Studies on the β-Turn of Peptides. VI.[1] Nuclear Magnetic Resonance Study on the Conformations of *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-Nitroanilides Related to the β-Turn Part of Gramicidin S

Tsutomu HIGASHIJIMA,† Kazuki SATO,* and Ukon NAGAI

Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194

† *Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Bunkyo-ku, Tokyo 113*

(Received April 21, 1983)

The conformations of *N*-(2,4-dinitrophenyl)-L-Leu-X-L-Pro-L-Val *p*-nitroanilides (**I**, X=D-Ala; **II**, X=Gly; **III**, X=L-Ala) were studied by nuclear magnetic resonance and circular dichroism spectroscopies. The L-Val-NH group of compound **I** takes part in an intramolecular hydrogen bonding, and the folded conformation is considered to be type II′ β-turn structure as in the case of [D-Ala$^{4,4'}$]-gramicidin S. The populations of β-turn conformer were considered to be in the order of **I**>**II**>**III**≈0 in methanol and *N*,*N*-dimethylformamide solutions, and decreased in dimethyl sulfoxide solution. Compound **II** in dimethyl sulfoxide and **III** in all the three solvents do not take a β-turn conformation. The data obtained from NMR and CD experiments showed a good correlation between the magnitudes of the Cotton effects (250—400 nm) and the populations of the β-turn conformation of these peptides. The proximity of the two chromophores was ascertained directly for compound **I** in *N*,*N*-dimethylformamide solution by nuclear Overhauser effects experiment. In addition, the β-turn preference of these peptides were in good accordance with the antibiotic activities of the gramicidin S analogs having the same tetrapeptide sequences at their β-turn parts.

Gramicidin S (GS) is a cyclic decapeptide antibiotic with the primary structure *cyclo*(-L-Val^1-L-Orn^2-L-Leu^3-D-Phe^4-L-Pro^5-L-Val$^{1'}$-L-Orn$^{2'}$-L-Leu$^{3'}$-D-Phe$^{4'}$-L-Pro$^{5'}$-).[2] Conformation of GS consists of the intramolecular antiparallel β-sheet with four hydrogen bonds between L-Val and L-Leu residues and two β-turns (type II′) around the D-Phe-L-Pro sequences (Fig. 1).[3] The characteristic feature of the conformation is the orientation of side chains in which the charged L-Orn side chains are on one side and the hydrophobic L-Val and L-Leu side chains on the other side of the molecule. The conformation of GS is considered to be stabilized not only by the four intramolecular hydrogen bonds but also by the stable β-turns formed by two D-Phe-L-Pro sequences. Izumiya *et al.* summarized the antibacterial activities of many GS analogs.[4] The activities of GS analogs were correlated to their conformations.[5] For example, the activities and the populations of GS-type β-sheet conformer were in the order of GS≈[D-Ala$^{4,4'}$]-GS>[Gly$^{4,4'}$]-GS≫[L-Ala$^{4,4'}$]-GS≈0.[6] It is of interest to study the β-turn preference of the amino acid sequences at the corner positions of GS analogs.

In the previous paper, we gave an outline of a new method to study the β-turn conformation of linear tetrapeptides.[7] *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-nitroanilides (Dnp-tetrapeptide-pNA's) exhibited characteristic CD spectra above 250 nm, and the Cotton effects were considered to be due to the exciton coupling of the transition moments in the two terminal chromophores. The magnitudes of the Cotton effects near 310 and 350 nm were expected to reflect well the β-turn preference of the peptides.

This paper describes ^{1}H and ^{13}C nuclear magnetic resonance (NMR) studies on the conformations of tetrapeptide derivatives related to the β-turn part of GS, Dnp-L-Leu-X-L-Pro-L-Val-pNA (**I**, X=D-Ala; **II**, X=Gly; **III**, X=L-Ala), to study a correlation between the Cotton effects and the conformations of these peptides (Fig. 2). ^{1}H NMR measurements were systematically carried out including (a) temperature dependence of NH proton chemical shifts in various solutions, (b) nuclear Overhauser effects on amide, CH, and aromatic proton resonances, and (c) solvent dependences of NH and CH proton chemical shifts and spin-coupling constants ($^3J_{NH-C^{\alpha}H}$).

