Enantiomer-Differentiating Ability of Cyclo(L-Phe–L-Pro)$_4$ Having a Rigid Skeleton for Phenylalanine Methylester Hydrochloride

Takashi ISHIZU* and Shunsaku NOGUCHI

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Sanzo Gakuen-cho 1, Fukuyama, Hiroshima 729–02, Japan. Received February 12, 1997; accepted March 19, 1997

The formation constant of the 1 : 1 complex of cyclo(L-Phe–L-Pro)$_4$ (1) with L-PheOMe·HCl was about 13.2 times that of the complex with D-PheOMe·HCl. The 1 : 1 complex of 1 with L-PheOMe·HCl formed three intermolecular hydrogen bonds between Pro^2 CO, Phe^1 CO and the amino group, and Phe^2 NH and the carbonyl group, whereas that with D-PheOMe·HCl formed only one between Phe^1 CO and the amino group.

Key words cyclopeptide; chiral recognition; hydrogen bond; NMR; formation constant

The design and synthesis of so-called chiral receptors, which display enantiomeric recognition, are of considerable interest. Various types of chiral receptors for amino acid derivatives have been synthesized.[1–5] We have been attempting to construct such receptors by the use of cyclopeptides containing amide bonds, which can bind to and include various guest compounds, often *via* intermolecular hydrogen bonds. We selected a cyclopeptide having a rigid skeleton, which might recognize chirality by forming different kinds of intermolecular hydrogen bond with D- and L-form molecules.

Kimura and Imanishi reported that cyclo(L-Phe–L-Pro)$_4$ (**1**) did not form a complex with metal ions due to the rigidity of its skeleton.[6] We showed that the 1 : 1 complex formation[7] of cyclo(L-Phe–L-Pro)$_4$ (**1**) with L-PheOMe·HCl did not alter the ^{13}C-NMR spectral pattern of **1**, indicating that the skeleton of **1** was too rigid to change its conformation.[8] This raised the possibility that **1** may act as a chiral receptor for PheOMe·HCl, and we investigated whether this is the case by means of NMR measurements in $CDCl_3$.

Results and Discussion

As shown in Fig. 1 the residues of **1** were named Phe^1, Pro^1, Phe^2, and Pro^2. It takes a C_2 symmetric conformation with an intramolecular hydrogen bond between Pro^1 CO and Phe^1 NH.[9] The assignments of the ^{1}H-NMR signals of **1** were based on the combination of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multi-quantum coherence (HMQC),[10] and heteronuclear multiple bond coherence (HMBC)[11] spectra. Each proton signal of Pro^2 in the ^{1}H-NMR spectrum was shifted upfield by 0.51—1.54 ppm in comparison with that of Pro^1 because of the shielding effect of the ring current of the phenyl group of Phe^2 (Table 1). This indicated that the benzene ring of Phe^2 was opposite the pyrrolidine ring of Pro^2 (Fig. 1). The assignments of the ^{13}C-NMR signals of **1** were reported previously.[8]

Information about the structural flexibility of **1** can be experimentally obtained from the relaxation times of the carbon resonances (T_1). The NT_1 values (N=number of attached protons, T_1=longitudinal relaxation time) are correlated directly with molecular mobility. The NT_1 values of all the α carbons [265 (Phe^1), 315 (Pro^1), 334 (Phe^2), and 333 (Pro^2) ms] were very similar, indicating the rigidity of the backbone. After the 1 : 1 complex formation with L-PheOMe·HCl, their values [410, 400, 335, and 376 ms, respectively] were a little larger and still similar. This suggested a slight decrease of the rigidity due to the change of the hydrogen bonding pattern, as described below. Interestingly the values of C_β of Phe^1 and Phe^2, 1752 and 1794 ms, were decreased to 760 and 584 ms, respectively by the complex formation, suggesting the approach of L-PheOMe·HCl to then in the cavity of **1**. Similar changes of NT_1 values upon complex formation with D-PheOMe·HCl were also observed.

Figures 2(a) and (b) show plots of the changes in the chemical shifts [172.29 (Pro^1 CO), 171.30 (Pro^2 CO), 170.33 (Phe^2 CO), 168.85 ppm (Phe^1 CO)] of the four carbonyl carbons of **1** *vs.* the molar ratio of L- and D-PheOMe·HCl in the ^{13}C-NMR measurements. Each complex was determined by the molar method to be 1 : 1. The formation constants[12] of the 1 : 1 complex of **1** with

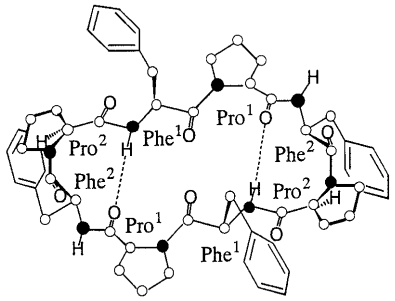

Fig. 1. Schematic Representation of Cyclo(L-Phe–L-Pro)$_4$ (**1**)

