Pressure Effect on the Conformational Equilibrium of [Leu]$^5$-Enkephalin in Water

A Shimizu$^{1*}$, T Takekiyo$^{2*}$, M Kato$^3$, Y Taniguchi$^3$, and Y Yoshimura$^2$

$^1$ Department of Environmental Engineering for Symbiosis, Soka University, 1-326 Tangi-cho, Hachioji, Tokyo, 192-8577, Japan
$^2$ Department of Applied Chemistry, National Defence Academy, 1-10-20 Hashirimizu, Yokosuka, Kanagawa, 239-8686, Japan
$^3$ Department of Applied Chemistry, Ritsumeikan University, 1-1-1, Nojihigashi, Kusatsu, Shiga, 525-8577, Japan

E-mail: $^{1*}$ shimizu@soka.ac.jp, $^{2*}$ take214@nda.ac.jp

Abstract. The conformational stability of [Leu]$^5$-enkephalin, Tyr-Gly-Gly-Phe-Leu, in water have been investigated under high pressure by FTIR spectroscopy. Three peaks at 1638, 1650, and 1680 cm$^{-1}$ were determined by second derivative FTIR spectra in the amide I' region of [Leu]$^5$-enkephalin. The peaks at 1637 and 1680 cm$^{-1}$ are assigned to the $\beta$-strand and turn structures, respectively. These peaks mean that [Leu]$^5$-enkephalin takes a $\beta$-hairpin-like structure in water. Moreover, the absorbance at 1638 cm$^{-1}$ increases with increasing pressure, and this change shows a sigmoidal curve. Thus, we concluded that [Leu]$^5$-enkephalin has the $\beta$-hairpin-like and disordered structures in water. From the FTIR profile at high pressures, the $\beta$-hairpin-like structure of [Leu]$^5$-enkephalin is stabilized by a high pressures. Our result shows that the folded structures such as $\alpha$-helix and $\beta$-hairpin structures of short peptide such as [Leu]$^5$-enkephalin are stabilized at high pressures.

1. Introduction
Monomeric $\beta$-structure peptides, which are $\beta$-turn and $\beta$-hairpin, provide a good model system of anti-parallel $\beta$-sheet structure, in that these consist of two $\beta$-strands linked by a short loop [1,2]. Recently, many groups have shown that short peptides can fold to a $\beta$-structure in aqueous solution. Thermodynamic study of short $\beta$-structure peptides can provide basic information for understanding protein unfolding [1-4]. Although there have been investigated the thermal structural stability of $\beta$-structure peptides in water [1-4], no studies on the structural stability under high pressure was reported.

According to our previous high-pressure FTIR study of helical peptide (AK16:YGAAKAAA KAAAACKAA-NH$_2$) [5, 6], the population of $\alpha$-helical structure of AK16 increases with increasing pressure, and the $\alpha$-helical structure was stabilized at high pressures. It would be interesting to investigate whether the $\beta$-structure of short peptides is stabilized at high pressures or not.

Here, we focused on the conformational stability under high pressure of [Leu]$^5$-enkephalin, which has the sequence of YGGFL, in water. Previous Raman [7], MD theoretical [8], and FTIR [9] results suggested that [Leu]$^5$-enkephalin takes the $\beta$-turn and extended structures in water, however the structure of [Leu]$^5$-enkephalin in water is still unclear.
In this paper, we have investigated the conformational equilibrium of [Leu]$^5$-enkephalin in water by high-pressure FTIR spectroscopy. Our result shows that [Leu]$^5$-enkephalin takes the β-hairpin-like and disordered structures in water, but the population of β-hairpin-like structure is low at room temperature. Besides, the β-hairpin-like structure of [Leu]$^5$-enkephalin is stabilized by a high pressure.

2. Methods

[Leu]$^5$-Enkephalin (Purity: 98%) was purchased from Peptide Institute Inc. and used without further purification. Deuterium oxide (D$_2$O) (Purity: 99.9%, CEA (France)) was used without further purification. The concentration of the sample was 1.7 weight %. The solute-solute interaction is negligible under this condition [9].

The details of FTIR spectral measurement were described in the previous papers [5, 9]. For the pressure experiments, the sample solutions were placed together with a small amount of powdered α-quartz in a 1.0 mm diameter hole of a 0.05 mm thick Hasteloy C-276 gasket mounted on a diamond anvil cell (DAC). The α-quartz was used as a pressure calibrant [10].

3. Results and Discussion

The amide I’ vibration in FTIR spectra, primarily C=O stretching mode of the amide linkage, appears in the region from 1620 to 1690 cm$^{-1}$. The frequency position and the intensity pattern are characteristic of the dominant secondary structure of the peptide. Therefore, this mode has provided a monitor sensing the formation of β-sheet structures of peptides and proteins [11].