Experimental

Syntheses of **I**—**III** were reported previously.[8] The solvent DMSO-d_6 (99.8%) was obtained from Commissariat a l'Energy Atomique and CD_3OH (99.5%) and DMF-d_7 (99.5%) from E. Merck.

^{1}H NMR spectra were recorded on a Bruker WH-270 spectrometer (270 MHz for ^{1}H), equipped with a Bruker B-ST-100/700 temperature control unit. ^{13}C NMR spectra were recorded on a JEOL FX-90Q (22.5 MHz for ^{13}C),

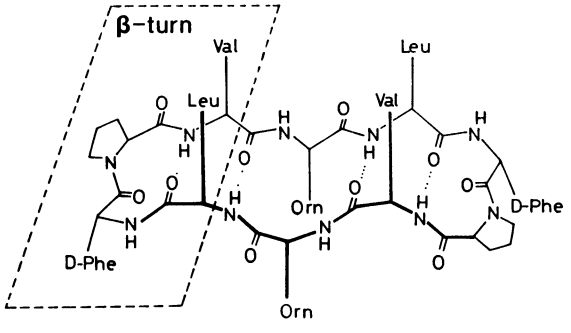

Fig. 1. β-Sheet conformation of gramicidin S.

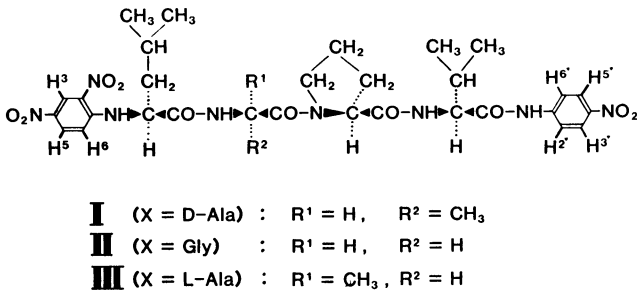

Fig. 2. Primary structures of Dnp-tetrapeptide-pNA's (**I**—**III**).

with a variable-temperature unit. Internal references for chemical shift were DSS for ^1H and TMS for ^{13}C. Nuclear Overhauser effect (NOE) data were also recorded on a Bruker WH-270 instrument at the concentration of 5 mM (1 M=1 mol dm^{-3}) at 23 °C. The FID's were collected after applying a 5-s low power saturation pulse in alternating blocks of 16 on-resonance and 16 off-resonance spectra using a SW of 3000 Hz with 16K data points for 512 spectral acquisitions each, Fourier transformed and subtracted to obtain the difference spectra.

CD spectra were measured on a JASCO J-40A spectropolarimeter at the concentration of 0.1 mM at room temperature (23±2 °C).

Results

CD Measurements. CD spectra of **I**—**III** were measured in DMF and DMSO solutions, and the results are summarized in Fig. 3 together with those measured in MeOH solutions.[7] Compound **I** having D-Ala at the second position showed the largest Cotton effects in DMF and DMSO solutions as well as in MeOH solution of the three compounds. In DMSO solution, however, the Cotton effects decreased to about 40% of those in DMF and MeOH solutions. Compound **III** showed no significant CD band near 310 nm and 350 nm in any solutions, and **II** showed intermediate CD spectra between **I** and **III**. The strong CD bands of **I** are not due to intermolecular interaction, since CD spectra of **I** showed no concentration dependences at the range from 5 mM to 0.1 mM. Accordingly, the strong CD bands are attributed to the intramolecular interaction between the two terminal chromophores in **I**.

Assignments of ^1H Resonances. The ^1H NMR spectra of **I**—**III** were assigned with the aid of spin-decoupling, H–D exchange of NH protons, and saturation transfer (between the two conformers) in CD_3OH, DMSO-d_6, and DMF-d_7 solutions as shown in Table 1. All the spectra showed two sets of resonances for most of the protons due to *cis-trans* isomerism for the X–L-Pro bonds. Chemical shifts of aromatic protons are shown in Fig. 4, and those of the other protons are listed in Table 1 together with spin-coupling constants ($^3J_{NH-C^\alpha H}$). In all the solutions, aromatic protons of major conformer were shifted to higher field in the order of **I**>**II**>**III**, while those of minor conformers did not show such significant sequence dependences (Fig. 4). In DMSO-d_6 solution, the L-Pro-C$^\alpha$H proton of the major conformer of **I** showed higher field shift (4.41 ppm) than that of the minor conformer (4.87 ppm) suggesting that major conformer had *trans* D-Ala–L-Pro bond. This assignment was ascertained by ^{13}C NMR study as described in the next section. In the other solutions and also in the other compounds (**II** and **III**), the L-Pro–C$^\alpha$H protons of the major conformers showed the similar chemical shifts to that of the major conformer of **I** in DMSO-d_6 solution. The D-Ala residue in major conformer of **I** showed small spin-coupling constants ($^3J_{NH-C^\alpha H}$) of 3.9 and 4.0 Hz in CD_3OH and in DMF-d_7 solutions, respectively, suggesting that the ϕ angle of D-Ala residue is certainly fixed in these solutions, whereas the L-Ala residue of **III** showed spin-coupling constants

