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Short communication

Structure in Solution of *Diploptera punctata* Allatostatin-2

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Small peptides, allatostatins inhibit the biosynthesis of juvenile hormone-III, a kind of sesquiterpenoids which plays an important role in the breeding of insects.[1] More than seventy allatostatins have been discovered, which can be classified as cockroach type, cricket type, and moth type.[2] Of those, cockroach type allatostatins were found from *Diploptera punctata* first. Based on the studies on their precursor, thirteen *D. punctata* allatostatins (DpASTs) were known.[3] They are composed of Tyr(Phe)-Xaa-Phe-Gly-Leu(Ile)-NH_2 in *C*-terminal.[4] Since most peptides belonging to allatostatin family inhibit the biosynthesis of hormone-III, it is thought that their inhibitory effects (allatostatic effect) are caused by the sequence similarity.[5] Even the peptides isolated from tobacco hornworm *Manduca Sexta* contain Pro-Ile-Ser-Cys-Phe-OH in their *C*-terminal, however, they show allatostatic effect.[6,7] On the contrary, schstostatin-2 isolated from locust *Schstocerca gregaria* is composed of the same *C*-terminal as DpASTs, but it does not show allatostatic effect.[8] Therefore, it can be considered that allatostatic effect may be caused by the three-dimensional structure of the peptides. Of the 13 DpASTs, structural studies of DpAST-5, DpAST-7, DpAST-8, and DpAST-9 were reported previously.[9] Even though DpAST-2 is the longest peptide which is composed of 18 amino acids, its structure in solution has not been reported yet. We tried to determine its solution structure which may give information about the relationships between allatostatic effect and the structure of the peptide.

Synthetic DpAST-2 was purchased as a lyophilized powder from BACHEM (Bubendorf, Swiss). Since its purity was 98%, no further purification was performed. Its structural study was carried out using NMR spectroscopy. All NMR measurements were performed on a Bruker Avance 400 spectrometer (9.4 T) and a Bruker DRX 600 spectrometer (14.1 T). The NMR spectra were collected in the mixed solvent system (70% trifluoroacetic acid and 30% D_2O). The concentration of the sample was approximately 50 mM. The experimental procedure for structure determination were followed according to the methods reported previously by Yang and Lim.[9] All calculations for structure refinement were performed using InsightII software (Accerlys, San Diego, USA) on a Silicon Graphics O2 R12,000 workstation. The experimental methods in detail were stated in the previous work.[9]

First, the ^{1}H chemical shifts of DpAST-2 were assigned based on the interpretation of correlated spectroscopy (COSY), total correlated spectroscopy (TOCSY), and nuclear Overhauser exchanged spectroscopy (NOESY). Because there are four tyrosine residues in the peptide, their chemical shifts except for aH and bH could not be assigned completely. As listed in Table 1, total assignments of the ^{1}H NMR data were obtained. In order to determine the three-dimensional structure using NMR spectroscopy, constraints among the protons were applied. For this purpose, cross peaks showing nuclear Overhauser effect (nOe) were collected. According to the distances between inter residues, the sequential map was obtained as shown in Fig. 1, where the strong line shows strong nOe data and denotes the distance between two protons to be placed between 1.5Å and 2.5Å. Likewise, the medium and weak lines denote their distances between 2.5Å and 3.5Å, and 3.5Å and 5Å, respectively. Based on the nOe sequential map, this peptide has a week helix group because a step is observed between 4th residue and 10th residue.

Using the distances calculated from nOe data the simulated annealing was carried out. The peptide was subjected to energy minimization and molecular dynamics where the temperature was elevated from 300 K to 1,000 K by 100 K step, 1 atm for 5 ps and dropped in like manner for 25 psec. The total energy at the final step ranged within 10 kcal/mol. The structure with the lowest total energy was chosen and evaluated using PROCHECK. Among the 118 structures showing at least 2.0Å resolution, the best structure was analyzed. The statistical analysis of Ramachandran plot (Fig. 2) obtained from PROCHECK showed that 42.9% of the residues are in the most favored region, 50.0% in the additional allowed region, 7.1% in the generously allowed region, and 0% in the disallowed region, where two end-residues, Ala^1 and Leu^{18}, and glycine and proline residues were excluded for the analysis.

