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Studies on the *β*-Turn of Peptides. III[1]. Syntheses and Conformational Properties of *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-Nitroanilides Related to *β*-Turn Preferring Sequences in Proteins

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(Received September 1, 1982)

To confirm the general applicability of CD spectra of *N*-(2,4-dinitrophenyl(Dnp))tetrapeptide *p*-nitroanilides (pNA's) to the analysis of *β*-turn preference of the tetrapeptide sequences, Dnp-pNA derivatives of some tetrapeptides related to *β*-turn preferring sequences in proteins were synthesized and subjected to CD and ^{1}H NMR measurements. CD spectra of Dnp–Gly–L-Pro–L-Asn–Gly–pNA (**5a**) were just the mirror image of those of gramicidin S-model peptide, Dnp–Gly–D-Ala–L-Pro–Gly–pNA (**6**). This suggested that **5a** strongly preferred the *β*-turn and the type of turn was different from that of **6**. The relative intensities of Cotton effects of the tetrapeptide with the general structure Dnp–Gly–L-Pro–Y–Gly–pNA (Y=L-Asn, Gly, L-Ala, or L-Gln) roughly agreed with the bend potentials of the amino acid residues at the third position of *β*-turns in proteins reported by Chou and Fasman. The effects of changing the amino acid residues at the second position of *β*-turn were also examined.

In the previous paper, we gave an outline of a new method to study the *β*-turn conformation of linear tetrapeptides.[2] *N*-(2,4-Dinitrophenyl)tetrapeptide *p*-nitroanilides (Dnp–tetrapeptide–pNA's)[3] exhibited characteristic CD spectra above 300 nm when they took *β*-turn conformations (Fig. 1). The Cotton effects were considered to be due to the interaction of the two terminal chromophores, namely, the exciton coupling of the transition moments in the two terminal chromophores, and the magnitude of the Cotton effects were shown to reflect the *β*-turn preference of the peptides. *β*-Turn preferences of the tetrapeptide derivatives has strong correlation with antibiotic activities of the gramicidin S (GS) analogs containing similar tetrapeptide sequences at their *β*-turn part.[1] This paper deals with the details of syntheses and conformational properties of Dnp-pNA derivatives of some tetrapeptides related to the *β*-turn preferring sequences in proteins to confirm the general applicability of the CD spectra of the tetrapeptide derivatives to the analysis of their *β*-turn conformation.

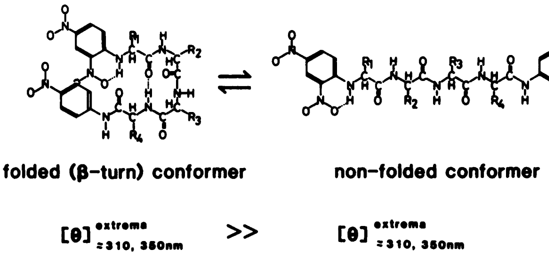

Fig. 1. Models of folded (*β*-turn) and non-folded conformers of a Dnp–tetrapeptide–pNA.

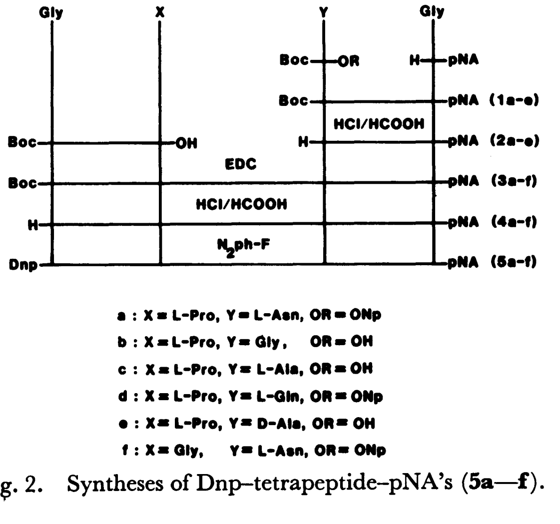

Fig. 2. Syntheses of Dnp–tetrapeptide–pNA's (**5a**—**f**).

