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ORIGINAL CONTRIBUTIONS

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Effect of hydrophobic amino acids on the conformational change of decapeptides in micellar environments

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Abstract A series of peptides containing various hydrophobic amino acids [methionine (Met), leucine (Leu), norleucine (Nle), phenylalanine (Phe), 2-aminooctanoic acid (Aoc), and 2-aminodecanoic acid (Ade)] were synthesized and their conformations were studied using circular dichroism (CD) spectroscopy in different solvents such as water, methanol, and aqueous solution of ammonium tetradecanesulfonate. Peptides containing hydrophobic amino acids with linear side chains formed β-sheets in water and methanol. Electrostatic interaction between the charged side chain (lysine) and a micelle consisting of an anionic surfactant, ammonium tetradecanesulfonate, is necessary for the formation of α-helices in micellar environments. The conformational transition from α-helix to β-sheet structure required moderate hydrophobicity and linear side chains. This conformational transition depended on the surfactant concentration.

Keywords Peptide · Micelle · α-Helix · β-Sheet · Norleucine

Introduction

α-Helices and β-sheets are the major secondary structures that organize the three-dimensional configuration of proteins. β-Sheet structures are associated with a number of diseases, such as Alzheimer's and scrapie, and contain more hydrophobic amino acids than α-helices [1, 2]. There has been great interest in a hydrophobic amino acid, leucine, which has an isobutyl group side chain and is strongly correlated to the conformation of protein (peptide) [3–5]. Investigations on the effect of the side chains of various hydrophobic amino acids have been reported recently, using Monte Carlo simulation and thermodynamic analysis [6, 7]. Nozaki and Tanford reported the free energies of transfer of amino acid side chains from water to organic solvents [8].

Water-soluble protein, which is composed of 25–30% hydrophobic amino acids, can self-assemble to form a defined structure. In general, hydrophobic amino acids lie in the interior region of the protein to avoid exposure to water. However, the natural conformation is highly influenced by solvent and is in equilibrium between folded and unfolded structures. The formation of β-sheets is similar to micelle formation, and is also comparable to the process that takes place in an aqueous medium for natural or synthetic proteins [9–13].

The hydrophobic effect of a counter ion in a micellar system has been studied [14–17]. These studies have shown that the hydrophobicity of a short alkyl chain depends on the number of carbon atoms. In a recent report, we investigated the effect of the chain length of the counter ion on the partial molal volume of alkanesulfonates [17]. For the hydrophobic interaction between

micelle and counter ion, a side chain length longer than that of an *n*-butyl group is needed. In general, a straight-chain hydrocarbon is more hydrophobic than a branched hydrocarbon.

Since decapeptides are sufficiently long, they can easily form a β-sheet, and also possibly an α-helix structure. Such structures are retained in the energetic balance between the exposure of side chains to water and to the peptide backbone. This balance is easily affected not only by hydrophobicity but also by charged residues in surfactant solution [18–20].

In this study, we investigated whether a series of peptides containing hydrophobic amino acids can form a particular conformation under the influence of the hydrophobic interaction between their side chain and the hydrocarbon chains of a micelle. Nonionic and ionic surfactants were used to investigate the effects of hydrophobic and electrostatic interactions on peptides. The conformation of the peptides was analyzed by circular dichroism (CD) spectroscopy. Here, we describe the characterization of decapeptides containing hydrophobic amino acids in various solutions and discuss the interesting results.

Experimental section

Design and synthesis of peptides

The synthetic peptides 2-9X4K consist of ten amino acid residues. 2-9X4K were designed to locate two hydrophobic amino acid residues (X = Met, Leu, Phe, Nle, Aoc, or Ade) in the same region of an α-helical wheel, as shown in Fig. 1. The other amino acids are alanine, which forms a stable helix in water [21], and lysine, which has an ε-NH_2 group in its side chain. In the β-sheet structure, the amino acids X face in opposite directions.

