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Oligopeptide-Mediated Helix Stabilization of Model Peptides in Aqueous Solution

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Abstract: Oligopeptide-mediated helix stabilization of peptides in hydrophobic solutions was previously found by NMR and CD spectroscopic studies. The oligopeptide included the hydrophobic amino acids found in its parent peptide and were interposed by relevant basic or acidic amino acids. The strength of the interactions depended on the amino acid sequences. However, no helix-stabilizing effect was seen for the peptides in phosphate buffer solution, because the peptides assumed a random-coil structure. In order to ascertain whether the helix-stabilizing effect of an oligopeptide on its parent peptide could operate in aqueous solution, model peptides EK17 (Ac-AEAAAAEAAAKAAAAKA-NH_2) and IFM17 (Ac-AEAAAAEIFMKAAAAKA-NH_2) that may assume an α-helix in aqueous solutions were synthesized. Interactions were examined between various oligopeptides (EAAAK, KAAAE, EIFMK, KIFME, KIFMK and EYYEE) and EK17 or IFM17 in phosphate buffer and in 80% trifluoroethanol (TFE)–20% H_2O solutions by CD spectra. EAAAK had little effect on the secondary structures of EK17 in both buffer and TFE solutions, while KAAAE, which has the reverse amino acid sequence of EAAAK, had a marked helix-destabilizing effect on EK17 in TFE. EIFMK and KIFME were found to stabilize the α-helical structure of EK17 in phosphate buffer solutions, whereas KIFMK and EYYEE destabilized the α-helical structure of EK17. EIFMK and KIFME had no effect on IFM17, because unexpectedly, IFM17 had appreciable amounts of β-sheet structure in buffer solution. It was concluded that in order for the helix-stabilizing effects to operate effectively, the following factors should be satisfied: (1) the model peptide, the α-helical conformation of which is to be stabilized, should essentially assume an α-helical structure by nature, and (2) the hydrophobicity of the side-chains of the oligopeptide should be high enough for the oligopeptide to perform stable specific side chain–side chain intermolecular hydrophobic interactions with the model peptide.

Keywords: α-helix; β-sheet; oligopeptide; CD spectroscopy; helix stabilization; amyloid; hydrophobic interactions; side chain–side chain interaction

INTRODUCTION

Generally, the stability of an α-helical structure of a peptide or protein is considered to depend on the following factors: (1) the intrinsic helix propensities of the constituent amino acids [1,2], (2) the presence of appropriate *N*- and *C*-caps that flank the helix termini the side chains of which can form hydrogen bonds with the initial four helix amide groups and the final four helix carbonyl groups [3–5], (3) charged group-helix dipole interactions [6–8], (4) specific side chain–side chain electrostatic interactions including salt bridges [9,10] and hydrogen bonds [11,12], and (5) specific side chain–side chain hydrophobic interactions [13–16]. All these factors are intramolecular in origin. Previously, it was found that the pentapeptide KIFMK stabilized the α-helical conformation of MP-1A (Ac-GGQDIFMTEEQK-NH_2) in 80% TFE–20% H_2O solutions [17]. This helix-stabilizing

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effect by KIFMK is intermolecular in origin. Further, the amino acid sequence-dependent helix-stabilizing effects were studied in detail [18]. It was found that (1) the hydrophobic IFM (Ile-Phe-Met) residues are absolutely necessary for both peptides, (2) the conformation and the lengths of the side chains of amino acids located on either side of the IFM residues are important factors, and (3) pentapeptides are the most effective number of amino acids in the oligopeptide. The helix-stabilizing effect appears to originate from both the mutual hydrophobic interactions between the two IFM residues and the electrostatic interactions (salt bridges) of two Lys residues in KIFMK with Asp and Glu of MP-1A, respectively.