The first compound synthesized was Dnp–Gly–L-Pro–L-Asn–Gly–pNA (**5a**). The sequence was expected to take the *β*-turn to a high degree because of the high bend potential of each component amino acid residue at the respective position in the table proposed by Chou and Fasman on the basis of statistical analysis of the X-ray crystallographic data of 29 globular proteins.[4] Kopple and Go,[5] and Fermandjian *et al.*[6] reported that Z–Gly–L-Pro–L-Asn–Gly–NHNH–Boc and H–Gly–L-Pro–L-Asn–Gly–OH, respectively, formed type-I *β*-turn by NMR analysis. Synthesis of **5a** is outlined in Fig. 2. Boc–L-Asn–Gly–pNA (**1a**) was prepared by coupling of Boc–L-Asn–ONp and H–Gly–pNA, and was deprotected with hydrogen chloride in formic acid to afford H–L-Asn–Gly–pNA·HCl (**2a**·HCl). Coupling of Boc–Gly–L-Pro–OH[7] and **2a** by EDC–HOBt method

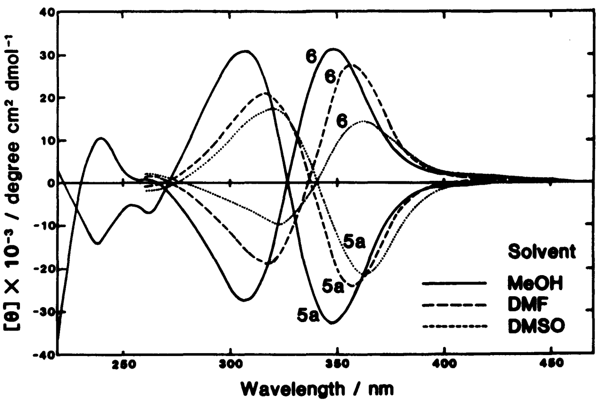

Fig. 3. CD spectra of Dnp–Gly–L-Pro–L-Asn–Gly–pNA (**5a**) and Dnp–Gly–D-Ala–L-Pro–Gly–pNA (**6**).

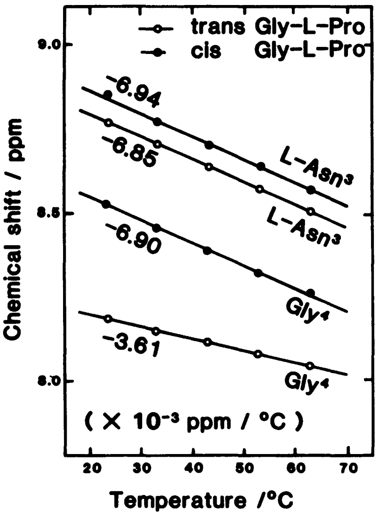

Fig. 4. Temperature dependences of the chemical shifts of the α-amide protons of **5a** in DMSO-d_6 solution.

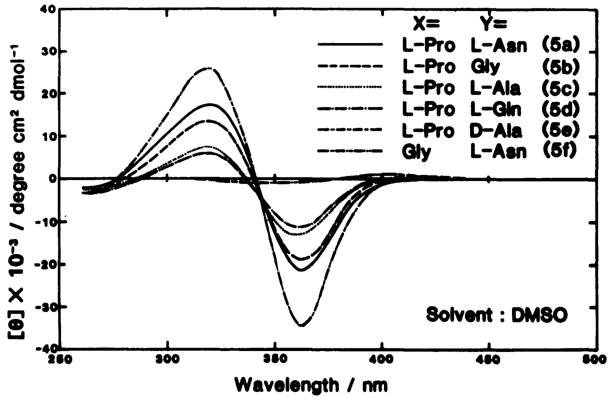

Fig. 5. CD spectra of Dnp-Gly-X-Y-Gly-pNA's (**5a**—**f**) in DMSO.

afforded Boc-Gly-L-Pro-L-Asn-Gly-pNA (**3a**), which was converted into the Dnp derivative, Dnp-Gly-L-Pro-L-Asn-Gly-pNA (**5a**), by deprotection and subsequent treatment with 1-fluoro-2,4-dinitrobenzene. Homogeneities of the synthetic peptides were confirmed by thin-layer chromatography and elemental analysis.

