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Short communication

Structures of Conopressin-G and -S

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A nanopeptide, conopressin is one of the vasopressin/oxytocin superfamilies. Vasopressin-related peptides that have been identified in several invertebrate phyla as well as insects, mulluses, and annelids.[1-4] Oxytocin contracts uterine muscle and vasopressin to cause antiurea and high blood pressure. Vasopressin and oxitocin, the neuropeptides, are nerve-transfer materials or peptides such as pituitary peptides, circulating hormones, gut hormones, opioid peptides and hypothalamin releasing hormones. Those produced in the brain take part in the control of secretary of nerve system, digestion of stimulation of inhibition, and relaxation of muscle contraction.[5] Therefore, the studies on conopressin are important in understanding the roles of neuropeptide in living systems. There are four known types of conopressins-S, -S', -G, and -G'. All of them are composed of 9 amino acids with a disulfide bond between the 1st and 6th residues and 2 invariant residues; especially, C-terminals are amidated. The primary sequence of conopressin-S(-S') and -G(-G') are as follows[1]: Cys-Ile-Ile-Arg-Asn-Cys-Pro-Arg(Lys)-Gly-NH_2 and Cys-Phe-Ile-Arg-Asn-Cys-Pro-Lys(Arg)-Gly-NH_2, respectively. In order to investigate the structural change by switching the second residue, NMR experiments of conopressin-S and -G were carried out.

Conopressin-S and conopressin-G were purchased from Bachem Co. Ltd (Bubendorf, Switzerland). The deuterated solvent for NMR experiments was purchased from Aldrich Co. Ltd (St. Louis, MO). Samples were prepared in 99.9% D_2O or in 50% D_2O/50% H_2O. All NMR spectra were obtained on a Bruker DPX 400 (9.4T, Karlsruhe, Germany). One mg of sample was dissolved in 0.5 m*l* aqueous solvent. All experiments followed the methods reported previously by Yang and Lim.[6] The peptides are composed of a disulfide bond and its length is short, so it is thought that the distances obtained from nuclear Overhauser effect (nOe) are not important for structure determination. As a result, in this experiment, only the dihedral angles between HN and Ha were considered. The calculation method for the determination of dihedral angles was based on the method proposed by Pardi as follows:[7]

$$^3J_{HN\text{-}H\alpha} = 6.4\cos^2(\Phi - 60^\circ) - 1.4\cos(\Phi - 60^\circ) + 1.9$$

where Φ denotes torsion angle in NH and Ha.

In order to obtain the three-dimensional structures of the peptides, dihedral angles were given as constrains for energy minimization and molecular dynamics. All calculations for structure refinement were performed using InsightII Software (Accerlys Inc., San Diego, USA) on a Silicon Graphics O2 R12000 Workstation. The experimental methods in detail followed the previous work by Yang and Lim.[6]

The sequence-specific resonances of amino acid in aliphatic region were identified by correlated spectroscopy (COSY) and total correlated spectroscopy (TOCSY) spectra.[8,9] The total assignments of ^1H chemical shifts of conopressin-S are listed in Table 1. In like manner, the total assignments of ^1H chemical shifts of conopressin-G are listed in Table 2. Coupling constants used for dihedral angles calculation are listed in the last column of the tables.

In order to obtain the refined structure from the NMR data, the simulated annealing was carried out with the constraints of dihedral angles. The peptide was subjected to energy minimization and molecular dynamics. After annealing, the structure with the lowest total energy was chosen and the refined structure was validated by PROCHECK. In the case of conopressin-S, based on the structures showing at least 2.0Å resolution, the statistical analysis of Ramachandran plot showed that 66.7% of the residues are in the most favored region, 33.3% in the additional allowed region, and 0% in the generously allowed region and,the disallowed region where two end-residues, glycine and proline residues, were excluded. In like manner, PROCHECK results of conpressin-G showed that 33.3% of the residues are in the most favored region, 66.7% in the additional allowed region, and 0% in the generously allowed region and the disallowed region.

