Complex Formation of Cyclo(L-Phe–L-Pro)$_4$ with Noradrenaline Hydrochloride

Takashi ISHIZU,* Jungo HIRAYAMA, and Shunsaku NOGUCHI

Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Sanzo Gakuen-cho 1, Fukuyama, Hiroshima 729–02, Japan. Received November 4, 1993; accepted January 12, 1994

The ^{13}C-NMR spectrum of cyclo(L-Phe–L-Pro)$_4$ (1) and DL-noradrenaline hydrochloride (DL-NA·HCl) in a mixture of $CDCl_3$ and CD_3OD suggested the formation of a complex, which was demonstrated to be 1:1 from examination of the titration curves. The complex retained the C_2-symmetric conformation of 1 containing two *cis*-peptide bonds, and was linked through hydrogen bonds between the carbonyl groups of the Phe^1 and Pro^2 residues, and the ammonium moiety of NA·HCl.

Keywords cyclic peptide; ^{13}C-NMR spectrum; noradrenaline hydrochloride; hydrogen bond

Complexes between host and guest, such as the binding of a substrate to its receptor, are common in biology. Direct observation of their interactions is difficult owing to the low molar concentration obtainable and the complexity of protein structure. Therefore, we have sought to design and synthesize artificial receptors using various peptides.

Noradrenaline (NA), a neurotransmitter of postganglionic neurons in the sympathetic nervous system, binds to the β-adrenergic receptor. Even a small amount of the L-enantiomer of NA has physiological activity.

This paper focuses on the complex formation of the proline-containing cyclic peptide, cyclo(L-Phe–L-Pro)$_4$ (**1**) with noradrenaline hyrochloride (NA·HCl). The Pro residue enhances the lipophilicity of the peptide and allows a *cis–trans* isomerization of the peptide bond. The latter feature increases the number of available conformations of the peptide, which should be favorable for complex formation.

Results and Discussion

Cyclo(L-Phe–L-Pro)$_4$ (**1**) was prepared previously and shown to take a C_2-symmetric conformation containing two *cis* peptide bonds at Pro^2 residues in $CDCl_3$ and CD_3OD, with 1–4 intramolecular hydrogen bonds as shown in Fig. 3.[1] We carried out ^{1}H–^{13}C long-range shift correlation (COLOC) spectral analysis to elucidate the assignment of the carbonyl carbon signals of **1** in the ^{13}C-NMR spectrum (Fig. 1). Phe^{2}NH, Pro^{2}H$_\alpha$, Pro^{2}H$_\beta$, and Pro^{2}H$_\delta$ were correlated with Pro^{1}CO, Pro^{2}CO, Pro^{2}CO, and Phe^{2}CO, and the four carbonyl signals appearing at 172.60, 171.77, 171.52, and 168.88 ppm were assigned to Pro^{1}CO, Pro^{2}CO, Phe^{2}CO, and Phe^{1}CO, respectively (Table I).

A solution of 1 eq of DL-NA·HCl (racemic form) in CD_3OD was added to a solution of **1** in $CDCl_3$, and the mixed solution was examined by ^{13}C-NMR measurement. The spectrum displayed split signals for the following carbons of DL-NA·HCl: C_p, C_γ, C_o, $C_{o'}$, C_β, and C_α (Table II). The splitting of the asymmetric carbon C_β (0.60 ppm) was largest. Such spectra result from the formation of diastereomeric pairs of complexes, *i.e.*, the complexes of **1** with D-NA·HCl and L-NA·HCl. Previously Deber *et al.* reported similar diastereomeric pairs of complexes of cyclo(Gly–L-Pro)$_n$ ($n=3$ or 4) with D, L mixtures of amino acid salts in $CDCl_3$, and the splitting of the ^{13}C-NMR signals.[2] A schematic representation of the complex of **1**

TABLE I. ^{13}C-NMR Spectral Data (ppm) of Cyclo(L-Phe–L-Pro)$_4$ (**1**) [A], and of **1** in the Complex with NA·HCl [B][a]

Carbon	A	B	Carbon	A	B
Pro^1 CO	172.60	172.49	Pro^2 C_α	60.65	60.85
Pro^2 CO	171.77	172.31	Pro^1 C_α	59.69	60.07
Phe^2 CO	171.52	171.52	Phe C_α	54.85	55.20
Phe^1 CO	168.88	169.29		54.85	54.73
Phe C_γ	138.26	138.17	Pro^1 C_δ	47.74	48.04
	135.74	135.65	Pro^2 C_δ	46.83	46.72
Phe C_o	129.72	129.78	Phe C_β	38.00	37.85
	128.89	129.11		35.42	35.27
Phe C_m	128.61	128.95	Pro^2 C_β	30.60	30.48
	128.46	128.63	Pro^1 C_β	29.44	29.54
Phe C_p	127.78	127.77	Pro^1 C_γ	25.80	25.93
	126.76	126.97	Pro^2 C_γ	21.53	21.53

a) The solvent was a mixture of $CDCl_3$ (500 μl) and CD_3OD (150 μl) in both cases.