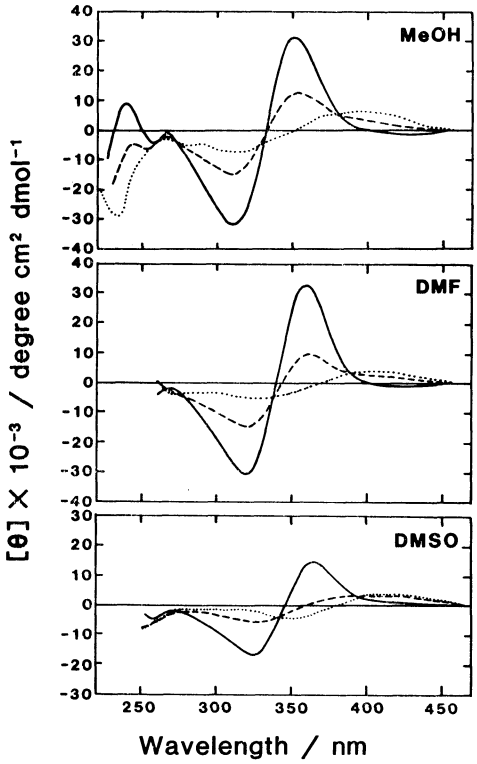

Fig. 3. CD spectra of Dnp-L-Leu-X-L-Pro-L-Val-pNA (**I**—**III**).
——: **I** (X=D-Ala), - - -: **II** (X=Gly), ……: **III** (X=L-Ala). Curves in MeOH solution were reproduced from Ref. 7 by kind permission of John Wiley & Sons, Inc.

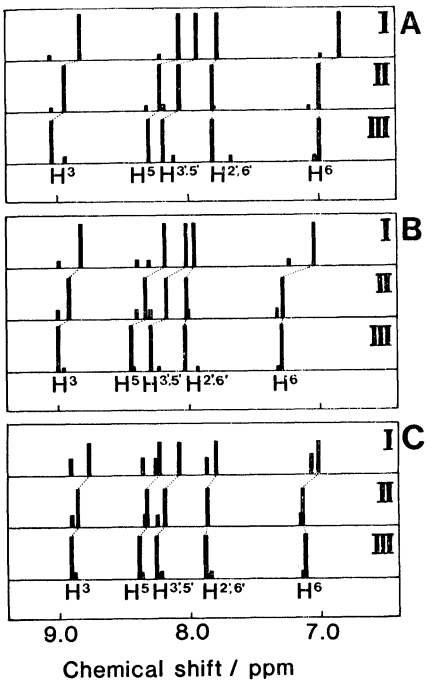

Fig. 4. Chemical shifts of aromatic protons of **I**—**III** in (A) CD_3OH in (B) DMF-d_7 and in (C) DMSO-d_6 solutions. The large bars and the small bars show the major and the minor resonances, respectively (for notation, see Fig. 2).

