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Effect of solvent on the *cis–trans* conformational equilibrium of a proline imide bond of short model peptides in solution

Masae Sugawara, Kenji Tonan, Shun-ichi Ikawa *

Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

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Abstract

Proton NMR spectra of proline-containing short peptides with *N*-terminal sequences of *N*-acetyl-prolyl- (Ac-Pro-) *N*-*tert*-butoxycarbonyl-phenylalanyl-prolyl- (Boc-Phe-Pro-) and *N*-*tert*-butoxycarbonyl-leucyl-prolyl- (Boc-Leu-Pro-) were measured in mixed solvents of hexadeuterodimethylsulfoxide and deuterochloroform ($CDCl_3$). Population ratios of *cis* and *trans* conformers with respect to the proline imide bond and chemical shifts of NH protons were obtained as a function of a $CDCl_3$ fraction of solvent. With increasing fraction of $CDCl_3$, the *trans* percentages of the Ac-Pro- imide bonds increased. On the other hand, those of Boc-Phe-Pro- decreased, and those of Boc-Leu-Pro- exhibited middle tendency. From the solvent-dependent variation of the chemical shifts of the NH protons, intramolecular hydrogen bonds that stabilize the *trans* form of Ac-Pro- and the *cis* form of Boc-Phe-Pro- were discussed. For the Ac-Pro- peptides, only the *trans* forms are found to the compatible with 7-, 10-, and 13-membered hydrogen-bonded rings that would be similar to the ordinary secondary structures, γ- and β-turns and α-helix, respectively. For the *cis* form of Boc-Phe-Pro-R (R = O-methyl or glycyl-O-ethyl), the hydrogen-bonded structure is found to be similar to the type-VIa β-turn. On the other hand, for Boc-Phe-Pro-Pro-Leu-Gly-NH_2, it has been suggested that two different hydrogen bonds, which are different from that of the type-VIa β-turn, support each other and cooperatively stabilize the *cis* form. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Peptides; Proline; *Cis–trans*; Intramolecular hydrogen bond; NMR

1. Introduction

The peptide bond preceding a prolyl residue, which is called a proline imide bond, is unique among other peptide bonds in proteins. The peptide bonds that do not involve Pro residues take predominantly the *trans* form and scarcely occur in the *cis* form in the folded structure of proteins,

* Corresponding author. Present address: Biomolecular, NMR Laboratory, Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, Quebec, Canada, H4P 2R2. Tel.: +81-11-7062707; fax: +81-11-7064924.

E-mail address: sikawa@sci.hokudai.ac.jp (S.-i. Ikawa).

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where the probability of occurrence of the *cis* form is less than 0.05% [1,2]. On the contrary, about 5% of the proline imide bond have been found to occur in the *cis* form in the folded proteins. For unstructured peptides, about 30% of the imide bonds adopt the *cis* form [1,3] and, for short peptides in solution, the amount of the *cis* form can be even comparable to that of the *trans* form, depending on amino acid sequences and solvent conditions [4–6]. This unusual distribution of the proline imide bonds can be explained by the unique structure of proline. The pyrrolidine ring, which covalently connects the side chain to the backbone nitrogen atom of proline, yields steric hindrance which is unfavorable to the *trans* form and, consequently, reduces significantly the energy difference between the *trans* and *cis* forms [1,2]. As a result, the *cis-trans* equilibrium of the proline imide bond becomes sensitive to environmental conditions, being different form that of other peptide bonds.

The above-mentioned difference in the *cis*/*trans* ratio between the folded and unfolded structure of proteins indicates that some change in environmental conditions, which occurs on the folding pathway, must shift the conformational equilibrium to the *trans* form. Solvent condition should be one of the important factors that influence conformation of local segments of he peptide chains. In order to study mechanism of the solvent effect, it is useful to examine short peptides because they are free from various long-range interactions, which would give rise to complicated effect on the conformation of polypeptide chains.

It is known that the fraction of the *trans* form of short model peptides is higher in less polar or non-polar solvents than in polar solvents [4,5]. In the recent IR and NMR study making use of mixed solvents of hexadeuterodimethylsulfoxide (DMSO-d_6) [6], we have found that both intramolecular hydrogen bonding and *cis*-to-*trans* isomerization are promoted by increased in fraction of $CDCl_3$ in the mixed solvents. It was concluded that the *cis*-to-*trans* isomerization is facilitated by formation of 10 membered or 13-membered intramolecular hydrogen-bonded rings that include the carbonyl group precedent to the prolyl residue.

It should be noted, however, that the above-mentioned results were only for peptides with an N terminus of an *N*-acetyl-Pro- (Ac-Pro-) or *N*-*tert*-butoxycarbonyl-Pro- (Boc-Pro-) type. If another residue exists before the prolyl residue, situation could be changed. For imide bonds in a sequence of -Xaa-Pro-, it has been found that the occurrence of the *cis* form depends on the type of the residue Xaa [1,7]. In addition, it has been suggested that the *cis* form could be stabilized by interaction between the pyrrolidine ring and the aromatic side chain of the preceding residue or both the preceding and following residues [8,9].

However, effect of solvent on the *cis–trans* equilibrium of -Xaa-Pro- has not been reported so far to our knowledge. In the present study, we have performed NMR measurements on two series of peptides; one includes peptides with an *N*-terminus of Ac-Pro- and the other includes Boc-Xaa-Pro- where Xaa is Phe or Leu residue. We have used mixtures of DMSO-d_6 and $CDCl_3$ at various mixing ratios as solvents for the NMR measurements. These mixed solvents are particularly useful for characterization of the *cis–trans* equilibrium and the intramolecular hydrogen bonding [6].