TABLE II. ^{13}C-NMR Spectral Data of DL-NA·HCl in the Complex with Cyclo(L-Phe–L-Pro)$_4$ (**1**)[a]

Cyclic peptide	Form	Chemical shifts δ (ppm) of DL-NA·HCl							
		C_p	$C_{m'}$	C_γ	C_o	C_m	$C_{o'}$	C_β	C_α
No cyclic peptide		145.45	145.43	132.59	117.90	115.84	113.38	69.81	46.78
1	D	145.25	145.30	132.49	117.58	115.57	113.40	69.42	46.67
	L	145.38	[b]	132.56	117.94	[b]	113.02	70.02	46.74

a) The solvent was a mixture of $CDCl_3$ (500 μl) and CD_3OD (150 μl). b) No splitting of the signal.

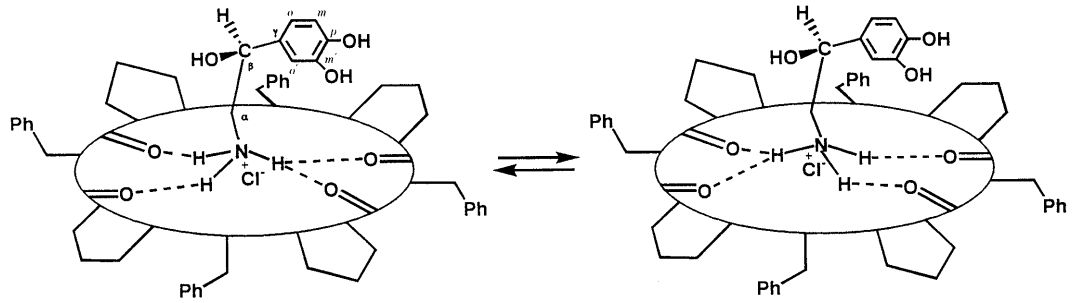

Chart 1

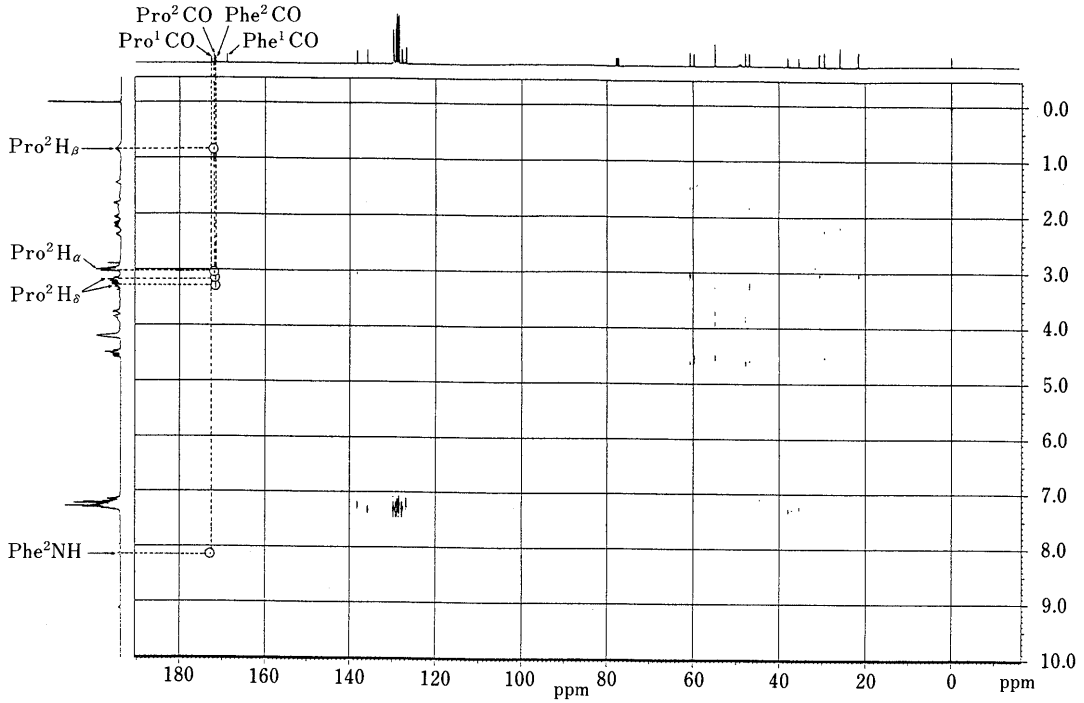

Fig. 1. ^{1}H–^{13}C COLOC Spectrum of Cyclo(L-Phe–L-Pro)$_4$ (**1**)

The solvent was a mixture of $CDCl_3$ (500 μl) and CD_3OD (150 μl).

