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Complexation of peptide with Cu^{2+} responsible to inducing and enhancing the formation of α-helix conformation

Jin Zou[1,2] & Naoki Sugimoto[1,3,*]
[1]*Department of Chemistry, Faculty of Science, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan*
[2]*Department of Biological Engineering, School of Chemical Engineering, Hebei University of Technology, Tianjin 300130, P.R. China*
[3]*High Technology Research Center, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan*
[*]*Author for correspondence (Tel: (81)(78)435-2497 ; Fax: (81)(78)435-2539; E-mail: sugimoto@konan-u.ac.jp)*

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Abstract

Role of some metal ions on the conformations of peptides was examined by using a series of short alanine-based peptides with single Trp-His (**W**-**H**) interaction in different environments. Circular dichroism (CD), Trp (**W**) fluorescence emission, and Fourier transform infrared (FTIR) spectroscopy revealed that there is a conformational role of Cu^{2+} in inducing and enhancing the formation of α-helix conformation. The complexation of the peptide with Cu^{2+} is responsible to the conformational effect because the chelation is able to stabilize peptide with an α-helix conformation. The possible factors affecting the role of Cu^{2+} are discussed in the paper. The results in this paper are useful to understand the important structural role of Cu^{2+} in protein folding and the possible mechanism in some neurodegenerative diseases such as Alzheimer's disease.

Introduction

It is known that most of biological processes involve specific interactions between proteins and other macromolecules, and the specific structures of proteins are important to these interactions. In some cases, a certain length of polypeptides, typically at least 50 amino acid residues, is necessary for folding up into an appropriate structure by itself (Berg & Shi 1996). On the other hand, metal ions play an important structural role to induce the folding of an α-amino acid sequence or stabilize existing secondary structures within proteins by metal complexation. It has been recently found that some domains in zinc-finger proteins are too small to fold by themselves but fold stably when they bound with zinc ion (Berg & Shi 1996; Fekkes *et al.* 1999). Both α-helix and β-sheet conformation of peptide can be induced and stabilized by some metal ions (Ghadiri & Fernholz 1990; Schneider & Kelly 1995). Despite of recent advances in strategies employing metal complexation to a nucleate or stabilizing secondary structure of proteins, there were few reports about the interaction between metal ions and polypeptides with single Trp-His (**W**-**H**) interaction in different environments.

It is found that some metal ions such as Zn^{2+}, Cu^{2+}, and so on are essential traces with important fundamental roles in biochemistry of human life (Berg & Shi 1996; Fekkes *et al.* 1999). Some neurodegenerative diseases including prion disease and Alzheimer's disease (AD) are characterized pathologically with the conformational transition and deposition of certain proteins such as prion protein (PrP) and amyloid β-peptide in brain, and metal ions play an important role to the aggregation of proteins. Recently, it was observed that Cu^{2+} has a high selectivity to bind the octapeptide repeat in N terminal segment of prion protein, PrP, which affects the conformation of PrP (Miura *et al.* 1996; Miura *et al.* 1999; Stöckel *et al.* 1998). The different effects of Zn^{2+} and Cu^{2+} on

the aggregation of amyloid β-peptide are also demonstrated and some possible mechanisms are reported by some researchers (Atwood *et al.* 1998; Liu *et al.* 1999; Miura *et al.* 2000).

Short alanine-based peptides are useful as simple models for studying the interactions that contribute to the peptide conformation and the folding of globular proteins (Blondelle *et al.* 1997; Huyghues-Despointes & Baldwin 1997; Padmannabhan *et al.* 1998). They were also used in our previous works to investigate the conformational transition between α-helix and β-sheet (Sugimoto *et al.* 1999) and the effect of Trp-His (**W**-**H**) interaction on peptide conformations (Zou & Sugimoto 2000), respectively. The results indicated that the conformation of short alanine-based peptides is relative to not only the type of Trp-His (**W**-**H**) interaction, that is, its interaction at $(i, i + 4)$ spacing in α-helix or at $(i, i + 2)$ spacing in β-sheet, but also its position in peptides. Both α-helix $(i, i + 4)$ and β-sheet $(i, i + 2)$ Trp/His (**W**-**H**) interaction at C terminus play an important role as a conformational switch to induce and stabilize a certain conformation in the corresponding short alanine-based peptides. It was observed in our previous study that there is a conformational effect of Cu^{2+} on the short alanine-based peptides with single Trp-His (**W**-**H**) interaction in different environments (Zou & Sugimoto 1999). In this paper, we have examined and compared the conformational effects of different metal ions including Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cs^{+}, Cu^{2+}, La^{3+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} with short alanine-based peptides in different environments. It has been revealed that the complexation of peptides with Cu^{2+} is responsible to inducing and enhancing α-helix conformation. The results in this paper are useful to understand the conformational role of Cu^{2+} in protein folding and the possible mechanism in some neurodegenerative diseases such as Alzheimer's disease.