Figure 1 shows the FTIR spectra in the amide I’ region of [Leu]$^5$-enkephalin in water at 0 and 80 ºC. Three peaks at 1637, 1650, and 1680 cm$^{-1}$ in the amide I’ region at 0 ºC were determined by the second derivative spectra. According to our recent study [12], the peaks around 1635 and 1680 cm$^{-1}$ of β-hairpin peptide such as Trpzip1 peptide (SWTWEGNKWTWK) are assigned to the β-strand and turn structures, respectively. The absorbance ($A_{1635 \text{ cm}^{-1}}$) around 1635 cm$^{-1}$ depends on the number of the intramolecular hydrogen bond (intra. H-bond) in the β-strand structure of Trpzip1 peptide. The $A_{1637 \text{ cm}^{-1}}$ of Trpzip1 peptide decreases with increasing temperature due to the disruption of the intra. H-bond [12]. A remarkable point is that the peaks at 1637 and 1680 cm$^{-1}$ of [Leu]$^5$-enkephalin are similar to those around 1635 and 1680 cm$^{-1}$ of Trpzip1 peptide. Besides, the $A_{1637 \text{ cm}^{-1}}$ of [Leu]$^5$-enkephalin decreases on going from 0 °C to 80 °C. This behavior is consistent with that of Trpzip1 peptide. Thus, the decrease of $A_{1637 \text{ cm}^{-1}}$ of [Leu]$^5$-enkephalin shows that the increase of temperature causes the disruption of the intra. H-bond (β-strand) structure of [Leu]$^5$-enkephalin. However, the value of $A_{1637 \text{ cm}^{-1}}$ of [Leu]$^5$-enkephalin is smaller than that of $A_{1635 \text{ cm}^{-1}}$ of Trpzip1 peptide. This is probably due to the difference in the intra. H-bond numbers between Trpzip1 peptide and [Leu]$^5$-enkephalin.

Figure 1. FTIR and the 2nd-derivative spectra of [Leu]$^5$-enkephalin in water at 0 and 80 °C. The solid and dashed lines represent the FTIR spectra at 0 and 80 °C, respectively.
The maximum hydrogen bond number of Trpzip1 peptide is five [12] whereas that of [Leu] 5-enkephalin is two [9]. On the basis of above results, two peaks at 1637 and 1680 cm\(^{-1}\) of [Leu] 5-enkephalin correspond to the \(\beta\)-strand and turn structures, which show the \(\beta\)-hairpin-like structure. The peak at 1650 cm\(^{-1}\) corresponds to the disordered (unfolded) structure. Accordingly, we suggest that [Leu] 5-enkephalin has the \(\beta\)-hairpin-like and disordered structures in water. The peak absorbances at around 1637 and 1680 cm\(^{-1}\) of [Leu] 5-enkephalin at 0 \(\degree\)C are lower than those of Trpzip1 [12]. Hence, it is difficult to detect the \(\beta\)-hairpin-like structure of [Leu] 5-enkephalin in water using NMR method [13, 14].

To discuss more about the conformational equilibrium, we measured pressure dependence of FTIR spectra of [Leu] 5-enkephalin. If [Leu] 5-enkephalin in water has the conformational equilibrium, pressure shifts the equilibrium to the conformation with a smaller partial molar volume (PMV) [6]. Otherwise, the value of absorbance should be a monotonous change meaning that [Leu] 5-enkephalin in water takes a single conformation. Figure 2 (a) shows the FTIR spectra in the amide I’ region of [Leu] 5-enkephalin in water as a function of pressure. The absorbances at 1637 and 1680 cm\(^{-1}\) increase with increasing pressure. The pressure dependence of the absorbance \((A_{1637\,cm^{-1}})\) at 1637 cm\(^{-1}\) of [Leu] 5-enkephalin was shown in Fig. 2(b). A remarkable result in Fig. 2(b) is that the pressure dependence of the change of \(A_{1637\,cm^{-1}}\) is not monotonous, and shows a sigmoidal curve showing the existence of above two species. Therefore, we conclude that [Leu] 5-enkephalin has the \(\beta\)-hairpin-like and disordered structures in water.

Next, we discuss the pressure stability for the secondary and local structures of [Leu] 5-enkephalin, as this is important for understanding the structural stability of peptides. As seen in Fig.2, the \(\beta\)-hairpin-like structure of [Leu] 5-enkephalin does not unfold even at 1.3 GPa. As mentioned in the Introduction, the \(\alpha\)-helical structures of AK16 did not unfold at high pressures. Generally, short- and oligo-peptides is fully hydrated with solvent water molecules [6]. The high stability of folded structures such as \(\alpha\)-helical and \(\beta\)-hairpin structures of short- and oligo-peptides under high pressure may be caused by the hydration effect with water molecules.

