Possible Interpretation of Inactivity of Novel Gramicidin S Analogs by CD Spectral Analysis of Tetrapeptide Derivatives Related to the Analogs[1)]

Kazuki SATO, Ukon NAGAI,* and Makoto TAMAKI†
Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194
†*Department of Chemistry, Faculty of Science, Toho University, Miyama, Funabashi, Chiba 274*
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Synopsis. CD spectral analysis of chromophoric derivatives of tetrapeptides related to the β-turn part of [D-Val$^{1,1'}$]- and [D-Val$^{1,1'}$, L-Phe$^{4,4'}$]-gramicidin S indicated that they had very low preference for β-turn formation. The results suggested that neither analog could take gramicidin S-like β-sheet conformation. It seems noteworthy that the side-chain bulkiness of the 4th amino acid of a tetrapeptide affects the stability of β-turn.

In the previous papers, we reported that the CD spectra of *N*-(2,4-dinitrophenyl) tetrapeptide *p*-nitroanilides reflected well the β-turn preference of the tetrapeptide sequences.[2-4)] β-Turn preferences of the tetrapeptides related to the β-turn part of gramicidin S (GS), *cyclo*(-L-Val$^{1,1'}$-L-Orn$^{2,2'}$-L-Leu$^{3,3'}$-D-Phe$^{4,4'}$-L-Pro$^{5,5'}$-)$_2$ (Fig. 1),[5)] had good correlation with antibiotic activities of the GS analogs having similar tetrapeptides sequences at their β-turn parts.[3)]

Recently, one of the authors (M. Tamaki) and his co-workers synthesized [D-Val$^{1,1'}$]- and [D-Val$^{1,1'}$, L-Phe$^{4,4'}$]-GS and found that both analogs were inactive against Gram-positive bacteria.[6)] In this paper, we attempted to explain the inactivity of the analogs by β-turn preference of their partial sequences. First, we synthesized Dnp-L-Ala-D-Ala-L-Pro-D-Ala-pNA (**2D**) and Dnp-L-Ala-L-Ala-L-Pro-D-Ala-pNA (**2L**) as models of the partial sequences of [D-Val$^{1,1'}$]- and [D-Val$^{1,1'}$, L-Phe$^{4,4'}$]-GS, respectively. Especially, **2D** was of interest due to the LDLD sequence which have never been examined yet.

Figure 2 shows the CD spectra of **2D** and **2L** together with those of Dnp-L-Leu-D-Ala-L-Pro-L-Val-pNA (**1D**)[2)] and Dnp-L-Leu-L-Ala-L-Pro-L-Val-pNA (**1L**)[2)], which are model peptides for [D-Ala$^{4,4'}$]-GS,[7,8)] and [L-Ala$^{4,4'}$]-GS,[8)] respectively. Compound **2D** sbowed strong Cotton effect above 250 nm typical for the exciton coupling between the two terminal chromophores, though it was a little weaker than that of GS-model peptide **1D**. So, β-turn preference of the sequence in **2D** seems to be high enough to allow the GS-analog containing the similar sequence to take GS-like conformation. On the other hand, **2L** did not show any particular CD patterns above 250 nm suggesting that **2L** took random conformation. The CD spectrum seems to be explained by the summation of CD of a Dnp-L-amino acid and a D-amino acid *p*-nitroanilide as in the case of **1L**. So, the inactivity of [D-Val$^{1,1'}$, L-Phe$^{4,4'}$]-GS seems to be due to the difficulty of taking GS-like β-sheet conformation as in the case of [L-Ala$^{4,4'}$]-GS.[8)]

Though CD spectra of **2D** suggested that [D-Val$^{1,1'}$]-GS had some possibility of taking GS-like β-sheet conformation, the analog was inactive and showed different CD spectra from that of GS.[6)] Based on the molecular model study, we considered that the bulky D-Val side chain might destabilize the β-turn conformation because of large steric hindrance with carbonyl oxygen of the L-Pro residue (cf. Fig. 1). So, we

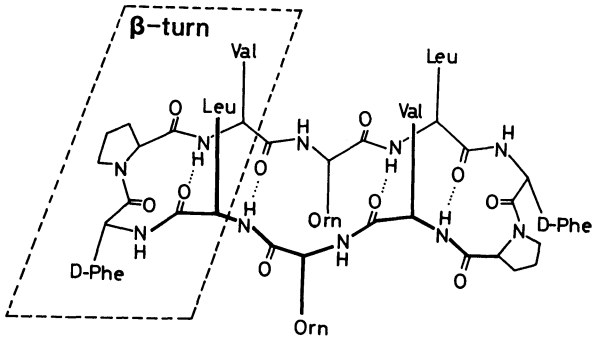

Fig. 1. β-Sheet conformation of gramicidin S.

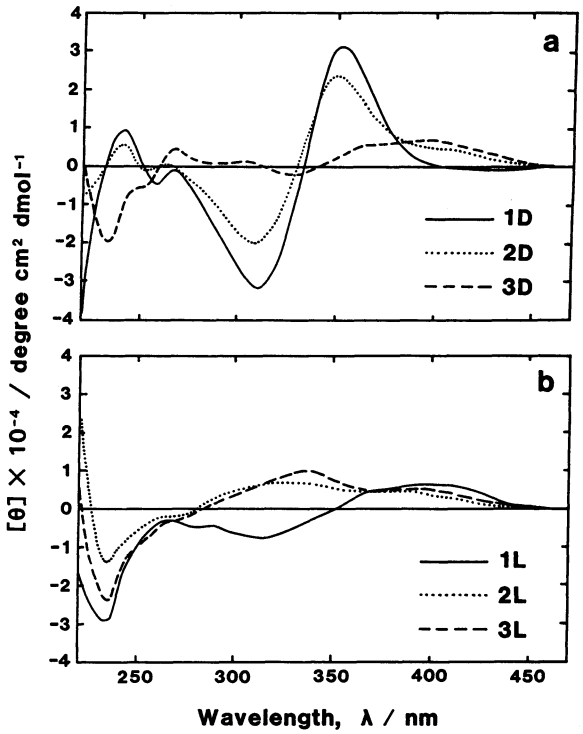

Fig. 2. CD spectra of (a) Dnp-X-D-Ala-L-Pro-Y-pNA and (b) Dnp-X-L-Ala-L-Pro-Y-pNA in MeOH. ——: X=L-Leu, Y=L-Val (**1D**, **1L**); ·····: X=L-Ala, Y=D-Ala (**2D**, **2L**); ------: X=L-Leu, Y=D-Val (**3D**, **3L**).