Experimental

Materials

The peptides listed in Table 1 were synthesized with Fmoc method on a Pioneer Peptide Synthesis System (Perseptive Biosystems Inc., USA) (Sugimoto *et al.* 1999; Zou & Sugimoto 2000). The synthesized peptides were cleaved from the resin by reacting with 2% (v/v) m-cresol, 6% (v/v) 1,2-ethanedithiol, 12% (v/v) thioanisole and 80% (v/v) trifluoroacetic acid (TFA) at room temperature for 1 h, precipitated from ice-cold diethyl ether and lyophilized. The crude peptides were purified on YMC C18 reverse phase column at 50 °C by a UV-8020/CCPM-II high performance liquid chromatography (TOSOH Co., Japan). The mobile phase consisted of Liquid A (0.1% (v/v) TFA in water) and Liquid B (0.08% (v/v) TFA in acetonitrile). The purified peptides (>95% according to HPLC analysis) were characterized by a Voyager-DE mass spectroscopy (Perseptive Biosystems Inc., USA) using a α-cyano-4-hydroxycinnamic acid matrix. Concentrations of the peptide stock solutions were determined from the absorption of the Trp (**W**) residue in each peptide at 278 nm with an UV/VIS/NIR spectrophotometer (JASCO Co. Ltd., Japan) (extinction coefficient $\varepsilon_{278} = 5500\ M^{-1}\ cm^{-1}$) (Sugimoto *et al.* 1999; Zou & Sugimoto 2000). Samples were prepared by diluting the stock solution with appropriate buffers containing 1 mM each of sodium citrate, sodium phosphate, sodium borate, and 10 mM sodium chloride which were adjusted to different pHs by HCl or NaOH (Chakrabartty *et al.* 1993; Fernandez-Recio *et al.* 1997). The chloride salts including Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cs^{+}, Cu^{2+}, La^{3+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} were used with 1:20 molar ratio of peptide to metal ion to examine the effect of various metal ions on the conformations of peptides unless otherwise noted.

Table 1. Peptide sequence and its notation[a]

Sequence	Notation
1. Ac-KAAAAA**W**AAA**H**AAAAK-NH_2	KA_5**W**A_3**H**A_4K
2. Ac-KAAAAAAAAAAA**W**A**H**K-NH_2	KA_{11}**W**A**H**K
3. Ac-**W**KAAAAAAAAAAAAA**H**K-NH_2	**W**KA_{12}**H**K
4. Ac-KAAAAAAA**W**AAAAAAK-NH_2	KA_7**W**A_6K
5. Ac-**W**KAAAAAAAAAAAAAK-NH_2	**W**KA_{13}K
6. Ac-K**W**AAA**H**AAAAAAAAK-NH_2	K**W**A_3**H**A_9K
7. Ac-KAAAAAAAAA**W**AAA**H**K-NH_2	KA_9**W**A_3**H**K

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

Circular dichroism measurement and analysis

Circular dichroism (CD) spectra of the peptides were obtained by using a JASCO J-600 spectropolarimeter (JASCO Co. Ltd., Japan) with 0.1 cm path length quartz cell at 2 °C, and interfaced to a Dell OptiPlex GXi computer. The instrument was calibrated by using (+)-10-camphorsulfonic acid. 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. The CD spectrum was the average of three scans made at 0.1 nm interval from 260 to 190 nm. The concentration of the peptides was 50 μM, prepared by diluting the stock solution with the appropriate buffers. The conformation of a peptide of 50 μM was monitored with the mean residue ellipticity, $[\theta]$, (deg cm^2 dmol^{-1}) at characteristic peaks in a CD spectrum, that is, the peaks of 208 and 222 nm for α-helix and that of 218 nm for β-sheet. And the relative helicity change of peptides upon the addition of various metal ions was approximately expressed by the relative percentage change of $[\theta]_{222}$, $\Delta[\theta]_{222}$, shown as following:

$$\Delta[\theta]_{222}(\%) = ([\theta]_2 - [\theta]_1) \times 100/[\theta]_1,$$

where $[\theta]_1$ and $[\theta]_2$ are the intensities of circular dichroism at 222 nm in the absence and presence of a certain metal ion, respectively. 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 measuring their CD spectra. Since an accurate determination of helical content with CD spectra is particularly difficult for peptides containing aromatic residues (Chakrabartty *et al.* 1993), we considered the effect of Trp (**W**) residue on the CD spectra of helical peptides as the error of CD measurement. Similar problem has been encountered and analogous method has been used in the study of Trp-His (**W-H**) interaction by other researchers (Fernandez-Recio *et al.* 1997).

Fluorescence measurement and analysis

The effects of metal ions on the conformation of peptides were also monitored with the fluorescence emission spectra of Trp (**W**) residue that is present in all the peptides used in this paper. Fluorescence spectra of peptides were measured in an F-3010 Fluorescence Spectrophotometer (Hitachi Co. Ltd., Japan) with 1.0 cm path length quartz cell at 2 °C. In all cases, the wavelength of 278 nm was used for the excitation of Trp (**W**) residue and the emission intensity was measured in the range from 320 nm to 500 nm, where there was an emission peak near the wavelength of 350 nm. Fluorescence emission intensity was shown in arbitrary units. The temperature of cell holder was controlled by 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. All measurements were carried out in 5 μM of peptide concentrations prepared by diluting the stock solution with an appropriate buffer. The concentration of Cu^{2+} was changed in different molar ratio of peptide to Cu^{2+} to show the concentration dependence of peptide binding with Cu^{2+}. The association constants (K_a) of peptide with Cu^{2+} were estimated with a curve fitting procedure by MacCurveFit version 1.4 of Kevin Raner Software in the following equation (Wendt *et al.* 1997; Nakano & Sugimoto 1998):

$$\begin{aligned}\Delta F = \; & \Delta F_{\max}\{1 + K_a[P]_0 + K_a[M]_0 - \\ & [(1 + K_a[P]_0 + K_a[M]_0)^2 - \\ & 4K_a^2[P]_0[M]_0]^{1/2}\}/2K_a[P]_0,\end{aligned}$$

where ΔF is the observed fluorescence change, ΔF_{max} is the maximum fluorescence change at saturation, K_a is the association constants of peptide with metal ions, and $[P]_0$ and $[M]_0$ are the initial concentrations of peptide and metal ion, respectively.