On the other hand, the frequency shifts at 1617 and 1517 cm\(^{-1}\) resulting from the side-chain vibration of Tyr residues were monitored to investigate the stability of local structures of [Leu] 5-enkephalin under high pressure. The frequency shifts of 1515 and 1617 cm\(^{-1}\) \((\nu_{\text{Tyr}})\) of proteins allow one to monitor the protein unfolding at this specific site (local structure) [15].

Figure 3 shows the pressure dependence of the frequency shifts of 1617 and 1515 cm\(^{-1}\) \((\nu_{\text{Tyr}})\) of [Leu] 5-enkephalin. Generally, the \(\nu_{\text{Tyr}}\) of proteins shifts to higher frequency as the pressure increases, and this indicates the exposure of Tyr residue to the solvent water [15]. The \(\nu_{\text{Tyr}}\) frequencies of [Leu] 5-enkephalin also shift to higher frequency with increasing pressure as in the case of proteins [16]. An interesting result is that both \(\nu_{\text{Tyr}}\) frequency shifts are not monotonous against the pressure, and show sigmoidal curves. This result means that Tyr residue of [Leu] 5-enkephalin forms a specific interaction such as a hydrophobic interaction with other residues, and the pressure disrupts this specific...
interaction. Rudolph-Böhner et al.[17] suggested that the proximity of the aromatic rings of Tyr and Phe residues of [Leu]$^5$-enkephalin indicates a folded structure. Thus, we can suggest that Tyr residue of [Leu]$^5$-enkephalin may form the specific interaction with Phe residue at room temperature and normal pressure. Further study such as a substitution effect from Phe residue to other aromatic residues in [Leu]$^5$-enkephalin will help us in understanding more details of the specific interaction of Tyr residue. We found that the population of β-hairpin-like structure (secondary structure) of [Leu]$^5$-enkephalin increases as the pressure increases. The pressure causes the disruption of the specific interaction of Tyr residue. This result may resemble the structural character of the intermediate state of peptides and proteins. If [Leu]$^5$-enkephalin forms the intermediate state at high pressures, short peptide such as [Leu]$^5$-enkephalin may be a good model system for understanding the intermediate state in the pressure denaturation process of proteins.

In summary, we have investigated the conformational equilibrium of [Leu]$^5$-enkephalin in water using high-pressure FTIR spectroscopy. From the FTIR spectral analysis of the amide I' region at normal and high pressures, we can conclude that [Leu]$^5$-enkephalin takes the β-hairpin-like structure in water, but this population is low at room temperature. Besides, like in the case of helical structure of oligopeptide, the β-hairpin-like structure of [Leu]$^5$-enkephalin does not unfold at high pressures. Further study of longer peptide such as Trpzip peptides will help us to understand more details of pressure stability of β-structure forming peptides.

References
[1] Chitnumsub P, Fiori W R, Lasuel H A, Diaz H, Kelly J W 1998 Bioog. Med. Biol. 7, 39
[2] Gellman S H 1998 Curr. Opin. Chem. Biol. 2, 717
[3] Nesloncy C L, Kelly J W 1996 Bioog. Med. Biol. 4, 739
[4] Hughes R M, Waters M L 2006 Curr. Opin. Chem. Biol. 16, 514
[5] Takekiyo T, Shimizu A, Kato M, Taniguchi Y 2005 Biochim. Biophys. Acta 1750, 1
[6] Takekiyo T, Imai T, Kato M, Taniguchi Y 2006 Biochim. Biophys. Acta 1764, 355
[7] Han S L, Stimson E R, Maxfield F R, Scherage H A 1980 Int. J. Pept. Protein Res. 16, 173
[8] Aburi M, Smith P E 2002 Biopolymers 64, 177
[9] Takekiyo T, Kato M, Taniguchi Y 2005 J. Mol. Liquids 119, 147
[10] Wong P T T, Moffat D 1989 J. Appl. Spectrosc. 43, 1279
[11] Bandeker J 1992 Biochim. Biophys. Acta 1120, 123
[12] Takekiyo T, Wu L, Yoshimura Y, Shimizu A, Keiderling T A 2009 Biochemistry 48, 1543
[13] Zetta L, Cabbasi F 1982 Eur. J. Biochem. 122, 215
[14] Gupta G, Sarma M H, Sarma R H, Dinhgra M M 1986 FEBS Lett. 198, 245
[15] Smeller L, Meesman F, Heremans K 2006 Biochim. Biophys. Acta 1764, 497
[16] Yu S, Venyaminov N, Kalnin N 1990 Biopolymers 30, 1243
[17] Rudolph-Böhner S, Quarzago D, Czisch M, Ragnarsson U, Moroder L 1998 Biopolymers 41, 591