All chemical reagents for peptide synthesis were purchased from Watanabe Chemical Co. (Hiroshima). Peptides were synthesized by the solid-phase method using Fmoc (fluoren-9-ylmethoxycarbonyl)-amino acids and *p*-alkoxybenzylalcohol resin (Alko resin) [22]. The synthesized peptides were purified by reverse phase HPLC on a reverse phase Tosoh semipreparative column TSK-GEL ODS-120T (7.8×300 mm, 120 Å pore size, Tosoh Co., Tokuyama). The purity of the peptides was analyzed by analytical HPLC, and it was found that the purities of all the peptides were more than 98%. The peptides were further characterized by FAB-MS.

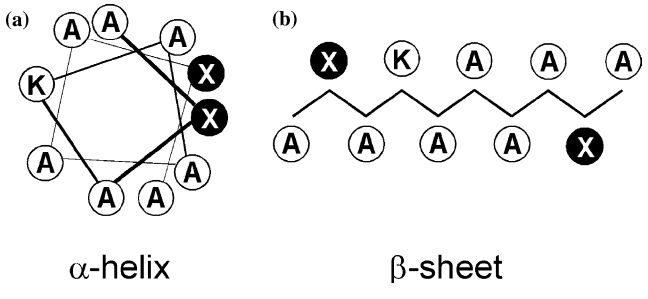

Fig. 1 Schematic representation of decapeptides containing hydrophobic amino acids (X: Met, Leu, Nle, Phe, Aoc, or Ade). **a** α-Helix structure, **b** β-sheet structure

Preparation of surfactant

Ammonium tetradecanesulfonate (C14SA) and dodecyldimethyl phosphineoxide (C12DPO) were prepared as described earlier [23–25]. Hexadecanetrimethylammonium bromide (C16TAB) was prepared using the same method as described in reference [24]. The three kinds of surfactants, C14SA, C12DPO, and C16TAB, have r.t. critical micelle concentrations (CMCs) of 2.36, 0.325 [26], and 0.92 [27] mM, respectively. The CMC of C14SA was measured using the conductivity versus the concentration at 30 ± 0.01 °C.

Circular dichroism spectroscopy

Circular dichroism measurements were performed on a Jasco J-720 spectropolarimeter (Tokyo, Japan) equipped with a thermoregulator at 30.0 ± 0.1 °C and a quartz cell of 0.1 mm pathlength. Spectra were obtained at 0.2 nm intervals from 190 to 250 nm. The samples were prepared by mixing surfactant solution and stock peptide solution.

Results and discussion

Circular dichroism spectroscopy

Circular dichroism spectra of peptides are helpful in determining their secondary structure. The amide group has an $n?\pi^*$ transition at ca. 220 nm and a $\pi?\pi^*$ transition at ca. 190 nm, so the spectra can be divided into three components: α-helices, β-sheets and random coils. Their components were calculated by the use of curve fitting with the least-squares method according to the literature [28].

Contribution of hydrophobic amino acids to conformation

We examined the effects of hydrophobic amino acids at position X in decapeptides AXAKAAAAXA, and aimed to determine whether the hydrophobicity of side

chains has an effect on the overall conformation of the peptide. CD analysis data on the conformation of peptide 2-9X4K are summarized in Table 1. The solubility of the peptide gradually decreased with increasing side chain hydrophobicity in pure H_2O; however, it increased up to 1 mg ml^{-1} in 4 mM HCl solution. In acidic water, when X was Aoc or Ade, 2-9X4K showed a strong preference for forming β-sheets; in the other cases it exhibited random structures.