In previous research [17,18], although the oligopeptide-mediated helix-stabilizing effects for MP-1A in 80% TFE–20% H_2O solution were seen, no appreciable helix-stabilizing effect was seen in aqueous solution. This is because MP-1A assumed a random coil structure. In this work, model peptides Ac-AEAAAAEAAAKAAAAKA-NH_2 (EK17) and Ac-AEAAAAEIFMKAAAAKA-NH_2 (IFM17) that may assume an α-helix in aqueous solution were synthesized. In the model peptides, the charged group-helix dipole interactions would be favourable, because there is a Glu close to the *N*-terminus (E2) and a Lys close to the *C*-terminus (K16). Also, an E7–K11 i, $i+4$ salt bridge or an ion pair may be formed that could stabilize an α-helical structure [9]. Ala was chosen to fill the space between the acidic and basic amino acids, since it has a small side chain and should not interfere with side chain interactions between other residues [19]. Thus EK17 and IFM17 should assume an α-helical structure even in aqueous solution. In IFM17, IFM (Ile-Phe-Met) residues were substituted for AAA8–10 to increase the hydrophobicities between E7 and K11. In an oligopeptide defined as αxyzβ, the 'xyz' part should be composed of the hydrophobic amino acids that are found in its parent model peptide [17,18]. They are interposed by relevant basic or acidic amino acids of 'α' and 'β' so as to form intermolecular salt bridges with the corresponding acidic/basic amino acids (Glu or Lys) of their parent model peptides. Interactions were studied of the model peptides (EK17 and IFM17) with various oligopeptides (EAAAK, KAAAE, EIFMK and KIFME) or with unrelated control oligopeptides (EYYEE and KIFMK) to see whether these oligopeptides have a helix-stabilizing effect on their parent model peptides in aqueous solution.

MATERIALS AND METHODS

Peptide Synthesis

All the peptides were synthesized automatically by solid-phase methods, using Fmoc chemistry on an Applied Biosystems 433A peptide synthesizer; their *N*-termini were acetylated (denoted by Ac-), and their *C*-termini were amidated (denoted by -NH_2). They were purified on a reverse-phase C_{18} high-performance liquid chromatography column using a gradient 90% A, 10% B to 40% A, 60% B, where A is 0.1% trifluoroacetic acid (TFA) in water and B is 0.1% TFA in acetonitrile; the rate of decrease in A was 50%/50 min. The peptides were characterized by ion spray mass spectrometry on a Perkin Elmer SCIEX API III mass spectrometer. EK17, Ac-AEAAAAEAAAKAAAAKA-NH_2: m/z calcd 1496.79 (monoisotope), 1497.68 (av.), found 1497.5 (MH^+); IFM17, Ac-AEAAAAEIFMKAAAAKA-NH_2: m/z calcd 1674.88 (monoisotope), 1675.97 (av.), found 1675.5 (MH^+); EAAAK, Ac-EAAAK-NH_2: m/z calcd 529.29 (monoisotope), 529.60 (av.), found 529.5 (MH^+); KAAAE, Ac-KAAAE-NH_2: m/z calcd 529.29 (monoisotope), 529.60 (av.), found 529.5 (MH^+); EIFMK, Ac-EIFMK-NH_2: m/z calcd 707.37 (monoisotope), 707.89 (av.), found 707.6 (MH^+); KIFME, Ac-KIFME-NH_2: m/z calcd 707.37 (monoisotope), 707.89 (av.), found 707.6 (MH^+); EYYEE, Ac-EYYEE-NH_2: m/z calcd 772.29 (monoisotope), 772.77 (av.), found 772.5 (MH^+); KIFMK, Ac-KIFMK-NH_2: m/z calcd 706.42 (monoisotope), 706.95 (av.), found 707.5 (MH^+).

Circular Dichroism (CD) Experiment

Stock peptide solutions (1 mM) were prepared by dissolving samples in 310 mOsm phosphate buffer (150 mM, pH 7.0), unbuffered water (pH 7.0), or 80% TFE–20% H_2O solutions (pH 7.0). Each stock peptide solution was pipetted equally and then it was diluted with an appropriate solvent to give a final concentration of 50 μM in each peptide solution or in each model peptide–oligopeptide mixed solution. In the unbuffered aqueous solutions, the pH was adjusted by adding small amounts of NaOH (pH 7.0). CD spectra (190–250 nm) were measured on a Jasco J-820 at room temperature after the instrument was calibrated with D-camphor-10-sulfonate. A 0.5 mm pathlength quartz cell was used for a 50 μM sample solution. Four scans were averaged for each sample; the averaged blank spectra were subtracted. In the CD spectra of model

peptide–oligopeptide mixed solutions or in the CD spectra of model peptide and oligopeptide solutions observed separately and summed by computer, the mean residue ellipticities were calculated by considering the number of amino acids to be 22. The helicity (α) was determined from the mean residue ellipticity [Θ] at 222 nm according to the relation $\alpha = ([\Theta]_{222} + 2000) \times 100/-30\,000$ (%) [20]. The percentages of each secondary structure were analysed by using CONTIN/LL within the CDPro software package [21,22].