Fourier transform infrared (FTIR) measurement and analysis

Infrared absorption, and particularly Fourier transform infrared (FTIR) spectroscopy, has also been proven to be one of the valuable tools for probing the secondary structure of biopolymers in the steady state (Haris & Chapman 1995). FTIR spectra were measured and recorded with a Nicolet N-750B FTIR spectrometer (Nicolet Instrument Corp. USA) in this work. A demountable liquid cell with KBr window (JASCO Co. Ltd., Japan) was used in the measurement. Typically, 512 scans for each, background and sample, were collected and the spectra were obtained with a resolution of 2 cm^{-1} in a transmission mode. The temperature in all experiments was kept at 25 °C. Background spectra were recorded under the same conditions with the media containing no peptides.

Results

Peptide design and characterization

Short alanine-based peptides shown in Table 1 were designed and synthesized *de novo* on a Pioneer Peptide Synthesis System by Fmoc method. The Trp (**W**) residue was introduced in the peptides and has an interaction with His (**H**) residue. The two Lys (K) residues were placed at the two termini of a peptide

to make it soluble in water. The peptides were acetylated (Ac-) at the N termini and amidated (-NH_2) at the C termini to decrease destabilising interactions of the helix dipole.

A Trp-His (**W**-**H**) interaction was introduced in different geometrical spacings and positions as a 'guest' to monitor the interaction in different environments. This paper used $(i, i + 4)$ Trp-His (**W**-**H**) as a possible α-helix geometrical space for the Trp-His (**W**-**H**) interaction on the basis of a regular α-helical structure of 3.6 residues per turn. A pair of $(i, i + 4)$ Trp-His (**W**-**H**) residues was introduced in the middle, N terminus and C terminus of the short alanine-based peptides to form three guest peptides, Sequence **1** (KA_5**W**A_3**H**A_4K), Sequence **6** (K**W**A_3**H**A_9K) and Sequence **7** (KA_9**W**A_3**H**K), respectively. A pair of $(i, i+2)$ Trp-His (**W**-**H**) was used as a possible β-sheet geometrical space for the Trp-His (**W**-**H**) interaction and introduced at the C terminus of a peptide to form Sequence **2** (KA_{11}**W**A**H**K). A Trp (**W**) residue was introduced at N terminus and a His (**H**) residue at C terminus of the same peptide forming Sequence **3** (**W**KA_{12}**H**K), where there is a pair of Trp-His (**W**-**H**) residues in neither $(i, i + 4)$ nor $(i, i + 2)$. The amino acid residues of Sequences **1**, **2**, **3**, **6**, and **7** are identical and the difference among them is the geometrical spacing and position of Trp-His (**W**-**H**) residues in the peptides. Only a Trp (**W**) residue was introduced in the different positions of peptides to study the effect of its position on peptide conformation and also use as a reference for comparing with other peptides. There is a Trp (**W**) residue in the middle of Sequence **4** (KA_7**W**A_6K) and a Trp (**W**) residue at the N terminal of Sequence **5** (**W**$KA_{13}K$), respectively. The amino acid residues of Sequences **4** and **5** are identical and the difference between them is only the position of a Trp (**W**) residue in the peptides.

The results in our previous study indicated that the Trp-His (**W**-**H**) interaction plays an important role in the conformation of short alanine-based peptides (Zou & Sugimoto 2000). The conformation of the peptides is relative to not only the type of Trp-His (**W**-**H**) interaction, that is $(i, i+4)$ in α-helix and $(i, i+2)$ in β-sheet, but also its position in the peptides. Both α-helix $(i, i + 4)$ and β-sheet $(i, i + 2)$ Trp-His (**W**-**H**) interactions at C terminus acted as a conformational switch which resulted in a typical α-helix conformation of **7** and β-sheet conformation of **2**, respectively.

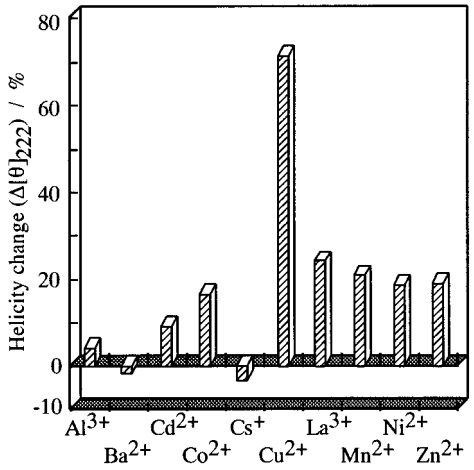

Figure 1. The helicity changes of **1** upon the addition of various metal ions. CD measurement was performed at 50 μM of peptide and pH 7. Other conditions are described in the text.

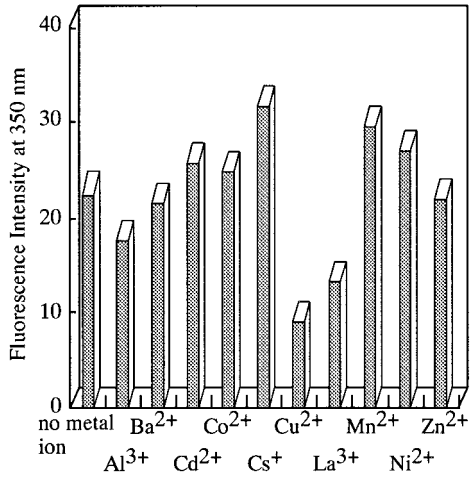

Figure 2. Trp (**W**) fluorescence emission intensity of **1** in the absence and presence of various metal ions. Fluorescence emission intensity was monitored at 350 nm and shown in arbitrary units.

