Sequence Effects on Helix-Sheet Conformational Transitions of Designed Amphiphilic Peptides

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Four 18-residue amphiphilic peptides were designed and synthesized, and their helix-sheet conformational transitions were investigated by circular dichroism (CD) in aqueous solution at different pH, peptide concentration and ionic strength as well as in a water/HFIP mixture. The α-helical conformations existed as monomeric states, while the β-sheet structures were formed by the aggregation of approximately 6 monomeric peptides for Peptide I, II, and III. The conformational transitions were provided by the self-association and dissociation of each peptide. The aggregated β-sheet structures would be stabilized by intermolecular hydrophobic interactions and complementary ionic bonds in addition to the conventional hydrogen bonds. In contrast, the formation of α-helical conformations would be caused by intramolecular electrostatic interactions between charged residues and the intrinsic high α-helix propensities of constituent amino acids in addition to hydrogen bonds. These peptides could be useful models for studying the factors governing the stability of secondary and tertiary structures as well as conformational transitions for comprehending the principles of how proteins fold into native states.

Since the syntheses of proteins and peptides by chemical and genetic techniques have made remarkable progress, many novel designed proteins can been synthesized. However, the design of novel proteins is a difficult task because the relationship between the protein tertiary structure and its amino acid sequence is not completely understood.[1,2] De novo design has recently developed for constructing novel proteins with specified tertiary structures and functions, as well as understanding the mechanism of protein folding. Amphiphilic peptides have frequently been utilized as well-defined structural units in de novo designed proteins, such as α-helix bundle proteins,[3] barrel proteins,[4] and β-sandwich proteins,[5] because they fold themselves into typical secondary and tertiary structures by mainly intermolecular hydrophobic interactions in aqueous solution.

On the other hand, another approach to understand folding involves the characterization of the conformational transitions and folding intermediates.[6,7] The conformational transitions of polypeptides and oligopeptides have been extensively investigated as model system of folding.[8—11] In particular, reversible helix-sheet conformational transitions, are induced by changing the experimental conditions, such as the solvent,[12] pH,[13] and redox reactions[14] for de novo designed amphiphilic oligopeptides, which have been recently reported.

We recently reported on an artificial amphiphilic peptide (peptide I in Fig. 1) which exhibits reversible helix-sheet conformational transitions by varying the peptide concentration, pH or ionic strength in aqueous solution.[15] Since the secondary structures of the peptide were significantly affected by a change of the hydrophobic as well as electrostatic interactions, such as ionic and hydrogen bonds, the conformational transitions were observed under various conditions. In addition, because the α-helical conformation existed as a monomeric structure and the β-sheet conformation was formed by an aggregation of the peptide, the conformational transition was induced due to a major contribution of the intermolecular interactions to self-association and dissociation. The peptide is an example of effective models to clarify the factors governing the stability of secondary and tertiary structures as well as the conformational transitions for understanding the principles of protein folding. Therefore, useful information concerning protein folding would be given by a conformational investigation of various amino acid sequences.

In the present study, by using the secondary structural propensities for amino acids, described by Chou and Fasman,[16] as well as amphiphilic sequences with alternating hydrophilic and hydrophobic residues,[17,18] four peptides with three different amino acid sequences of the same overall composition (peptide I, II, and III in Fig. 1), or some other composition (peptide IV in Fig. 1), were designed in order to understand the relationship between the sequence or the composition and the interactions among amino acid residues of the peptides for the formation of specific secondary structures. We describe the characterization of the structural properties of these four peptides in aqueous solution under various pH, ionic strengths or peptide concentrations as well as a water/hexafluoro-2-propanol (HFIP) mixture by circular dichroism (CD) measurements.

Experimental

Materials. Peptide synthesis was performed on an Applied Biosystems 431A Peptide Synthesizer using an automatic step-

a) **Peptide I** : NH_2-G E L E L E L E Q Q K L K L K L K G-COOH
Peptide II : NH_2-G E L K L E L K Q Q K L E L K L E G-COOH
Peptide III : NH_2-G E L E L K L K Q Q E L E L K L K G-COOH
Peptide IV : NH_2-G E L E A E L E Q Q K L K A K L K G-COOH

