Intramolecular Hydrogen Bonding and Conformation of Small Peptides: Variable-Temperature FTIR Study on *N*-Acetyl-L-Pro-L-Leu-Gly-NH_2 and Related Compounds

Kenji Tonan* and Shun-ichi Ikawa*

Contribution from the Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, Japan

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Abstract: Intramolecular hydrogen bonding and conformation of tripeptide amides in $CDCl_3$ solutions have been examined by variable-temperature FTIR spectroscopy. Absorption in the NH stretching region of *N*-acetyl-L-Pro-L-Leu-Gly-NH_2 was decomposed into some component bands by least-squares fitting. Two broad bands at around 3350 and 3290 cm^{-1} were assigned to intramolecularly hydrogen-bonded NH groups in different conformers. The other relatively sharp bands were assigned to hydrogen-bond-free NHs of the three different amide groups by referring to spectra of analogous amide compounds. From temperature dependence of the band intensities, it is concluded that the C-terminal amide group takes part in the hydrogen bonding in both hydrogen-bonded conformers. From comparison with spectra of *N*-trifluoroacetyl-L-Pro-L-Leu-Gly-NH_2 and L-Pro-L-Leu-Gly-NH_2, the 3290-cm^{-1} band has been assigned to a 13-membered ring with the hydrogen bond between NH of the C-terminal amide group and CO of the N-terminal acetyl group. The 3350-cm^{-1} band, on the other hand, has been assigned to a 10-membered hydrogen-bonded ring between the C-terminal amide NH and a prolyl carbonyl group. A van't Hoff analysis shows that the 13-membered hydrogen-bonded ring is enthalpically more favorable but entropically less favorable than the 10-membered hydrogen-bonded ring.

Introduction

The tertiary structure of a protein molecule is formed along the folding pathway, which seems to be determined by its amino acid sequence and its physical and chemical environment. Through the folding, some residues which are far apart in the sequence are put spatially close to one another and provide a specific site for interaction with ligands or other biomolecules. Thus the protein molecules acquire their unique biological functions. For a long polypeptide chain to fold into a compact and globular structure, formation of secondary structures such as α-helix and β-turn is an essential process. The α-helix shortens the overall length of a polypeptide backbone, while the β-turn reverses its direction. Both the α-helix and β-turn are formed by hydrogen bonding of carbonyl groups to amide NH groups located upward by 3 or 4 residues.[1−4] Through further folding, these secondary structural segments associate with or separate from each other by various interactions, such as hydrogen bonding, hydrophobic and hydrophilic interactions, and solvation effects, and finally form the tertiary structure of proteins.

The folding pathway has been one of the most important subjects of the study of proteins and a wide variety of studies have been performed.[5] Nevertheless, it still remains largely mysterious. In order to reveal a scheme of the folding, it is useful to examine the mechanism of the secondary structure formation for relatively small peptides,[6−8] which can be studied in detail by spectroscopic methods.[9,10] Infrared spectra are particularly useful for detecting and characterizing the hydrogen bonds[11,12] which are related to the α-helix, β-turn, and so on. Recently Gellman and co-workers[13−16] have reported IR and NMR studies on intramolecular hydrogen bonding for series of diamides and depsipeptides in dichloromethane solutions. They have examined various types of hydrogen-bonded rings and discussed their thermodynamic stabilities by use of synthetic compounds with the constitutionally restricted possibility of hydrogen bonding. Winningham and Sogah[17] have measured IR spectra of phenoxathiin derivatives as model compounds of β-turn nucleators and specified the hydrogen-bonding sites. These results provide a useful guide to a study of secondary structure formation of polypeptides. However, measurements of real peptides also will be indispensable for further looking into the secondary structures.

In this study, we have measured the infrared spectra of three kinds of tripeptide amides, *N*-acetyl-L-Pro-L-Leu-Gly-NH_2(Ac-PLG-NH_2), *N*-trifluoroacetyl-L-Pro-L-Leu-Gly-NH_2(Tfa-PLG-NH_2), and L-Pro-L-Leu-Gly-NH_2(H-PLG-NH_2) (Chart 1), and