RESULTS

The CD Spectra of Peptides

As shown in Figure 1A and B, the CD spectra of EK17 (Ac-AEAAAAEAAAKAAAAKA-NH_2) in phosphate buffer and in 80% TFE–20% H_2O solutions showed double negative maxima at 206 and 222 nm, and a strong positive maximum at 190 nm, indicating that the overall secondary structure was assuming an α-helical structure [23]. The CDPro calculations revealed high α-helix and low β-sheet contents (Table 1). On the contrary, IFM17 showed a negative band near 218 nm and a positive band between 195 and 200 nm, indicating that IFM17 involved appreciable amounts of β-sheet structure [23]. Every oligopeptide (EAAAK, KAAAE, EIFMK, KIFME, KIFMK and EYYEE) in buffer solution essentially assumed random coil structures accompanied by a β-sheet (Figure 1C). The CDPro calculations clearly showed that the oligopeptides including the IFM residues have higher β-sheet (lower random coil) content than those including the AAA residues (Table 1). In TFE solutions, EIFMK, KIFME and KIFMK contained appreciable amounts of both α-helix and β-sheet structures, whereas EAAAK, KAAAE and EYYEE contained only β-sheet structures (Figure 1D). Evidently, in the 'ExyzK' and 'KxyzE' types of oligopeptides in TFE, the α-helical content was higher in the cases where the 'xyz' part was IFM than where the 'xyz' part was AAA (Table 1). Thus, it is interesting to note that IFM residues stabilized β-sheets in buffer, while they stabilized α-helices in TFE for the oligopeptides.