Effects of metal ions on the conformation of **1**

To evaluate the specificity of the metal ions, at first, we examined CD spectra of **1** in the absence and presence of Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cs^{+}, Cu^{2+}, La^{3+}, Mn^{2+}, Ni^{2+}, or Zn^{2+} and compared the conformational effect of various metal ions with the relative helicity change, $\Delta[\theta]_{222}$. As shown in Figure 1, the conformational effect of the metal ions is evidently different. Cu^{2+} has an apparent promoting effect on enhancing the α-helix conformation of **1** with a high relative helicity change ($\Delta[\theta]_{222} = 71.4\%$) in pH 7. And there is a definite increase of α-helix conformation of **1** in the presence of Cd^{2+}, Co^{2+}, La^{3+}, Mn^{2+},

Ni^{2+}, or Zn^{2+}. On the other hand, Al^{3+}, Ba^{2+}, or Cs^{+} has no apparent effect on the conformation of **1**. Second, the interaction between metal ions and **1** was also monitored with Trp (**W**) fluorescence spectra in the absence and presence of various metal ions. Figure 2 indicated that the addition of Cu^{2+} resulted in a strong quenching of fluorescence intensity of Trp (**W**) residue at 350 nm among the various metal ions used in this paper. Thus, it is released that there is a high selectivity of the binding site for Cu^{2+} in **1**, which results in an apparent enhancement of α-helix conformation. And the high selectivity of the binding site for Cu^{2+} in **1** may respond to the interaction between Trp (**W**) residue and Cu^{2+}. Figure 2 also indicated that there is a certain interaction between La^{3+} and the Trp (**W**) residue of **1** which result in an increase of helicity of **1** with the addition of La^{3+} shown in Figure 1. But it is not stronger than that between Cu^{2+} and the Trp (**W**) residue of **1**.

High selectivity of the binding site for Cu^{2+} in the peptides

The selectivity of metal ions in peptides has an expected consequence of site-specific metal-ligand interaction, which generally depends on the affinity of metal ion toward the ligands employed, the compatibility of metal ion geometry, and the coordination sphere of the ligands with a certain conformation (Ghadiri & Choi 1990). To study high selectivity of the binding site for Cu^{2+} in peptides under different coordination environments, this paper examined the CD spectra of a series of short alanine-based peptides with single Trp (**W**) residue or Trp-His (**W**-**H**) pair in the absence and presence of Cu^{2+}, respectively.

Figure 3 shows CD spectra of **1**–**7** in the absence and presence of Cu^{2+}. Table 2 lists the corresponding relative helicity changes, $\Delta[\theta]_{222}$, of **1**–**7** upon the addition of Cu^{2+}, calculated from the spectra in Figure 3. As shown in Figure 3a, the characteristic α-helix spectra of **1** were observed with two minimum peaks at 208 and 222 nm in both absence and presence of Cu^{2+}. The addition of Cu^{2+} resulted in an apparent effect on enhancing the α-helix conformation of **1** with a high relative helicity change ($\Delta[\theta]_{222} = 71.4\%$) in pH 7.0. As reported in our previous work (Zou & Sugimoto 2000), $(i, i + 2)$ Trp-His (**W**-**H**) interaction at C terminus of short alanined-based peptide acted as a β-sheet conformational switch to form a typical β-sheet conformation of **2** as shown in Figure 3b. And an evident concentration dependence of CD spectra was only observed in **2** in the absence of Cu^{2+}. But the formation of amyloid fibril was not observed with ThT fluorescence assay. In this work, an evident conformational change of **2** was also observed with the high relative helicity change ($\Delta[\theta]_{222} = 138\%$) in Table 2 and Figure 3b upon the addition of Cu^{2+}. The concentration dependence of CD spectra of **2** in the presence of Cu^{2+} was also examined and a comparison between the absence and presence of Cu^{2+} was performed in this work. As shown in Figure 4, the concentration dependence of CD spectra in **2** was apparently decreased in the presence of Cu^{2+} comparing that in the absence of Cu^{2+}. The effect of Cu^{2+} on the conformation of **2** indicated that the presence of Cu^{2+} is also useful to the formation of α-helix conformation even in the peptide in which there is a potential to form β-sheet conformation in the absence of Cu^{2+}.

The strong effect of Cu^{2+} on inducing and enhancing the formation of α-helix conformation of short alanined-based peptides was also obtained in **3** ($\Delta[\theta]_{222} = 122\%$) and **4** ($\Delta[\theta]_{222} = 57.8\%$) with Table 2, and Figures 3c and 3d, respectively. On the contrast, CD spectra and relative helicity changes evidently indicated that there is not a strong effect of Cu^{2+} on inducing and enhancing the formation of α-helix conformation in **5** and **6** shown in Table 2, and Figures 3e and 3f. As shown in Table 1, there is a Trp (**W**) residue at N terminus of **5** and a pair of Trp-His (**W**-**H**) residues at N terminus of **6**, respectively. The effect of Cu^{2+} was not evidently observed in **7** from Figure 3g and its relative helicity change in Table 2 also.