TABLE 1. ^{1}H NMR DATA OF Dnp-L-Leu-X-L-Pro-L-Val-pNA (I—III) AT 23 °C

Compound	Solvent	Conformer[a] %		Chemical shift from DSS ppm							$^3J_{NH-C^\alpha H}$ Hz		
				L-Leu		X		L-Pro	L-Val		pNA			
				NH	$C^\alpha H$	NH	$C^\alpha H$	$C^\alpha H$	NH	$C^\alpha H$	NH	L-Leu	X	L-Val
I	CD_3OH	*t*. 90		8.68	4.35	8.87	4.57	4.44	7.52	4.17	9.22	7.0	3.9	8.6
		c. 10												
	DMF-d_7	*t*. 83		8.83	4.66	9.19	4.69	4.45	7.45	4.30	9.57		4.0	8.8
		c. 17												
	DMSO-d_6	*t*. 65		8.72	4.55	8.90	4.59	4.41	7.59	4.20	10.00	7.8	6.1	8.3
		c. 35		8.79	4.55	8.79		4.87	8.54		10.79	8.0	7.5	8.5
II	CD_3OH	*t*. 88		8.70	4.63	8.69	4.08	4.47	7.96	4.24	9.95	7.3	13.6[b]	7.8
		c. 12		8.68		8.58			8.44					
	DMF-d_7	*t*. 78		8.92	4.76	8.83	4.21	4.54	7.92	4.83	10.27		9.2[b]	8.1
		c. 22				8.62								
	DMSO-d_6	*t*. 73		8.76	4.60	8.65	4.03	4.48	8.04	4.28	10.49	7.3	9.8	8.3
		c. 27		8.75		8.59			8.48				10.3[b]	
III	CD_3OH	*t*. 86		8.67	4.50	8.56	4.64	4.39	8.19	4.27	10.40	7.3	6.4	7.3
		c. 14		8.63		8.59			8.51		10.26			
	DMF-d_7	*t*. 88		8.94	4.73	8.76	4.77	4.59	8.07	4.44	10.73		7.0	
		c. 12												
	DMSO-d_6	*t*. 85		8.74	4.56	8.67	4.61	4.48	8.06	4.30	10.68	7.8	7.4	7.8
		c. 15												

a) *t*, *trans* X-L-Pro bond; *c*, *cis* X-L-Pro bond. b) Sum of *J* for two methylene protons.

typical to free rotation.

Assignments of ^{13}C Resonances. ^{13}C NMR is useful for the study of *cis-trans* isomerism of the X–L-Pro peptide bond.[9] Chemical shifts of the L-Pro–C^β and C^γ carbons of **I**—**III** in DMSO-d_6 solutions were determined in order to assign the two sets of resonances to the conformers with *trans* or *cis* X–L-Pro bond. Though the resonances of the L-Pro–C^γ carbons were overlapped with those of L-Leu–C^γ and C^δ carbons, those of the L-Pro–C^β carbons were clearly observed; the major and the minor resonances were 28.99 and 31.85 ppm for **I**, 29.10 and 31.97 ppm for **II**, and 28.75 and 31.56 ppm for **III**, respectively. The results indicate that major resonances can be attributed to the conformers with *trans* X–L-Pro bond.

Temperature Dependences of Proton Chemical Shifts. The temperature dependences of chemical shifts of amide proton resonances are useful for distinguishing between "exposed" and "intramolecularly hydrogen-bonded" amide proton;[10] exposed protons exhibit larger temperature coefficients than hydrogen-bonded amide protons. The temperature dependences of amide proton chemical shifts are shown in Fig. 5 for major conformers of **I**—**III**. The L-Val–NH proton of **I** showed much smaller temperature dependences (-2.72×10^{-3} and -2.13×10^{-3} ppm °C^{-1}) than the D-Ala–NH proton (-7.06×10^{-3} and -5.93×10^{-3} ppm °C^{-1}) in CD_3OH and in DMF-d_7 solutions, respectively, indicating that the L-Val–NH proton of **I** was involved in stable hydrogen bonding. In DMSO-d_6 solution, the L-Val–NH proton of **I** also showed smaller temperature dependence (-3.44×10^{-3} ppm °C^{-1}) than the D-Ala–NH proton (-4.59×10^{-3} ppm °C^{-1}), though the difference between the two NH protons was not so clear as that in CD_3OH or DMF-d_7 solution. The L-Val–NH proton of **II** showed a little smaller temperature dependence than the Gly–NH proton in CD_3OH and in DMF-d_7 solutions, though the values (-6.85×10^{-3} and -5.00×10^{-3} ppm °C^{-1}, respectively) were too large for strongly hydrogen-bonded species. The values for the L-Val–NH and Gly–NH protons of **II** were reversed in DMSO-d_6 solution. The L-Val–NH proton of **III** always showed larger temperature dependences than the L-Ala–NH proton. The temperature dependences of L-Val–NH protons were in the order of **I**<**II**<**III** in any solvents. Most of the protons other than amide ones showed little temperature dependences, suggesting that the conformations of **I**—**III** did not change significantly in the temperature range of these experiments.