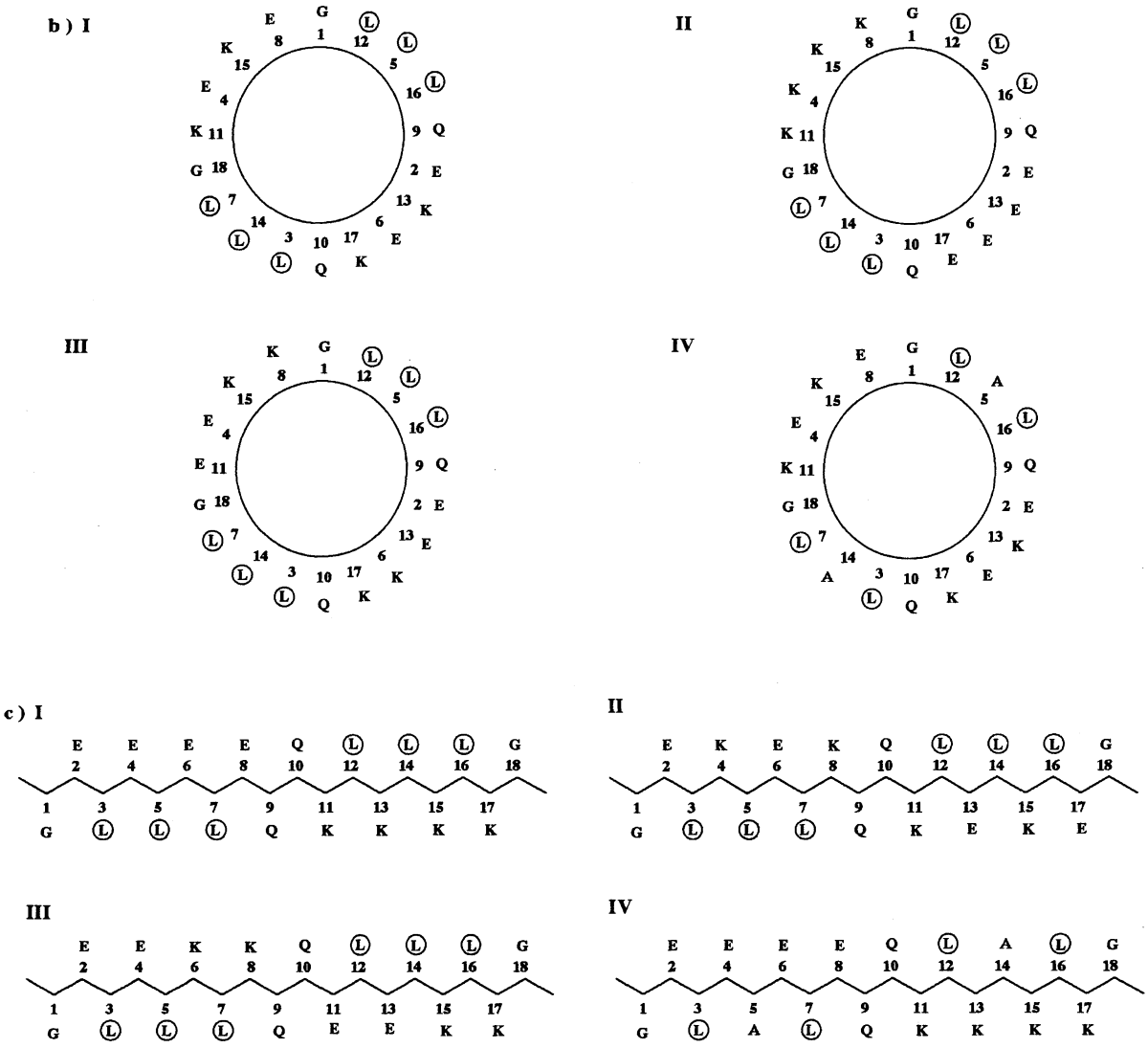

Fig. 1. a) Primary sequences of designed peptides. b) α-Helical wheel diagrams of the peptides. c) Schematic representation of β-sheet conformations of the peptides. Hydrophobic leucine residues are circled. G, glycine; E, glutamic acid; L, leucine; Q, glutamine; K, lysine; A, alanine.

wise solid-phase procedure involving an *N*-(fluoren-9-ylmethoxycarbonyl) (Fmoc) amino acid 1-hydroxybenzotriazol esters coupling/piperidine deprotection strategy. Fmoc–Gly, Fmoc–L-Leu, Fmoc–L-Gln, Fmoc–L-Glu(O*t*Bu), Fmoc–L-Lys(Boc), Fmoc–L-Ala, [*t*Bu, *t*-butyl; Boc, *t*-butoxycarbonyl], and other reagents for peptide synthesis were purchased from Applied Biosystems. All other chemicals and supplies were of high purity and were obtained from commercial sources.

High-performance liquid chromatography (HPLC) was carried out with a Waters 600E system controller liquid chromatograph equipped with a Waters 486 tunable absorbance detector and a Waters 741 data module. Analytical reverse-phase HPLC (RP-HPLC) was carried out on a Waters μ-Bondasphere C_{18} (3.9 mm×15 cm, 100 Å) column. Crude and purified peptides were chromatographed in the presence of 0.1% trifluoroacetic acid (TFA), and elution was conducted using a 40 min gradient of 5—60% aqueous acetonitrile containing 0.1% TFA at a flow rate of 1 ml min^{-1}.

The amino acid composition of the peptide was determined using a Hitachi 835 amino acid analyzer. The sample was hydrolyzed in an evacuated, sealed tube with 6 M hydrogen chloride at 110 °C for 24 h. The hydrolyzed peptide was derivatized by ninhydrin.

Peptide Synthesis and Purification. The peptides synthesized for this study are illustrated in Fig. 1. Peptide I was synthesized and purified as described before,[15] and the syntheses of Peptide, II, III, and IV were performed in a similar manner on a 0.25-mmol scale starting with 4-(hydroxymethyl)phenoxymethyl

(polystyrene/1% divinylbenzene) resin as the solid support, which gave the C-terminal carboxylic acid. These peptides were cleaved from the resin by reacting with 9.5 ml of TFA and 0.50 ml of deionized distilled water for 1.5 h at room temperature, as suggested by Applied Biosystems protocol. The crude peptides were purified in a single step by semipreparative RP-HPLC on a 1.9 cm by 15 cm Waters μ-Bondasphere C_{18} (100 Å) column with a linear 40 min gradient of 15 to 35% aqueous acetonitrile containing 0.1% TFA at a flow rate of 10 ml min^{-1}, the effluent being monitored at 214 nm. The final yields of the purified peptides were approximately 10—15% on the basis of the loading of the first amino acid to the resin. The purity of the peptides was checked by analytical HPLC and amino acid analysis.