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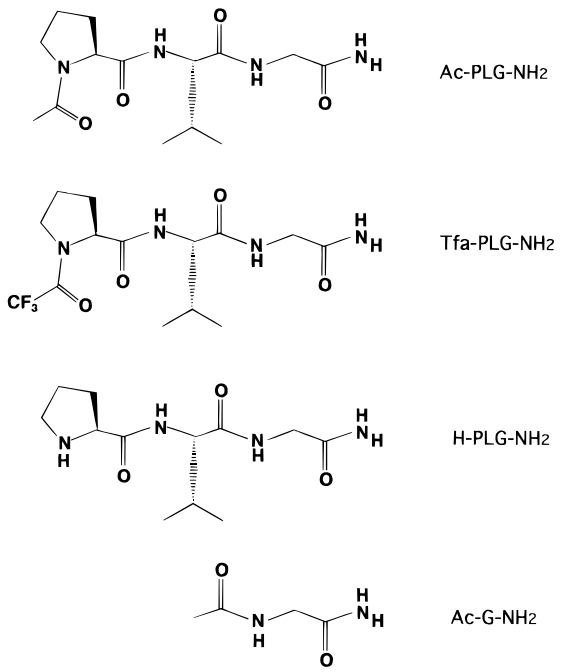
Chart 1



related compounds in chloroform solutions at various temperatures. Two types of intramolecularly hydrogen-bonded conformers with 10- and 13-membered rings, respectively, have been found. In addition, ultraviolet CD spectra were measured and found to be correlated with the infrared bands assigned to the 13-membered ring.

Experimental Section

Reagents. L-Pro-L-Leu-Gly-NH_2(H-PLG-NH_2) was purchased from Sigma Chemical Co. Deuteriochloroform from CEA (France) was used as solvent for IR measurements after being dried over molecular sieves 4A for more than 48 h. All other reagents except those mentioned below were of the highest grade commercially available and were used without further purification.

***N*-Acetyl-L-Pro-L-Leu-Gly-NH_2 (Ac-PLG-NH_2).** Three hundred and fifty milligrams (1.25 mmol) of H-PLG-NH_2 was dissolved in a mixed solvent of 40 mL of *N*,*N*-dimethylformamide, 80 mL of dichloromethane, and 4 mL of triethylamine. To this solution was added a total of 12 mL (125 mmol) of acetic anhydride in 24 portions at room temperature with stirring, which was continued for 1 h. Volatiles were removed with a rotary evaporator. The resulting residue was washed with a total of 40 mL of diethyl ether and the final product obtained was 282 mg (862 μmol, 69% yield). The melting point was measured to be 164−165 °C and purity was confirmed by a single peak of reverse-phase HPLC. Amino acid composition: Pro (1), Leu (1.03), and Gly (1.02). Elemental Anal. Found: C (54.8%), H (7.9%), N (16.9%). Calcd: C (55.2%), H (8.0%), N (17.2%). The FAB low-resolution mass spectrometric result, *m/e* 327, agreed with an expected value for $C_{15}H_{26}N_4O_4$.

***N*-Trifluoroacetyl-L-Pro-L-Leu-Gly-NH_2 (Tfa-PLG-NH_2).** One hundred milligrams (352 μmol) of H-PLG-NH_2 was dissolved in 21 mL of the mixed solvent mentioned above. A total of 0.1 mL (800 μmol) of *S*-ethyl trifluorothioacetate was added in 10 portions to the solution at room temperature with stirring. After the solution was stirred for 30 min, volatiles were removed with an evaporator and the residue was washed with a total of 50 mL of diethyl ether to afford 52 mg (137 μmol, 39%) of the desired compound. Melting point: 174−179 °C. Elemental Anal. Found: C (46.2%), H (6.3%), N (12.8%), F (16.3%). Calcd: C (47.4%), H (6.1%), N (14.7%), F (15.0%). The FAB low-resolution mass spectrometric result, *m/e* = 381, agreed with an expected value for $C_{15}H_{23}N_4O_4F_3$.

***N*-Isopropylacetamide.** Seventeen milliliters (200 mmol) of isopropylamine and 28 mL (200 mmol) of triethylamine were diluted with 200 mL of dichloromethane. To this solution was added a total of 14.2 mL (200 mmol) of acetyl chloride in 70 portions at 0 °C with stirring. After 30 min, dichloromethane was removed by evaporation at room temperature and the resulting slurry was suspended in 100 mL of ethyl acetate. The filtrate was left standing overnight. The supernatant was collected and concentrated by evaporation at 70 °C so that ethyl acetate was completely removed. The residual liquid was finally obtained (14 mL). ^{1}H NMR (90 MHz, in $CDCl_3$): δ 1.14 (doublet, J = 6.6 Hz, 6H), 1.94 (singlet, 3H), 4.05 (multiplet, J = 6.6 Hz, 1H), and 5.66 (broad, 1H). Any impurity signals were negligibly small.

IR Measurements. Infrared spectra were measured with a BOMEM DA3 Fourier-transform spectrometer at 2-cm^{-1} resolution. A variable-temperature cell used was described previously.[18] Windows used were optically polished CaF_2 discs and sample path lengths were fixed at 8 mm. Absorbance of samples was obtained by subtracting the pure solvent spectrum measured at the same temperature.

