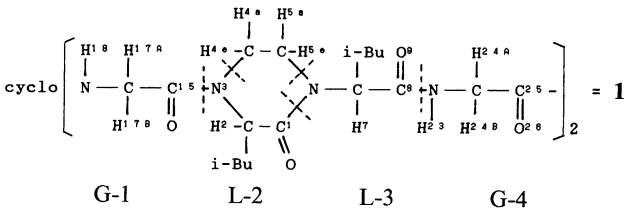
Two Conformers and Aggregation of Cyclic Octapeptide, *cyclo*(G-eLL-G)$_2$, in Chloroform

Hiroyuki MIYAKE,* Yoshitane KOJIMA,* Tetsushi YAMASHITA,and Akio OHSUKA
Department of Chemistry, Faculty of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558
(Received September 13, 1991)

Synopsis. In chloroform, *cyclo*(G-eLL-G)$_2$ exists as an equilibrium of two conformers, and aggregates from the monomer to the oligomer with an increase in the peptide concentration, owing to the intermolecular hydrogen bonds formed between the amide hydrogen and the oxygen.

The solution conformations of various cyclic peptides[1-4] have been studied widely, since they are important in the research of both naturally occurring and synthetic peptides, enzyme model, ionophore and chiral recognition etc. Recently, the authors have reported that a cyclic octapeptide, *cyclo*(G-eLL-G)$_2$ (**1**), containing glycine (G) and *N*,*N*′-ethylene-bridged (*S*)-leucyl-(*S*)-leucine(eLL) can distinguish (*R*)- and (*S*)-amine salts[5,6] and effectively bind alkaline earth metal ions.[7]

In this article, **1** was examined by NMR, FTIR, and molecular-weight measurements in order to understand its behavior in organic solvents. It was found from these results that **1** exists in equilibrium between two conformers in chloroform, referring to the ^{1}H NMR data of its unit (Boc-G-eLL-OH=**2**, Boc: *t*-butoxycarbonyl), though **1** has only one conformer in CD_3CN[7] and dimethyl sulfoxide(DMSO)-d_6. Moreover, in chloroform, one conformer of **1** aggregates each other with intermolecular hydrogen bonds as the concentration increases. The structures and numberings of **1** are shown as follows:



Experimental

Cyclic peptide **1** and its unit **2** were prepared according to the method[5] reported earlier. The ^{1}H and ^{13}C NMR measurements of **1** were carried out in $CDCl_3$ and DMSO-d_6 at 28—55 °C using TMS as an internal standard on JEOL GX-400. The concentrations were 1—40 mmol dm^{-3}. All of the signals were assigned by ^{1}H NMR, changing the measured temperature, and by two-dimensional (COSY and NOESY) and selective decoupling methods.[6,7] The FTIR spectra of **1** were obtained in spectrometric-grade chloroform at 28 °C through a range of concentrations (0.5—20 mmol dm^{-3}) in an NaCl cell with a Nicolet 5ZDX-FTIR. The molecular weight of **1** was measured in chloroform at 36 °C using benzil as a reference by means of a Knauer vapor pressure osmometer.

Results and Discussion

Two Conformers of 1. Table 1 shows the ^{1}H NMR chemical shifts (ppm) of **1**. Peptide **1** is in equilibrium between **1A** (5.63 and 3.68 ppm for H^2 and H^{4e}, respectively) and **1B** (4.62 ppm for both of H^2 and H^{4e}), whose peptide bonds between G-1 and L-2 are trans and cis, respectively, comparing the data obtained for **2**.[6,8] As shown in Fig. 1, **1A** exists predominantly, compared to **1B**. Moreover, the ratios of **1B** to **1A** become higher with the elevation of temperature, as shown by the change in the amide proton(H^{18} and H^{23}) signals. The nuclear overhauser effect (NOE) signals observed between H^7 and H^{23} of **1A** was useful for establishing the assignment of the signals of **1A**.