Nuclear Overhauser Effects. Nuclear Overhauser effects (NOE's) are useful for the conformational analysis of peptides, since the effects are inversely proportional to the sixth power of the distance between the irradiated and observed protons. NOE's were observed for **I**—**III** in DMF-d_7 solutions at 23 °C as shown in Table 2. It is noteworthy that NOE's were observed for Dnp–H^3 resonance and D-Ala–$C^\alpha H$ proton resonance by the irradiation of pNA aromatic protons (Fig. 6A) and L-Pro–$C^\alpha H$ proton (Fig. 6B) resonances, respectively. On the other hand, the NOE enhancement was not observed for **III** between Dnp and pNA aromatic protons.

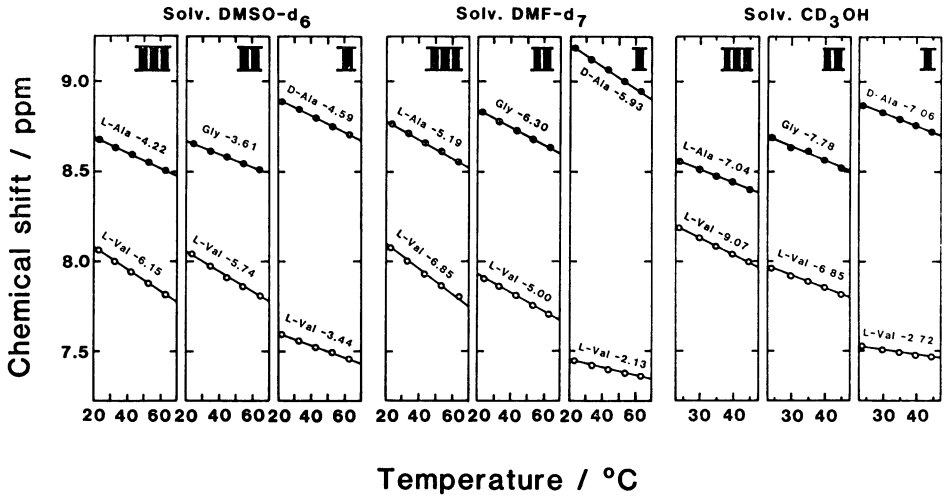

Fig. 5 Temperature dependences of amide proton chemical shifts of major resonances of **I**—**III**. Numbers on the lines refer to $10^3 \times$ temperature coefficient in ppm $°C^{-1}$. ○: L-Val⁴-NH, ●: X²-NH.

TABLE 2. NOE OBSERVATIONS FOR Dnp-L-Leu-X-L-Pro-L-Val-pNA (**I**—**III**) IN DMF-d_7 AT 23 °C[a]