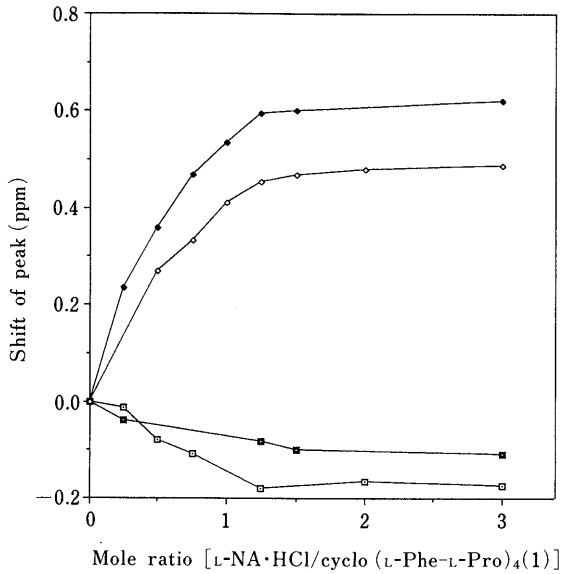

Fig. 2. The Shifts of the Signals of the Carbonyl Carbons of Cyclo(L-Phe–L-Pro)$_4$ (**1**) in the ^{13}C-NMR Spectra upon Addition of L-NA·HCl

The formation constant of the complex between **1** and L-NA·HCl was calculated by a non-linear least-squares method as 1.260×10^6 M^{-1}. —◆—, Pro²CO; —◇—, Phe¹CO; —□—, Phe²CO; —⊡—, Pro¹CO.

with one enantiomer of DL-NA·HCl is shown in Chart 1. It is suggested that the ammonium moiety of NA·HCl is bound to the carbonyl groups of **1** through hydrogen bonds in the cavity. Adrenaline hydrochloride did not form such a complex, implying that the primary amine of NA·HCl is necessary for the complex formation.

Table I shows ^{13}C-NMR spectra of **1** in the free state and as the complex with DL-NA·HCl in a mixture of $CDCl_3$ and CD_3OD. Little difference was apparent. It is suggested that essentially no conformational change of **1** occurs upon complex formation, and **1** still takes a C_2-symmetric conformation containing two *cis* peptide bonds in these solvents.

Figure 2 shows a plot of the changes of chemical shifts of the carbonyl carbons of **1** in the ^{13}C-NMR spectra *vs.* the mole ratio of L-NA·HCl to **1**. Saturation of each titration curve is attained at a mole ratio of about 1 : 1. It was suggested that the stoichiometry of the complex of **1** with L-NA·HCl is 1 : 1. The formation constant of the complex was calculated by a non-linear least-squares method as 1.260×10^6 M^{-1}. Furthermore, the shifts of the signals of Pro²CO and Phe¹CO are large, while those of Phe²CO and Pro¹CO are relatively small. This implies that the hydrogen bonds were formed between the car-

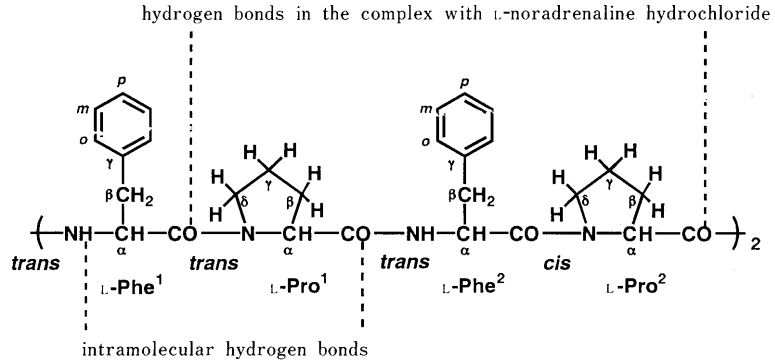

Fig. 3. The Positions of the Hydrogen Bonds in the Complex of Cyclo(L-Phe–L-Pro)$_4$ (**1**) with L-NA·HCl

bonyl groups of Pro^2 and Phe^1 residues of **1**, and the ammonium moiety of L-NA·HCl (Fig. 3).

Experimental

^{13}C-NMR and COLOC spectra were determined with a Bruker AM-400 in a mixture of $CDCl_3$ and CD_3OD at 25 °C using tetramethylsi lane (TMS) as an internal standard.

Complex Formation of Cyclo(L-Phe–L-Pro)$_4$ (1) with DL-NA·HCl A solution of 1 eq of DL-NA·HCl (racemic form) (3.085 mg, 1.50×10^{-5} mol) in CD_3OD (150 μl) was added to a solution of cyclo(L-Phe–L-Pro)$_4$ (**1**) (14.640 mg, 1.50×10^{-5} mol) in $CDCl_3$ (500 μl).

Acknowledgements We thank Miss A. Imajyuku, Miss K. Emori, Mr. Y. Kawai, Miss M. Kawahara, Mr. A. Yasuhara, and Miss T. Yamazaki for experimental assistance.

References and Notes

1) S. Kimura, Y. Imanishi, *Biopolymers*, **22**, 2191 (1983).
2) C. M. Deber, E. R. Blout, *J. Am. Chem. Soc.*, **96**, 7566 (1974).