2. Experiments

The model peptides used for measurements are shown in Fig. 1, where amino acid residues are indicated by the usual one-letter abbreviations. These are classified into two types. The peptides in one group have a proline as the first residue with an acetyl group at the N-terminus. In the other group, peptides have a proline as the second residue and a Boc group as an N-terminal substituent. These compounds were synthesized by the ordinary liquid-phase method [10] and impurities were found to be negligible by infrared and NMR spectra. Starting compounds for the syntheses were commercially available Boc-Xaa-OH (Xaa = Pro, Phe, Leu, Gly), H-Pro-OMe (Me = methyl), H-Leu-OMe, H-Gly-OEt (Et = ethyl), and H-Pro-Leu-Gly-NH_2. Condensation reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and anti-racemization reagent 1-hydeoxybenzotri-

azole from Watanabe Chemical Industries, Ltd. were used. Deprotection of Boc-peptides was performed with 4 M HCl in 1,4-dioxane. Solvents used for spectroscopic measurements were $CDCl_3$ and DMSO-d_6 from CEA (France) and dried over molecular sieves 4A for 48 h before the preparation of sample solutions.

Proton NMR spectra of 1.25 mM peptide solutions in DMSO-d_6/$CDCl_3$ mixed solvents were measured with a JEOL EX400 Fourier-transform NMR spectrometer at 298 K. Chemical shifts were standardized by a residual proton of DMSO

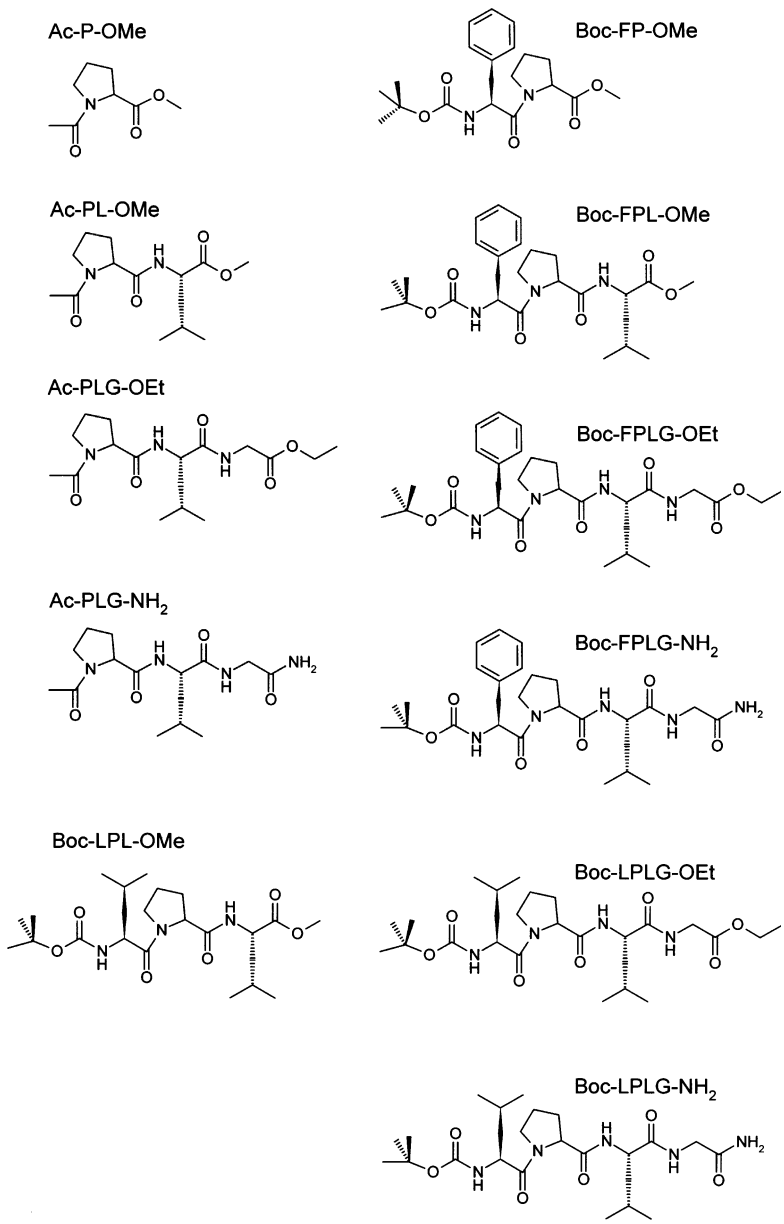

Fig. 1. Peptides used in the present study. Ac: acetyl, Boc: *tert*-butoxycarbonyl, P: Pro (prolyl), G: Gly (glycyl), L: Leu (leucyl), F: Phe (phenylalanyl), Me: methyl, Et: ethyl.

for solutions in DMSO-containing solvents and by a residual $CHCl_3$ proton for solutions in pure $CDCl_3$. The observed NMR signals were assigned with COSY (two-dimensional correlated spectroscopy) and NOESY (nuclear Overhauser enhancement-exchange spectroscopy) spectra. These two-dimensional spectra were measured with a JEOL ALPHA600 Fourier-transform NMR spectrometer at 298 K. Each data matrix size collected with 24–64 scans was 512×1024 and the spectrum was Fourier-transformed in both dimensions using the squared sine-bell window function.

Infrared spectra were measured with a Perkin-Elmer System-2000 Fourier-transform spectrometer at 2 cm^{-1} resolution and at a room temperature. A cell with CaF_2 windows and a sample path-length of 6 mm was used. Sample concentrations were 1.25 mM in $CDCl_3$.

3. Results

Two different signals, assigned to the *cis* and *trans* forms, were observed for each proton of the peptides. Among these signals, clearly separated pair for a Pro-α proton or a Lue-NH or Phe-NH proton were integrated to obtain the population ratios of the *cis* and *trans* forms. Fig. 2 shows the resulting percentages of the *trans* form plotted against the $CDCl_3$ fraction of the mixed solvents.