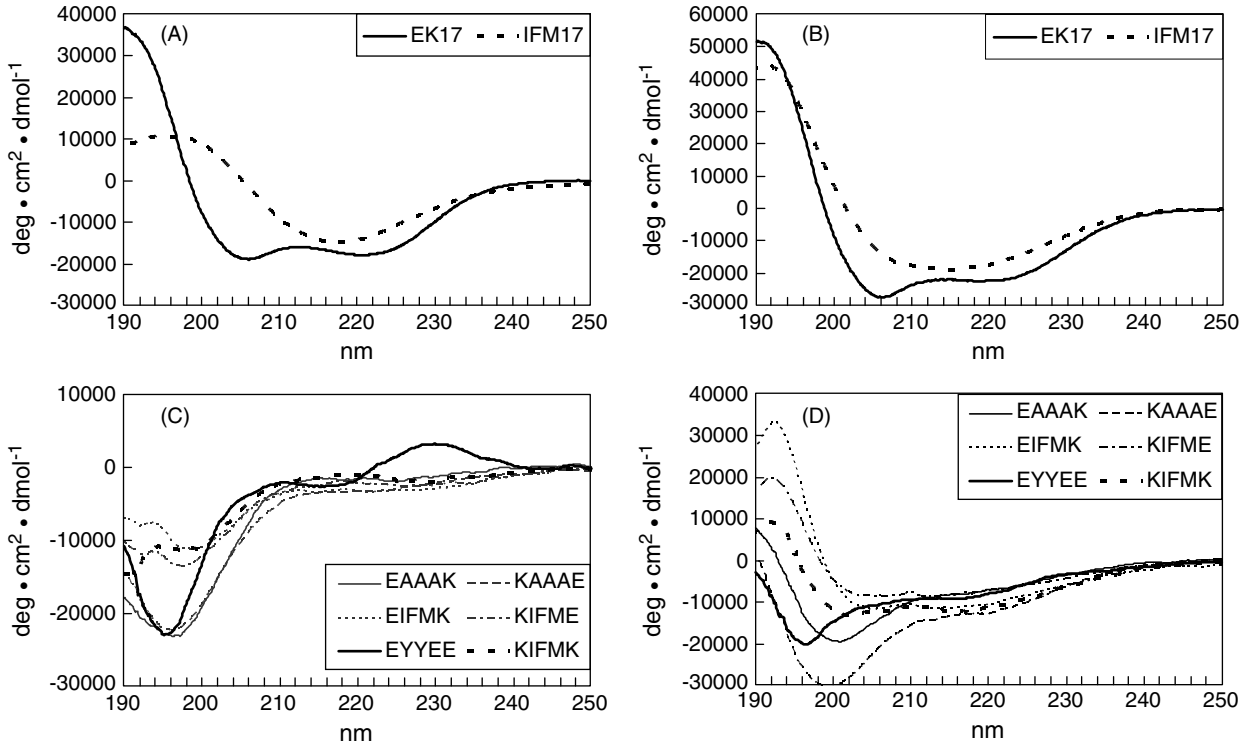

Figure 1 CD spectra of EK17 and IFM17 (A and B) and EAAAK, KAAAE, EIFMK, KIFME, EYYEE and KIFMK (C and D) in aqueous buffer solutions (A and C) and in 80% TFE–20% H_2O solutions (B and D). The concentrations of all of the peptides were 50 μM.

Table 1 Calculation of Secondary Structure Contents (%) for the CD Spectra of Model Peptides and Oligopeptides[a]

Peptide	In buffer		In 80% TFE	
	α-helix (%)	β-sheet (%)	α-helix (%)	β-sheet (%)
EK17	41.5	8.8	61.8	3.0
IFM17	8.2	36.9	48.5	11.8
EAAAK	6.1	17.7	10.5	26.9
KAAAE	5.9	17.1	11.8	16.7
EIFMK	7.5	29.3	37.7	26.0
KIFME	6.1	26.4	19.1	27.2
EYYEE	1.7	34.0	5.2	35.3
KIFMK	6.6	25.6	19.9	20.9

[a]The secondary structure contents were calculated by using CONTIN/LL within the CDPro software package [21,22].

Interactions between EK17 and EAAAK or KAAAE

Figure 2 shows the CD spectra of EK17 and oligopeptides in phosphate buffer (A and C) and in 80% TFE–20% H_2O (B and D) solutions. The thin lines show the sum of the CD spectra of EK17 and the oligopeptide observed separately, while the thick lines show the CD spectra of EK17 and the oligopeptide mixed solutions. If an oligopeptide really stabilized the α-helical conformation of EK17, the increase in the helical content should be reflected in the CD spectra. However, the CD spectra showed that neither EAAAK nor KAAAE stabilized the α-helical conformation of EK17 in both kinds of solvent. KAAAE significantly destabilized the α-helix of EK17 in both phosphate buffer (Figure 2C) and TFE (Figure 2D) solutions. From the changes in the ellipticity at 222 nm, changes in the helical content of EK17-KAAAE solution were estimated, these are summarized graphically in Figure 3. The KAAAE decreased the helical content by 5.5% in buffer and by 15.9% in TFE. It is considered that KAAAE disrupted the intramolecular salt bridge between E7 and K11 of EK17 by forming intermolecular salt bridges between K and E7, and between E and K11 of KAAAE. Unrelated control peptides tried (EYYEE, KIFMK) also reduced the α-helical contents by 3%–6% in both kinds of solution (Figure 3).