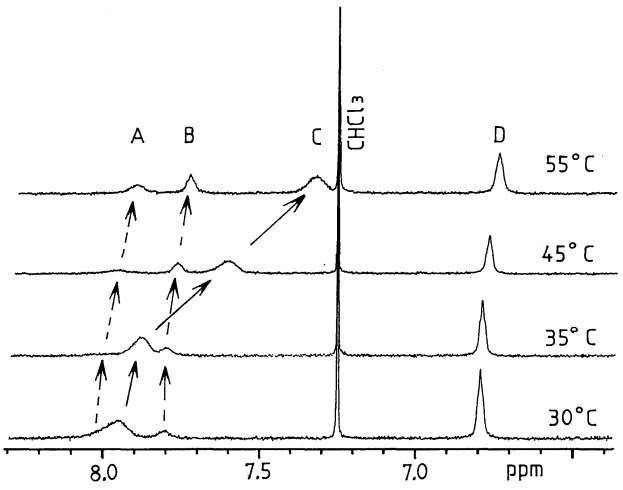


Fig. 1. NMR spectra of amide protons of **1** at various temperatures in $CDCl_3$. [**1**]=10 mmol dm^{-3}. H^{23} of **1B** (A), H^{18} of **1B** (B), H^{23} of **1A** (C), H^{18} of **1A** (D).

Table 1. ^{1}H NMR Chemical Sifts of *cyclo*(G-eLL-G)$_2$ in $CDCl_3$ at 50 °C

Form	Chemical shift, δ/ppm										
	H^{18}	H^{17A,B}		H^2	H^{4a}	H^{4e}	H^{5a}	H^{5e}	H^7	H^{23}	H^{24A,B}	
1A	6.75	3.90		5.63	3.44	3.68	3.55	3.36	5.27	7.48	4.31	3.53
1B	7.74	4.43	3.48	4.62	3.07	4.62	3.39	3.24	5.40	7.92	4.43	3.32

Also, the NOESY spectra shows the chemical exchange between each H^{18} (H^{17A}, H^{17B}, H^{2}, H^{4e}, H^{7}, H^{23}, H^{24A}, and H^{24B}) signals of **1A** and **1B**, supporting the existence of the equilibrium between **1A** and **1B**. Moreover, the chemical shifts of H^{17A} and H^{17B} indicate one signal (3.90 ppm) for **1A**, and two signals (4.43 and 3.48 ppm) for **1B** (see Table 1), so that the G-1 methylene group of **1B** is more rigid than that of **1A**.

From the above results and Corey–Pauling–Koltun (CPK) modeling, it is suggested that all peptide bonds of **1A** and **1B**, except for the peptide bond between G-1 and L-2 of **1B**, are trans, and that **1A** and **1B** each have different C_2 symmetry structures.

Aggregation of 1. Previously, other workers[2,3,9,10] examined the molecular conformations and aggregation of cyclic peptides in chloroform at various temperatures and concentrations, since the peptide–solvent interaction is weaker than the peptide–peptide one. Therefore, the temperature and concentration dependence of **1** was examined in $CDCl_3$ by NMR measurements. As shown in Fig. 1, an amide proton (H^{23}=C) of **1A** shifts up markedly with elevation of temperature, suggesting that this proton forms an intermolecular hydrogen bond with the amide oxygen. The temperature coefficients[9] (ppm/deg) of the amide protons of **1A** and **1B** derived from Fig. 1 by a least-squares method are -2.66×10^{-3} and -26.31×10^{-3} for H^{18} and H^{23} of **1A**, and -3.62×10^{-3} and -5.37×10^{-3} for those of **1B**, respectively. On the other hand, the temperature coefficients of cyclic peptide **1** were -3.68×10^{-3} and -5.92×10^{-3} for two amide protons, when the ^1H NMR measurement was carried out in DMSO-d_6 whose hydrogen-bond formation ability is stronger than that of $CDCl_3$. These values of **1** are almost identical with those of the other amide compounds[11] having no intramolecular hydrogen bonds, suggesting a possibility to exclude the existence of intramolecular hydrogen bonds. Figure 2 reveals the chemical shift changes of the amide protons and carbons of **1A** vs. various concentrations in $CDCl_3$. H^{23} shows the marked down-field shift with an increase in the concentration, due to the intermolecular hydrogen bond. Also, Fig. 2 indicates that two amide carbons (C^8 and C^{25}) shift down. The down-field shift of C^8 may not be attributed to the direct participation of the amide oxygen (O^9) in the intermolecular hydrogen bond, but the neighboring effect of H^{23}. Accordingly, another amide oxygen (O^{26}) is considered to form the intermolecular hydrogen bond with H^{23}, as supported by CPK modeling.