CD Measurements. CD measurements were carried out with a JASCO J-500A spectropolarimeter. A quarts cell with a 1-mm sample path length was used and its temperature was controlled at 20 °C. Solvents used were 20 mM 2-(*N*-morpholino)propanesulfonate (MOPS) buffer at pH 7.0, trifluoroethanol (TFE), and $CDCl_3$. The spectral results were given as mean residue ellipticity (deg cm^2/dmol) after subtracting a pure solvent spectrum.

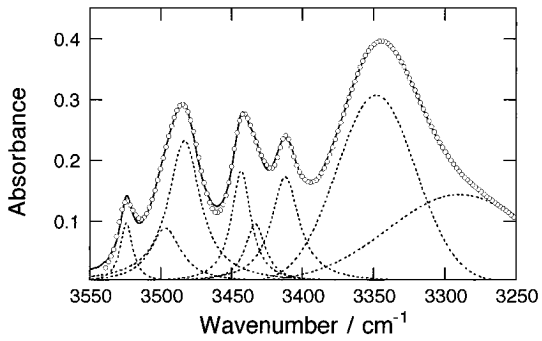


Figure 1. Infrared spectrum of 1.25 mM Ac-PLG-NH_2 in $CDCl_3$ at 25 °C obtained by subtracting the pure $CDCl_3$ spectrum at the same temperature: observed intensity (open circle), calculated total intensity (solid line), and calculated component intensities (dotted line).

Results

Figure 1 shows an IR spectrum of 1.25 mM Ac-PLG-NH_2 in $CDCl_3$ at 25 °C. Four distinct peaks at frequencies higher than 3400 cm^{-1} are assigned to hydrogen-bond-free (HB-free) NH groups, and a broad band at around 3350 cm^{-1} and its low-frequency shoulder are assigned to intramolecularly hydrogen-bonded (HBd) NH groups. These overlapping bands have been resolved by least-squares fitting with a computer. It should be noted here that curve fitting, in general involves some arbitrariness. To manage the problems of how many components should be taken into account and how to get reliable band parameters, we have performed curve fitting in several steps. At first, the calculation was carried out on the limited wavenumber range from 3550 to 3485 cm^{-1} and parameters of three Lorentzian functions were obtained. Next, the calculation range was extended to the lower wavenumbers in several steps, and parameters of newly added components were adjusted at each step. Finally, taking all the parameter values thus obtained as initial values, a least-squares calculation was performed on the whole wavenumber region 3550−3250 cm^{-1} to refine the parameter values. The baseline was taken to be a flat line estimated from a higher-frequency region which is free from the solute absorption. A Lorentzian function is used for

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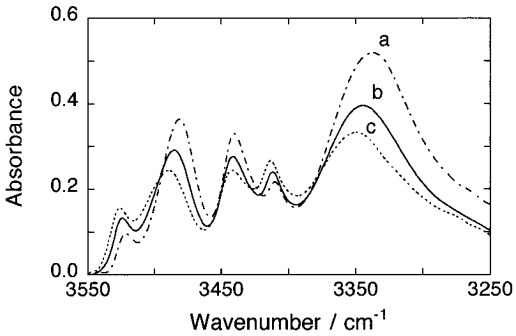


Figure 2. Infrared spectra of 1.25 mM Ac-PLG-NH_2 in $CDCl_3$ at (a) − 20, (b) 25, and (c) 70 °C.

representing each of the HB-free bands, in view of the fact that single HB-free bands of acetamide and its *N*-alkyl derivatives were well reproduced by a Lorentzian function. Each of the HBd bands, on the other hand, is represented by a Gaussian function which is adequate to reproduce an inhomogeneously broadened band, such as the HBd bands. In addition to four Lorentzian functions for the four distinct bands, two more Lorentzian functions were needed to reproduce the observed spectra. The resulting fit is shown in Figure 1.

The four distinct HB-free NH bands have been assigned by referring to spectra of simple amide compounds in $CDCl_3$ solutions. Acetamide is a structural analogue of the C-terminal part of Ac-PLG-NH_2. The asymmetric and symmetric NH stretching bands of this compound are observed at 3534 and 3416 cm^{-1}, respectively. These frequencies are in good agreement with those of the 3524- and 3412-cm^{-1} bands of Ac-PLG-NH_2, which are then assigned to the asymmetric and symmetric NH stretchings of the C-terminal amide group. Peak frequencies of NH stretching bands of *N*-methylacetamide, *N*-ethylacetamide, and *N*-isopropylacetamide are measured at 3469, 3454, and 3442 cm^{-1}, respectively. This indicates that the frequencies of the NH stretching become lower with an increase in the number of substituent methyl groups on the carbon atoms adjacent to the NH groups. From this fact, it can be inferred that the more carbon atoms attached to the α-carbon, the lower the frequency of the adjacent NH stretching in peptides. Therefore, it is reasonable to assign the lower 3443-cm^{-1} band to HB-free stretching of Leu-NH and the higher 3484-cm^{-1} band to that of Gly-NH. Assignments of 3496- and 3432-cm^{-1} bands will be given later.