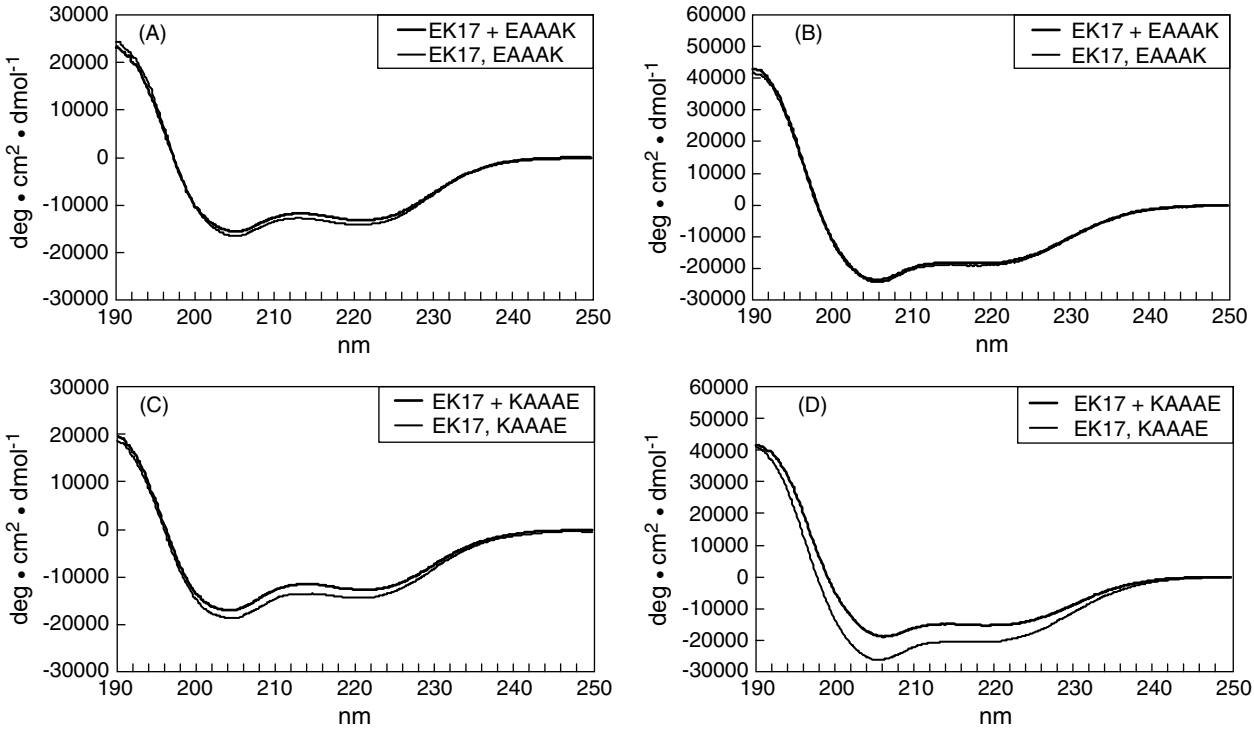

Figure 2 CD spectra of EK17 (50 μM) and oligopeptides (50 μM) systems in aqueous buffer solutions (A and C) and in 80% TFE–20% H_2O solutions (B and D). Oligopeptides are EAAAK (A and B) and KAAAE (C and D). Thick lines indicate CD spectra for EK17-oligopeptides mixed solutions and thin lines indicate the sum of those for EK17 and oligopeptides by computer.

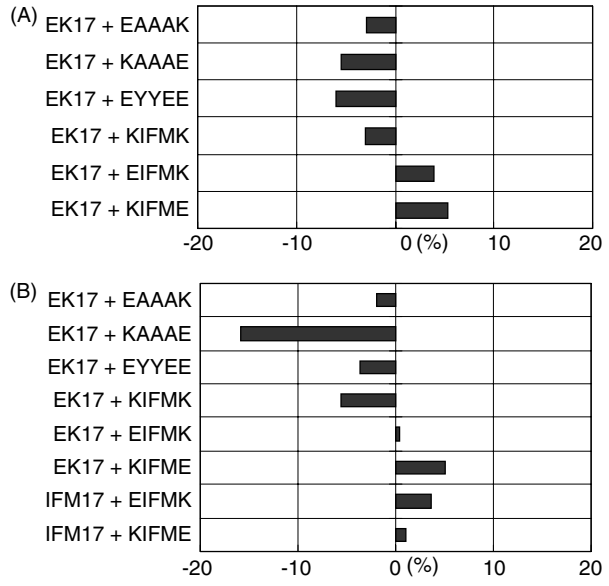

Figure 3 Changes in the helical contents (%) of the model peptide–oligopeptide systems in aqueous buffer solutions (A) and in 80% TFE–20% H_2O solutions (B). The helical contents were estimated from the ellipticity at 222 nm.

Interactions between EK17 and EIFMK or KIFME

It is expected that the interaction between an oligopeptide and its parent model peptide would be reinforced with increasing hydrophobicity in the 'xyz' part of the oligopeptide, 'αxyzβ'. This expectation may especially be true for peptides in aqueous solution, since the hydrophobic interactions would be reinforced in water than in alcohol. Thus EAAAK and KAAAE were modified as EIFMK and KIFME, respectively. The IFM (Ile-Phe-Met) residues were tentatively chosen to increase the hydrophobicities of the 'xyz' part of the oligopeptide, since it has already been used in our previous work [17,18]. Interactions between EK17 and EIFMK or KIFME in phosphate buffer and in 80% TFE–20% H_2O solutions were investigated (Figure 4). The CD spectra showed that EIFMK and KIFME stabilized the α-helix of EK17 in buffer solutions (Figure 4A and C) by 3.9% and 5.3%, respectively (Figure 3A). In TFE, EIFMK showed no appreciable effect on the secondary structure (Figure 4B), whereas KIFME increased the α-helical content of the KIFME-EK17 solution by 5.0% (Figures 3B and 4D).