The CD spectra of **5a** were just the mirror image of those of GS-model peptide Dnp-Gly-D-Ala-L-Pro-Gly-pNA (**6**)[1] (Fig. 3). The result indicates that **5a** strongly prefers the β-turn, and the type of the turn would be different from that of the GS model peptide. Certainly, GS is known to take a type II′ β-turn, however **5a** cannot take a type II′ β-turn because of five-membered ring structure of L-Pro residue at the second position. Though it is difficult to discriminate exactly the type of the turn, type II β-turn seems probable for **5a** because of enantiomeric feature of the CD spectra. The ^{1}H NMR spectrum of **5a** in DMSO-d_6 showed two sets of resonances for most of the protons due to *cis-trans* isomerism for the Gly-L-Pro bond. The *cis-trans* ratio was estimated to be 3 : 7 according to the relative peak area of the same proton. Chemical shifts of aromatic protons on the Dnp chromophore of the *trans* isomer (H-3, 8.78 ppm; H-5, 8.25 ppm; H-6, 7.09 ppm) were shifted to higher field compared with those of the *cis* isomer (H-3, 8.78 ppm; H-5, 8.27 ppm; H-6, 7.15 ppm), suggesting the approach of the two terminal chromophores in the *trans* isomer. The Gly^4-NH proton of the *trans* isomer showed smaller temperature dependence than the other NH protons, indicating the presence of 4→1 hydrogen bond essential for β-turn conformation (Fig. 4).[8] Most of the protons other than CO-NH protons show little temperature dependences, suggesting that the conformation of **5a** did not change significantly in the range of this experiment (23—63 °C).

To study the sequence-conformation relationship, a series of compounds having a general structure Dnp-Gly-L-Pro-Y-Gly-pNA (**5b**, Y=Gly; **5c**, Y=L-Ala; **5d**, Y=L-Gln; **5e**, Y=D-Ala) were examined. Syntheses of **5b**—**e** were carried out by a similar manner to that described for the synthesis of **5a** except that EDC coupling was used for the preparation of **1b**, **c**, and **e** (Fig. 2). The CD spectra of **5a**—**e** in DMSO are shown in Fig. 5. They all showed a pair of strong Cotton effects near 310 and 350 nm, and their intensities decreased in the order, L-Asn (**5a**)>Gly (**5b**)>L-Ala (**5c**)>L-Gln (**5d**), which corresponds roughly to that of decreasing bend potentials at the third position in the table of Chou and Fasman.[4] The results suggest that the bend potentials in proteins may be valid also for oligopeptides. When Y was D-Ala (**5e**), the highest ellipticities were observed suggesting that the presence of an D-amino acid at the third position is favorable for the β-turn conformation. Certainly, ^{1}H NMR study of **5e** also indicated that **5e** had higher population of β-turn conformer than **5a**. The ^{1}H NMR spectrum of **5e** in DMSO-d_6 showed two sets of resonances and the *cis-trans* ratio for the Gly-L-Pro bond was about 3 : 7, which is similar to that of **5a**. However, in the *trans* isomer of **5e**, aromatic protons on the Dnp-chromophore showed chemical shifts at higher field (H-3, 8.77 ppm; H-5, 8.20 ppm; H-6, 7.02 ppm) than those of **5a**, and the Gly^4-NH proton showed smaller temperature dependence (-3.27×10^{-3} ppm °C^{-1}) than that of **5a**. This means that CD spectra of Dnp-pNA derivatives are good measures of β-turn preference of tetrapeptide sequences besides GS-model peptides.

To examine the contribution of L-Pro at the second position of a tetrapeptide to the β-turn formation, Dnp-Gly-Gly-L-Asn-Gly-pNA (**5f**) was synthesized similarly (Fig. 2). The CD spectrum of **5f** measured in DMSO was compared with those of **5a** and **5b** (Fig. 5). High β-turn preference of the Gly-L-Pro-L-Asn-Gly sequence was not affected so much by replacement of L-Asn^3 with Gly but decreased significantly by replacement of L-Pro^2 with Gly. The results emphasized the importance of L-Pro residue at the second position for the β-turn formation as expected from remarkably high bend potential of the residue at the second position.[4]

It is interesting to note that the β-turn preference of amino acid residues in tetrapeptides well reflects that in proteins. The fact suggests that such a small fragment conformation of a protein is largely governed by short-range interactions of the amino acid residues involved.

