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Pressure Stability of the α-Helix Structure in a De Novo Designed Protein $(\alpha\text{-l-}\alpha)_2$ Studied by FTIR Spectroscopy

Takahiro Takekiyo,[1*] Naohiro Takeda,[1] Yasuhiro Isogai,[2] Minoru Kato,[1] Yoshihiro Taniguchi[1]

[1] *Department of Applied Chemistry, Ritsumeikan University 1-1-1, Noji-higashi, Kusatsu, Shiga 525-8577, Japan*

[2] *RIKEN Spring-8 Center, Harima Institute, 1-1-1, Koto, Sayo Hyogo 679-5148, Japan*

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ABSTRACT:

The pressure-induced structural changes of a de novo designed four-helix bundle protein, $(\alpha\text{-l-}\alpha)_2$, in aqueous solution have been investigated by FTIR spectroscopy. Changes in the amide I' band intensity show that pressure induces disruption of tertiary interactions and stabilizes the solvated α-helical form. This may suggest that the exposure of the hydrophobic core to the solvent by pressure is not a sufficient condition for pressure-induced unfolding of the α-helices of proteins. © *2006 Wiley Periodicals, Inc. Biopolymers 85:185–188, 2007.*

Keywords: helix bundle protein; pressure effect; α-helix structure; FTIR spectroscopy

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INTRODUCTION

The stability of de novo-designed proteins has recently emerged as an attractive research subject for studying the structural stability, function of proteins, and exploring the amyloid formation.[1] Recently, Kammerer et al.[2] designed a de novo helix bundle-type protein having a simplified sequence, which transforms into the amyloid fibrils at an elevated temperature. Thus, this approach critically tests our understanding of the principles of protein folding and amyloid formation.[1,2] Especially, α-helix is one of the most basic structures in proteins, and three- and four-helix bundle proteins have been studied as a common native folding protein motif.

Generally, it has been reported that the α-helices of helix-rich proteins such as myoglobin[3] and α-lactalbumin[4] collapse with increasing pressures. Recently, we reported FTIR study of the pressure effect on the helix-coil equilibrium of Ala-rich peptide (AK16: YGAAKAAAAKAAAAKA-NH_2), which does not have the tertiary structure (e.g., hydrophobic interaction, S—S bond, etc.).[5,6] The α-helix conformer of AK16 was stabilized under high pressure, and this suggested that the presence of the tertiary structure influences on the difference in the pressure stability between AK16 peptide and helical proteins.

Here, we focus on the pressure effect on the structural stability of a de novo designed four-helix bundle protein, $(\alpha\text{-l-}\alpha)_2$, which has the α-helix structure and forms the tertiary structure. The topology of $(\alpha\text{-l-}\alpha)_2$, whose monomer is designed to be helix-loop-helix, is constructed by dimerization of a 63-residue polypeptide, and held mainly by the hydrophobic interactions between hydrophobic side chains at the interhelical packing interfaces.[7] Figure 1 shows the amino acid sequence of $(\alpha\text{-l-}\alpha)_2$.

In this study, we measured the pressure-induced structural changes of $(\alpha\text{-l-}\alpha)_2$ in aqueous solution by FTIR spectroscopy. We discuss the contribution of the tertiary structure to the stability of the α-helix structure under high pressure.

MATERIALS AND METHODS

The artificial gene encoding the designed protein with optimal codons of *Escherichia coli* was synthesized and cloned into a pRSET-C vector (Invitrogen). The artificial gene in the vector was transformed into a *E. coli* strain BL21 (DE3) and was expressed under

Correspondence to: Yoshihiro Taniguchi; e-mail: taniguti@se.ritsumei.ac.jp
*Present address: Department of Applied Chemistry, National Defense Academy, Yokosuka, Kanagawa 239-8686, Japan.

FIGURE 1 Amino acid sequence of four-helix bundle protein, $(\alpha\text{-l-}\alpha)_2$.

the control of the T7 promoter using isopropyl 1-thio-β-D-galactopyranoside (IPTG). The protein was obtained from a soluble fraction of lysed cells and was purified using a reverse-phase HPLC. Additionally, $(\alpha\text{-l-}\alpha)_2$ was dialyzed overnight against 10 m*M* Tris-HCl buffer (pH 7.6), and then lyophilized. The sample solution was prepared by dissolving $(\alpha\text{-l-}\alpha)_2$ in 50 m*M* Tris-DCl buffer (pD7.6) with 100 m*M* NaCl immediately prior to the FTIR measurements. The protein concentration was 25 mg/ml. All exchangeable backbone amide protons in $(\alpha\text{-l-}\alpha)_2$ were completely deuterated within 10 min after dissolving it in D_2O buffer at room temperature.