The refined structure obtained from the simulated annealing is shown in Fig. 3. As expected based on the nOe sequential map, the peptide shows a short helix between Ser^3 and Lys^9. Because the structure is compact, the distance between the *N*-terminal and the *C*-terminal is 17.4Å. The ribbon representation shows that there is a turn at Val^{13}. Nine residues between Arg^{10} and Leu^{18} are close to each other, where as all side chains except Arg^{10} and Leu^{11} are face outside. Connolly surface of the peptide gives information about hydrophobicity (Fig. 4).

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Table.1 The assignment of ^{1}H NMR chemical shifts of allatostatin B in 70% trifluoro acetic acid and 30% water mixture

Residue	Chemical shift/ppm			
	NH	$C_\alpha H$	$C_\beta H$	others
Ala[1]		4.21	1.72	
Tyr[2]	8.45	4.77	3.12, 3.21	δ^{ND}, ε^{ND}
Ser[3]	8.12	4.67	3.99, 4.07	
Tyr[4]	8.10	4.71	3.14, 3.30	δ 7.34, ε^{ND}
Val[5]	7.81	4.14	2.28	γ 1.18
Ser[6]	8.00	4.50	4.06, 4.17	
Glu[7]	8.13	4.41	2.18	γ 2.52
Tyr[8]	8.03	4.65	3.12, 3.28	δ^{ND}, ε^{ND}
Lys[9]	7.76	4.40	1.99, 2.05	γ 1.58, 1.62, δ 1.88, å 3.19
Arg[10]	7.95	4.57	2.00, 2.20	γ 1.82 1.87, δ 3.38, NH 7.29
Leu[11]	7.80	4.89	1.94	γ 1.68, δ 1.19 1.16
Pro[12]		4.61	2.18, 2.36	γ 2.25, δ 3.38 4.02
Val[13]	7.38	4.21	2.24	γ 1.04
Tyr[14]	7.84	4.69	3.10, 3.19	δ^{ND}, ε^{ND}
Asn[15]	7.97	4.81	2.79, 2.91	NH_2 7.37
Phe[16]	8.03	4.68	3.27, 3.42	δ 7.45, ε^{ND}, ζ^{ND}
Gly[17]	8.19	4.00, 4.16		
Leu[18]	7.93	4.56	1.84	γ^{ND}, δ 1.10, 1.15

ND: not detected.

Fig. 1. The nOe sequential map of DpAST-2. Strong line: 1.5Å-2.5Å, medium line: 2.5Å-3.5Å, weak line: 3.5Å-5Å.