Amino acid analyses of Peptide II, III, and IV showed a close correlation with the theoretical composition, as indicated [observed (theory)]: Peptide II: Gly 1.95 (2), Glx 5.94 (6), Leu 6.12 (6), Lys 3.99 (4). Peptide III: Gly 1.92 (2), Glx 5.97 (6), Leu 6.05 (6), Lys 4.06 (4). Peptide IV: Ala 1.95 (2), Gly 1.97 (2), Glx 5.92 (6), Leu, 4.08 (4), Lys 4.08 (4).

Circular Dichroism (CD) Measurements. The CD spectra were obtained at 20 °C under a constant flow of nitrogen on a Jovin Ivon CD6 spectropolarimeter equipped with an interface and a personal computer using a quartz cuvette of 1 mm pathlength. The instruments were calibrated with an aqueous solution of ammonium *d*-camphorsulfate.[19] The observed ellipticity was expressed as the mean residue ellipticity $[\theta]$, which was normalized to units of degrees centimeter squared per decimole. The α-helical and β-sheet contents were calculated by dividing the experimental ellipticities at 222 and 217 nm from the CD spectra by the reported values of molar ellipticities of 100% α-helical and β-sheet peptides, $[\theta]$ (222 nm) = −31000 and $[\theta]$ (217 nm) = −20000 deg cm^2 $dmol^{-1}$, respectively.[20,21] Peptide stock solutions were prepared at 2580 μM (5.4 mg mL^{-1}) and various aqueous pHs. The precise peptide concentrations of the stock solutions were determined by a ninhydrin analysis of a hydrolyzed peptide sample.

In pH-induced conformational transition measurements, 5 μL of the peptide stock solution dissolved in deionized, distilled water at 2580 μM of peptide concentration was mixed with 495 μL of an acid or alkaline solution. The pH was adjusted with HCl and NaOH and measured with a Horiba pH meter (F-16) before the CD measurements. The peptide had a maximal solubility of 5000 μM (about 10 mg mL^{-1}) in neutral water and 1300 μM in pH 12.0 water, respectively. CD measurements were not made beyond pH 13.0 because of the significantly decreasing solubility for the peptide under strong alkaline aqueous conditions.

CD samples for conformational transition measurements, induced by changing the peptide concentration, were prepared by diluting stock peptide solutions in water at various pH.

For salt-induced conformational transition measurements, 5 μL of the peptide stock solutions at 2580 μM of peptide concentration were mixed with 495 μL of a salt solution in water at various pH.

5 μL of the peptide stock solutions at 2580 μM of peptide concentration were mixed with 245 μL of water at pH 7.0 and 250 μL of HFIP for HFIP-induced conformational transition measurements.

Size Exclusion Chromatography. Size exclusion chromatography of the peptide was carried out using a combination of 0.74 cm by 30 cm Ultrahydrogel 250 and 0.74 cm by 30 cm Ultrahydrogel 500 columns (Waters). The eluent was monitored by measuring the absorbance at 214 nm. The peptide or protein standards were applied to the column in 0.15 M NaCl, 0.2 M phosphate buffer, (1 M = 1 mol dm^{-3}), pH 7.0, and eluted with the same buffer at a flow rate of 1.0 mL min^{-1}. The peptide was applied onto the column at a concentration of approximately 500 μM, and the apparent molecular weights were determined by interpolation from the standard curve.

Results and Discussion

Peptide Design. We have now designed three peptide analogues (Fig. 1) of Peptide I, which have shown helix-sheet conformational transitions upon changing the peptide concentration, pH or ionic strength for investigating the relationship between the amino acid sequences and secondary structures. Figure 1 shows an α-helical wheel diagram and a schematic representation of the β-sheet structures for recognizing the working sites of electrostatic and hydrophobic interactions for four designed peptides. All of the peptides have a glycine residue at both termini for breaking an α-helix and β-sheet structure of the peptides and for facilitating the connection of two peptides. In addition, glutamine residues were located at the 9th and 10th positions for all peptides; therefore, the glycine and glutamine residues would not affect the potentials for the formation of secondary structures among these four peptides.

Peptide I, II, and III consist of the same amino acid composition; also these peptides have leucine residues as a hydrophobic residue at the same positions, whereas the lysine and glutamic acid residues, as charged residues, were aligned at different positions, as shown in Fig. 1. Therefore, there would be no difference in the contribution of hydrophobic interactions to the stabilization of an α-helical and β-sheet conformations for these peptides, though useful information might be given to understand the relationship between the electrostatic interactions and the stabilization of secondary structures.