For the Ac-Pro-Leu- compounds shown in Fig. 2a, the ratios of the *trans* forms increase with increasing fraction of $CDCl_3$, while the *cis–trans* ratio of Ac-Pro-OMe is approximately independent of the solvent mixing ratio. This is consistent with the previous results for different Ac-Pro-Gly- and Boc-Pro-Gly- compounds [6]. The increase of the *trans* population has been found to be parallel to increase in intramolecular hydrogen bonding of the peptides. We have concluded in the previous paper that formation of the 10-membered or 13-membered intramolecular hydrogen-bonded ring that includes the carbonyl group precedent to the prolyl residue facilitated the *cis–trans* isomerization. These hydrogen-bonded rings would correspond to those of the usual secondary structures, the β-turn and α-helix, respectively.

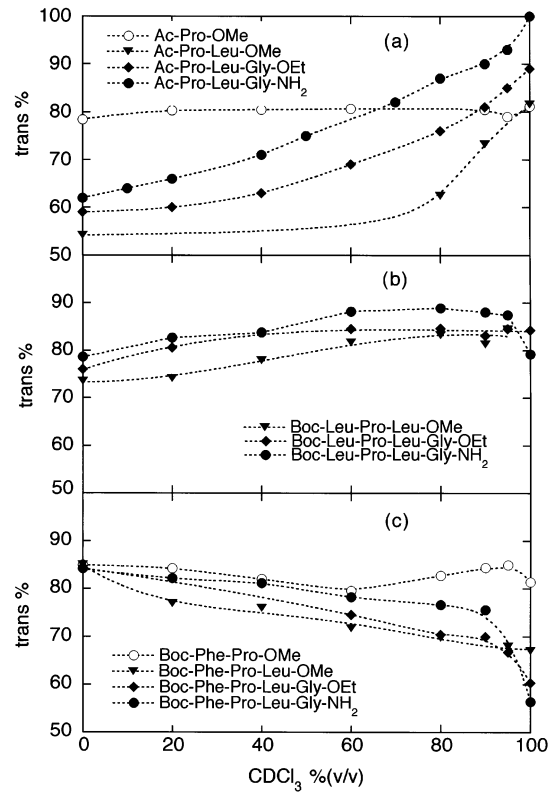

Fig. 2. The *trans* percentages of proline imide bonds plotted against $CDCl_3$ fractions of the DMSO-d_6-$CDCl_3$ mixed solvents for (a) Ac-Pro- compounds, (b) Boc-Leu-Pro-Leu- compounds, and (c) Boc-Phe-Pro- compounds.

For Boc-Phe-Pro-Leu- compounds, on the contrary, relative ratio of the *trans* forms decrease with increasing $CDCl_3$ fraction as shown in Fig. 2c, and those for the Boc-Leu-Pro-Leu- compounds exhibit middle tendency as shown in Fig. 2b. The contrast between the Ac-Pro-Leu- and Boc-Phe-Pro-Leu- compounds are clearly shown in Fig. 3, where solvent dependent changes in the *trans* percentages are compared among related compounds of the three types. It is intriguing how the *cis–trans* isomerization is related to the intramolecular hydrogen bonding for the Boc-Phe-Pro-Leu- compounds. A simple logical extension of the previous results [6] might suggest that the intramolecular hydrogen bonds that stabilize the *trans* form could be disturbed as the $CDCl_3$ fraction increases. Another possibility is existence of

some hydrogen-bonded structure that stabilizes the *cis* form, a candidate of which would be the type-VIa β-turn-like structure [7]. To elucidate hydrogen-bonded structures, infrared spectra and NMR chemical shifts are useful.

Figs. 4 and 5 show observed infrared NH stretching absorption of Ac-Pro-and Boc-Phe-Pro-compounds. The broad bands with peak wave numbers below 3400 cm^{-1} are assigned to the intramolecularly hydrogen-bonded NH groups while bands at higher than 3400 cm^{-1} are assigned to hydrogen-bond-free NH groups [6,10,11]. The broad peaks at around 3350 cm^{-1} are assigned to the β-turn like structure with a 10-membered hydrogen-bonded ring, while the peaks or shoulders at around 3300 cm^{-1} are assigned to a 7-membered hydrogen-bonded ring. For Ac-Pro-Leu-Gly-NH_2 and Boc-Phe-Pro-Leu-Gly-NH_2, the bulge at around 3300 cm^{-1} can be assigned to a 13-membered hydrogen-bonded ring, which resembles one turn of the α-helix [10], instead of the 7-memebered hydrogen-bonded ring. This is supported by the chemical shift data that will be discussed later.

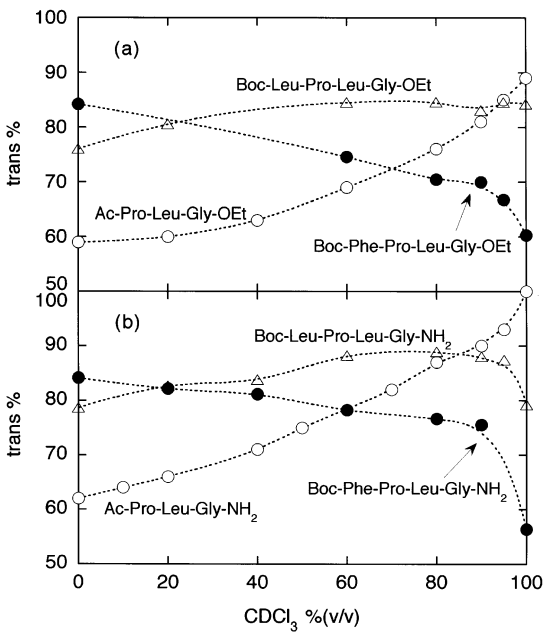

Fig. 3. Comparison of solvent-dependent changes of the *trans* percentages among Ac-Pro-, Boc-Phe-Pro- and Boc-Leu-Pro-compounds with (a) -Leu-Gly-OEt and (b) -Leu-Gly-NH_2, respectively, at the C-terminus.