Figure 2 shows spectra of 1.25 mM Ac-PLG-NH_2 at −20, 25, and 70 °C. With increasing temperature, the HB-free bands at 3524 and 3412 cm^{-1} that are assigned to the C-terminal amide group increase in intensity, while all the other band intensities apparently decrease. This fact indicates that Ac-PLG-NH_2 in the $CDCl_3$ solution exists in equilibrium among a few conformers and that the C-terminal amide group takes part in the intramolecular hydrogen bonds. This is confirmed by estimating temperature coefficients of the component band intensities, $(1/I)(dI/dT)$. Examination of the temperature coefficients is useful for further looking into the HB-free bands of different NH groups.

The temperature coefficients of the band intensities are thought to be affected by two factors. One is the temperature-dependent shift in the conformational equilibrium and the other is the thermal expansion of the solution. For a NH group which does not take part in the hydrogen bonds, the bands of both the HB-free and HBd conformers will almost completely overlap and provide a single band. Therefore, its apparent band intensity will be nearly independent of the temperature-dependent shift in equilibrium. Then, the temperature dependence of the band intensity is given only by the thermal expansion of the solution. The temperature coefficient of band intensities due to this effect is estimated to be -1.3×10^{-3} K^{-1}, which is given by the thermal expansion coefficient[19] of the solvent, $CDCl_3$. Observed temperature coefficients of symmetric and asymmetric NH stretching bands of acetamide, and the NH stretching bands of *N*-methylacetamide, *N*-ethylacetamide, and *N*-isopropylacetamide in $CDCl_3$ solutions, which were dilute enough to neglect intermolecular hydrogen bonding, are -3.7×10^{-3}, -3.2×10^{-3}, -2.9×10^{-3}, -4.2×10^{-3}, and -2.9×10^{-3} K^{-1}, respectively. Differences between these values and the above-mentioned value, -1.3×10^{-3} K^{-1}, are attributed to change in the Polo−Wilson internal-field effect[20] and in the solvent effect on the transition moments, both of which will decrease with decreasing solvent density. Then, the value of -1.3×10^{-3} K^{-1} can be taken as the upper limit of the temperature coefficient for HB-free bands that are irrespective of the HBd−HB-free conformational equilibrium. A band with a temperature coefficient larger than -1.3×10^{-3} K^{-1} is, therefore, assigned to a HB-free band of the NH group that is concerned with the hydrogen bonding, because the population of the HB-free conformer will increase with increasing temperature. Conversely, the temperature coefficient of a HBd band should be definitely smaller than -1.3×10^{-3} K^{-1}.

The HB-free bands of C-terminal NH_2 of Ac-PLG-NH_2 at 3524 and 3412 cm^{-1} exhibit positive temperature coefficients, 1.6×10^{-2} and 6.6×10^{-3} K^{-1}, respectively. Therefore, the proportion of the HB-free state of C-terminal NH_2 increases with increasing temperature. This fact clearly indicates that the C-terminal amide group takes part in the intramolecular hydrogen bonds.

The temperature coefficient of the Gly-NH band at 3484 cm^{-1} was -2.7×10^{-3} K^{-1}. This value is close to that of *N*-methylacetamide mentioned above and indicates that the Gly-NH is not involved in the hydrogen bonding. The temperature coefficients of the Leu-NH 3443-cm^{-1} band and its low-frequency neighbor the 3432-cm^{-1} band, being -0.61×10^{-3} and -8.9×10^{-3} K^{-1}, respectively, are distinctly larger and smaller than those of the Gly-NH and *N*-ethyl- and *N*-isopropylacetamide NH bands. The former value is even larger than -1.3×10^{-3} K^{-1}. This fact suggests that the Leu-NH group is in equilibrium between two different HB-free states. The frequency of the Leu-NH group, even if in the HB-free state, will be more or less dependent on whether the neighboring prolyl carbonyl group acts as a hydrogen bond acceptor or not. Therefore, the 3443- and 3432-cm^{-1} bands may be assigned to HB-free Leu-NH groups of conformers without and with, respectively, the hydrogen bond that involves the prolyl carbonyl group. It will be shown later that the prolyl carbonyl group takes part in a 10-membered hydrogen-bonded ring. A band at 3496 cm^{-1} is probably assigned to a HB-free NH of the C-terminal NH_2 group, the other NH of which is hydrogen bonded. This assignment is supported by the considerably large negative temperature coefficient of this band, -6.9×10^{-3} K^{-1}, which indicates this band is given by a HB-free NH of the HBd conformers.