*Comparison of FTIR of **2** in the absence and presence of Cu^{2+}*

There are some amide mode vibrations, the amide I being the most useful for peptide structural analysis. The amide I absorption band, arising primarily from the C=O stretching vibrations of the carbonyls of peptide backbone, is a particularly good indicator of secondary structural changes because of its marked sensitivity to hydrogen bonding and to structure dependent vibrational coupling (Williams *et al.* 1996). The amide I absorption occurs in the region of 1600–1700 cm^{-1}. In general, the conformation of the main chain backbone of a peptide affects the observed frequency of the amide vibrations because the various motions of the peptide structure (primarily the C=O stretch, the C-N stretch, and the NH wag) couple with one another via dipolar, electronic, and kinematic mechanisms that are

Table 2. Effect of Cu^{2+} on the conformation of various peptides[a]

Sequence	**1**	**2**	**3**	**4**	**5**	**6**	**7**
$[\theta]_1$	−11.2	−4.0	−5.5	−11.6	−13.9	−9.3	−22.6
$[\theta]_2$	−19.2	−9.5	−12.2	−18.3	−15.6	−9.6	−23.3
$\Delta[\theta]_{222}$(%)	71.4	138	122	57.8	12.2	3.23	3.10

[a] $[\theta]_1$ and $[\theta]_2$ are the CD ellipticities (10^3 deg cm^2 $dmol^{-1}$) of peptides at 222 nm in the absence and presence of Cu^{2+}, respectively. The CD ellipticity ($[\theta]$) was measured and the helicity change ($\Delta[\theta]_{222}$) was calculated as described in the text.

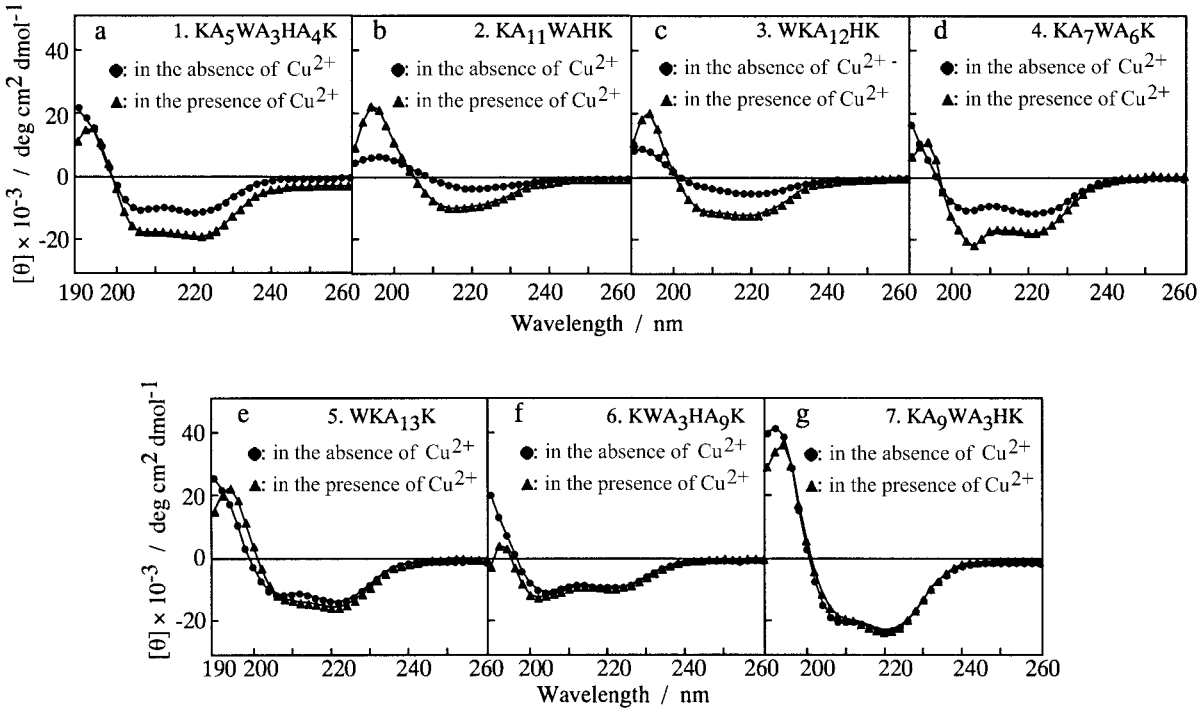

Figure 3. CD spectra of the short alanine-based peptides **1**–**7** in the absence and presence of Cu^{2+}. CD measurement was performed at 50 μM of peptide and pH 7. Other conditions are described in the text.

conformationally dependent. Consequently, the frequency and width of the amide I band is sensitive to secondary structural changes (Bauer *et al.* 1994).

In order to monitor the conformational role of Cu^{2+} in **2**, the FTIR spectra of **2** were measured in the absence and presence of Cu^{2+}, respectively. As shown in Figure 5, there were two amide I components at 1610 cm^{-1} and 1666 cm^{-1} in the absence of Cu^{2+}, respectively. On the contrast, only one amide I component with a frequency of 1643 cm^{-1} was observed in the presence of Cu^{2+}. According to the observed values (Bauer *et al.* 1994) and the calculated values (Miyazawa & Blout 1960) of the frequencies of amide I component in various conformations, the 1610 cm^{-1} component was assigned to a β-sheet conformation and 1666 cm^{-1} component was relative to random coil conformation in the absence of Cu^{2+}. The component of 1643 cm^{-1} was assigned to an α-helix conformation in the presence of Cu^{2+}. The results of FTIR examination in **2** also reveal that Cu^{2+} is useful to induce and enhance the formation of α-helix conformation even in the peptide in which there is a potential to form β-sheet conformation in the absence of Cu^{2+}. The result is corresponding well to the results obtained with CD examination.