The FTIR spectra were measured using a FTIR-680 plus spectrometer (JASCO, Tokyo) equipped with a liquid nitrogen cooling MCT detector. Each spectrum was obtained by coadding 256 scans at a spectral resolution of 2.0 cm^{-1}. The infrared beam was condensed by a zinc selenide lens system onto the sample in the diamond anvil cell. The completion of hydrogen-deuterium exchange was confirmed from no further changes in the amide II band. This amide band around 1550 cm^{-1} shifts to around 1450 cm^{-1} as a result of deuteration of the backbone amide protons. For the pressure-tuning experiments, the sample solution was placed together with a small amount of powdered $BaSO_4$ in a 1.0-mm diameter hole of a 0.1-mm thick SUS-301 gasket mounted on a diamond anvil cell. $BaSO_4$ was used as a pressure calibrant.[8]

RESULTS AND DISCUSSION

The amide I' vibrational mode consists of the C═O stretching, C—N stretching, and C—C—N deformation modes, and appears in the region from 1620 to 1690 cm^{-1}. This mode has been known to be highly sensitive to the secondary structures of polypeptides and proteins so that it has served as an indicator of the α-helix and/or β-sheet structures.[9,10]

Figures 2a and 2b show the original and second derivative FTIR spectra of the amide I' region of $(\alpha\text{-l-}\alpha)_2$ in aqueous solution at 0.1 MPa and 298 K. The second derivative FTIR spectra show four peaks at 1632, 1643, 1652, and 1673 cm^{-1}. Recently, it was reported that the peak at around 1635 cm^{-1} of oligopeptides is typical for the solvent-exposed α-helix structures.[11–15] The solvent-exposed α-helix give peak frequencies to be lower than the characteristic frequencies for the buried α-helix (the peak at around 1650 cm^{-1}).[15–17] Zhu et al.[18] reported that the peak around 1630 cm^{-1} of three-bundle helix protein (α_3D) is assigned to the solvated α-helix. Therefore, we assigned the peaks at 1632 and 1652 cm^{-1} of $(\alpha\text{-l-}\alpha)_2$ to the solvated and buried α-helices, respectively. Moreover, the amide I' bands of the turn structure of polypeptides and proteins have been observed in the region from 1670 to 1680 cm^{-1}.[18,19] The present peak observed at 1673 cm^{-1} corresponds to the above criterion. Generally, the peak

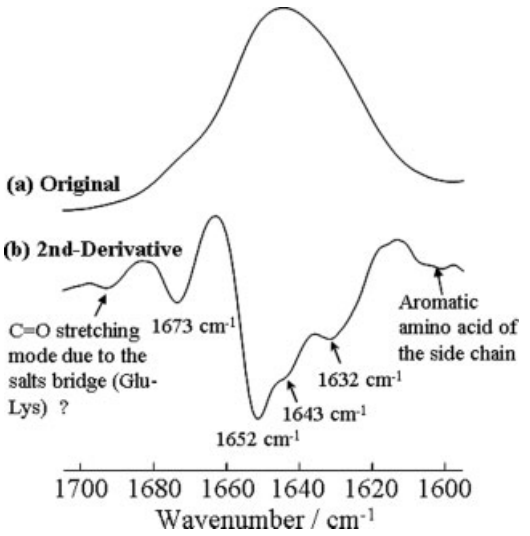

FIGURE 2 Original (a) and second derivative (b) FTIR spectra of the amide I' region of $(\alpha\text{-l-}\alpha)_2$ in aqueous solution at 0.1 MPa and 298 K.

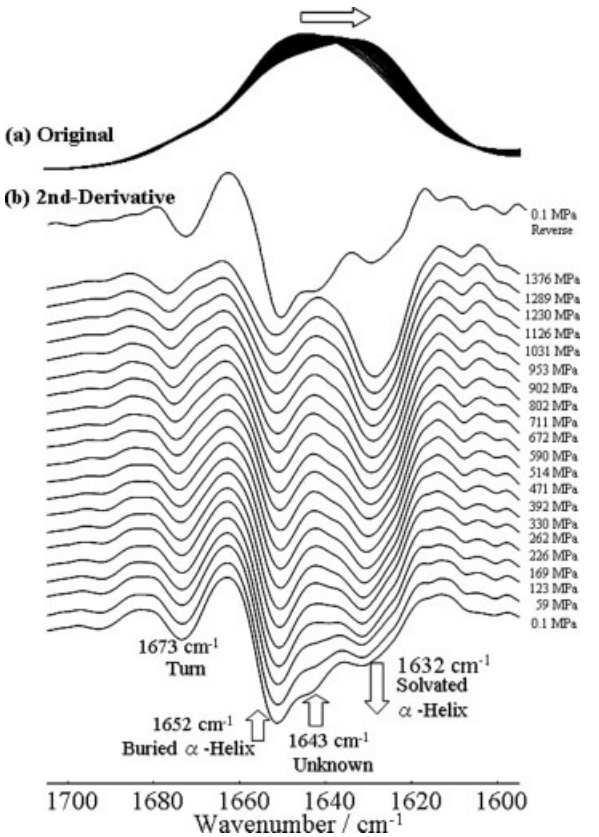

FIGURE 3 Original (a) and second derivative (b) FTIR spectra of the amide I' region of $(\alpha\text{-l-}\alpha)_2$ in aqueous solution as a function of pressure at 298K.