As shown in Figure 3, the spectra of Ac-PLG-NH_2 exhibit concentration dependence. Spectra of 1.25 and 10 mM solutions are considerably different in shape at the low-frequency region at −20 °C, though they agree fairly well with each other at 70 °C. The spectral shapes of 2.5 and 5 mM solutions were found to be almost the same as that of the 1.25 mM solution at both temperatures. Therefore, intermolecular hydrogen bonding

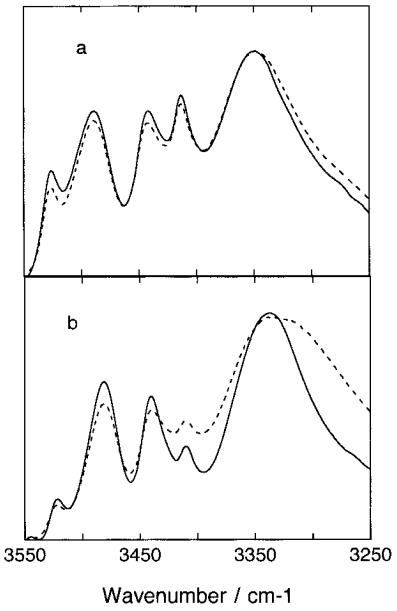


Figure 3. Infrared spectra of 1.25 (solid line) and 10 mM (broken line) Ac-PLG-NH_2 in $CDCl_3$ at (a) 70 and (b) −20 °C.

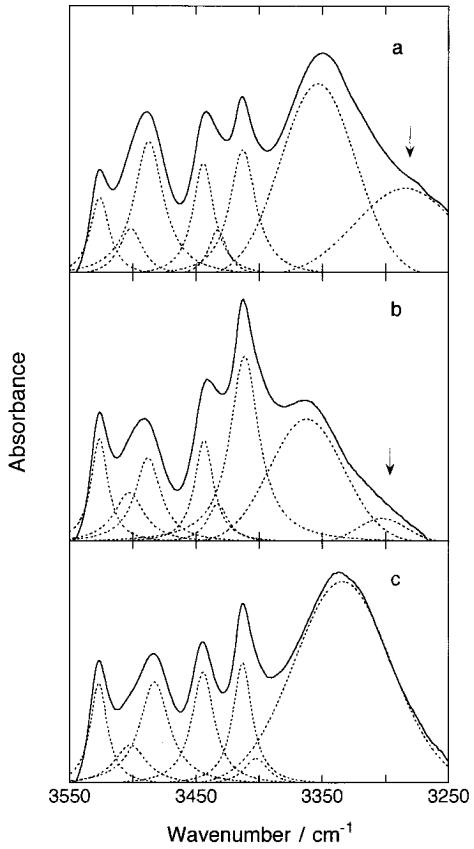


Figure 4. Infrared spectra (solid line) and the component bands (dotted line) calculated by the least-squares method for 1.25 mM (a) Ac-PLG-NH_2, (b) Tfa-PLG-NH_2, and (c) H-PLG-NH_2 in $CDCl_3$ at 70 °C.

becomes appreciable at high concentrations and low temperatures, and the 1.25 mM concentration is dilute enough to neglect the intermolecular hydrogen bonding in the experimental temperature range.

Figure 4 shows spectra of 1.25 mM solutions of the three peptides, Ac-PLG-NH_2, Tfa-PLG-NH_2, and H-PLG-NH_2, at 70 °C. The component band of the lowest frequency, which is assigned to the hydrogen-bonded C-terminal NH for Ac-PLG-NH_2, noticeably decreases in intensity for Tfa-PLG-NH_2 and disappears for H-PLG-NH_2. This fact indicates that the