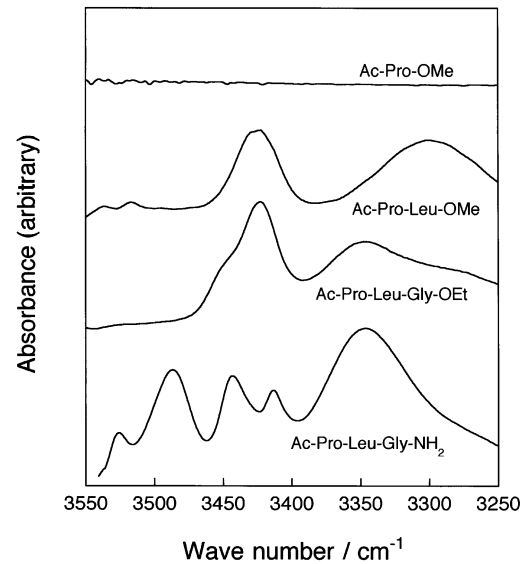

Fig. 4. Infrared absorption in the NH stretching region of 1.25 mM solutions of Ac-Pro- compounds in $CDCl_3$.

It should be noted that corresponding compounds of the Ac-Pro- and Boc-Phe-Pro- groups exhibit similar infrared NH stretching absorption

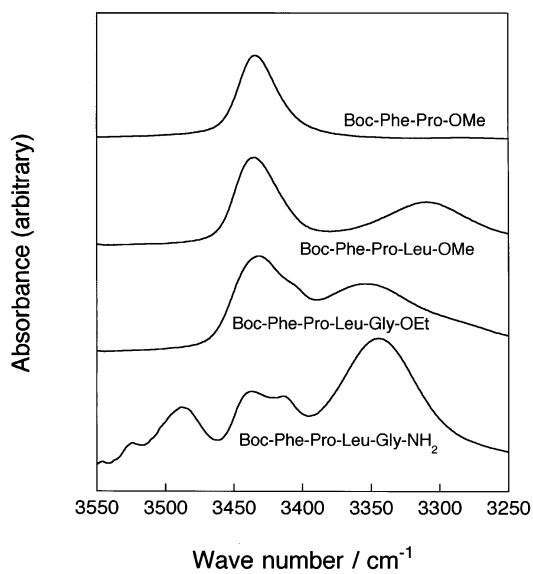

Fig. 5. Infrared absorption in the NH streching region of 1.25 mM solutions of Boc-Phe-Pro-compounds in $CDCl_3$.

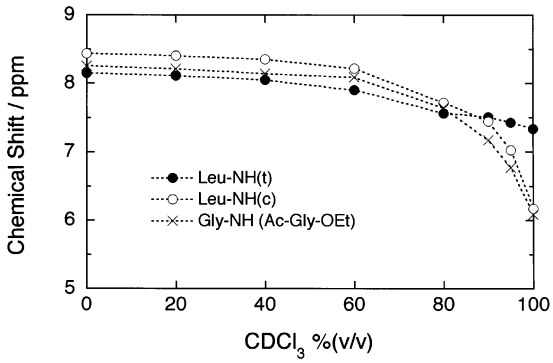

Fig. 6. Chemical shifts plotted against $CDCl_3$ fractions in the DMSO-$CDCl_3$ mixed solvents for NH-protons of Ac-Pro-Leu-OMe and Ac-Gly-OEt.

to each other except for Ac-Pro-OMe and Boc-Phe-Pro-OMe. Although Boc-Phe-Pro-OMe has a Phe-NH proton, it proves to be free from intramolecular hydrogen bonds like Ac-Pro-OMe that has no NH proton. Then the degrees of the intramolecular hydrogen bonding of the Boc-Phe-Pro- compounds are similar to the corresponding Ac-Pro- compounds. Infrared spectra, however, can not distinguish the *cis* and *trans* forms, and therefore, can not provide information about correlation between the hydrogen-bonded structures and the *cis*–*trans* ratios. To obtain further insight, we need to know to what degree each of the NH protons of the peptides takes part in the intramolecular hydrogen bonding. For this purpose, the NMR chemical shifts are useful [6].

Fig. 6 shows an example of how the chemical shifts of NH protons vary with the $CDCl_3$ fraction in the DMSO–$CDCl_3$ mixed solvents. The large chemical shift variation for the Gly-NH proton of Ac-Gly-OEt is interpreted as follows. Since DMSO is a strong hydrogen-bond acceptor, the Gly-NH proton is considered to be hydrogen-bonded to DMSO in the DMSO-rich solvents. These hydrogen bonds will decrease as the $CDCl_3$ fraction increases and completely disappear in 100% $CDCl_3$ solvent, which does not act as a hydrogen-bond acceptor but a very weak hydrogen-bond donor. As a result, signals of the Gly-NH proton show a large high-field shift as the $CDCl_3$ fraction increases from 0 to 100%. This will be also the case for a Leucyl amide proton of the *cis* form [designated by Leu-NH(c)] of Ac-Pro-Leu-OMe because its chemical shift shows a very similar variation. On the contrary, Leu-NH(t) shows distinctly smaller chemical shift variation. This proton is able to form an intramolecular hydrogen bond with the acetyl carbonyl group in the *trans* configuration. The Leu-NH(c) proton, on the other hand, can not from sterically any intramolecular hydrogen bond. Then the infrared hydrogen-bonded band of Ac-Pro-Leu-OMe seen in Fig. 4 is assigned to the *trans* form, and moreover, the small chemical shift variation of Leu-NH(t) can be assumed to be caused by formation of the intramolecular hydrogen bond in the $CDCl_3$-rich solvent. That is to say, the decay of the hydrogen bonds between DMSO and Leu-NH(t) may be compensated by the intramolecular hydrogen bonds between DMSO and Leu-NH(t) may be compensated by the intramolecular hydrogen bonding, and the variation of the chemical shift is significantly reduced. Thus, the chemical shift variations in the DMSO–$CDCl_3$ mixed solvents are useful to characterize the NH protons by their ability to form intramolecular hydrogen bonds.

