide in geometry and environment. Therefore, it may be expected that the positional effect of both amino acid residues and the interactions between side-chain residues on the conformation of peptides and the folding of globular proteins is one of the important molecular factors which one has to consider in order to understand and control the conformation and its transition of a peptide or protein. It is likely that a wider substructural search regarding the positional effect of both amino acid residues and the interactions between side-chain residues will yield a better understanding of the conformation of peptides and the folding of globular proteins. Such knowledge will be useful in the evolution of new protein folding by accumulations of simple mutations.

Conclusion

The results in this paper demonstrate that the Trp–His interaction has a different effect on the conformation of peptide depending on its different geometrical spacing and positions in short alanine-based peptides. The role of the Trp–His interaction in the conformational switch between the α-helix and the β-sheet of peptides was indicated by the finding that the Trp–His pairs with (i, $i + 4$) and (i, $i + 2$) spacing at the C terminus resulted in the α-helix and β-sheet conformations of the peptides, respectively. The possible factors contributing to the important role of the Trp–His interaction as a conformational switch to control and induce the formation of α-helix or β-sheet conformation of peptides have also been discussed. The role of the Trp–His interaction in the conformational switch between the α-helix and the β-sheet of peptides found in this paper is useful in understanding the conformation of peptides and the folding of globular proteins, and in the development of new protein folding schemes by accumulations of simple mutations.

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