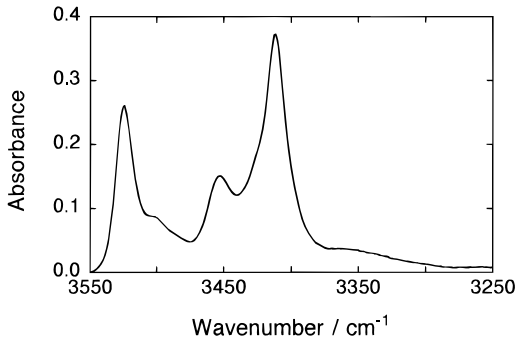
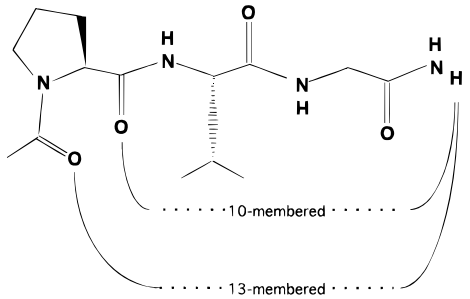


Figure 5. Infrared spectrum of 1.25 mM Ac-G-NH_2 in $CDCl_3$ at 25 °C.

Chart 2



hydrogen bond related to this band is between the C-terminal NH and the carbonyl oxygen of the N-terminal acetyl group, forming a 13-membered ring (Chart 2). Trifluorination of the acetyl group will largely reduce the proton-accepting ability of its carbonyl oxygen owing to the considerable electron-withdrawing property of the trifluoromethyl group. Therefore, the hydrogen bond concerned is weaker in Tfa-PLG-NH_2 than in Ac-PLG-NH_2. On the other hand, H-PLG-NH_2 has no carbonyl group at the N-terminus and no possibility of forming the 13-membered hydrogen-bonded ring.

Figure 5 shows the spectrum of a 1.25 mM solution of acetylglycinamide (Ac-G-NH_2, Chart 1) in $CDCl_3$ at room temperature. This compound has an equivalent constitution to the C-terminal half of Ac-PLG-NH_2 and has the possibility of forming a 7-membered hydrogen-bonded ring. Obviously bands to be assigned to hydrogen bonds are not appreciable except for a minor swelling, and this spectral feature was found to be nearly independent of temperature. Therefore, the 7-membered ring will not be appreciable for Ac-PLG-NH_2 as well as for Ac-G-NH_2 at the experimental temperatures. In conclusion, the hydrogen-bonded bands at around 3350 cm^{-1}, which are observed in common for Ac-PLG-NH_2, Tfa-PLG-NH_2, and H-PLG-NH_2, are assigned to a 10-membered ring with a hydrogen bond between the C-terminal amide NH and the prolyl carbonyl group (Chart 2). This is consistent with the temperature behavior of the HB-free bands of the Leu-NH group mentioned previously.

It is well-known that the hydrogen bonds in the α-helix and in the β-turn of polypeptides form 13- and 10-membered rings, respectively, which are similar to those of the tripeptide amides of the present study. The ultraviolet CD spectrum is one of the most useful tools for studying specific secondary structures of polypeptides or proteins. But for short peptides such as Ac-PLG-NH_2 or its analogues, it is unknown whether the discrimination of secondary structures can be obtained from CD or not, because only minimal CD data have been accumulated. In view of this, we have measured ultraviolet CD spectra and compared them with the IR spectra. The resulting CD spectra of Ac-PLG-NH_2, Tfa-PLG-NH_2, and H-PLG-NH_2 are shown in Figure

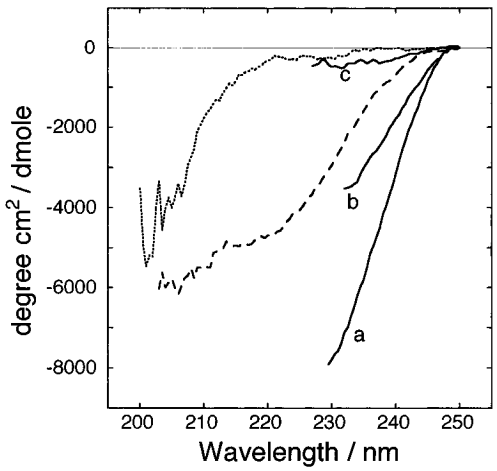


Figure 6. CD spectra of 1.25 mM Ac-PLG-NH_2 (a), Tfa-PLG-NH_2 (b), and H-PLG-NH_2 (c) in $CDCl_3$ (solid line) and Ac-PLG-NH_2 in TFE (broken line) and in a MOPS (pH 7) buffer solution (dotted line).