Compound	Signal irradiated	Signal observed (%)
I	L-Leu-C^αH(+D-Ala-C^αH)	Dnp-H^6 (19.4), pNA-$H^{2'-6'}$ (<1), L-Leu-$C^{\beta,\gamma}$H (2), L-Leu-C^δH (<1)
	D-Ala-C^αH	Dnp-H^6 (14.5), L-Pro-C^δH (2.7), L-Pro-C^δH′ (1.7), L-Leu-$C^{\beta,\gamma}$H (2)
	L-Pro-C^αH	L-Pro-C^βH (5.7), L-Val-NH (2.3)
	L-Val-C^αH	L-Val-C^βH (6.1), L-Val-C^γH (<1)
	L-Pro-C^δH[b]	D-Ala-C^αH (>3.7), L-Pro-C^δH′ (7.5), L-Pro-C^βH (2.2)
	L-Pro-C^δH′ [b]	D-Ala-C^αH (>3.2), L-Pro-$C^{\beta,\gamma}$H (1.8)
	D-Ala-NH(+pNA-NH)	L-Leu-C^αH (6.3), D-Ala-C^αH (3.4), pNA-$H^{2',6'}$ (2.8), L-Val-C^αH (2)
	Dnp-H^6	Dnp-H^5 (18.9), L-Leu-C^αH (10.3)
	pNA-$H^{2',3',5',6'}$	Dnp-H^3 (2.1)
II	L-Leu-C^αH	Dnp-H^6 (18.7), L-Leu-C^αH (2.1)
	Gly-C^αH	L-Pro-C^δH (2.8), L-Pro-C^δH′ (4.7)
	L-Pro-C^αH	L-Val-NH (4.6)
	L-Val-C^αH	pNA-$H^{2',6'}$ (<1), L-Val-NH (3), L-Val-C^βH (4.7)
	L-Pro-C^δH	Gly-C^αH (small)
	L-Pro-C^δH′	Gly-C^αH (2.5)
	L-Leu-NH+pNA-NH+Dnp-H^3	pNA-H^2 (3.8), L-Leu-C^αH (3.3), L-Val-C^αH (4.5)
	Dnp-H^6	Dnp-H^5 (25), Dnp-H^3 (<1), L-Leu-C^αH (9.5)
	Dnp-H^5	Dnp-H^6 (6.8)
	pNA-$H^{2',6'}$	Dnp-H^3 (1.9)
	pNA-$H^{3',5'}$	Dnp-H^3 (3.8)
III	L-Leu-C^αH(+L-Ala-C^αH)	Dnp-H^6 (16.9), L-Ala-NH (4.3), L-Pro-C^δH (2.2), L-Leu-C^βH (2)
	L-Ala-C^αH(+L-Leu-C^αH)	L-Pro-C^δH (3.3), L-Leu-C^βH (3.8), Dnp-H^6 (14.7), L-Ala-NH (3.4)
	L-Pro-C^αH	L-Val-NH (>4.3), L-Pro-$C^{\beta,\gamma}$H (small)
	L-Val-C^αH	L-Val-C^βH (4.2)
	L-Ala-C^βH	L-Ala-C^αH (>5.5)
	L-Pro-C^δH	L-Ala-C^αH (>11.6), L-Pro-$C^{\alpha,\gamma}$H (small)
	L-Val-NH	L-Pro-C^αH (5.9), L-Val-C^αH (3)
	Dnp-H^6	Dnp-H^5 (16.2), Dnp-H^3 (1), L-Leu-C^αH (>6.7)
	Dnp-H^5	Dnp-H^3 (2), Dnp-H^6 (6.7)

a) Signals of minor conformer with *cis* X-L-Pro bond are omitted. b) C^δH and C^δH′ are a lower and a higher field signals, respectively.

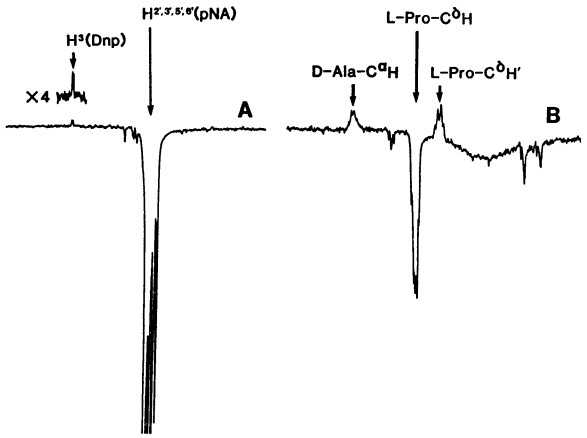

Fig. 6. NOE observations of **I** in DMF-d_7 solution. Signals irradiated were (A) pNA-$H^{2',3',5',6'}$ protons and (B) L-Pro-$C^{\delta}H$ proton.

Discussion

Cis-trans *Isomerism for X-L-Pro Bonds.* Major resonances of **I**—**III** in DMSO-d_6 solutions were attributed to the conformers with *trans* X–L-Pro bonds by ^{13}C NMR resonances. In CD_3OH and DMF-d_7 solutions, also, major resonances of **I**—**III** are considered to be due to the *trans* isomer, since all the major L-Pro–$C^{\alpha}H$ proton resonances showed similar chemical shifts to those in DMSO-d_6 solution. Populations of *trans* conformers of **I** and **II** increased in the order of in DMSO-d_6<DMF-d_7<CD_3OH solutions (Table 1). On the other hand, those of **III** did not show such large solvent dependences. It seems that the *cis* conformers of **I**—**III** are not in the fixed conformations in all solvents examined, since they showed similar chemical shifts of aromatic and some other protons for the three compounds (Fig. 4 and Table 1). These are ascertained by the large temperature dependences of the L-Val and X (X=D-Ala, Gly, or L-Ala) NH proton chemical shifts for the *cis* conformers. In contrast, the *trans* conformers of **I**—**III** showed large sequence and solvent dependences of chemical shifts for most of the NH and $C^{\alpha}H$ protons, indicating that their *trans* conformations depend on sequence and solvent. Detailed discussions are described hereafter for the conformations of *trans* (major) isomer of **I**—**III**.