Fig. 7 shows chemical shifts of four different types of NH protons of Boc-Phe-Leu-OMe plotted against the $CDCl_3$ percentage of the DMSO-$CDCl_3$ mixed solvents. It is found that both Phe-NH(t) and Phe-NH(c) show large variation of about the same magnitude. This is reasonable because the Phe-NH proton has no chance to

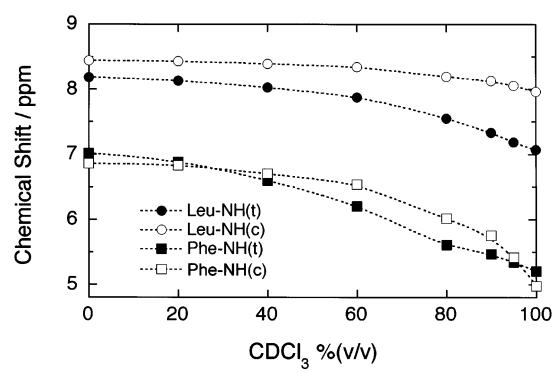

Fig. 7. Chemical shifts plotted against $CDCl_3$ fractions in the DMSO-$CDCl_3$ mixed solvents for NH-Protons of Boc-Phe-Pro-Leu-OMe.

make any intramolecular hydrogen bond both in the *trans* and *cis* configurations. On the other hand, variations for Leu-NH(t) and Leu-NH(c) are significantly smaller, and moreover, Leu-NH(c) shows distinctly smaller variation than Leu-NH(t). These facts indicate that the Leu-NH proton takes part in some intramolecular hydrogen bonds both in the *trans* and *cis* forms, and intriguingly the hydrogen-bond of the *cis* form could be more stable than that of the *trans* form. The hydrogen-bonded structure of the trans conformer is thought to be the 7-membered hydrogen-bonded ring formed by the Leu-NH(t) and the Phe-carbonyl group. This type of hydrogen-bonded structure, is, however, sterically incompatible with the *cis* configuration. Then the hydrogen bond partner of the Leu-NH(c) is restricted to the Boc-carbonyl group, and they form 10-membered hydrogen-bonded ring. This structure, being compatible with the *cis* configuration, has the same hydrogen-bonded pattern as that of the type-VIa β-turn.

4. Discussion

The observed chemical shifts of all the compounds studied are listed in Table 1. The first five compounds have no or little possibility of intramolecular hydrogen bonding in the whole range of the mixed solvents. Therefore, their chemical shift variations, $\Delta\delta = \delta(\mathrm{DMSO}) - \delta(\mathrm{CDCl_3})$, are considered to be solely due to change in the intermolecular DMSO-peptide hydrogen bonds. Consequently, these values can be adopted as standards for estimating the degree to which each of the amide protons of peptides is involved in the intramolecular hydrogen bonding in pure $CDCl_3$ solvent. Since the chemical shift variations may more or less depend on structural configuration close to the NH group concerned, in addition to the intramolecular hydrogen bonding, we have adopted several standards for different NH protons. The ratio of the chemical shift variation of each amide proton of the peptides to that of a suitable standard, $\Delta\delta/\Delta\delta_{\mathrm{Ref}}$, is indicated in the fifth column of Table 1.

It proved that the values of the NH protons for the *cis* forms of Ac-Pro-Leu-compounds are closed to 1. This is consistent with the steric incompatibility of intramolecular hydrogen bonding with the *cis* configuration for these compounds, as mentioned before for an example of Ac-Pro-Leu-OMe. For Ac-Pro-Leu-Gly-NH_2, $\Delta\delta$ values for the *cis* form were not obtained because the *cis* form completely disappears in the pure $CDCl_3$ solvent as shown in Fig. 2a. On the other hand, the $\Delta\delta/\Delta\delta_{\mathrm{Ref}}$, values of the NH protons for the *trans* form of Ac-Pro-Leu-compounds are distinctly smaller than unity. This fact indicates that these protons are involved in some intramolecular hydrogen bonds. The degrees of the involvement in hydrogen bonding for different NH protons could be compared by using magnitudes of the $\Delta\delta/\Delta\delta_{\mathrm{Ref}}$ values; smaller the values the larger the involvement.

For Ac-Pro-Leu-Gly-OEt, both Leu-NH(t) and Gly-NH(t) are involved in the intramolecular hydrogen bonding. The former is thought to make the same type of hydrogen-bonded structure, the 7-membered ring, as that of Ac-Pro-Leu-OMe. On the other hand, the hydrogen-bonded structure for the Gly-NH(t) is considered to be a 10-membered ring with the acetyl carbonyl group. Another possibility of Gly-NH(t) for forming 7-membered ring with prolyl carbonyl group can be neglected, because the 7-membered ring that does not cross a prolyl residue is not stable enough [6]. The fact that the $\Delta\delta/\Delta\delta_{\mathrm{Ref}}$ values of Gly-NH(t) is smaller than that of Leu-NH(t) suggests that the 7-membered ring might be more stable than the 10-membered ring. However, the difference in the $\Delta\delta/\Delta\delta_{\mathrm{Ref}}$ values seems not large enough to discuss conclusively.