For Peptide I, in a β-sheet structure, electrostatic repulsion between the same charge residues within a β-sheet would exist, whereas it is expected that an α-helical conformation would possess an intramolecular electrostatic attraction between the opposite-charged residues. For Peptide II, intramolecular electrostatic repulsion and attraction would be present in a β-sheet and α-helical conformations, respectively. For Peptide III, the α-helical conformation might be stabilized because ionic pairs exist at the optimal spacing (i, i+4) between the members of a pair, where the side chains of the two amino acids forming the ionic pair are spaced by three amino acid.[22] The β-sheet structure would exhibit both intramolecular electrostatic attraction and repulsion. On the other hand, it would be difficult to predict how intermolecular electrostatic interactions would be formed.

Peptide IV has a different amino acid composition from the other peptides. The primary structure of Peptide IV contains a Leu to Ala exchange in the position corresponding to residues 5 and 14 in Peptide I to examine the significance of hydrophobic interactions for the formation of a β-sheet structure. It seems that the β-sheet structure is destabilized by the prevention of an intermolecular hydrophobic interaction for Peptide IV, because an alanine is less hydrophobic than a leucine residue, and an α-helical conformation is also presumed to be destabilized in analogy with Peptide I.

Peptide Synthesis. The peptides shown in Fig. 1 were synthesized by stepwise solid-phase peptide synthesis, and were purified to apparent homogeneity by a single-step procedure using reverse-phase HPLC. Amino acid analytical data concerning the analogues are agreement with the theoretical values. The purities of the analogues based on analytical HPLC were >90%.

Dependence of Conformation on pH. Figure 2 shows the CD spectra of Peptide I in aqueous solution at different pH values. The CD spectra at pH 1.0, 3.0, and 7.0 are characterized by a strong negative Cotton effect centered at around 217 nm and a strong positive Cotton effect at around 197 nm, which indicates that the peptide adopts predominantly β-sheet structures. The β-sheet contents are calculated based on the ellipticities at 217 nm and by using poly(L-lysine) with a β-sheet structure as a reference,[21] which are approximately 90, 60, and 100% at pH 1.0, 3.0, and 7.0, respectively. The CD spectra of Peptides I at pH 2.0 and 12.0 are characteristic of an α-helical conformation showing double minima at around 208 and 222 nm and a maximum at 193 nm. The helical contents, are calculated based on the ellipticity at 222 nm, which is 30 and 20% at pH 2.0 and 12.0, respectively, because the signal/noise ratio of the CD spectra increases along with the wavelength, and the ellipticities at 222 nm of the other secondary structures are remarkably lower than that of a helix.

Figure 3 shows the CD spectra of the four designed peptides in aqueous solution at pH 7.0. Peptide II and III adopt β-sheet structures and their β-sheet contents are approximately 97 and 87%, respectively, while the CD spectrum of Peptide IV displays the features of peptides in a substantially unordered conformation.

Table 1 lists the secondary structure and its content for four peptides at various pH. Peptide I, II, and III also exist as β-sheet structures in aqueous solution at pH 1.0 as well as from pH 3.0 to 10.0 (data not shown), whereas their CD spectra at pH 2.0 and 12.0 are indicative of α-helical conformations. On the other hand, Peptide IV adopts an unordered conformation at pH 1.0 as well as from 3.0 to 10.0 (data not shown), but exhibits an α-helical conformation at pH 2.0 and 12.0. The dependences of the β-sheet content on the pH for Peptide I, II, and III are shown in Fig. 4. The β-sheet contents of the peptides follow the order Peptides I > II > III under these conditions. For Peptide I, the content is almost independent of the pH from pH 4.0 to 10.0, but at pH 3.0 it decreases drastically to 60% and reverts to approximately 90% at pH 1.0. In analogy with Peptide I, the β-sheet contents of Peptides II and III decrease steeply upon changing the pH from 4.0 to 3.0, and increase again at pH 1.0. However, from pH 4.0 to 10.0, in contrast to Peptide I, the β-sheet contents of Peptides II and III are somewhat dependent on

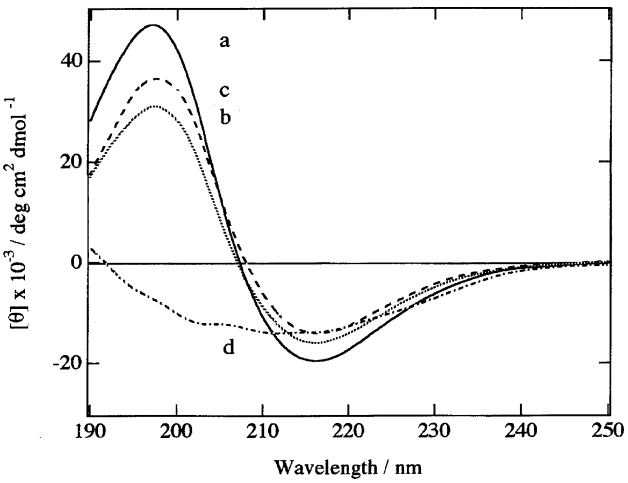

Fig. 3. CD spectra of four peptides in aqueous solution at pH 7.0 at a concentration of 34.4 μM. (a) Peptide I, (b) Peptide II, (c) Peptide III, and (d) Peptide IV.