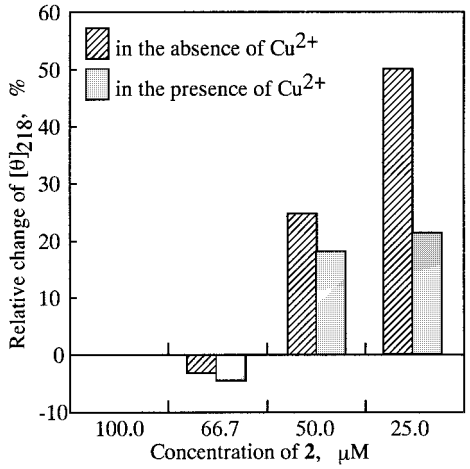

Figure 4. Concentration dependence of the mean residue ellipticity of **2** at 218 nm. The mean residue ellipticity at 100 μM was used as the reference and the relative changes, $\Delta[\theta]$ (%), at different concentrations were calculated as following: $\Delta[\theta](\%) = ([\theta]_2 - [\theta]_1) \times 100/[\theta]_1$, in which $[\theta]_1$ was $[\theta]$ at 100 μM, and $[\theta]_2$ was that at 100 μM, 66.7 μM, 50 μM, and 25 μM, respectively.

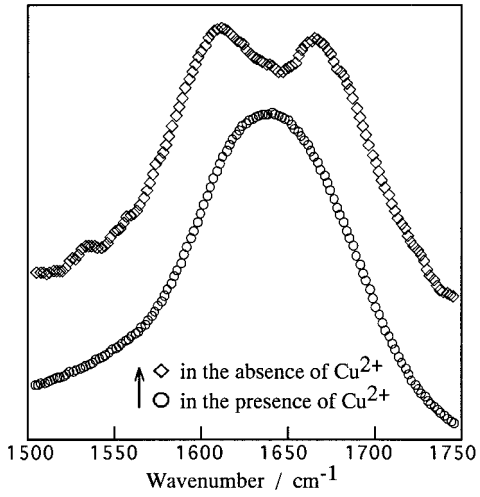

Figure 5. FTIR spectra of **2** in the absence and presence of Cu^{2+}. The measurement was performed at 0.907 mM of **2** and pH 7. The ratio of peptide to Cu^{2+} was 1:5 molar ratio and other conditions are described in the text.

Stability of peptides binding with Cu^{2+}

To evaluate the stability of peptides binding with copper ion, we determined the association constants (K_a) and calculated the free energy change (ΔG_2^0) of **1**–**4** binding with Cu^{2+}, in which evident conformational changes were observed upon the addition of Cu^{2+} as described above. The fluorescence intensities of the peptides were monitored with Cu^{2+} in different molar radios and the association constants (K_a) were estimated with a curve-fitting procedure. As shown in Table 3, the stable **3** binding with Cu^{2+} was obtained with its association constants, ($K_a = 2.44 \pm 0.26 \times 10^5$ M^{-1}, and the free energy change, ($\Delta G_2^0 = -6.8 \pm 0.1$ kcal mol^{-1}). The difference of these values among **1**–**4** is not large. Figure 6 shows that there is a strong quenching of the fluorescence spectroscopy of **3** with Cu^{2+} and the quenching of Trp fluorescence emission reaches a saturation state at higher molar ratio of Cu^{2+} to peptide. It is indicated that peptide binding with Cu^{2+} gives a conformation change of short alanine-based peptides and the influence of Cu^{2+} on the conformation of peptides is concentration dependent. The results of Trp (**W**) fluorescence spectroscopy corresponded well to that of CD spectra about the effect of Cu^{2+} on the conformation of peptides.

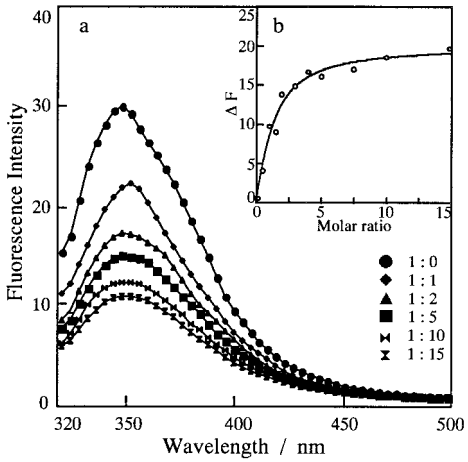

Figure 6. (a) Fluorescence spectra of Trp (**W**) in the absence and presence of Cu^{2+} with different molar ratio. Fluorescence emission intensities are shown in arbitrary units. (b) Relative decrease of Trp (**W**) fluorescence emission intensity at 350 nm in the presence of Cu^{2+} with different molar ratios.

Table 3. Stability of peptide binding with Cu^{2+} at 2 °C[a]

Sequence	K_a (10^4 M^{-1})	ΔG_2^0 (kcal mol^{-1})
1	5.3 ± 0.8	-5.9 ± 0.1
2	10.7 ± 1.3	-6.3 ± 0.1
3	24.4 ± 2.6	-6.8 ± 0.1
4	8.7 ± 2.7	-6.2 ± 0.2

[a] K_a was estimated by the curve fitting procedure of fluorescence intensities with Cu^{2+} in different molar ratios. ΔG_2^0 was calculated as following: ΔG_2^0 = -RTLnKa.

