Effect of pressure on the α-helix structure of tetrameric coiled-coil peptides

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Abstract. The effect of pressure on the α-helix structure of tetrameric coiled-coil peptides has been investigated using Fourier-transform infrared spectroscopy. To examine the influence of the hydrophobic core on pressure stability of the α-helices, the present study targeted GCN4-pLI and its variants (L9S, L9A, and L9G), which have different cavity in size. The amide II band was used to examine the stability of the hydrophobic core. From monitoring the amide I' band, it was shown commonly for all the peptides that the solvent-inaccessible α-helix decreases with increasing pressure while the solvent-accessible α-helix increases with increasing pressure. It was strongly suggested that the hydration of the helices is a significant factor for the pressure-induced folding. From further detailed analyses of pressure dependence of the amide I' band intensities, it was found that there is a positive correlation between the cavity size and the pressure-induced unfolding of the solvent inaccessible α-helix for the variants.

1. Introduction

It has long been a question how applying pressure leads to folding/unfolding of proteins [1,2]. As an essential step to elucidate this problem, we have investigated model peptides forming α-helix, which is the most common structural motif of proteins. An previous Fourier-transform infrared spectroscopic (FTIR) study [3] on an alanine-rich peptide, AK20 (Ac-AA(AAKAA)₃AAY-NH₂), demonstrated that the α-helix is stabilized by pressure. Recently, we have also found that α-helices of GCN4-p1, which is a prototype of a coiled coil consisting of two amphipathic α-helices (dimer), is also stabilized by pressure [4]. These results are quite different from generally observed pressure denaturation of proteins.

In the present study, to elucidate influence of hydrophobic core on pressure resistivity, we employed GCN4-pLI and its variants (L9S, L9A, and L9G) as target molecules, which are derivatives of GCN4-p1. GCN4-pLI and the variants form tetrameric coiled-coil structure (a helical wheel diagram of the tetrameric coiled-coil is shown in figure 1) and have various sizes of cavities.

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2. Materials and methods
GCN4-pLI and its variants (L9S, L9A, and L9G) (the peptide sequences are shown in table 1), were synthesized on PS3 automated peptide synthesizer (Protein Technologies, Inc.) by the solid-phase method using standard 9-fluorenlymethoxy carbonyl chemistry, purified by reversed-phase high-pressure liquid chromatography (JASCO Corp.), and identified by matrix-assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF-MASS) (Voyager, Applied Biosystems Inc.). The residual trifluoroacetic acid (TFA) from peptide synthesis, which has a strong infrared absorbance at \( \approx 1672 \ \text{cm}^{-1} \) [6], was removed by a lyophilization against 10 mM HCl solution. The lyophilized peptides were dissolved into pure water and the pH was adjusted to neutral by adding an appropriate amount of 0.1 M NaOH. Then, the peptide solutions were dialyzed against pure water to remove the NaCl salt produced in the above process, and lyophilized. GCN4-pLI was dissolved in 2 mM Tris-DCI buffer to be 4 mM and pD 7.6 (The pD values were estimated by adding 0.4 to a value taken from a pH meter). L9A was dissolved in 2 mM Tris-DCI buffer to be 2 mM and pD 5.6. L9S and L9G were dissolved in 50 mM Tris-DCI to be 2 mM and pD 7.4. For FTIR measurements under acidic pD, GCN4-pLI was dissolved in 15 mM DCI solution to be 2 mM and pD 1.8. Then, the sample was completely-deuterated by incubation at about 1 GPa and 85 °C. The peptide concentrations were determined from the ultraviolet absorbance of the tyrosine residue (\( \varepsilon_{274} = 1450 \ \text{M}^{-1} \ \text{cm}^{-1} \)). FTIR spectra were recorded at 25 °C on FTIR 6100 spectrometer (JASCO Corp.) equipped with a MCT liquid nitrogen cooled detector. For pressure-variable measurements, diamond anvil cell with a gasket of 100 \( \mu \text{m} \) thickness was used. Barium sulfate was added to the samples as an internal calibrant [7]. The sample chamber was purged with nitrogen gas for 20 minutes prior to each measurement. 256 interferograms were collected to obtain a spectrum with a resolution of 2 cm\(^{-1}\). Spectral analyses were performed using GRAMS Research™ for System2000 FTIR Version 3.01B (Galactic Industries Corp.).