The refined structures of conopressin-S and -G are shown in Figs. 1A and 1B, respectively. Because the 8th residues in the two peptides are different from each other, the side chain of Arg8 of conopressin-S and that of Lys8 of conopressin-G were compared. As shown in Fig. 1, while Arg8 of conopressin-S is close to the ring formed by a disulfide bond, Lys8 of conopressin-G faces outward from the ring. The distance between amine group of Arg8 and Ha of Ile3 in conopressin-S is 3.6Å, and that between amine group of Lys8 and Ha of Ile3 in conopressin-G is also 3.6Å. In addition, C-terminal of conopressin-S faces outward from the ring and the distance between amine group of Arg8 and amine group of Gly9 is 7.6Å. C-terminal of conopressin-G is inward and the distance between Lys8 and Gly9 is 10.9Å. That is, the direction of C-terminal causes the difference. Two peptides contain Arg4 and Asn5. In conopressin-S, Arg4 and Asn5 look toward outside and inside, respectively. On the contrary, in conopressin-G, the reverse is the case.

Because of the change from Ile to Phe of 2-position, the

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Table 1. ^{1}H chemical shifts of conopressin-S in aqueous solvent at 298 K

Residue	Chemical Shift (ppm)				$^3J_{NH\alpha}$ (Hz)
	NH	CαH	CβH	CγH and others	
Cys	-[a]	4.36	3.54, 3.35	-	-[a]
Ile	7.99	4.21	2.0	CγH 1.49, 1.23 CδH 1.00, 0.93	6.31
Ile	8.85	4.46	2.06	CγH 1.43, 1.25; CδH 1.00, 0.95	8.8
Arg	8.65	4.33	1.85	CγH 1.67; CδH 3.26; NH1 7.25; NH2 7.58, 6.92	6.59
Asn	8.38	4.73	2.83	-	8.3
Cys	-[a]	4.83	3.30, 3.02	-	-[a]
Pro	-	4.49	2.34	CγH 2.08 1.96; CδH 3.82, 3.74	-
Arg	8.26	4.11	1.91	CγH 1.85,1.64; CδH 3.25, 3.04; NH1 7.23; NH2 7.51, 7.11	-[a]
Gly	8.45	4.95	-	-	8.25

[a]not observed.

Table 2. ^{1}H chemical shifts of conopressin-G in aqueous solvent at 298 K

Residue	Chemical Shift (ppm)				$^3J_{NH\alpha}$ (Hz)
	NH	CαH	CβH	CγH and others	
Cys	-[a]	4.17	3.38,3.18	-	-[a]
Phe	-[a]	4.80	3.20, 3.06	-[a]	-[a]
Ile	7.80	4.12	1.87	CγH 1.20, 0.90; CδH 0.80	8.46
Arg	8.06	4.08	1.75	CγH 1.60, 1.50; NH 7.23, 7.07	3.70
Asn	8.35	4.70	2.90	NH 7.46, 6.90	7.67
Cys	-[a]	4.80	3.24, 2.94	-	-[a]
Pro	-	4.40	2.30, 1.90	CγH 2.00; CδH 3.70	-
Lys	8.60	4.25	1.87, 1.77	CγH 1.47,1.42; CδH 1.70, 1.65; CεH 2.99, 2.96	-[a]
Gly	8.44	3.91	-	-	4.82

[a]not observed.

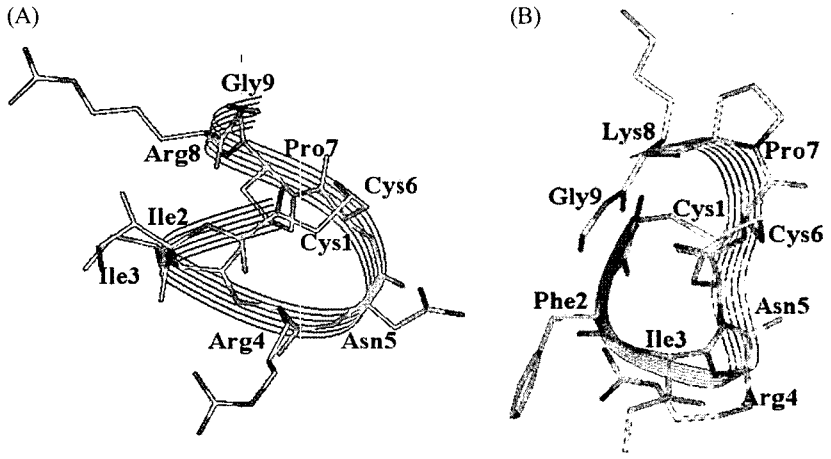

Fig. 1. Heavy atom ribbon representation of conopressin-S (A) and -G (B).