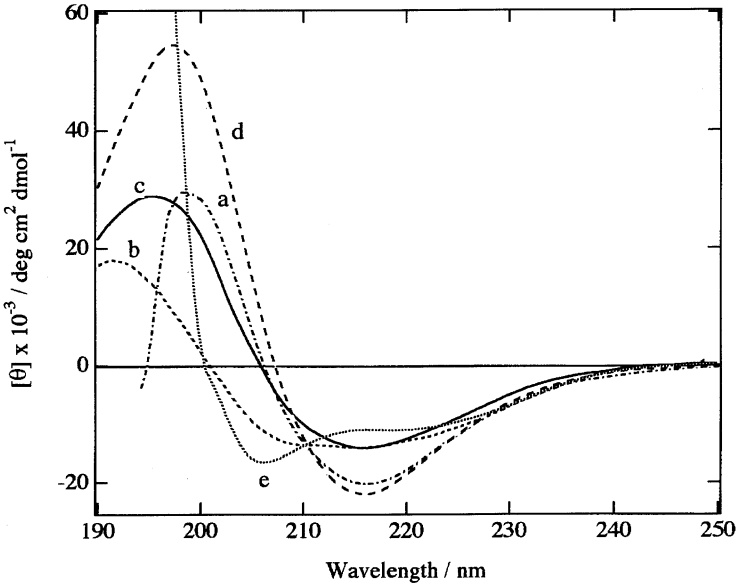

Fig. 2. CD spectra of Peptide I at a concentration of 34.4 μM in aqueous solution at different pH values. pH values: (a) 1.0, (b) 2.0, (c) 3.0, (d) 7.0, and (e) 12.0.

Table 1. Secondary Structure and the Content under Various Conditions at a Concentration of 34.4 μM for Four Peptides Based on CD Spectra[a)]

Peptide	pH 7.0	pH 2.0	pH 12.0	pH 2.0 100 mM NaCl	pH 7.0 Water/HFIP =50/50
I	β-sheet (100%)	α-helix (30%)	α-helix (20%)	β-sheet (76%)	α-helix (75%)
II	β-sheet (97%)	α-helix (18%)	α-helix (10%)	β-sheet (68%)	α-helix (60%)
III	β-sheet (87%)	α-helix (26%)	α-helix (17%)	β-sheet (59%)	α-helix (90%)
IV	Random coil	α-helix (14%)	α-helix (8%)	Random coil	α-helix (75%)

a) The content is given in parentheses.

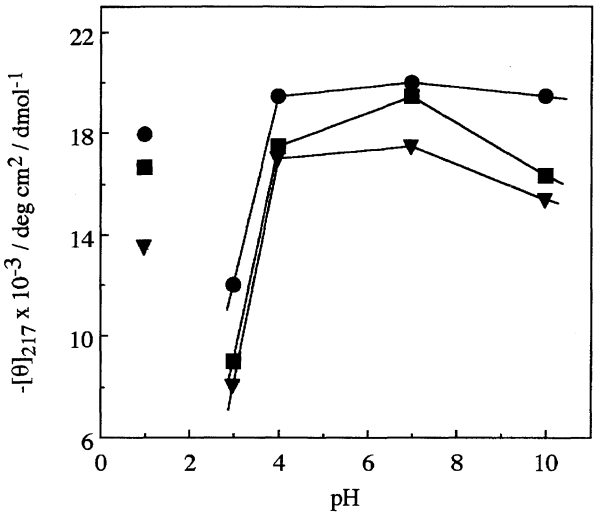

Fig. 4. Dependences of ellipticities at 217 nm on pH. (●): Peptide I, (■): Peptide II, and (▼): Peptide III.

the pH, and the contents decrease slightly at pH 4.0 and 10.0 compared to that at pH 7.0.

Peptides I, II, and III exhibit pH-induced helix-sheet conformational transitions at around pH 1.0, 2.0, and 12.0, while random coil-helix conformational transitions takes place at around these pHs for Peptide IV because it cannot form a β-sheet structure from pH 3.0 to 10.0. Peptide IV is given by the substitution of each leucine residue at positions 5 and 14 for Peptide I with an alanine residue. Therefore, hydrophobic interactions between leucine residues would play a key role in the formation of β-sheet structures because a leucine residue has a higher hydrophobicity than does an alanine residue.

The strength of hydrophobic interactions is significantly independent of the pH, while the strength of electrostatic interactions varies with the pH due to a change in the charged states of glutamic acid and lysine residues with the pH. Peptide I would not have electrostatic attractive interactions between negatively charged glutamic acid and positively charged lysine residues within a β-sheet, based on a consideration of its amino acid sequence; however, it would have intersheet electrostatic attractive interactions between oppositely charged residues. On the other hand, Peptides II and III could possess intrasheet as well as intersheet electrostatic attractive interactions. It therefore is likely that the formation of β-sheet structures is responsible for intersheet, rather than intrasheet, electrostatic interactions.

All four peptides adopt α-helical conformations at pH 2.0 as well as at pH 12.0; the α-helical contents follow the order Peptides I > III > II > IV at both pHs. Since glutamic acid has a pK_a of 4.3 and lysine has a pK_a of 10.5, glutamic acid and lysine residues would be neutralized at pH 2.0 and 12.0, respectively. Therefore, intersheet ionic bonds between negatively charged glutamic acid and positively charged lysine cannot form at pH 2.0 or 12.0 for the stabilization of β-sheet structures. It is presumed that these ionic bonds would be an important factor for the formation of β-sheet structures.