![Hydrophobic core](image)

**Figure 1.** Helical-wheel diagram of a tetrameric coiled-coil. The seven positions in heptad are denoted as a-b-c-d-e-f-g.

### Table 1. Peptide sequence of GCN4-pLI, its variants and GCN4-pL1.

| Peptide  | sequence |
|----------|----------|
| GCN4-pLI | abcdefg  |
| L9S      | abcdefg  |
| L9A      | abcdefg  |
| L9G      | abcdefg  |
| GCN4-pL1 | abcdefg  |

3. Results and discussion

3.1. Effect of pressure on amide I' and amide II band of GCN4-pLI and its variants
Figure 2 shows pressure dependence of FTIR spectra in the amide II region for GCN4-pLI, L9S, L9A, and L9G at neutral pD. In the figure (a), an amide II peak appears at \( \approx 1550 \ \text{cm}^{-1} \) at 0.1 MPa and 25 °C, indicating that the amide protons in the solvent inaccessible region, e.g. the hydrophobic core, of GCN4-pLI are protected from the exchange by deuterium. Applying pressure results in a decrease in
the amide II intensity, suggesting that the protected amide protons inside the coiled coil is exposed to the solvent. In contrast to GCN4-pLI, none of the variants have an amide II peak around 1550 cm\(^{-1}\) at 0.1 MPa and 25 °C, suggesting that all the variants are loosely packed even at ambient pressure.

Figure 2. Pressure dependence of FTIR spectra in the amide II region of GCN4-pLI (a), L9S (b), L9A (c), and L9G (d) at neutral condition (a: pD 7.6, b: pD 7.4, c: pD 5.6, d: pD 7.4).

Figure 3 shows pressure dependence of FTIR spectra and the second derivative spectra of GCN4-pLI and the variants under neutral pD condition. Peaks observed at ~1630 cm\(^{-1}\) and ~1650 cm\(^{-1}\) are assigned to the solvent accessible \(\alpha\)-helix and the solvent inaccessible \(\alpha\)-helix, respectively [8]. The second derivative spectra of the variants at 0.1 MPa after decompression are similar to the spectra

Figure 3. Pressure dependence of FTIR spectra (bottom) and the second derivative spectra (top) of GCN4-pLI (a), L9S (b), L9A (c), and L9G (d) at neutral condition (a: pD 7.6, b: pD 7.4, c: pD 5.6, d: pD 7.4). Spectra lines are displayed in increments of about 100 MPa for convenience.
at 0.1 MPa, indicating that the structural changes by pressure are almost reversible. The reversibility of GCN4-pLI cannot be confirmed from the present experiment because the H/D exchange of amide protons occurs with increasing pressure and then it affect the spectra.

As described above, the amide protons of GCN4-pLI are not completely deuterated even over 1 GPa under neutral pH condition. For more accurate analyses of the secondary structure of proteins, completely deuterated samples should be used. To examine the effect of pressure on the secondary structure of GCN4-pLI, we measured the FTIR spectra of GCN4-pLI under acidic condition. Figure 4 shows pressure dependence of the FTIR spectra and the second derivative spectra of GCN4-pLI at pH 1.8. The second derivative spectra of GCN4-pLI at 0.1 MPa after decompression are similar to the initial spectra at 0.1 MPa, indicating that the structural change by pressure is reversible. In the following section, we use this data as well to compare the effect of pressure on the secondary structure between GCN4-pLI and variants.

3.2. Comparison of the secondary structure changes of GCN4-pLI and the variants induced by pressure

In this section we compare the pressure effects on the secondary structure of GCN4-pLI and the variants. Figure 5 shows the pressure dependence of the second derivative intensities per 1 mM at 1650 cm\(^{-1}\) (assigned to solvent inaccessible \(\alpha\)-helix) and at 1635 cm\(^{-1}\) (assigned to solvent accessible \(\alpha\)-helix). For all the peptides, the solvent accessible \(\alpha\)-helices and the solvent inaccessible \(\alpha\)-helices increase and decrease, respectively, with increasing pressure. The pressure-induced unfolding of the solvent inaccessible \(\alpha\)-helices is in agreement with the pressure denaturation of proteins. On the other hand, the present pressure-induced folding of the solvent accessible \(\alpha\)-helices is in agreement with the cases of monomeric model peptides, AK20 [3] and AK16 [9], which are the solvent accessible \(\alpha\)-helices. Thus, it is highly possible that the solvation of helices is a key factor for the pressure-induced folding of \(\alpha\)-helices.