Figure 3 indicates the NH absorption spectra of **1** obtained for various concentrations with IR. The intermolecular hydrogen bonded NH absorption (3312 cm^{-1})[10,12] becomes larger as the concentration increase and disappears significantly below the dilute concentration (5×10^{-4} mol dm^{-3}), supporting the hydrogen bond between the amide hydrogen and oxygen of **1** at the concentrations over ca. 10^{-3} mol dm^{-3}.

The molecular weight measurement of **1** indicated that the mean molecular numbers of aggregation of **1** are 1.4, 1.9, 2.0, and 2.8 for the concentrations of 10.2, 20.0, 25.0, and 40.0 mmol dm^{-3}, respectively. As shown schematically in Fig. 4, these results suggest that **1A** aggregates from the monomer to the dimer, and then the oligomer,

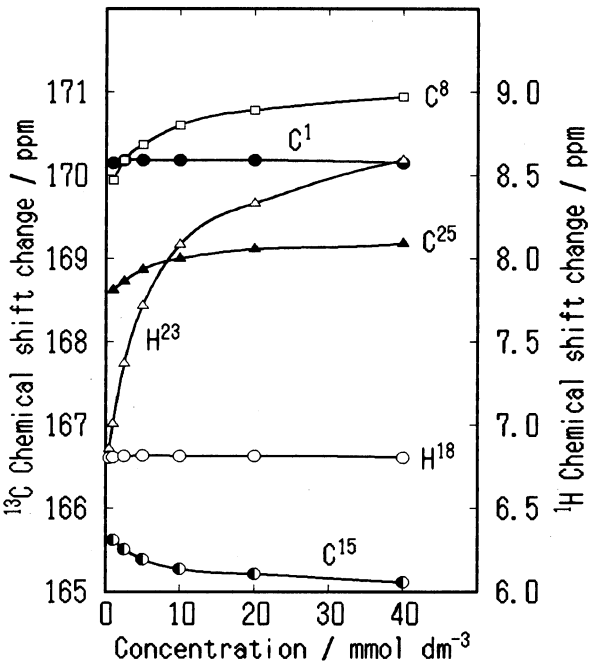


Fig. 2. Chemical shift changes of amide protons (H^{18}, H^{23}) and carbons (C^{15}, C^1, C^8, and C^{25}) of **1A** for the various concentrations in $CDCl_3$ at 28 °C.

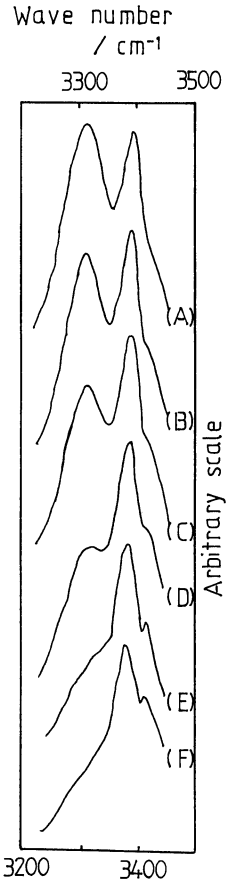


Fig. 3. Amide NH absorptions of **1** in chloroform at 28 °C. The concentrations of (A), (B), (C), (D), (E), and (F) are 2×10^{-2}, 1×10^{-2}, 6×10^{-3}, 3×10^{-3}, 1×10^{-3}, and 5×10^{-4} mol dm^{-3}, respectively.