6. Ellipticity could not be measured at wavelengths shorter than 230 nm in $CDCl_3$ solutions due to strong absorption of $CDCl_3$ in this region. Therefore, we did not observe the double minimum of mean residue ellipticity at 222 and 208 nm, characteristic of the α-helix. However, the CD spectrum of Ac-PLG-NH_2 in $CDCl_3$ shows a rapid increase in negative ellipticity at wavelengths shorter than 250 nm. This spectral feature diminished for Tfa-PLG-NH_2 and disappeared for H-PLG-NH_2. Interestingly enough, the difference in the CD spectrum among the three peptides is consistent with that in the IR intensity of the 3290-cm^{-1} band that is assigned to the 13-membered hydrogen-bonded ring. In spite of its ability to form the 10-membered ring, H-PLG-NH_2 exhibits almost a flat CD spectrum in the same wavelength region. These findings suggest that the increase in the negative ellipticities at wavelengths shorter than 250 nm in $CDCl_3$ solutions of Ac-PLG-NH_2 and Tfa-PLG-NH_2 becomes a useful sign of a specific conformation of the 13-membered ring. As seen in Figure 6, this spectral feature of Ac-PLG-NH_2 disappears in the aqueous MOPS solution, while the TFE solution exhibits intermediate ellipticity between those in the $CDCl_3$ and aqueous solution. In the aqueous environment, Ac-PLG-NH_2 may adopt a random structure. Of course, the CD spectra alone indicates nothing about the α-helical nature of the 13-membered rings. However, the hydrogen-bonded sites determined from the IR spectra for the present 13-membered rings are exactly the same as those of the α-helix. It is, therefore, reasonable to suggest that the 13-membered ring may have a structure similar to that of a one-loop segment of the α-helix.

Discussion

Thermodynamic properties of the HBd conformers are obtained from analysis of the band intensities as a function of temperature.[12,21] Figure 7 shows van't Hoff plots for Ac-PLG-NH_2 in $CDCl_3$. Intensity ratios of the 3412-cm^{-1} band, which is assigned to the HB-free NH of the C-terminal amide, to each of the two hydrogen-bonded bands, I_f/I_b, were plotted against inverse absolute temperature. Here, two-state conformational equilibrium has been assumed between the HB-free conformer and each of the HBd conformers with the 10- and 13-membered rings. Usually, the slopes of the logarithmic plots provide the enthalpy changes, ΔH, for formation of the 10- and 13-membered hydrogen-bonded rings from the HB-free conformer.

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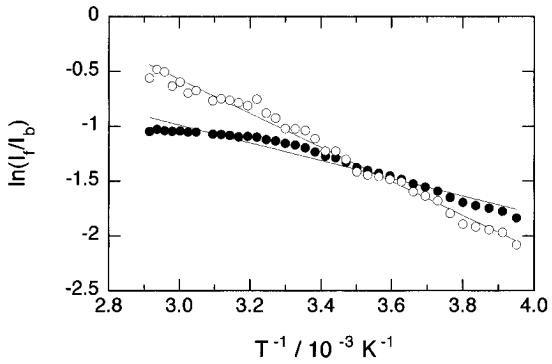

Figure 7. Logarithmic plots of the intensity ratios $I_{f(3412)}/I_{b(3350)}$ (closed circle) and $I_{f(3412)}/I_{b(3290)}$ (open circle) against inverse absolute temperature for 1.25 mM Ac-PLG-NH_2 in $CDCl_3$.

Table 1. IR Spectroscopic Results of the Thermodynamic Properties for the Intramolecular Hydrogen Bonding of Three Tripeptides

	ΔH^a (kJ mol^{-1})		ΔS^b (J mol^{-1} K^{-1})	ΔG^c (kJ mol^{-1})
compds	[free → 10]	[free → 13]	[10 → 13]	[10 → 13]
Ac-PLG-NH_2	−8.1 ± 0.8	−12 ± 3	−22 ± 2	2.5 ± 2.9
Tfa-PLG-NH_2	−4.4 ± 0.6	−4.7 ± 2.9	−34 ± 5	9.7 ± 3.5
H-PLG-NH_2	−8.1 ± 1.3			

[a] Mean values of ΔHs obtained by using the upper limit and the lower limit of c_b. [b] Errors are three times the standard deviations. [c] Values at 295 K.

However, we have to take account of the fact that the molar integrated intensities depend on temperature in somewhat different ways particularly between HB-free and HBd NH bands. In this case, the logarithm of the intensity ratio can be written as[21]

$$\ln(I_f/I_b) = \Delta H/RT + (c_f - c_b)T + \text{constant} \qquad (1)$$

where c_f and c_b are the temperature coefficients of the molar integrated intensities of HB-free and HBd bands, respectively. Since the concentration of the conformers changes with temperature, the values of c_f and c_b cannot be obtained from the observed band intensities of the molecule concerned. We have assumed, therefore, that the intrinsic c_f value of the 3412-cm^{-1} band is given by that of the symmetric NH stretching of acetamide, -3.7×10^{-3} K^{-1}. For estimation of the c_b value, it is difficult to find a model compound with an analogous intramolecular hydrogen bond but without the HBd−HB-free equilibrium. However, we can take the observed c_b value of the band concerned as the lower limit of the intrinsic c_b value, while the upper limit is given by the previously mentioned value, -1.3×10^{-3} K^{-1}. With this restriction, the ΔH value has been obtained by least-squares fitting of eq 1 to the observed plots. The same analysis has been carried out for Tfa-PLG-NH_2 and H-PLG-NH_2. The resulting mean values are listed in Table 1 together with the errors estimated from the both limits of c_b.