around 1640 cm^{-1} in proteins is assigned to a random structure.[10,19] The peak shape of the random structure would be very broad, but that of the current peak at 1643 cm^{-1} is sharp. Therefore, it would be difficult to assign the peak to the random structure.

The FTIR spectra of the amide I' mode of (α-l-α)$_2$ have been measured up to 1380 MPa. Figures 3a and 3b show the original and second derivative FTIR spectra of the amide I' region of (α-l-α)$_2$ in aqueous solution at various pressures. The band intensity of the solvated α-helix significantly increases with increasing pressure. The band intensity of the buried α-helix slightly decreases with increasing pressure from 0.1 to 330 MPa, and seems to significantly decrease above 330 MPa. The unknown peak at 1643 cm^{-1} disappears at 330 MPa. The band intensity of the turn structure seems to slightly decrease as pressure increases. Recently, Chapeaurouge et al.[20] reported the pressure-induced unfolding of (α_2)$_2$ four-helix bundle protein, which has nearly the same primary structure constructing the hydrophobic core of (α-l-α)$_2$, using the spectral center of mass of Trp fluorescence. They concluded that the pressure of about 350 MPa induces reversible dissociation of dimmer of (α_2)$_2$ to partially folded monomer. This pressure is close to the pressure at which the peak at 1643 cm^{-1} of (α-l-α)$_2$ disappears. It suggests that the behavior of the peak intensity at 1643 cm^{-1} with increasing pressure correlates with the dissociation of (α-l-α)$_2$.

The peak position (1632 cm^{-1}) of the solvated α-helix band shifts to the lower frequency with increasing pressure. As described in the previous section, the C=O stretching mode of the peptide backbone mainly contribute to the amide I' mode. On the other hand, the peak position (1652 cm^{-1}) of the buried α-helix shifts to the higher frequency with increasing pressure. A recent simulation study of a AK peptide by Paschek et al.[21] showed that the pressure-induced increase in strength of solvent hydrogen-bonding to the peptide carbonyls significantly contributes to the lower frequency shift of the amide I whereas the contribution of the internal helical hydrogen-bond is minor. Thus, the lower frequency shift of the solvated α-helix means that the hydrogen bond between the carbonyl oxygen and water become stronger with increasing pressure. Since the buried α-helix is located in the interior of protein, it does not form the intermolecular hydrogen bond between the carbonyl oxygen and water molecules. Therefore, the higher frequency shift means that the intramolecular hydrogen bond between the C=O and N—H in a buried α-helix becomes weaker with increasing pressure.

The decrease in the intensity of the peak at 1643 cm^{-1} suggests that the interhelical surfaces are more exposed to solvent water under high pressure. The exposure of the interhelical surfaces to the solvent is expected to increase the population of the solvated α-helix. Bands of the solvated and buried α-helix structures remain distinct at 1380 MPa. Recently, FTIR study by Desai et al.[17] reported that the α-helices of trp-repressor, which has high α-helix content, are stabilized under high pressure. The present result is similar to that of trp-repressor, and the α-helices of (α-l-α)$_2$ are not unfolded even at 1380 MPa. Hence, these results suggest that there seems to be no positive correlation between the pressure unfolding of the secondary structure and the pressure-induced disruption of tertiary interaction of (α-l-α)$_2$. However, this does not always support that the same conclusion is possible to the case of proteins. It is because the tertiary interactions are not critical for forming the secondary structure of highly stable proteins. Further extensive studies using peptide having the same level of helical propensity are required to be done.

In summary, we investigated the pressure-dependence of the α-helix structures of four-helix bundle protein (α-l-α)$_2$ in aqueous solution using FTIR spectroscopy. From the pressure-dependence of the band intensities of the amide I' mode, it was confirmed that (α-l-α)$_2$ does not unfold even at 1380 MPa. The α-helix of (α-l-α)$_2$ is hydrated more strongly under high pressure. The present study demonstrated that the exposure of the hydrophobic core to the solvent by pressure does not always cause the unfolding of α-helical proteins.

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