In methanol, the Leu-type peptide kept a random structure, and the other peptides almost entirely formed β-sheets.CD spectra of β-sheets show negative and positive bands at around 218 and 195–200 nm, respectively. In the CD spectra where X = Nle, Aoc, or Ade, all of which have straight hydrocarbon chains, a twisted β-sheet structure was shown. At the positive band near 195 nm, the spectrum of a slightly twisted β-sheet is red-shifted toward the long wavelength and increases in intensity to ca. 30,000 deg cm^2 $dmol^{-1}$ in the antiparallel β-sheets [29]. In the CD spectra for X = Phe or Met, the strength of the signal around 195 nm decreased. These results represent a decrease in the β-sheet component. However, the positive peak (λ_{max}) blue-shifted to below 195 nm when X was Phe. This shift comes from the formation of a flatter β-sheet structure [29]. As mentioned above, 2-9X4K could not form β-sheets when X was Leu, which has a branched side chain. It seems that the major factor influencing a twisted β-sheet is a linear shape, rather than hydrophobicity in the side chain.

Micelle-induced conformational transition of 2-9X4K

Figure 2 shows the CD spectral changes of 2-9X4K with different species of hydrophobic amino acids obtained just after dissolution (a) and after 24 h of incubation (b) in C14SA aqueous solution. In contrast to the results (see Table 1) obtained in H_2O (acidic), the spectra of 2-9X4K containing Met, Leu, Nle, and Aoc exhibited α-helical structures. The presence of double negative bands at around 220 and 205 nm shows an α-helical conformation. In particular, when X was Aoc, the helix–helix interaction was exhibited [30, 31], and the absolute value of molecular ellipticity $[\theta]_{222}$ was larger than that of $[\theta]_{208}$. This result suggests that some helices aggregate in micellar solution. On the other hand, the spectral pattern of the peptide containing Ade displayed a β-sheet structure. Taking into consideration the results in H_2O, it appears that the peptide containing Ade is able to form a β-sheet structure without micelles. The spectrum of the peptide containing Phe gave an ambiguous pattern, representing a mixture of all three structural types (α-helix, β-sheet, and random).

After 24 h of incubation, 2-9X4K containing Nle, Aoc, or Phe underwent a conformational transition to β-sheets. More importantly, the Nle result represents a strongly twisted sheet. The 2-9X4K with Met was unable to change from an α-helix to a β-sheet. Although the length of the thioether group in Met is similar to that of *n*-butyl in Nle, the conformation was different. Meanwhile, the α-helix structure of the Leu-type peptide was stable for at least two weeks after the addition of C14SA. This result indicates that the conformational change to β-sheet depends on the structure of the side chain on the amino acid residue X.

The molecular ellipticity of a series of peptides at 205 nm ($[\theta]_{205}$) and the free energy of the shift from water to an organic solvent ($\mu\,^{\circ}_{org}$–$\mu\,^{\circ}_{w}$) are shown in Fig. 3. Open columns represent the molecular ellipticities after 3 min and shaded columns represent the values after 24 h of incubation in 17 mM C14SA solution. The negative/positive bars represent the α-helix or β-sheet characters, respectively. As the free energy of the side chain decreased, the structure of the peptides with Phe and Nle changed dramatically from α-helix to β-sheet. In

Table 1 Conformation of 2-9X4K in H_2O and methanol. 2-9X4K: Ala- Xaa-Ala-Lys-Ala-Ala-Ala-Ala- Xaa-Ala

Amino acid Xaa	Side chain -R	H_2O[a]	Methanol[c]			$\mu\,^{\circ}_{org}$ - $\mu\,^{\circ}_{w}$ (cal mol^{-1})
				λ_{max}(nm)	$[\theta]_{max}$ (deg cm^2 $dmol^{-1}$)	
Methionine	$-CH_2CH_2SCH_3$	Random	β-Sheet	197	16,000	−1,300[d]
Leucine	$-CH_2CH(CH_3)_2$	Random	Random	–	–	−1,800[d]
Phenylalanine	$-CH_2C_6H_5$	Random	β-Sheet	194	22,000	−2,500[d]
Norleucine	$-CH_2CH_2CH_2CH_3$	Random	β-Sheet	197	26,000	−2,600[d]
2-Aminooctanoic acid	$-CH_2CH_2CH_2CH_2CH_2CH_3$	Random / β-Sheet[b]	β-Sheet	197	26,000	–
2-Aminodecanoic acid	$-CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$	β-Sheet	β-Sheet	197	26,000	–