Three amide protons of Ac-Pro-Leu-Gly-NH_2, Leu-NH(t), Gly-NH(t) and *anti*-NH(t), show considerably smaller $\Delta\delta/\Delta\delta_{\mathrm{Ref}}$ values than unity, and are thought to significantly take part in the intramolecular hydrogen bonding. Among others the *anti*-NH(t) is most heavily involved in the hydrogen bonding. According to the previous study [10], it forms mainly a 13-membered hydrogen-bonded ring with the acetyl-carbonyl group, and to a less extent a 10-membered ring with the prolyl carbonyl group. The Leu-NH(t) and Gly-

Table 1
Chemical shifts of the amide protons of peptides[a]

Compound proton	δ (DMSO)	δ ($CDCl_3$)	$\Delta\delta$	$\Delta\delta/\Delta\delta$ (Ref.)
Ac-Leu-OEt				
Leu-NH	8.17	5.78	2.39	(1)
Ac-Gly-OEt				
Gly-NH	8.25	6.08	2.17	(2)
Ac-Leu-NHMe				
Leu-NH	7.92	6.02	1.90	(3)
Aac-Gly-NHMe				
Gly-NH	8.06	6.22	1.84	(4)
Ac-NH_2				
Anti-NH	7.25	5.38	1.87	(5)
Syn-NH	6.97	5.24	1.73	(6)
Ac-Pro-Leu-OMe				
Leu-NH (t)	8.15	7.33	0.82	0.34 (1)
Leu-HN (c)	8.43	6.17	2.26	0.95 (1)
Ac-Pro-Leu-Gly-OEt				
Leu-NH (t)	7.89	7.11	0.78	0.41 (3)
Leu-NH (c)	8.21	6.30	1.91	1.01 (3)
Gly-NH (t)	8.13	6.82	1.31	0.60 (2)
Gly-NH (c)	8.37	6.35	2.02	0.93 (2)
Ac-Pro-Leu-Gly-NH_2				
Leu-NH (t)	8.02	7.03	0.99	0.52 (3)
Leu-NH (c)	8.28	–	–	
Gly NH (t)	7.92	7.07	0.85	0.46 (4)
Gly-NH (c)	8.09	–	–	
Anti-NH (t)	7.08	6.63	0.45	0.24 (5)
Anti-NH (c)	7.19	–	–	
Syn-NH (t)	7.08	5.25	1.83	1.06 (6)
Syn-NH (c)	7.06	–	–	
Boc-Phe-Pro-Leu-OMe				
Phe-NH (t)	7.02	5.202	1.82	0.96 (3)
Phe-NH (c)	6.86	4.97	1.89	0.99 (3)
Leu-NH (t)	8.18	7.07	1.11	0.46 (1)
Leu-NH (c)	8.44	7.96	0.48	0.20 (1)
Boc-Phe-Pro-Leu-Gly-Oet				
Phe-NH (t)	7.05	5.17	1.88	0.99 (3)
Phe-NH (c)	6.82	5.11	1.71	0.90 (3)
Leu-NH (t)	7.90	6.54	1.36	0.72 (3)
Leu-NH (c)	8.17	7.58	0.59	0.31 (3)
Gly-NH (t)	8.17	6.87	1.30	0.60 (2)
Gly-NH (c)	8.30	7.05	1.25	0.58 (2)
Boc-Phe-Pro-Leu-Gly-NH_2				
Phe-NH (t)	7.14	5.00	2.14	1.13 (3)
Phe-NH (c)	6.94	5.23	1.71	0.90 (3)
Leu-NH (t)	8.13	6.63	1.50	0.79 (3)
Leu-NH (c)	8.44	6.89	1.55	0.82 (3)
Gly-NH (t)	8.06	7.29	0.77	0.42 (4)
Gly-NH (c)	8.01	7.51	0.50	0.27 (4)

Table 1 (*Continued*)

Compound proton	δ (DMSO)	δ ($CDCl_3$)	$\Delta\delta$	$\Delta\delta/\Delta\delta$ (Ref.)
Anti-NH (t)	7.21	6.59	0.62	0.33 (5)
Anti-NH (c)	7.20	6.55	0.65	0.35 (5)
Syn-NH (t)	7.18	5.21	1.97	1.14 (6)
Syn-NH (c)	7.16	5.21	1.95	1.13 (6)
Boc-Leu[1]-Pro-Leu[2]-Ome				
Leu^1-NH (t)	6.88	5.12	1.76	0.93 (3)
Leu^1-NH (c)	6.73	5.12	1.61	0.85 (3)
Leu^2-NH (t)	8.13	7.21	0.92	0.38 (1)
Leu^2-NH (c)	8.40	8.14	0.26	0.11 (1)
Boc-Leu[1]-Pro-Leu[2]-Gly-Oet				
Leu^1-NH (t)	6.92	5.03	1.89	0.99 (3)
Leu^1-NH (c)	6.50	4.99	1.51	0.79 (3)
Leu^2-NH (t)	7.84	7.01	0.83	0.44 (3)
Leu^2-NH (c)	–	7.45	–	
Gly-NH (t)	8.16	6.67	1.49	0.69 (2)
Gly-NH (c)	–	–	–	
Boc-Leu^1-Pro-Leu^2-Gly-NH_2				
Leu^1-NH (t)	6.95	4.98	1.97	1.04 (3)
Leu^1-NH (c)	–	5.10		–
Leu^2-NH (t)	8.11	7.04	1.07	0.56 (3)
Leu^2-NH (c)	-	6.84	–	
Gly-NH (t)	7.97	7.24	0.73	0.40 (4)
Gly-NH (c)	–	7.75		
Anti-NH (c)	–	6.61	–	
Anti-NH (c)		6.63	–	
Syn-NH (t)		5.23	–	
Syn-NH (t)		5.19	–	

[a] Chemical shifts were standardized by a residual proton of DMSO or a residual $CHCl_3$ proton. Alphabets in parentheses mean *trans* (t) and *cis* (c).