The results of the α-helical contents of Peptides, I, II, and III would indicate that the α-helical contents increase with increasing electrostatic repulsion between the same charged residues within a β-sheet and with increasing electrostatic attraction between opposite charged residues within an α-helix. In addition, because a glutamic acid residue is positioned near to the C-terminal for Peptide II, and thus a helix dipole-charge interaction is not formed, the α-helical content for Peptide II would also be lower than those for Peptides I and III. It is likely that these peptides could exhibit α-helical conformations when β-sheet structures are destabilized due to a decrease in the electrostatic attraction for the formation of β-sheet structures, or an increase in the electrostatic repulsion for the destruction of β-sheet structures, because these peptides comprise amino acids with high α-helical propensities.

Peptide IV has a lower α-helical content compared with those of the other peptides. This result might be caused by a lower α-helical propensity of an alanine residue than that of a leucine residue.[23)]

Because the stabilities of α-helical and β-sheet conformations are influenced by an amino acid sequence, hydrophobic as well as electrostatic interactions among amino acid residues other than conventional hydrogen bonds would be dominant factors for secondary structural formation.

Dependence of Conformation of Peptide Concentration. Figure 5 shows the CD spectra of Peptide I at two kinds of concentrations in aqueous solution at pH 2.0. Table 2 lists the secondary structure and its content at two concentrations at pH 2.0 and 12.0 for four peptides. The secondary structure for Peptide IV is independent of its concentration at pH 2.0 and 12.0, which is an α-helical conformation. Since the α-helical content is not also dependent on its concentration, the α-helical conformation would exist as a monomeric state.

At pH 2.0 and 12.0, Peptides II and III show α-helical conformations under lower peptide concentration, whereas they exist as β-sheet structures under higher peptide concentration. Peptide I exhibits helix-sheet conformational transitions at pH 2.0 due to a change in the peptide concentration, while the secondary structure is independent of its concentration

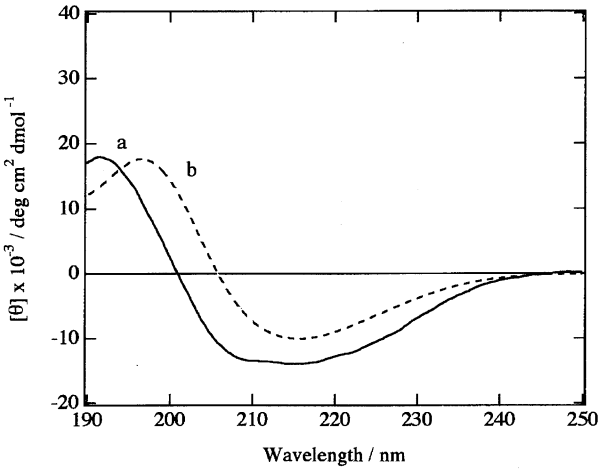

Fig. 5. CD spectra of Peptide I as a function of peptide concentration in aqueous solution at pH 2.0. peptide concentrations: (a) 34.4 μM and (b) 344 μM.

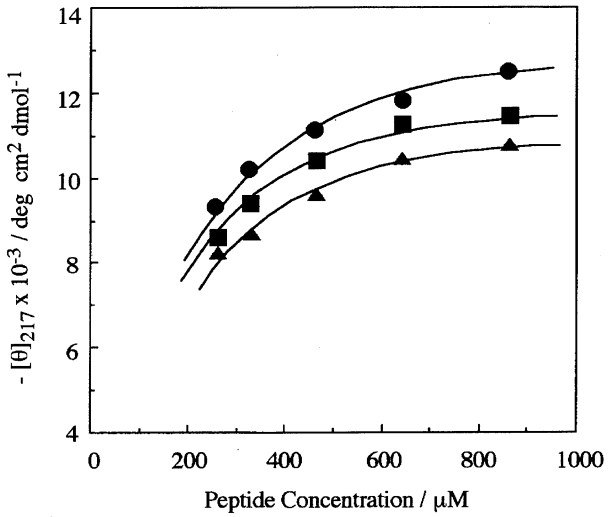

Fig. 6. Dependences of ellipticities at 217 nm on peptide concentration at pH 2.0. (●): Peptide I, (■): Peptide II, and (▲): Peptide III.

Table 2. Secondary Structure and the Content at Two Concentrations of 34.4 μM and 344 μM for Four Peptides Based on CD Spectra[a)]

Peptide	pH 2.0		pH 12.0	
	34.4 μM	344 μM	34.4 μM	344 μM
I	α-helix (30%)	β-sheet (53%)	α-helix (20%)	α-helix (20%)
II	α-helix (18%)	β-sheet (49%)	α-helix (10%)	β-sheet (54%)
III	α-helix (26%)	β-sheet (45%)	α-helix (17%)	β-sheet (67%)
IV	α-helix (14%)	α-helix (14%)	α-helix (8%)	α-helix (8%)

a) The content is given in parentheses.

at pH 12.0. Since the α-helical contents in acidic and alkaline solutions are independent of the peptide concentration for these three peptides, the α-helical conformations exist as monomeric peptides.