Discussion

During the past two decades a great deal of efforts is devoted to the characterization of the biological role of copper using combined or separate techniques of biology, biochemistry, and coordination chemistry. It is known that copper is an important component of various redox enzymes. The function of copper in biological systems was found primarily in redox reactions often associated with the reduction of oxygen to water or with the transfer of oxygen to a substrate (Sakurai *et al.* 1996; Multhaup *et al.* 1996). Free copper is also a toxic ion, as exemplified by its ability to inactivate proteins through tyrosine nitration, and both deficiency and excess of copper lead to diseases such as Wilson's disease, Menkes' syndrome, and possibly familial amyotrophic lateral sclerosis (FALS) (Waggoner *et al.* 1999). Recently, it was also indicated that Cu^{2+} has a high selectivity to bind an octapeptide repeat in N terminal segment of PrP that affects the conformation of PrP (Miura *et al.* 1996, 1999; Stöckel *et al.* 1998). The different effects of Cu^{2+} and Zn^{2+} on the aggregation of amyloid β-peptide were also demonstrated and some possible mechanisms were reported by some researchers (Atwood *et al.* 1998; Liu *et al.* 1999; Miura *et al.* 2000). It was observed in our previous study that there is a conformational effect of Cu^{2+} on the short alanine-based peptides with single Trp-His interaction in different environments (Zou & Sugimoto 1999). We have examined here the effect of some metal ions including Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cs^{+}, Cu^{2+}, La^{3+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} on the conformations of short alanine-based peptides with single Trp-His interactions in different environments. Our results have indicated that the conformational role of Cu^{2+} is responsible to its interaction with peptides, which is dependent on the geometrical spacings and positions of Trp/His (**W**/**H**) pair.

Roles of Cu^{2+} in the conformation of peptides

We studied the binding of peptide with Cu^{2+} and its effect on the peptides with different conformations. The relative helicity changes of **1** with the addition of Cu^{2+} indicated an apparent effect of Cu^{2+} on enhancing the formation of α-helix conformation of **1** with $(i, i + 4)$ Trp-His (**W**-**H**) pair in the middle of peptide. And a high selectivity of the binding site was observed with the association constants (K_a) and free energy change (ΔG) determined by the quenching of Cu^{2+} in the Trp (**W**) fluorescence spectroscopy with different molar ratios. Similar significant difference in conformation and Trp (**W**) fluorescence emission intensity was not obtained with the addition of other metal ions including Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cs^{+}, La^{3+}, Mn^{2+}, Ni^{2+}, or Zn^{2+} although a certain interaction between La^{3+} and the Trp (**W**) residue of **1** was observed.

The role of Cu^{2+} in the conformation of peptides was also reported by other researchers, recently. For example, the enhancing α-helix conformation of Cu^{2+} was discovered in an alanine-based peptide with an $(i, i + 4)$ His-His (**H**-**H**) pair at the C terminus and the formation of the complex between Cu^{2+} and His-His (**H**-**H**) pair is responsible to the increase of helicity (Ghadiri & Choi 1990). But the effect of Cu^{2+} on the conformation of peptides was not reported with a pair His-His (**H**-**H**) in different spacings and positions as described above. The effect of Cu^{2+} on enhancing α-helix conformation was also observed with the interaction between Cu^{2+} and PHGGGWGQ, which appears in the N terminal segment of PrP (Miura *et al.* 1996). The peptide does not assume any regular structure without Cu^{2+}, whereas Cu^{2+} binding to the HGGG segment induces the formation of α-helical structure on the C terminal side of the peptide chain. But the effect of Cu^{2+} of inducing and enhancing the formation of α-helix conformation was not reported in the peptide in which there is a potential to form β-sheet conformation in the absence of Cu^{2+} as **2** described in this work.

We observed the high selectivity of the binding site for Cu^{2+} in different coordination environment of peptides and its effect on peptide conformations. Our results demonstrated that the presence of Cu^{2+} resulted in not only an apparent effect on inducing and enhancing the formation of α-helix conformation in **4** with a Trp (**W**) residue in the middle of peptide as observed in **1**, but also in **2**, in which there is a potential to form β-sheet conformation in the absence of Cu^{2+}. Because of the presence of $(i, i + 2)$ Trp-His (**W**-**H**) pair at the C terminus of peptide as a conformational switch of β-sheet conformation, **2** has a typical β-sheet conformation and its CD spectra is evidently concentration dependence in the absence of Cu^{2+}. The relative helicity change, $\Delta[\theta]_{222}$, was increased and concentration dependence of its CD spectra was decreased upon the addition of Cu^{2+} evidently. The results of FTIR examination in **2** also reveal that Cu^{2+} is useful to induce and enhance the formation of α-helix conformation even in the peptide in which there is a potential to form β-sheet conformation in the ab-

sence of Cu^{2+}. The similar conformational effect of Cu^{2+} was also observed in **3**.