For quantitative comparison of the pressure dependence of variants, we estimate the initial slopes of the lines in figure 5 using data over the pressure range from 0.1 MPa to about 250 MPa. We summarized the results as a histogram as shown in figure 6. As to the solvent inaccessible \(\alpha\)-helix, there are significant differences between GCN4-pLI (5.2×10\(^{-7}\) cm\(^2\) mM\(^{-1}\) MPa\(^{-1}\) (neutral condition), 1.2×10\(^{-7}\) cm\(^2\) mM\(^{-1}\) MPa\(^{-1}\) (acidic condition)) and the variants (L9S: 2.0×10\(^{-8}\) cm\(^2\) mM\(^{-1}\) MPa\(^{-1}\), L9A: 2.5×10\(^{-8}\) cm\(^2\) mM\(^{-1}\) MPa\(^{-1}\), L9G: 5.8×10\(^{-8}\) cm\(^2\) mM\(^{-1}\) MPa\(^{-1}\)). Among the variants, there seems to be a positive correlation between the pressure dependence and the cavity volumes: The larger the cavity volume is, the larger the slope grows (the stability against pressure decreases). This correlation is in

![Figure 4. Pressure dependence of the IR absorption spectra (bottom) and the second derivative spectra (top) of GCN4-pLI at pH 1.8 and at every about 200 MPa.](image-url)
agreement with the commonly accepted theory that the cavity is a significant factor for the pressure denaturation (the loss of cavity volume accompanied by denaturation majorly contributes to the negative $\Delta V$ for denaturation). As to the solvent accessible $\alpha$-helix, the values are $-1.4 \times 10^{-7}$ cm$^2$ mM$^{-1}$ MPa$^{-1}$ for GCN4-pLI (neutral condition), $-1.2 \times 10^{-7}$ cm$^2$ mM$^{-1}$ MPa$^{-1}$ for GCN4-pLI (acidic condition), $-2.2 \times 10^{-7}$ cm$^2$ mM$^{-1}$ MPa$^{-1}$ for L9S, $-1.1 \times 10^{-7}$ cm$^2$ mM$^{-1}$ MPa$^{-1}$ for L9A, and $-2.3 \times 10^{-7}$ cm$^2$ mM$^{-1}$ MPa$^{-1}$ for L9G. These values are scattered within twice factor. There seems to be no significant correlation between the pressure dependence and the cavity volumes. This result is reasonable since the stability of the solvent accessible $\alpha$-helix is unrelated to the presence of the cavity.

**Figure 5.** Pressure dependence of the second derivative peak intensities assigned to the solvent accessible $\alpha$-helix [GCN4-pLI (○: pD 7.6, ●: pD 1.8), L9S (◆: pD 7.4), L9A (▲: pD 5.6) and L9G (▲: pD 7.4)] and to the solvent inaccessible $\alpha$-helix [GCN4-pLI (○: pD 7.6, ●: pD 1.8), L9S (◆: pD 7.4), L9A (▲: pD 5.6) and L9G (▲: pD 7.4)]. The solid lines indicated the initial slopes estimated using data over the pressure range from 0.1 MPa to about 250 MPa.

**Figure 6.** Comparison of the pressure dependence of the second derivative intensities assigned to solvent inaccessible $\alpha$-helix (red) and solvent accessible $\alpha$-helix (blue) from 0.1 MPa to about 250 MPa.
4. Conclusion
From the H/D exchange experiments, it is suggested that the coiled coil of GCN4-pLI is more packed compared to the variants possessing larger cavities in their hydrophobic cores. The solvent-inaccessible helices of all the peptide decrease with increasing pressure, while the solvent-accessible helices of those increase with increasing pressure. The pressure-induced unfolding of the solvent inaccessible α-helices is in agreement with the pressure denaturation of proteins. The pressure-induced folding of the solvent accessible α-helices is in agreement with that of monomeric model peptides which are solvated. It is highly possible that the solvation of helices is a significant factor for the pressure-induced folding of α-helices. There is a positive correlation between the cavity volume and the pressure-induced unfolding of the solvent-inaccessible helices of variants.

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