Figure 6 shows the dependences of the β-sheet structural contents on the peptide concentrations at pH 2.0 for Peptide I, II, and III from calculations based on the ellipticity at 217 nm. Since the β-sheet contents increase along with increasing peptide concentration for all three peptides, the formation of β-sheet conformations would be induced by an aggregation of each peptide. The β-sheet contents for these peptides follow the order Peptides I > II > III under these experimental conditions.

Similarly, at pH 12.0, the β-sheet contents increase with increasing peptide concentrations for Peptides II and III (data not shown); thus, the formation of β-sheet conformations would be required due to an aggregation of each peptide.

In addition, the formation of aggregated β-sheet structures is also supported from the results of size-exclusion chromatography for the three peptides described below. However, at pH 7.0, the β-sheet contents would not depend on the peptide concentrations for the three peptides because of approximately 90—100% β-sheet contents, even at the lowest measurable peptide concentrations by CD. It is assumed that such low peptide concentrations are not adequate to dissociate the aggregated β-sheet structures, and that these peptide concentration independences imply that the aggregated β-sheet structures are unusually stable.

The aggregated β-sheet structures would be stabilized by electrostatic interactions and hydrophobic interactions in addition to conventional hydrogen bonds. In general, since long-range intermolecular interactions become weaker along with a decrease in the peptide concentration, the aggregated structures are destabilized. However, the aggregated β-sheet structures stably form in aqueous solution at pH 7.0 by mainly strong complementary ionic bonds and hydrophobic interactions. In contrast, at pH 2.0 and 12.0, because the intersheet ionic bonds cannot act to stabilize the structures, hydrophobic and weaker electrostatic interactions would be required to overcome the electrostatic repulsion between the same charged residues within a β-sheet for the formation of β-sheet structures. At pH 2.0, for the three peptides, it is likely that hydrophobic and weaker electrostatic interactions can overcome the electrostatic repulsion between negatively charged glutamic acid residues within a β-sheet.

The difference in the peptide concentration-induced conformational transitions at pH 12.0 among Peptides I, II, and III would be considered based on the difference in the amino acid sequences of the peptides. The difference in the electrostatic interactions within a β-sheet would be predicted from the schematic representation of the β-sheet conformations in Fig. 1 for Peptides I, II, and III when they form β-sheet structures. Peptide I would be expected to have an electrostatic repulsion between the same charged residues for the destabilization of a β-sheet structure, whereas it appears that Peptides II and III have an electrostatic attraction for the stabilization of β-sheet structures. Since hydrophobic and weaker electrostatic interactions cannot overcome elec-

trostatic repulsion between negatively charged glutamic acid residues within a β-sheet for Peptide I, it cannot form a β-sheet structure at pH 12.0. On the other hand, because Peptides II and III have electrostatic attraction within a sheet, they would adopt β-sheet structures.

Dependence of Conformation on NaCl Concentration. Figure 7 shows the CD spectra of Peptide III at a peptide concentration of 34.4 μM in a pH 2.0 aqueous solution at different NaCl concentrations; Table 1 lists the secondary structures and their contents at different NaCl concentrations for the four peptides. The addition of NaCl induces an α-helix to the β-sheet conformational transitions of Peptides I, II, and III at pH 2.0, while Peptide IV salt-induced conformational transitions are not observed. The β-sheet contents follow the order Peptides I > II > III; this order is identical with that in aqueous solution from pH 3.0 to 10.0.

The β-sheet contents increase with increasing concentration of NaCl for these peptides, as previously reported (data not shown).[15] The mechanism of these salt-induced conformational transitions at pH 2.0 would be responsible for two possible effects which can stabilize the aggregated β-sheet structures. One is shielding of the electrostatic repulsion between positively charged lysine residues within a β-sheet; the other is an increase in the intermolecular hydrophobic interactions by affecting the water structure.

Dependence of Conformation on HFIP Concentration. Figure 8 shows the CD spectra of Peptide II at a peptide concentration of 34.4 μM in different solvents at pH 7.0; Table 1 lists the secondary structures and their contents at different solvents for the four peptides. Since all of the peptides adopt α-helical conformations in a water/HFIP mixture, Peptides I, II, and III display helix-sheet conformational transitions due to the addition of HFIP, and Peptide IV exhibits random coil-helix transitions due to its addition. These α-helical contents are much higher than those observed in aqueous solution at pH 2.0 or 12.0. The α-helical contents for these peptides follow the order Peptide III > I = IV > II under these experimental conditions.

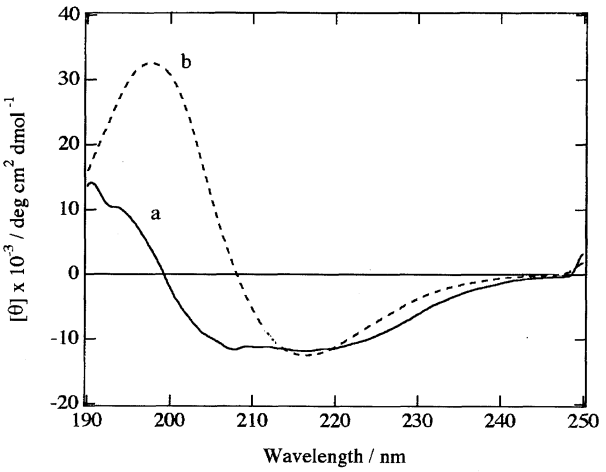

Fig. 7. CD spectra of Peptide III at a concentration of 34.4 μM in pH 2.0 aqueous solution at different NaCl concentrations. NaCl concentrations: (a) 0 mM and (b) 100 mM.