Table 1. Yields and Analytical Data of Synthetic Peptides

Compound	Yield %	Mp θ_m/°C	$[\alpha]_D^{23}$ a) °	R_f^1	R_f^2	R_f^3	Found (%) C	H	N	Calcd (%) C	H	N
4L	72	192—193	41.8	0.67	0.32	0.79	55.68	6.61	14.33	55.34	6.54	14.67
5D	65	115—118	67.2	0.63	0.24	0.77	54.52	6.59	15.20	54.73	6.62	15.32
5L	68	118—120	24.4	0.63	0.24	0.80	54.24	6.64	15.09	54.29	6.65	15.19[b]
2D	81	146—148	117.2	0.62	0.18	0.76	50.18	4.86	18.01	50.08	5.01	17.97[c]
2L	97	274—276	47.6	0.70	0.17	0.79	50.39	4.91	17.53	50.08	5.01	17.97[c]
6D	72	226—229	140.1	0.67	0.29	0.81	56.86	6.87	13.84	57.02	6.98	13.85
6L	82	100—104	41.1	0.69	0.25	0.88	56.58	6.87	13.69	57.02	6.98	13.85
7D	57	112—116	53.7	0.77	0.25	0.79	57.33	7.33	13.15	57.40	7.55	13.39[c]
7L	76	117—120	14.0	0.77	0.25	0.88	57.12	7.30	12.99	57.40	7.55	13.39[c]
3D	71	120—124	84.8	0.75	0.27	0.79	53.46	5.76	16.01	53.33	5.79	16.05[d]
3L	79	125—127	63.2	0.75	0.29	0.83	53.38	5.73	15.79	53.33	5.79	16.05[d]

a) *c* 1, MeOH. b) $0.25H_2O$. c) $0.5H_2O$. d) $0.75H_2O$.

synthesized Dnp-L-Leu-D-Ala-L-Pro-D-Val-pNA (**3D**) and Dnp-L-Leu-L-Ala-L-Pro-D-Val-pNA (**3L**) as closer models to the original sequences of the GS analogs. Tetrapeptide **3D** as well as **3L** showed weak CD bands above 250 nm (Fig. 2). The result suggested that **3D** did not prefer to take β-turn conformation and therefore [D-Val$^{1,1'}$]-GS did not take GS-like β-sheet conformation.

Consequently, inactivity of both [D-Val$^{1,1'}$]- and [D-Val$^{1,1'}$, L-Phe$^{4,4'}$]-GS seems to be caused by the reluctance of their partial sequences to take β-turn conformation. However, the reason of reluctance seems to be different; partial sequence of the former has some potential to take β-turn conformation except for the steric hindrance of bulky D-Val side chain, whereas, that of the latter has not such potential at all.

Present study proves that CD spectra of the chromophoric derivatives of peptides are useful for interpreting the structure-activity relationships of GS analogs, and suggests that the bulkiness of the 4th-position amino acids as well as their configurations[9] has a significant effect on β-turn formation.

Experimental

All the melting points were uncorrected. Thin-layer chromatographies were carried out on Merck silica gel 60 F_{254} plates with the following solvent systems: R_f^1, $CHCl_3$-MeOH (5:1, v/v); R_f^2, $CHCl_3$-MeOH-AcOH (95:5:1, v/v); R_f^3, *n*-BuOH-AcOH-pyridine-H_2O (4:1:1:2, v/v). Optical rotations were measured on an Union automatic polarimeter PM-201. CD spectra were recorded on a JASCO J-40A spectropolarimeter at the concentration of 0.1 mM (1 M=1 mol dm^{-3}) at 23 °C. All the peptides were synthesized according to schemes shown in Fig. 3 by similar manners as reported previously,[3] and the results are summarized in Table 1.

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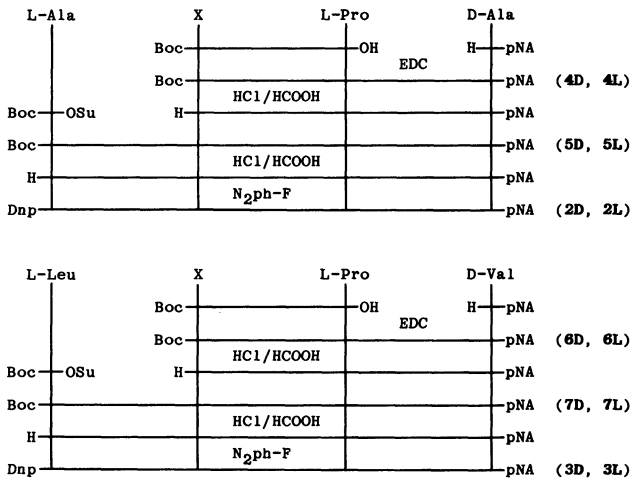

Fig. 3. Synthetic schemes of Dnp-tetrapeptide-pNA. Affix **D** or **L** means X=D-Ala or L-Ala, respectively.

References

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