Entropy difference between two conformers is given by[12]

$$\Delta S = \Delta H/T - R\{\ln(I_1/I_2) - \ln(\alpha_1/\alpha_2)\} \qquad (2)$$

if the ratio of the molar integrated intensities, α_1/α_2, is given. This ratio could be given by a slope of a I_1 vs I_2 plot, but unfortunately it is usually difficult to estimate owing to temperature dependence of the molar integrated intensities.[21] It is well-known that the molar integrated intensities of the N−H or O−H stretching bands increase remarkably when the NH or OH groups take part in hydrogen bonds.[11] In addition, the local environment of the NH or OH bonds changes with the hydrogen

bonding and so does the temperature coefficients of the intensities.[21] Therefore, it is difficult to estimate ΔS between HB-free and HBd conformers. However, between the two HBd conformers, it is possible to estimate ΔS. The states of the hydrogen bonds in the 10- and 13-membered rings will not be much different from each other, and we can assume $\alpha_1 = \alpha_2$ without introducing any significant error. Then the ΔS value can be obtained from the intercept of the van't Hoff plots. The values of entropy difference thus obtained and free energy difference, ΔG, between the conformers with 10- and 13-membered rings are listed in Table 1.

At 295 K, the ΔG for the change from the 10- to the 13-membered ring for Ac-PLG-NH_2 is 2.5 ± 2.9 kJ mol^{-1}, indicating that the 10-membered ring may be a little more stable than the 13-membered ring at 295 K. The values of ΔH for formation of the 13-membered ring from the HB-free conformer are larger than those for the 10-membered ring. Therefore, the 13-membered ring is enthalpically more favorable than the 10-membered ring. On the other hand, the ΔS value for change from the 10- to the 13-membered ring is negative, and consequently, the 13-membered ring is entropically less favorable than the 10-membered ring. These differences in enthalpy and entropy between two conformers can be explained by the difference in dipole−dipole interactions with solvent chloroform molecules. If the 13-membered ring has a similar structure to that of a one-loop segment of the α-helix, all the group dipole moments of the four peptide units point parallel,[22] and should be cumulative. As a result, the dipole moment of the 13-membered ring may be considerably larger than that of the 10-membered ring. Therefore, the 13-membered ring exhibits stronger dipole−dipole interaction with polar solvents and makes its solvation structure more rigid, as compared with the 10-membered ring. Thus the 13-membered ring is enthalpically more favorable and entropically less favorable than the 10-membered ring.

These findings imply that the secondary structure of polypeptides is easily transformed by a mild change in temperature or other surrounding conditions. We can, therefore, imagine that the polypeptides experience a large number of steps of formation and collapse of transient local secondary structures, through the folding pathway to the biologically active structure of proteins. Even after being fully folded, a protein molecule may alter its local secondary structure with an environmental change or fluctuation. Such a local conformational change may lead to a rather drastic change in the whole structure of the protein molecule, and may affect its biological activity. An intriguing example is a potential-dependent ion channel which consists of a protein molecule embedded in a cell membrane. Its secondary structure may vary with a change in the membrane potential, which results in the opening and closing of the ion channel.[23] Therefore, elucidating the mechanism of the formation and stabilization of secondary structures provides a key for understanding the biological function of the protein molecule.

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Conclusion

Infrared spectra of three kinds of tripeptide amides, Ac-PLG-NH_2, Tfa-PLG-NH_2, and H-PLG-NH_2, and related compounds have been measured in chloroform solutions at various temperatures. For Ac-PLG-NH_2 and Tfa-PLG-NH_2, the absorption in the NH stretching region is decomposed into six hydrogen-bond-free NH bands and two broad hydrogen-bonded NH bands. The latter two bands have been assigned to hydrogen-bonded conformers with 10- and 13-membered hydrogen-bonded rings, respectively. Thermodynamic properties of these conformers have been obtained from van't Hoff analyses of the temperature dependence of the component intensities. It is shown that the 13-membered ring is enthalpically more favorable but entropically less favorable than the 10-membered ring.

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