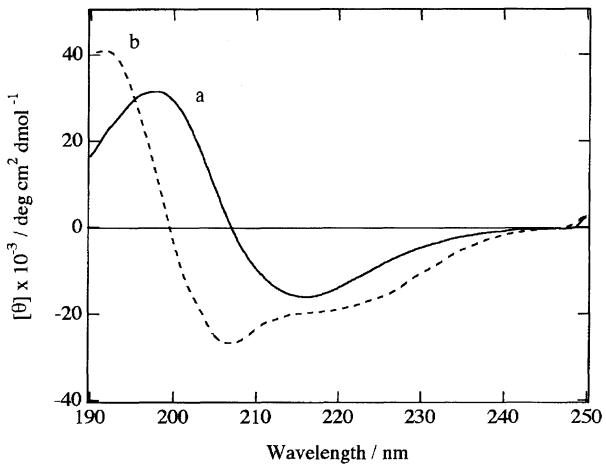

Fig. 8. CD spectra of Peptide II at a concentration of 34.4 μM in different solvents. solvents: (a) water pH 7.0 and (b) water/HFIP (50/50 vol%) at pH 7.0.

Because such an organic solvent as HFIP enhances hydrogen bonds as well as electrostatic interactions and reduce hydrophobic interactions, this solvent would generally destabilize the β-sheet structures for alternating amphiphilic polypeptides by collapsing the hydrophobic interactions.[24,25] Also, since these peptides comprise amino acids with high α-helical propensity, they would exhibit α-helical conformations. Because Peptide II has the highest electrostatic repulsion between the same charged residues in an α-helix as well as the highest electrostatic attraction in a β-sheet, it is likely that Peptide II would have the lowest α-helical content. These results would indicate that intramolecular electrostatic interactions play an important role in the formation of secondary structures in water/HFIP mixture systems.

Aggregation Numbers of the Aggregated β-Sheet Structures. Since the β-sheet structural contents for Peptides I, II, and III increase with increasing peptide concentrations (Fig. 6), the β-sheet structures would be formed by an aggregation of several peptides. The apparent molecular weights of the aggregated β-sheet structures were measured by size-exclusion chromatography in order to determine the aggregation numbers of the aggregated β-sheet structures for each peptide. Figure 9 shows the calibration curve in size exclusion chromatography of the aggregated structures in aqueous solution at pH 7.0. These experimental conditions give the formation of β-sheet structures for Peptides I, II, and III. The apparent molecular weights were approximately 13000, 12700, and 12400 for Peptides I, II, and III, respectively, and the aggregation number were approximately 6.20, 6.06, and 5.92. The aggregation numbers for these three peptides were almost of the same magnitude, and were consistent with the formation of the β-sheet hexamer. Therefore, the difference in their β-sheet contents for Peptides I, II, and III would not be caused by the aggregation numbers.

Peptides I, II, and III form aggregated β-sheet structures immediately after the preparation of a sample. In general, alternating amphiphilic polypeptides take up aggregated β-sheet structures after standing for several weeks or hours in

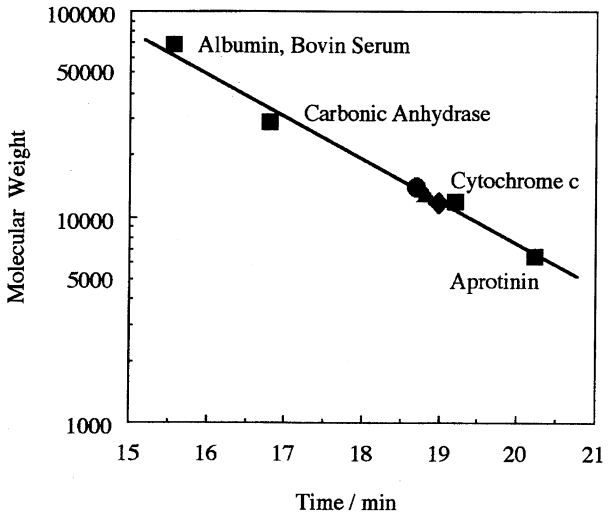

Fig. 9. Calibration curve for a combination of a 0.78×30 cm Ultrahydrogel 250 and a 0.78×30 cm Ultrahydrogel 500 columns used in the size exclusion chromatography of the peptide aggregates. (●): Peptide I, (■): Peptide II, and (◆): Peptide III.

an aqueous solution in the presence of salt. This slow β-sheet formation would be due to only intermolecular hydrophobic interactions, while these peptides form β-sheet structures rapidly by intermolecular hydrophobic and electrostatic interactions. This rapid β-sheet structural formation appears to lead to fast conformational transitions.

These experiments show that it is feasible to study the self-organization of the typical secondary structures and reversible conformational transitions as a function of hydrophobic interactions and electrostatic interactions. The availability of the stabilization and the destabilization of α-helical and β-sheet conformations in aqueous solution or a water/organic solvent mixture for amphiphilic peptides should contribute to the de novo designs of functional proteins. These peptides can also be good models for studying the mechanism of folding and actuators with fast responsibility.

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