Table 1. Yields and analytical data of synthetic peptides

Compound	Yield/%	Mp θ_m/°C	$[\alpha]_D^{22}$ [a]/°	Found(%)			Calcd(%)			R_f^1	R_f^2	R_f^3
				C	H	N	C	H	N			
1a	82	202—203	−14.0	49.76	5.48	16.97	49.87	5.66	17.11	0.39	0.09	0.75
1b	77	223—226	—	50.90	5.81	16.18	51.13	5.72	15.90	0.51	0.14	0.88
1c	74	202—203	+11.8	50.88	5.85	14.69	51.19[b]	6.17[b]	14.92[b]	0.71	0.30	0.88
1d	69	183—184	+10.8	51.35	5.90	16.78	51.06	5.95	16.54	0.37	0.09	0.80
1e	65	198—199	−10.8	52.82	5.51	15.23	52.45	6.05	15.29	0.75	0.34	0.88
2a·HCl	93	222—223	+2.0	41.98	4.42	20.49	41.69	4.66	20.26	0.05	0	0.60
2b·HCl	100	115—125	—	40.99	4.31	19.37	41.60	4.54	19.41	0	0	0.49
2c·HCl	100	221—224	+17.4	43.26	4.86	18.18	43.65	4.99	18.51	0.09	0	0.65
2d·HCl	97	204—205	−44.0	43.35	5.02	19.73	43.64	5.07	19.58	0.19	0	0.74
2e·HCl	84	236—237	−31.8	44.10	5.19	18.23	43.65	4.99	18.51	0.10	0	0.67
3a	63	154—156	−44.6	49.72	5.62	16.82	49.56[c]	6.07[c]	16.86[c]	0.43	0.06	0.67
3b	66	139—141	−42.2	51.97	5.95	16.46	52.17	5.97	16.59	0.56	0.12	0.85
3c	80	121—123	−33.4	52.06	6.18	15.82	52.16[b]	6.28[b]	15.87[b]	0.66	0.20	0.74
3d	52	136—138	−33.6	49.76	5.93	16.20	49.66[d]	6.33[d]	16.22[d]	0.47	0.06	0.85
3e	75	125—130	−41.6	53.05	6.26	15.94	53.07	6.20	16.15	0.59	0.18	0.85
3f	53	212—213	−0.4	47.87	5.78	18.90	48.18	5.58	18.73	0.18	0.03	0.71
5a	98	207—209	−84.8	45.68	4.18	19.35	45.73[d]	4.61[d]	19.20[d]	0.26	0.03	0.73
5b	82	157—162	−92.8	46.38	4.20	19.18	46.78[c]	4.44[c]	18.98[c]	0.60	0.10	0.81
5c	78	218—219	−79.2	48.17	4.46	18.50	48.39[b]	4.56[b]	18.81[b]	0.58	0.11	0.83
5d	91	160—161	−77.0	46.92	4.42	19.03	47.19[c]	4.72[c]	19.06[c]	0.31	0.03	0.67
5e	78	256—257	−154.6	47.68	4.34	18.67	47.68	4.67	18.54	0.52	0.13	0.85
5f	93	218—220	+8.0	44.51	3.94	20.57	44.15[b]	4.04[b]	21.06[b]	0.11	0	0.66

a) *c* 1, DMF. b) 1/2H_2O. c) H_2O. d) 3/2 H_2O.

Experimental

Synthesis of Peptides. All the melting points were measured on a Yanagimoto micro melting point apparatus and uncorrected. TLC's 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. Yields, physical constants, and the results of elemental analyses of the synthetic peptides are summarized in Table 1.

Boc-L-Asn-Gly-pNA (1a). To a solution of H-Gly-pNA (351 mg, 1.8 mmol) in DMF (8 ml) was added Boc-L-Asn-ONp (636 mg, 1.8 mmol). The reaction mixture was stirred overnight at room temperature, evaporated *in vacuo*, and the residue was dissolved in EtOAc. The solution was washed successively with 10% citric acid, 4% $NaHCO_3$, and water, dried (Na_2SO_4), and evaporated to leave crystals, which was recrystallized from EtOH; yield, 601 mg (82%).

Compound **1d** was prepared by a similar manner to that described for the preparation of **1a**.

Boc-L-Ala-Gly-pNA (1c). To a chilled solution of Boc-L-Ala-OH (378mg, 2 mmol) and H-Gly-pNA (390 mg, 2 mmol) in DMF (8 ml) was added EDC·HCl (383 mg, 2 mmol), and the mixture was stirred at 0 °C for 1 h and at room temperature overnight. The reaction mixture was treated as described for **1a**; yield, 542 mg (74%).

Compounds **1b** and **1e** were prepared by a similar manner to that described for **1c**.

H-L-Asn-Gly-pNA·HCl (2a·HCl). Compound **1a** (409 mg, 1 mmol) was dissolved in 0.1 M (1 M=1 mol dm^{-3}) hydrogen chloride in formic acid (12 ml). The solution was allowed to stand at room temperature for 30 min and evaporated *in vacuo*. The residue was crystallized by addition of ether; yield, 323 mg (93%).