NH(t) are thought to form a 7-membered ring and a 10-membered ring, respectively, with the acetyl carbonyl group. These hydrogen-bonded structures are considered to be similar to the γ-turn and the β-turn, respectively.

For Boc-Phe-Pro-Leu- compounds, in contrast to Ac-Pro-Leu- compounds, the NH protons of both *trans* and *cis* conformers take part in some intramolecular hydrogen bonds. The Leu-NH(c) proton as well as the Leu-NH(t) proton of Boc-Phe-Pro-Leu-OMe exhibits a significantly small $\Delta\delta/\Delta\delta_{Ref}$ value, which indicates existence of a stable intramolecular hydrogen bonds in the *cis* configuration. This is also the case for Boc-Leu^1-Pro-Leu^2-OMe in which the Leu^2-NH(c) proton is involved in the intramolecular hydrogen bonds. As mentioned previously, a hydrogen bond partner of Leu-NH(c) is restricted to the Boc carbonyl group and a likely candidate for the hydrogen-bonded structure is the type-VI β turn. A ball-and-stick model of this structure for Boc-Phe-Pro-Leu-OMe is examined on the basis of molecular mechanics calculation with a MM + force field [12], and the optimized structure is shown in Fig. 8. Comparison of the $\Delta\delta/\Delta\delta_{Ref}$ values suggests that the hydrogen bond in the *cis* configuration is more stable than that in the *trans* configuration. However, it does not mean that the *cis* form is more stable than the *trans* form. In fact, the *cis*/*trans* ratios of Boc-Phe-Pro-Leu-OME and Boc-Leu^1-Pro-Leu^2-OMe are 32/68 and 15/85, respectively, in $CDCl_3$. These facts indicate that non-hydrogen-bonded structures are less stable in the *cis* configuration than in the *trans*

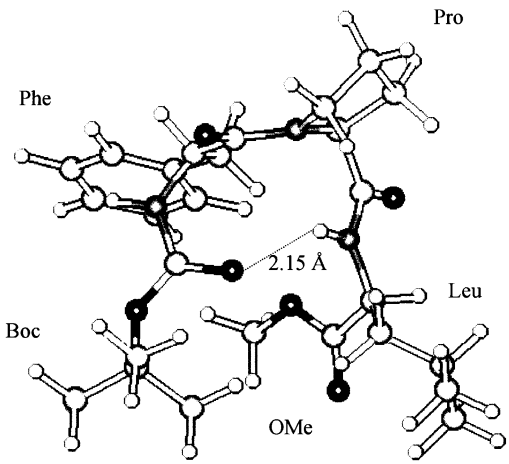

Fig. 8. A ball-and-stick model of type-VIa β-turn structure for Boc-Phe-Pro-Leu-OMe. The distance between the Boc-CO oxygen and the Leu-NH proton is 2.15 Å as shown in the figure.

configuration. As a result, the relative stability of the hydrogen-bonded structure is larger in the *cis* configuration than in the *trans* configuration. Nevertheless the *trans* form as a whole is more stable than the *cis* form.

For Boc-Phe-Pro-Leu-Gly-OEt, similarly, the hydrogen-bonded ring that involves the Leu-NH proton is more stable in the *cis* form than in the *trans* form in $CDCl_3$. The hydrogen bond partner of the Leu-NH(c) proton should be the Boc-carbonyl group as for Boc-Phe-Pro-Leu-OMe and a candidate for this *cis* hydrogen-bonded structure is also the type-VIa β-turn. Unfortunately, chemical shift of the Leu^2-NH(c) of Boc-Leu^1-Pro-Leu^2-Gly-OEt in 100% DMSO could not be obtained by overlap of other signals. However, this compound may have a similar *cis* hydrogen-bonded structure to that of Boc-Phe-Pro-Leu-Gly-OEt.

For Boc-Phe-Pro-Leu-Gly-NH_2, on the contrary, the Leu-NH proton is less involved in any intramolecular hydrogen bonding both in the *trans* and *cis* forms as compared with those of Boc-Phe-Pro-Leu-OMe and Boc-Phe-Pro-Leu-Gly-OEt mentioned above. Instead, mainly the Gly-NH and *anti*-NH protons take part in some hydrogen bonding both in the *trans* and *cis* forms. There are some possible hydrogen-bonded structures for these amide protons. Among those, the 7-membered hydrogen-bonded ring that does not cross a prolyl residue is not stable enough and can be neglected as mentioned before. In the *trans* configuration, therefore, the possible hydrogen bonded structures for Gly-NH(t) are 10- and 13-membered rings with Phe- and Boc-carbonyl groups, respectively. The *anti*-NH(t) proton has possibilities of forming 10-, 13- and 16-membered rings with Pro-, Phe-, and Boc-carbonyl groups, respectively. The 10- and 13-membered rings, which may be similar to the β-turn and α-helix structures, are quite common for short peptides in solution and probably coexist with each other in the present solutions. On the other hand, it is not clear whether the 16-membered ring exists to an appreciable degree or not.