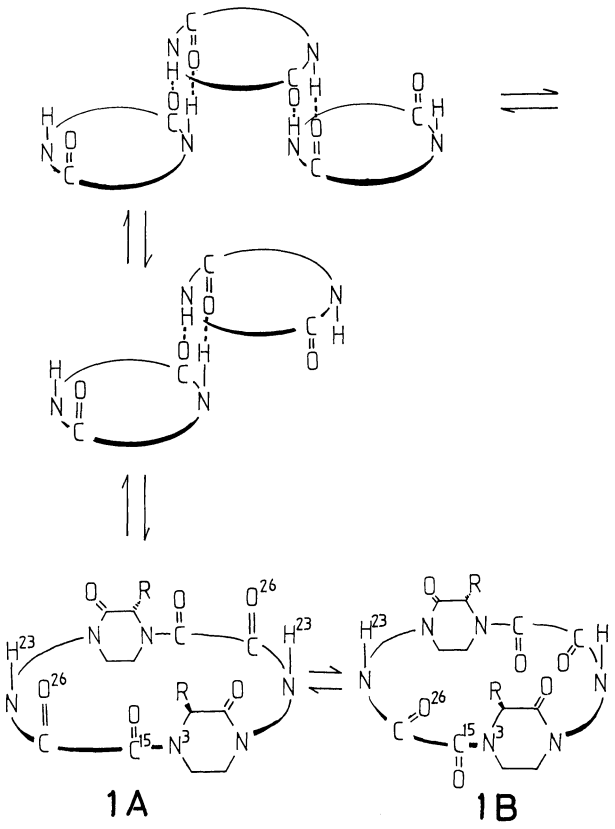


Fig. 4. Schematic representation of the equilibrium between **1A** and **1B**, and the aggregation of **1A** in chloroform.

with increasing the concentration, owing to the intermolecular hydrogen bonds formed between H^{23} and O^{26} of **1A**, as reported earlier by Shimizu et al.[10]

References

1) E. Ozeki, S. Kimura, and Y. Imanishi, *Int. J. Pept. Protein Res.*, **34**, 111 (1989).
2) K. D. Kopple, K. N. Parameswaran, and J. P. Yonan, *J. Am. Chem. Soc.*, **106**, 7212 (1984).
3) L. M. Gierasch, C. M. Deber, V. Madison, C.-H. Niu, and E. R. Blout., *Biochemistry*, **20**, 4730 (1981).
4) J.-I. Hong, S. K. Namgoong, A. Bernardi, and W. C. Still, *J. Am. Chem. Soc.*, **113**, 5111 (1991).
5) Y. Kojima, T. Yamashita, M. Washizawa, and A. Ohsuka, *Makromol. Chem., Rapid Commun.*, **10**, 121 (1989).
6) H. Miyake, K. Shibata, Y. Kojima, T. Yamashita, and A. Ohsuka, *Makromol. Chem., Rapid Commun.*, **11**, 667 (1990).
7) Y. Kojima, Y. Ikeda, H. Miyake, I. Iwado, K. Hirotsu, K. Shibata, T. Yamashita, A. Ohsuka, and A. Sugihara, *Polym. J.*, **23**, 1359 (1991).
8) Tripeptide **2** consists of two conformers (**2A** and **2B**) whose peptide bonds between G and eLL are trans for **2A** (5.03 and 3.77 ppm for H^2 and H^{4e}, respectively) and cis for **2B** (4.34 ppm for both of H^2 and H^{4e}) in $CDCl_3$, referring the previous data[6] of **2** in CD_3CN. The numbering of eLL of **2** follows that of **1**.
9) L. G. Pease and C. Watson, *J. Am. Chem. Soc.*, **100**, 1279 (1978).
10) T. Shimizu, Y. Tanaka, and K. Tsuda, *Int. J. Pept. Protein Res.*, **27**, 554 (1986).
11) M. Ohnishi and D. W. Urry, *Biochem. Biophys. Res. Commun.*, **36**, 194 (1969).
12) S.-I. Mizushima, T. Shimanouchi, M. Tsuboi, and T. Arakawa, *J. Am. Chem. Soc.*, **79**, 5357 (1957).