Interactions between IFM17 and EIFMK or KIFME

We further aimed at enhancing interactions by substituting AAA8–10 in the model peptide by IFM residues, expecting that the α-helical conformation for the model peptide would be more effectively stabilized than for EK17. However, this was not the case. In buffer solutions, neither EIFMK nor KIFME affected the secondary structure of IFM17 (data not shown). Evidently, these results were due to the high β-sheet content of IFM17 (Figure 1A). In contrast, EIFMK in TFE increased the α-helical content of IFM17-oligopeptide solution by 3.7%, as could be expected from the α-helical structure of IFM17 (Figure 3B). However, KIFME in TFE had no effect on the structure of IFM17.

Interactions between IFM17 and EAAAK, KAAAE, EIFMK, or KIFME in Unbuffered Aqueous Solution

Since IFM17 adopted a structurally well-defined β-sheet structure in buffered aqueous solution (Figure 1A) and also there was no appreciable change in the CD spectra by EIFMK or KIFME (data not shown), the CD spectra in unbuffered aqueous solution at pH 7.0 were evaluated. In the unbuffered solution, since there was no high concentration of salts as found in an isotonic buffer (150 mM), changes in the CD spectrum were expected resulting from the decreased β-sheets and possibly from increased α-helices. However, this was not what was observed. In unbuffered aqueous solution, IFM17 showed nearly an identical CD profile to that observed in the buffered solution. It was noticed, however, there were changes in the intensity of the CD band at 195 nm arising from the π-π* transitions of the amide groups [23] (Figure 5C and D). Although EAAAK and KAAAE had little effect on the secondary structure of IFM17, as expected (Figure 5A and B), EIFMK and KIFME appreciably reduced the intensity of the CD band at 195 nm, showing that these oligopeptides distorted the β-sheet structure of IFM17.

DISCUSSION

It was confirmed that the model peptide, EK17, assumed an α-helical structure in aqueous solution. The oligopeptides, EIFMK and KIFME, were found to stabilize the α-helical conformation of EK17. It was expected that IFM17 would also assume an α-helical structure in aqueous solution. In addition, its α-helical structure would be more stabilized than EK17 by the oligopeptides. However, this was not the case. IFM17 dominantly assumed a β-sheet structure (Figure 1A). Moreover, no appreciable