Intramolecular Hydrogen Bonding. The L-Val–NH proton of **I** is concerned in a strong hydrogen bonding in CD_3OH or DMF-d_7 solution as shown in Fig. 5. In DMSO-d_6 solution, also, the L-Val–NH group is hydrogen-bonded. The hydrogen bonding is intramolecular, since **I** showed no concentration dependence of CD spectra. On the other hand, the L-Val–NH proton chemical shifts of **III** showed such large temperature dependences as that of *N*-methylacetamide in all the three solvents, indicating that the L-Val–NH group is not involved in an intramolecular hydrogen bonding. The temperature coefficients of L-Val–NH chemical shifts of **II** are intermediate of **I** and **III** for the three solutions. Accordingly, it seems that the magnitudes of the hydrogen bonding of the L-Val–NH protons of **I**—**III** are in the order of **I**>**II**>**III**≈0 in CD_3OH and DMF-d_7 solutions and **I**>**II**≥**III**≈0 in DMSO-d_6 solution.

The two CO groups of L-Leu (β-turn) and D-Ala (γ-turn) are the candidate for the acceptor of the intramolecular hydrogen bonding of L-Val–NH proton for compound **I**. It is noteworthy that the coupling constant ($^3J_{NH-C^{\alpha}H}$) of D-Ala residue for compound **I** is as small as 3.9 Hz and 4.0 Hz in CD_3OH and DMF-d_7 solution, respectively. The coupling constant ($^3J_{NH-C^{\alpha}H}$) of L-Ala residue is 8.5 Hz for Ac–L-Ala–$NHCH_3$, and 7.1 Hz for Ac–L-Ala–(*trans*)-L-Pro–OH,[11] which do not have specific conformations. These indicate that the ϕ angle of D-Ala residue of compound **I** is fairly fixed. This holds for the β-turn conformation (L-Leu^1–CO···HN–L-Val^4) of compound **I**: The ϕ angle of the second [$(i+1)$th] residue of β-turn conformation is known to be −60° for L-amino acid or +60° for D-amino acid, which indicates that the coupling constant ($^3J_{NH-C^{\alpha}H}$) for the residue is *ca.* 3.5 Hz.

In this series of compounds, the weaker is the hydrogen bonding of L-Val residue, the larger is the coupling constant ($^3J_{NH-C^{\alpha}H}$) of X-residue (X=L-Ala or D-Ala). On the other hand, the ϕ angle will not be fixed in the case of γ-turn conformation (D-Ala^2–CO···HN–L-Val^4).

*Type of the β-Turn of **I** and **II**.* [D-$Ala^{4,4'}$]-GS, which has similar conformation to that of GS,[6] is a good measure to estimate the type of the β-turn of **I**, though it is difficult usually to discriminate exactly the type of the β-turn of such a linear peptide as **I**. The NH protons of **I** in CD_3OH and DMF-d_7 solutions showed similar chemical shifts, coupling constants and temperature dependences to those of [D-$Ala^{4,4'}$]-GS in DMSO-d_6 solution (chemical shifts: D-Ala, 8.92 ppm; L-Val, 7.40 ppm; $^3J_{NH-C^{\alpha}H}$: D-Ala 3.7 Hz; L-Val, 8.8 Hz; temperature dependences: D-Ala, -7.50×10^{-3} ppm $°C^{-1}$; L-Val, -2.52×10^{-3} ppm $°C^{-1}$). Furthermore, NOE observation between D-Ala–$C^{\alpha}H$ and L-Pro–$C^{\delta}H$ protons of **I** indicates the proximity of these two protons (Fig. 6B). These results suggest that **I** takes the same type II′ β-turn as that of [D-$Ala^{4,4'}$]-GS. Since the corresponding residue of **II** to the D-Ala of **I** for the β-turn conformation is Gly, the type of the β-turn of **II** is expected to be similar to that of **I** (type II′).