[a][Peptide] = 1 mM and [HCl] = 4 mM
[b]Mixed spectrum of random and β-sheet
[c][Peptide] = 1 mM
[d]Free energy of the shift to an organic solvent from water, taken from Nozaki and Tanford [8]

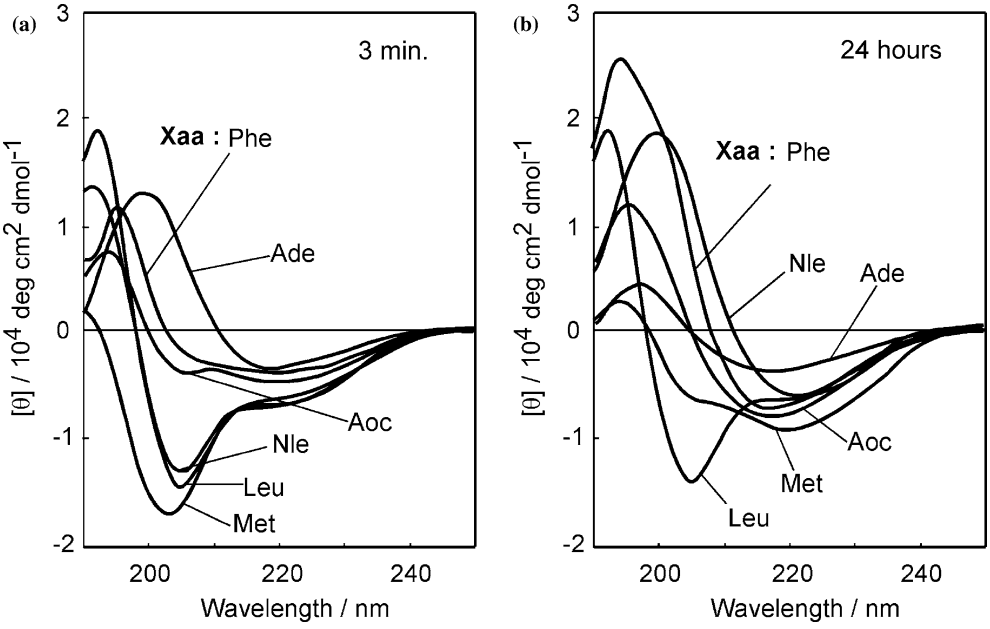

Fig. 2 Circular dichroism spectra of 2-9X4K in C14SA solution **a** after 3 min incubation, **b** after 24 h incubation. [surfactant] = 17 mM, [peptide] = 1 mM and [HCl] = 4 mM, at 30 °C