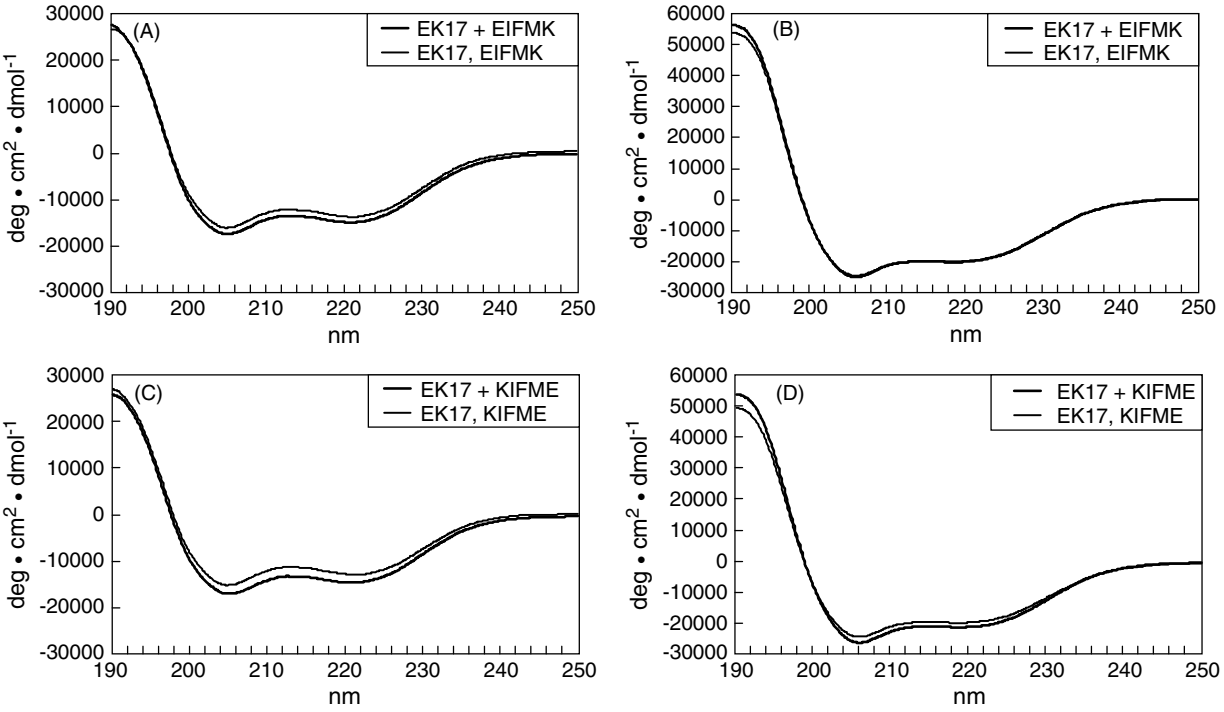

Figure 4 CD spectra of EK17 (50 μM) and oligopeptides (50 μM) systems in aqueous buffer solutions (A and C) and in 80% TFE–20% H_2O solutions (B and D). Oligopeptides were EIFMK (A and B) and KIFME (C and D). Thick lines indicate CD spectra for EK17-oligopeptides mixed solutions and thin lines indicate sum of those for EK17 and oligopeptides by computer.

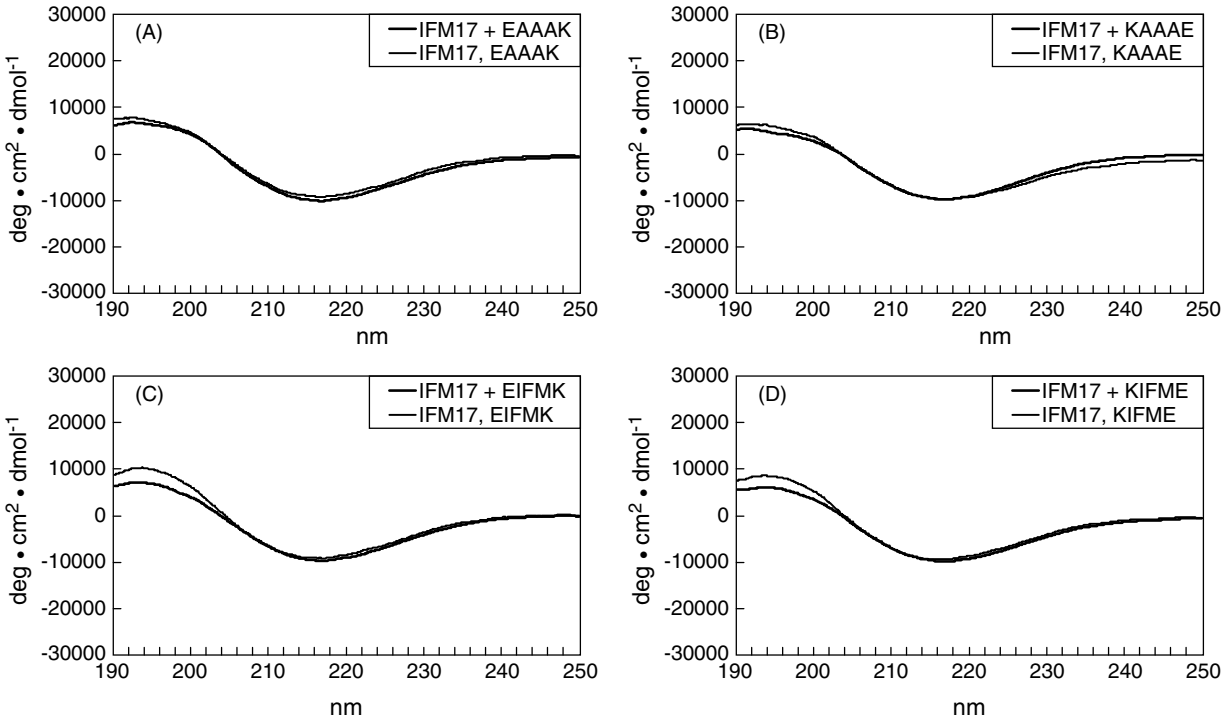

Figure 5 CD spectra of IFM17 (50 μM) and oligopeptides (50 μM) systems in unbuffered aqueous solutions at pH 7.0. Oligopeptides were EAAAK (A), KAAAE (B), EIFMK (C), and KIFME (D). Thick lines indicate CD spectra for IFM17-oligopeptides mixed solutions and thin lines indicate sum of those for IFM17 and oligopeptides by computer.