The Phe-carbonyl group of Boc-Phe-Pro-Leu-Gly-NH_2 in the *cis* form is difficult to make any intramolecular hydrogen bonds due to steric hindrance. Consequently, the hydrogen-bonding partner of the Gly-NH(c) is restricted to the Boc-carbonyl group, while the Pro-carbonyl group is excluded by the reason mentioned above. The C-terminal *anti*-NH(c) is able to make 10-membered ring with the Pro-carbonyl, while a possibility of a 16-membered ring with the Boc-carbonyl could not be excluded. At present we have not enough knowledge to conclusively discuss details of the hydrogen-bonded structure in the *cis* form. However, examination with a ball-and-stick model on the basis of molecular mechanics calculations suggests that a 10-membered hydrogen-bonded ring of *anti*-NH(c) and a 13-membered ring of Gly-NH(c) can be formed simultaneously. If the 10-membered ring is like the type-I β-turn, the Gly-NH(c) group is protruded from the ring toward sterically possible region for the Boc-carbonyl group in the *cis* form. This hydrogen-bonded structure of Boc-Phe-Pro-Leu-Gly-NH_2 optimized with the MM + force field is shown in Fig. 9. These two intramolecular hydrogen-bonded rings seem to support each other, and may cooperatively contribute to stabilization of the *cis* form of Boc-Phe-Pro-Leu-Gly-NH_2. Each of hydrogen bonds could be less stable than that of the type-VIa β-turn structure. However, molecular mechanics calculations indicated that the structure with the two hydrogen-bonded rings shown in Fig. 9 was considerably more stable than a type-VIa β-turn structure for this peptide. Al-

though, such cooperative actions of different hydrogen bonds on the structures of proteins and polypeptides are commonly observed [13], they have so far not been reported for such a short peptide as composed of four residues to our knowledge. It should be noted here that the above-mentioned cooperativity of hydrogen bonds is different in nature from the usual hydrogen-bond cooperativity that is essentially caused by electronic induction effect in a succession of hydrogen bonds [13]. The former may be called conformational cooperativity of hydrogen bonds.

Finally, we briefly discuss difference in the *cis*/*trans* ratio between imide bonds of the Phe-Pro and Leu-Pro sequences. As seen in Fig. 2 and Fig. 3, the *cis* form of Boc-Phe-Pro-Leu- compounds are more stable than those of Boc-Leu-Pro-Leu- compounds in $CDCl_3$-rich solvent. This fact indicates that the Phe residue stabilize more the *cis* imide bond than the Leu residue. This is consistent with a statistical preference of proline imide bonds in proteins. Probability of occurrence of the *cis* imide bond is somewhat larger in -Xaa-Pro- sequence, where Xaa indicates a residue with an aromatic side chain, than in other -Yaa-Pro- sequence [1,8]. The reason for this, however, seems not yet clear enough. Interaction between the aromatic side chain and the pyrrolidine ring has been sometimes suggested [1,7,9]. However, conformational study by Kang et al. [8] suggested that the interaction is not strong enough. The present molecular mechanics calculations also do not imply any specific interaction between the Phe-aromatic ring and the pyrrolidine ring. For Boc-Phe-Pro-Leu-OMe, another conformation with a different dihedral angle about the C_α–C_β axis of the Phe residue and a closer distance between the two rings is slightly higher in energy than the optimized structure shown in Fig. 8. The same is true for Boc-Phe-Pro-Leu-Gly-NH_2. Although this could be due to an insufficiency of the molecular mechanics force field used in the present study, we may need to take account of another factor such as solvent effect. In general, an aromatic side chain is considered to be somewhat stronger in interaction with solvent than an aliphatic side chain, probably owing to a smaller ionization potential of the aromatic ring than that of the aliphatic side chain. This will cause difference in interaction with solvent and could yield different conformational preference in solution.

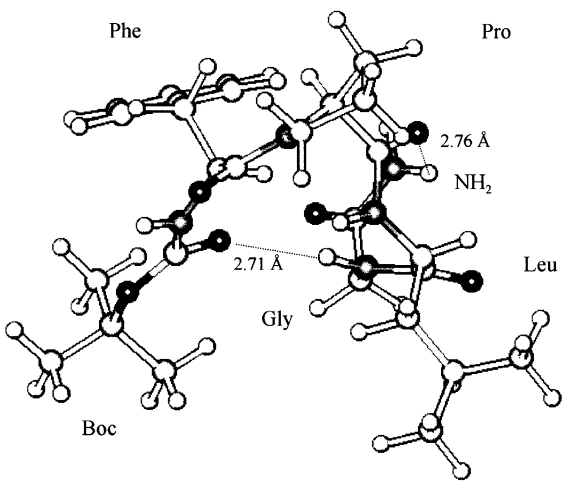

Fig. 9. A ball-and-stick model of hydrogen-bonded structure for the *cis* form of Boc-Phe-Pro-Leu-Gly-NH_2. The distance between the Boc-CO oxygen and the Gly-NH proton is 2.71 Å and that between the Pro-CO oxygen and the C-terminal *anti*-NH proton is 2.76 Å as shown in the figure.

5. Summary

This *cis*–*trans* conformational equilibrium of a proline imide bond of different short peptides in DMSO-$CDCl_3$ mixed solvents has been studied as a function of solvent mixing ratio by proton NMR. It is found that, with increasing fraction of $CDCl_3$, the *trans* percentage of Ac-Pro- imide bonds increases, but that of Boc-Phe-Pro- decreases and that of Boc-Leu-Pro- exhibits middle tendency. From the solvent-dependent change of chemical shifts of NH protons, intramolecular hydrogen bonds that stabilize the *trans* form of Ac-Pro and the *cis* form of Boc-Phe-Pro- were discussed. It is found that formation of 7-, 10-, and 13-membered intramolecular hydrogen-bonded rings stabilizes the *trans* form of Ac-Pro- imide bonds. On the other hand, the *cis* of Boc-Phe-Pro-R (R = OMe or Gly-OEt) is stabilized by formation of a 10-membered hydrogen-bonded ring similar to that of the type-VIa β-turn. For Boc-Phe-Pro-Leu-Gly-NH_2,

on the other hand, it is suggested that the *cis* form is stabilized by cooperative action of two different hydrogen-bonded rings, both of which are different from the type-VIa β-turn structure. Difference in the *cis*/*trans* ratio between the Boc-Phe-Pro- and Boc-Phe-Pro- sequences was briefly discussed.

Acknowledgements

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