Interaction between Dnp and pNA Chromophores. As the results of β-turn formation of peptide backbone, N- and C-terminal chromophores approach to each other. Certainly, higher-field shifts of aromatic proton chemical shifts of Dnp and pNA groups in *trans* isomer of **I** are due to ring-current effects, indicating the proximity of the two terminal chromophores (Fig. 4). On the other hand, aromatic protons of both isomers of **III** did not show large higher-field shifts but show the similar chemical shifts as those in *cis* isomer of **I**. Aromatic protons in the *trans* isomer of **II** showed intermediate higher-field shifts between those of the *trans* isomer of **I** and **III**. NOE observation between the aromatic protons of Dnp and pNA chromophores in the *trans* isomer of **I** is a direct evidence of the approach of these two chromophores (Fig. 6A).

Correlation between CD Spectra and β-Turn Conformations. The characteristic CD spectra of Dnp–tetrapeptide–pNA's are considered to be due to the exciton coupling of the transition moments in the two terminal chromophores. The order of Cotton effects of **I**—**III** shown in Fig. 3 is in a good accordance with the order of populations of β-turn conformers in **I**—**III** estimated from NMR measurements: In CD_3OH and in DMF-d_7 solutions, **I** has higher population of β-turn conformers than **II**, and **III** does not take a β-turn conformation. In DMSO-d_6 solution, **I** has some populations of β-turn conformer, though at a lower level than those in CD_3OH and in DMF-d_7 solutions, while **II** has little population of β-turn conformer as well as **III**. These good agreements suggest that the CD spectra of Dnp–pNA derivatives of tetrapeptides are good reflections of β-turn preference of the peptides.

Structure-activity Relationship of Gramicidin S Analogs. The β-turn preferences of **I**—**III** showed good correlation with the activities of the GS analogs having the same tetrapeptide sequences as **I**—**III** at their β-turn parts. This means that conformational preference of partial sequences of GS analogs seem to play a significant role for determining their biological activities as the results of stabilization of their active conformations.

We thank Professor Tatsuo Miyazawa of The University of Tokyo for helpful discussion.

References

1) Part V of this series: M. Kawai, K. Sato, R. Sugawara, and U. Nagai, *Bull. Chem. Soc. Jpn.*, **56**, 3005 (1983).

2) The abbreviations used in this paper are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations: Dnp, 2,4-dinitrophenyl; pNA, *p*-nitroanilide; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; TMS, tetramethylsilane; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; FID, free induction decay.

3) A. Stern, W. A. Gibbons and L. C. Craig, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 734 (1968); R. Schwyzer and U. Ludescher, *Biochemistry*, **7**, 2591 (1968).

4) N. Izumiya, T. Kato, H. Aoyagi, M. Waki, and M. Kondo, "Synthetic Aspects of Biologically Active Cyclic Peptides-Gramicidin S and Tyrocidines, "Kodansha, Tokyo, and Halsted Press, New York (1979), pp. 49—70.

5) Ref. 4, pp. 71—97.

6) M. Kawai and U. Nagai, *Biopolymers*, **17**, 1549 (1978); T. Higashijima, M. Tasumi,T. Miyazawa, M. Kawai, and U. Nagai, "Peptide Chemistry 1977; Proceedings of the 15th Symposium on Peptide Chemistry," ed by T. Shiba, Protein Research Foundation, Osaka (1978), pp. 97—102.

7) K. Sato, M. Kawai, and U. Nagai, *Biopolymers*, **20**, 1921 (1981).

8) K. Sato, M. Kawai, and U. Nagai, *Bull. Chem. Soc. Jpn.*, **56**, 1527 (1983).

9) C. Deber, V. Madison, and E. R. Blout, *Acc. Chem. Res.*, **9**, 106 (1976) and references therein.

10) M. Ohnishi and D. W. Urry, *Biochem. Biophy. Res. Commun.*, **36**, 194 (1969); K. D. Kopple, M. Ohnishi, and A. Go, *Biochemistry*, **8**, 4087 (1969).

11) V. F. Bystrov, "Progress in Nuclear Magnetic Resonance Spectroscopy, 10" ed by J. W. Emsley, J. Feeney, and L. H. Sutcliffe, Pergamon Press, Oxford (1976), pp.41—81.