change in the secondary structure by oligopeptides was noted. Although IFM residues consist of amino acids which are liable to form β-sheets [24,25], the present observation was in contrast to the secondary structure predictions by such as SOPMA (self-optimized prediction method) [26] and GOR IV [27]. They predicted exclusive α-helical structure throughout the whole amino acid sequence. At first, it was considered that the intramolecular salt bridge between E7 and K11 might be weakened in such a high salt concentration as found in an isotonic buffer. This appeared to be reflected in the difference in the α-helical contents between EK17 in buffer (41.5%) and that in TFE (61.8%) in the CDPro calculations (Table 1). As a result, the salt bridge in IFM17 would be disrupted and the E7-K11 moiety was made to adopt a β-sheet conformation obeying the intrinsic β-sheet propensities of IFM. However, IFM17 in unbuffered aqueous solution also adopted a structurally well-defined β-sheet structure. Thus the β-sheet structure of IFM17 appears to be intrinsically due to the IFM residues, but not due to the high salt concentrations. The β-sheet propensities of the IFM residues were also noted for the oligopeptides in buffer solution as described above.

Presently, two kinds of oligopeptides were used to force the intermolecular salt bridges to form with E7 and K11 of EK17. One was an 'ExyzK' type and the other was a 'KxyzE' type of oligopeptide. Among others, it turned out that the 'KxyzE' type of oligopeptide had a larger effect on the secondary structure of EK17 than the 'ExyzK' type of oligopeptide, regardless of helix-stabilizing or helix-destabilizing effects. These phenomena were observed in both kinds of solvent (Figures 2 and 4). This finding suggests that EK17 and the oligopeptide interact in a 'parallel' fashion with each other regarding their amino acid sequences. In contrast, in the case of IFM17, EIFMK had larger effects on the secondary structure of IFM17 than KIFME, implying that they are interacting in an 'antiparallel' fashion with each other. The β-sheet structure of IFM17 appears to have favoured the antiparallel interactions. Another finding was that in a case where 'xyz' was AAA, the oligopeptide destabilized, while in a case where 'xyz' was IFM, it stabilized an α-helix of EK17. Again, these phenomena were observed in both kinds of solvent. The specific side chain–side chain hydrophobic interactions [13–16] appeared to have operated intermolecularly between the β-branched (Ile) and bulky (Phe and Met) hydrophobic side chains and the methyl groups of AAA residues, resulting in stabilization of the α-helices of EK17. The higher content of random coil structure for the oligopeptides consisting of AAA than for those consisting of IFM may be a reason for the helix-destabilizing effect, although its mechanism cannot be explained. The experimental results indicate that an oligopeptide should adopt a definite secondary structure in order to stabilize an α-helical structure of a model peptide.

In conclusion, the helix-stabilizing effect which was mediated by oligopeptides depended on the following factors: (1) a model peptide, the α-helical conformation of which is to be stabilized, should essentially assume an α-helical structure by nature, and (2) the hydrophobicity of the side chains of the oligopeptide should be high enough to perform sufficient specific side chain–side chain hydrophobic interactions with the model peptide. If these conditions are fully satisfied, the oligopeptide can stabilize the α-helical conformation for another peptide regardless of the hydrophobicity of the media.

A common feature related to the pathogenic mechanisms of such neurodegenerative disorders as Alzheimer's and prion diseases is an abnormal protein deposition in brain tissues [28–31]. The proteins deposited are amyloid fibrils which are composed of proteins adopting β-sheet structures. Most of the structural parts adopting the β-sheets are produced by conformational changes from α-helices. Thus the design of helix stabilizing agents for the moieties adopting α-helices by nature could be amyloid-inhibiting therapeutics for neurodegenerative disorders. The present data could be a clue for designing these agents. Current studies are being undertaken to find helix stabilizing agents for prion disease [32].

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