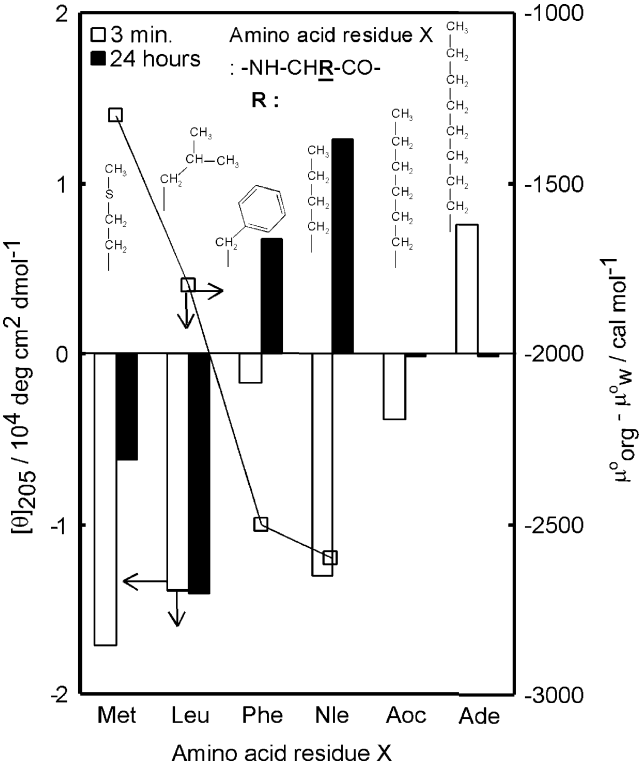

Fig. 3 Changes in molar ellipticity at 205 nm for amino acid residues X, taken from data in Fig. 2a, b. Open columns: after 3 min incubation, and shaded columns: after 24 h incubation. The free energy shift from water to an organic solvent ($\mu_{org} - \mu_w$) is shown by squares. [surfactant] = 17 mM, [peptide] = 1 mM and [HCl] = 4 mM, at 30 °C

the cases with Aoc and Ade, which have extended hydrocarbon chains, no significant changes in the α-helix to β-sheet transition were obserbed. This could be due to the interaction of the side chains of Aoc and Ade with C14SA micelles. As a result, β-sheet structure could be relaxed by the addition of surfactant micelle.

Effect of surfactant micelles on peptide

Circular dichroism spectra of 2-9X4K (X = Nle) in various surfactant solutions are shown in Fig. 4. Three kinds of surfactants, C14SA (anionic), C16TAB (cationic), and C12DPO (nonionic), were used for the measurements. It is expected that ionic and nonionic surfactants might be able to interact with peptides by electrostatic and hydrophobic effects, respectively. All samples were measured immediately after mixing the peptide stock solution and the surfactant. The spectral pattern differed according to the hydrophilic group of the surfactant. The peptide formed an α-helical structure in anionic C14SA solution, and a random structure in nonionic C12DPO solution. In the case of cationic C16TAB solution, the spectral pattern of the peptide exhibited unordered structure. After all, the conformational transition of the peptide was observed in only C14SA. It was unchanged in C12DPO and C16TAB solutions (data not shown). These results suggest that there is an electrostatic interaction between the sulfonate group (SO_3^-) of the C14SA molecule and the protonated amino groups (NH_3^+) of the Lys and N termini of the peptide, under acidic conditions (pH = 2–3). Electrostatic interaction was also required for α-helix formation in an ionic surfactant solution.

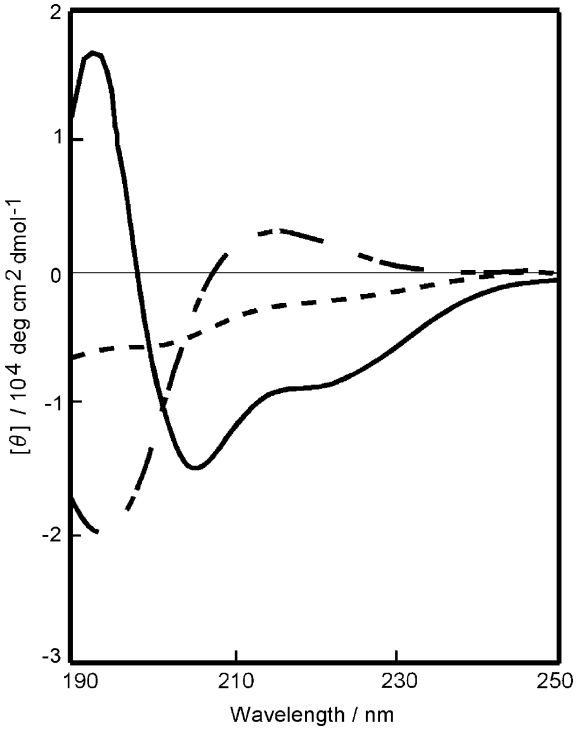

Fig. 4 Circular dichroism spectra of 2-9X4K (X = Nle) in various surfactant solutions; C14SA (*solid line*), C16TAB (*short dashed line*) and C12DPO (*dotted and dashed line*). [surfactant] = 17 mM, [peptide] = 1 mM and [HCl] = 4 mM, at 30 °C