Table 1. ^{1}H-NMR Spectral Data (ppm) for the Pro Residues of **1**

Residue	Proton	**1**[a]	Complex of **1** with L-PheOMe·HCl[b]	b—a
Pro^1	H_α	4.53	4.77	0.24
	H_β	2.20, 2.36	2.20, 2.36	0, 0
	H_γ	2.03, 2.20	2.03, 2.20	0, 0
	H_δ	3.76, 3.82	3.76, 3.82	0, 0
Pro^2	H_α	3.01	3.13	0.12
	H_β	0.90, 1.82	0.78, 1.73	−0.12, −0.09
	H_γ	0.81, 1.40	0.60, 1.30	−0.21, −0.10
	H_δ	3.25, 3.31	3.13, 3.13	−0.12, −0.18

a) Solution of **1** (9.76 mg) in $CDCl_3$ (550 μl). *b*) The above solution with L-PheOMe·HCl (2.15 mg) in $CDCl_3$ (550 μl).

* To whom correspondence should be addressed.

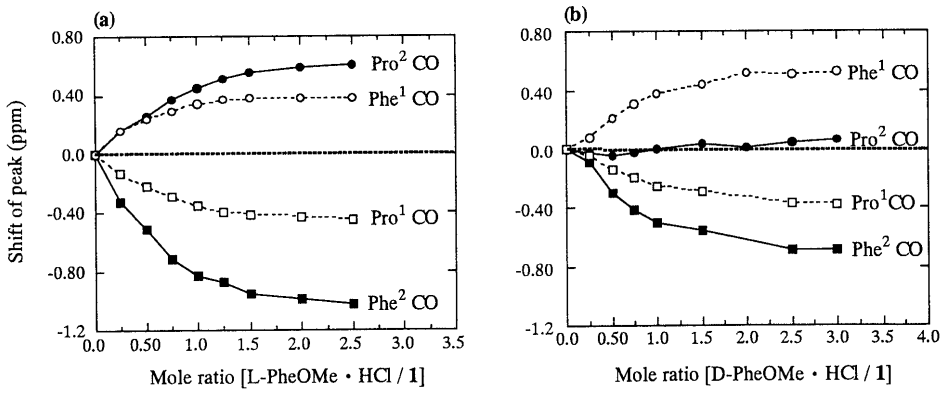

Fig. 2. The Shifts of the ^{13}C-NMR Signals of Carbonyl Carbons of **1** upon the Addition of L-PheOMe·HCl (a) and D-PheOMe·HCl (b)

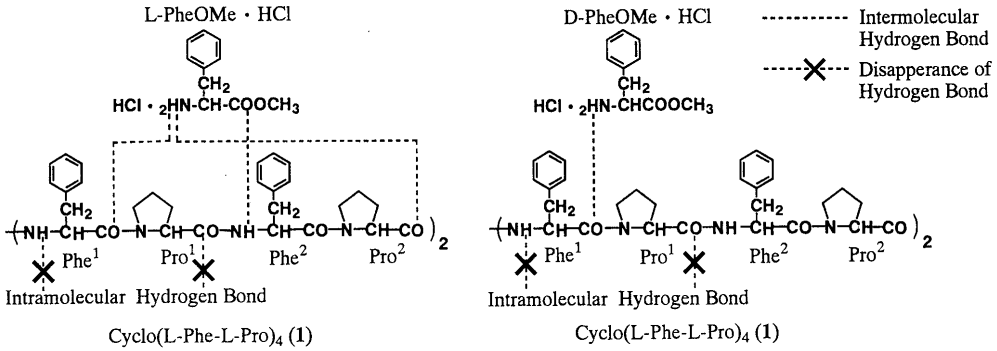

Fig. 3. Hydrogen Bonds between **1** and L-, D-PheOMe·HCl

D- and L-PheOMe·HCl were calculated by a non-linear least-squares method as 88 and 1160 mol^{-1}, respectively; the latter was about 13.2 times the former.

Generally speaking, an NMR signal is shifted downfield upon hydrogen bond formation. As shown in Fig. 2(a), the signals of Pro^2 CO and Phe^1 CO shifted downfield, indicating that these two carbonyl groups formed intermolecular hydrogen bonds with the amino group of L-PheOMe·HCl. On the other hand, only the signal of Phe^1 CO is shifted downfield in the case of D-PheOMe·HCl (Fig. 2(b)). The upfield shifts of Pro^1 CO observed in both Fig. 2(a) and (b) may be caused by the loss of the intramolecular hydrogen bond of **1**. Furthermore, those of Phe^2 CO may be due to the ring current of the benzene of PheOMe·HCl or Phe^2.

Upon complex formation, the carbonyl carbon of L-PheOMe·HCl was shifted downfield by 1.25 ppm in the ^{13}C-NMR spectrum, indicating the formation of the intermolecular hydrogen bond between the carbonyl group of L-PheOMe·HCl and Phe^2 NH. However, such a downfield shift was not observed on complex formation with D-PheOMe·HCl. The hydrogen bonding patterns in the 1 : 1 complexes of **1** with L- and D-PheOMe·HCl are summarized in Fig. 3. The complex of L-PheOMe·HCl formed three intermolecular hydrogen bonds, whereas that of D-PheOMe·HCl formed only one. This may be the reason why **1** can recognize the chirality of PheOMe·HCl.