On the contrast, the strong effect of Cu^{2+} on inducing and enhancing α-helix of short alanine-based peptides was not obtained in **5** and **6**, where there is a Trp (**W**) residue or a Trp-His (**W**-**H**) pair at the N termini of the peptides. The positive pole of macrodipole in α-helix (Armstrong & Baldwin 1993; Huyghues-Despointes & Baldwin 1997) is a possible factor which is generally near the N terminal of peptides and unfavourable to the interaction between Cu^{2+} and the ligand (Trp (**W**) residue or Trp-His (**W**-**H**) pair). The similar effect of Cu^{2+} was not also observed in **7** because there is an $(i, i+4)$ Trp-His (**W**-**H**) pair at the C terminus as a conformational switch of α-helix conformation which resulted in a strong α-helix conformation of **7** even in the absence of Cu^{2+} as shown in Figure 3g. Therefore, it was demonstrated that Cu^{2+} had an apparent effect on inducing and enhancing α-helix conformation of short alanine-based peptides with single Trp-His (**W**-**H**) interaction in different geometrical spacings and positions. And the effects of Cu^{2+} are dependent on the special coordination environment, that is the geometrical spacing and position of Trp-His pair (**W**-**H**).

Complexation of peptide with Cu^{2+}

As described above, the conformational effects of Cu^{2+} are dependent on the special coordination environment, that is the geometrical spacing and position of Trp/His (**W**/**H**) pair. Both His (**H**) and Trp (**W**-**H**) residues are responsible to the effect because the imidazole ring of His (**H**) and the indole ring of Trp (**W**) are good chelating ligand to Cu^{2+}. It is known that Cu^{2+} can coordinate to nitrogen, oxygen, or sulfur ligands in peptides and proteins to form some Cu^{2+} complexes of either square-planar or square-planar with weak axial ligands such as the coordination geometries of [3N, 1O] (three-nitrogen and one-oxygen) (Miura *et al.* 1999), [4N] (Valko *et al.* 1999), or [2N, 2O] (Wang *et al.* 1998). Recently, the studies indicate that PrP (prion protein) binds selectively to Cu^{2+} because of the complexation of Cu^{2+} with the imidazole ring of His, amide, and carbonyl groups of backbone in its conformationally flexible N terminal region (Stöckel *et al.* 1998; Miura *et al.* 1999).

Figure 7 shows a possible molecular model of the complexation of Cu^{2+} with $(i, i+4)$ Trp-His (**W**-**H**) in α-helix conformation of **1** according to the results described above. The molecular model was constructed

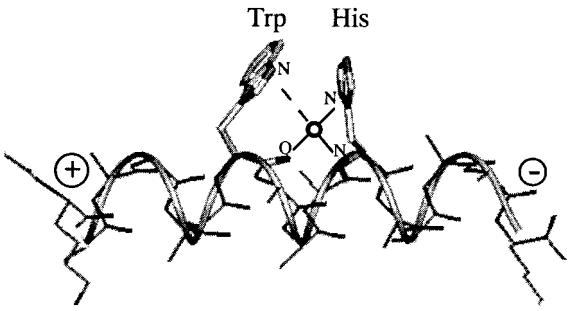

Figure 7. Molecular model of the complexation of **1** with Cu^{2+} in α-helix conformation. The positive and negative poles of macrodipole in α-helix conformation were also shown in this figure. The model was constructed with QUANTA 97.1/CHARMm 23.2 in an INDIGO 2 Silicon Graphics computer.

with QUANTA 97.1/CHARMm 23.2 on an INDIGO 2 Silicon Graphics computer. In the model, Nπ of imidazole ring of His(**H**)-11, N of deprotonated amide of His(**H**)-11, and O of carbonyl of Trp(**W**)-7 occupy three of the four tetragonal ligand positions of the Cu^{2+} complex. The forth ligand position may be N of imidazole ring of Trp(**W**)-7 to form the coordination geometry of [3N, 1O], or O of water to form coordination geometry of [2N, 2O]. It is evident that the complexation of Cu^{2+} with **1** is useful to induce and enhance the α-helix conformation of **1** as described above. It is also possible for the formation of similar complexation of Cu^{2+} with **2** or **3** in the coordination geometry of [2N, 2O] at the C terminus. As we showed recently, the interaction at C terminal is important to the conformation of short alanine-based peptides (Zou & Sugimoto 2000). Therefore, the complexation of peptide with Cu^{2+}as shown in Figure 7 is responsible to inducing and enhancing the formation of α-helix conformation.

Recently, the complexation of Cu^{2+} was also indicated to explain the role of some metal ions such as Cu^{2+} and Zn^{2+} in several neurodegenerative diseases including Alzheimer's disease (AD) and prion disease. The results of Raman spectra demonstrate the formation of complexation of amyloid β-peptide (1–40) with Cu^{2+} in the coordination geometry of [4N] in neutral and basic pHs (Miura *et al.* 2000). The chelation of Cu^{2+} is useful to stabilize the soluble form of amyloid β-peptide (1–40). Our results of both ThT fluorescence assay and atomic force microscope (AFM) also confirm the inhibitor role of Cu^{2+} in the aggregation of amyloid β-peptide (1–42) *in vitro*, which has a strong propensity to form pathogenic aggregates in brain (to be described in another paper).

Conclusions

Circular dichroism (CD), Trp (**W**) fluorescence emission, and Fourier transform infrared (FTIR) spectroscopy indicated that there is a conformational role of Cu^{2+} in inducing and enhancing the formation of α-helix conformation in short alanine-based peptides with single Trp/His (**W**/**H**) interaction in different environments. The complexation of Cu^{2+} with peptides is responsible to the conformational effect because the chelation is able to stabilize peptide with an α-helix conformation. The possible factors affecting the role of Cu^{2+} are discussed in the paper. The results in this paper are useful to understand the important structural role of Cu^{2+} in protein folding and the possible mechanism in some neurodegenerative diseases such as Alzheimer's disease.

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