dihedral angles between Ile2 and Ile3, Ile3 and Arg4, and Arg4 and Asn5 are -174.63, -141.90 and -178.24, respectively, on conopressin S; however, that of Phe2 and Ile3 is 149.51, Ile3 and Arg4, -131.20 and Arg4 and Asn5, -156.85 on conopressin G. These differences of residue on 2-position between conopressin G and conopressin S would affect $^3J_{NH\alpha}$ on Arg4. Connolly surface of the peptide gives information about hydrophobicity as well as its volume. Connolly surfaces of conopressin-S and -G are shown in Figs. 2A and 2B, respectively. The size of conopressin-S is 19.3Å × 14.3Å × 11.6Å, and that of conopressin-G is 20.8Å × 13.3Å × 8.9Å. Because of the direction of C-terminal, conopressin-G is lengthier than conopressin-S. As shown in Fig. 2A, two hydrophobic residues, Ile2 and Ile3 are in the depressed place between two hydrophilic residues, Arg4 and Arg8. In conopressin-G; however, two hydrophobic residues, Phe2 and Ile3, jut out. Conpressin G is known for acting on polysynaptic pathway on *Aplysia californica*, neurons in the branchial,

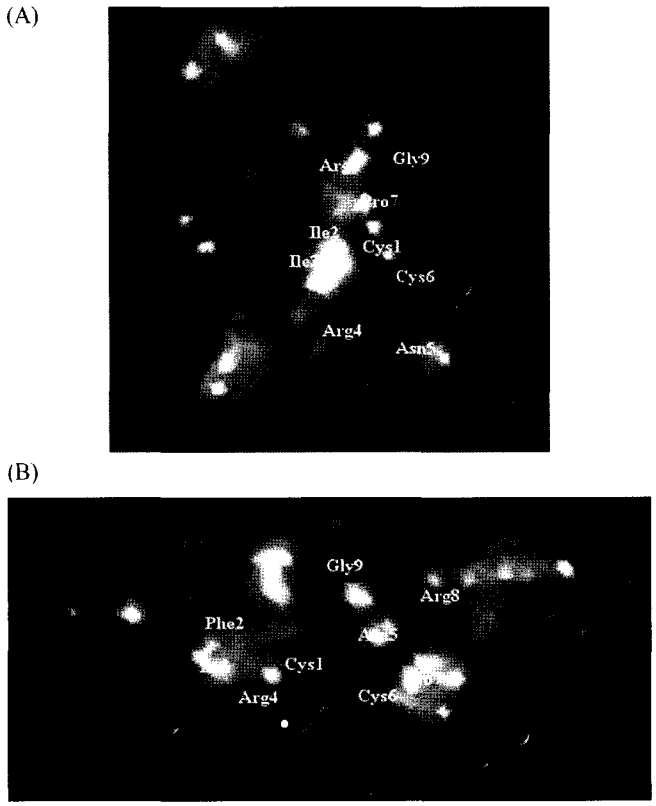

Fig. 2. Connolly surfaces for Conopressin-S (A) and -G (B). Red: the most lipophilic; blue: the most hydrophilic.

siphon nerves, and Ca^{2+}-dependent Cl currents in brain.[10-12] Conopressin S is similar with vasopressin and oxitoxine with effects such as contraction of the uterus in reproduction, the regulation of osmotic balance, and contraction of smooth muscle cells in arteries.[13-14] Combined, these reasons may explain why more hydrophobicity of Conopressin-G as compared to conopressin-S is caused.

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