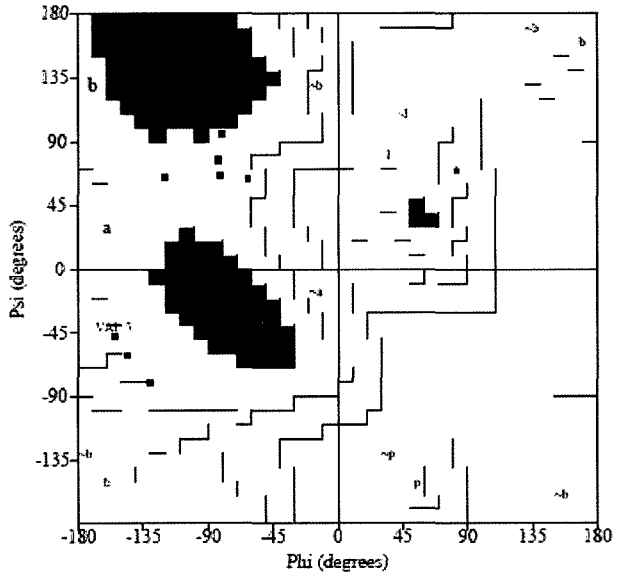

Fig. 2. Ramachandran plot of DpAST-2 obtained from PROCHECK. Tyr[2], Ser[3], Tyr[4], Val[5], Ser[6], Glu[7], Tyr[8], Lys[9], Arg[10], Leu[11], Val[13], Tyr[14], Asn[15], Phe[16] residues are presented because proline is not considered in PROCHECK.

While the *C*-terminal is hydrophobic, the *N*-terminal is hydrophilic. The peptides *D. punctata* allatostatins have

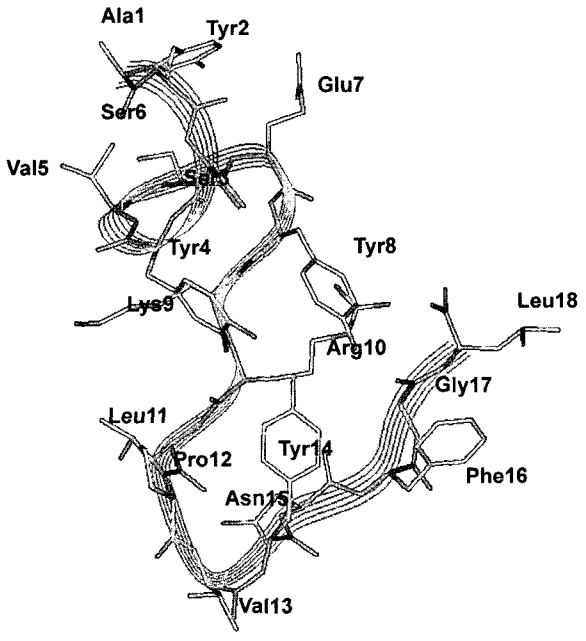

Fig. 3. The ribbon representation of DpAST-2. A short helix between Ser[3] and Lys[9] and a turn at Val[13].

common features such as amidated *C*-terminal and sequence, Tyr(Phe)-Xaa-Phe-Gly-Leu(Ile)-NH_2. Since the *C*-terminal showing hydrophobicity is conserved in allatostatins, it was expected that the allatostatic effect is caused by the conserved sequence. As mentioned above, however, schstostatin-2 isolated from locust *Schstocerca gregaria* comprising of the same *C*-terminal sequence does not have allatostatic effect; thus the *C*-terminal showing hydrophobicity may not play an

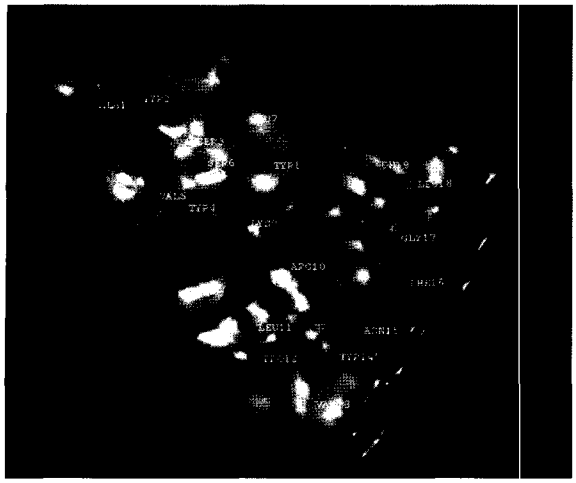

Fig. 4. Connolly surface of DpAST-2. Brown: the most hydrophobic; blue: the most hydrophilic; green: medium.

important role in the biological function. As a result, it can be considered that the molecule accepting DpAST-2 does not have hydrophobic interaction. Connolly surface of DpAST-2 suggested that the binding site of the molecule interacting with DpAST-2 may have hydrophilic site.

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