Futhermore, each proton of Pro^2 of **1** except for H_α was shifted upfield by 0.09—0.21 ppm because of the shielding effect of the ring current of the benzene of L- or D-PheOMe·HCl (Table 1). This suggested that the benzene

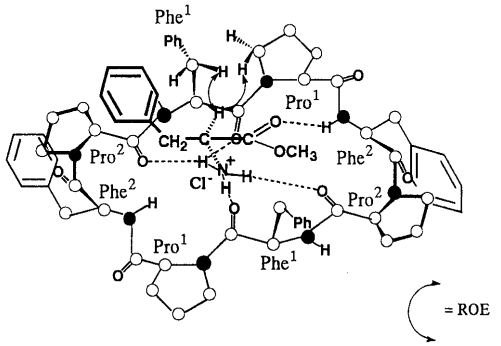

Fig. 4. Schematic Representation of the Complex of **1** with L-PheOMe·HCl

ring of PheOMe·HCl entered the hydrophobic space between Pro^2 and Phe^1 residues and lay close to the pyrrolidine ring of Pro^2 (Fig. 4). The existence of the intermolecular hydrogen bond of the carbonyl group of PheOMe·HCl was presumed to be due to this fixation of the benzene ring upon the face of the pyrrolidine ring of Pro^2. A schematic representation of the 1 : 1 complex of **1** with L-PheOMe·HCl is shown in Fig. 4. This was confirmed by the result of rotating-frame Overhauser enhancement spectroscopy (ROESY). Two important correlations were the intermolecular ROEs between H_β of Phe^1, H_δ of Pro^1 and H_α of L-PheOMe·HCl.

Experimental

NMR spectra were obtained on JEOL Lambda 500 spectrometers operating at 500.00 MHz for ^{1}H and 125.65 MHz for ^{13}C. Chemical shift values were expressed in ppm downfield from tetramethylsilane

(TMS) as an internal standard. Samples were dissolved in $CDCl_3$ (99.8% D, Aldrich Chemical Company, Inc.) in a 5 mm i.d. sample tube. The possibility of peptide aggregation of **1** was examined by recording the ^{1}H-NMR spectra of solutions containing 1.82—54.55 mM (**1**) in $CDCl_3$. No significant changes in chemical shifts or line widths were observed over this concentration range.

T_1 values were estimated using the standard inversionrecovery sequence to determine the null in signal intensity. ROESY experiments were done using a mixing time of 500 ms in the phase-sensitive mode.

References and Notes

1) Cram D. J., Helgeson R. C., Sousa L. R., Timko J. M., Newcomb M., Moreau P., deJong F., Gokel G. W., Hoffman D. H., Domeier L. A., Peacock S. C., Madan K., Kaplan L., *Pure Appl. Chem.*, **43**, 327 (1975).
2) Cram D. J., "Application of Biochemical Synthesis in Organic Systems, Part II," Vol. X, ed. by Jones J. B., Sih C. J., Perlmann D., Techniques of Chemistry, John Wiley and Sons, New York, 1976, p. 815.
3) Cram D. J., Cram D. M., *Acc. Chem, Res.*, **11**, 8 (1978).
4) Hong J.-I., Namgoog S. K., Bernardi A., Still W. C., *J. Am. Chem. Soc.*, **113**, 5111—5112 (1991).
5) *a*) Gala'n A., Andreu D., Echavarren A. M., Prados P., de Mendoza J., *J. Am. Chem. Soc.*, **114**, 1511—1512 (1992); *b*) Echavarren A. M., Gala'n A., Lehn J.-M., de Mendoza J., *J. Am. Chem. Soc.*, **111**, 4994—4995 (1991).
6) Kimura S., Imanishi Y., *Biopolymers*, **22**, 2383—2395 (1983).
7) *a*) Deber C. M., Blout E. R., *J. Am. Chem. Soc.*, **96**, 7566—7567 (1974); *b*) Madison V., Deber C. M., Blout E. R., *J. Am. Chem. Soc.*, **99**, 4788—4798 (1977).
8) Ishizu T., Hirayama J., Noguchi S., Iwamoto H., Hirose J., Hiromi K., *Chem. Pharm. Bull.*, **41**, 2029—2031 (1993).
9) Kimura S., Imanishi Y., *Biopolymers*, **22**, 2191—2206 (1983).
10) Bax A., Griffey R. H., Hawkins L. B., *J. Magn. Reson.*, **55**, 301—315 (1983).
11) Bax A., Summers M. F., *J. Am. Chem. Soc.*, **108**, 2093—2094 (1986).

12) The formation constants K (M^{-1}) were obtained by a non-linear least-squares method using the following equation: $R = KS/(1+KS)$ [$R = CS/CS_0$, CS = shift value (ppm), CS_0 = maximum shift value (ppm), S = free of PheOMe·HCl (M)].