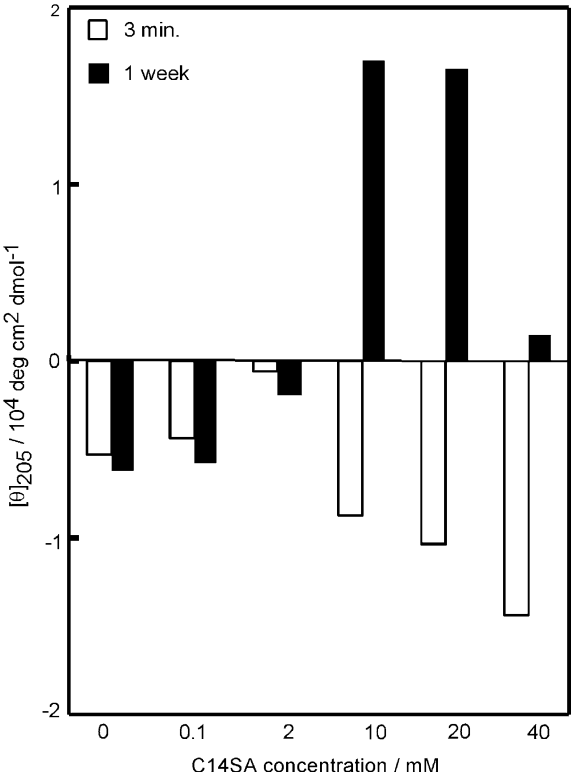

Fig. 5 Changes in molar ellipticity at 205 nm after 3 min (*open columns*) and 1 week (*shaded columns*) in the C14SA concentration range of 0–40 mM. [peptide] = 1 mM, [HCl] = 4 mM at 30 °C

Effect of surfactant concentration on peptide

To investigate the effect of surfactant concentration on the conformational change of the peptides, we measured CD spectra of the peptide 2-9X4K (X = Nle) at surfactant concentrations ranging from 0.1 to 40 mM. C14SA molecules are able to exist over this concentration range as the CMC of C14SA is 2.36 mM at 30 °C. The peptide concentration was constant at 1 mM. The data obtained are shown in Fig. 5. The molecular ellipticity after 3 min is represented by open columns and that after 1 week of incubation is represented by shaded columns. In this figure, the negative bars represent the formation of α-helical structure, while the positive bars represent β-sheet formation. A drastic change in the conformation of the peptide occurred when the C14SA concentrations were 10 and 20 mM. However, the spectral pattern of β-sheets was not strongly indicated when the C14SA concentration increased further, up to 40 mM. It seems that the conformational change depends on the ratio of the peptide to the micelle. The aggregation number of C14SA is about 80 because sodium tetradecanesulfonate has an aggregation number of 80 at 60 °C [32]. It is estimated that one or two molecules of 2-9X4K can exist in one micelle. Therefore, the conformational transition would not occur, since many peptide molecules cannot exist in a single micelle at a concentration above 40 mM. At the low C14SA concentration range, the sample solution was immediately turbid at 2 mM, which is approximately equal to the CMC. This turbidity is suspected to be coprecipitation of peptides with C14SA. Moreover, the peptide showed no change in the conformation below the CMC. It was found that the conformational transition of 2-9X4K (X = Nle) from α-helix to β-sheet depends on the number of peptides per micelle sphere.

Conclusion

A series of 2-9X4K peptides (X = Nle, Met, Phe, Aoc, and Ade), with the exception of X = Leu, preferentially formed β-sheet structures in methanol, and exhibited a conformational transition from α-helix to β-sheet in C14SA aqueous solution. In aqueous medium, an anionic surfactant (C14SA) was needed for the formation of α-helices. Moreover, the conformational change depends on the free energy shift from water to an organic solvent and the concentration of C14SA. Our findings suggest that electrostatic interaction between the peptide and micelle is necessary for α-helix formation, and that β-sheet structures can occur in the presence of hydrophobic, linear side chains.

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