Compounds **2b**—**e** were prepared as described for **2a**.

Boc-Gly-L-Pro-L-Asn-Gly-pNA (3a). To a chilled solution of Boc-Gly-L-Pro-OH[7] (54 mg, 0.2 mmol), **2a**·HCl (69 mg, 0.2 mmol), and TEA (0.028 ml, 0.2 mmol) in DMF (2 ml) were added HOBt (54 mg, 0.4 mmol) and EDC·HCl (38 mg, 0.2 mmol). The reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight and evaporated. After the addition of water, the solid was collected by filtration, washed successively with 10% citric acid, 4% $NaHCO_3$, and water, and dried *in vacuo* over P_2O_5. The crude product dissolved in MeOH (2 ml) was applied to a column (3×170 cm) of Sephadex LH-20 and eluted with MeOH. The fractions with the desired product detected by UV absorption and TLC were collected and evaporated, and the residue was solidified by addition of ether and recrystallized from MeOH-ether; yield, 71 mg (63%).

Compounds **3b**—**e** were prepared as described for **3a**.

Boc-Gly-Gly-L-Asn-Gly-pNA (3f). Boc-Gly-Gly-OH[9] (46 mg, 0.2 mmol) and **2a**·HCl (69 mg, 0.2 mmol) were coupled as described for **3a**. The crude product obtained by filtration and subsequent washing, was recrystallized from EtOH; yield, 55 mg (53%).

H-Gly-L-Pro-L-Asn-Gly-pNA·HCl (4a·HCl). Compound **3a** (56 mg, 0.1 mmol) was dissolved in 0.1 M hydrogen chloride in formic acid (1.2 ml). The solution was allowed to stand at room temperature for 30 min and evaporated to leave an oil. The product was used for the next reaction without further treatment; yield was quantitative.

Compounds **4b**—**f** were prepared as described for **4a**.

Dnp-Gly-L-Pro-L-Asn-Gly-pNA (5a). To a solution of **4a**·HCl (50 mg, 0.1 mmol) and TEA (0.042 ml, 0.3 mmol) in DMF (2 ml) was added N_2ph-F (37 mg, 0.2 mmol). The reaction mixture was stirred at room temperature for 3 h and evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (5 ml) and applied to a column (1.8×20 cm) of silica gel 60

(Merck), and the column was washed with $CHCl_3$. The desired product was eluted with a mixture of $CHCl_3$ and MeOH (5 : 1, v/v). The fractions containing the desired product were collected and evaporated to leave a solid, which was recrystallized from MeOH–ether; yield, 31 mg (98%).

Compounds **5b**—**e** were prepared as described for **5a**.

*Dnp-Gly-Gly-*L*-Asn-Gly-pNA (5f).* To a solution of **4f**·HCl (46 mg, 0.1 mmol) and TEA (0.042 ml, 0.3 mmol) in DMF (2 ml) was added N_2ph–F (37 mg, 0.2 mmol). The reaction mixture was stirred at room temperature for 3 h and evaporated *in vacuo*. After the addition of water, the precipitate was collected by filtration, washed successively with 10% citric acid, 4% $NaHCO_3$, and water, and dried *in vacuo* over P_2O_5. The crude product was recrystallized from DMF–ether; yield, 55 mg (93%).

CD Measurements. CD spectra were recorded on a JASCO spectropolarimeter model J-20 or J-40 in a 0.1 mM solution at room temperature (23±2 °C). Concentrations of peptide solutions were corrected by UV absorbances based on the fact that all the peptides showed the same molar absorptivity in the same solvent irrespective of the structures: UV_{max}; 326 nm (MeOH), 337 nm (DMF), 343 nm (DMSO).

^1H NMR Measurements. ^1H NMR spectra were recorded on a Bruker WH-270 spectrometer equipped with a Nicolet-1180 computer and with a Bruker B-ST-100/700 temperature control unit. The spectra were measured at 23±0.5 °C if not mentioned otherwise. Chemical shifts were measured from the internal standard of DSS.[3] The spectra were assigned by spin-decoupling, H–D exchange, and saturation transfer methods.

The authors wish to express their thanks to Professor Tatsuo Miyazawa of The University of Tokyo for helpful discussion. Thanks are also due to the members of Analitical Department of System Engineering Laboratory, Mitsubishi-Kasei Industries Ltd., for elemental analyses.

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