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Conformational Analysis of a Cyclic Heptapeptide, Pseudostellarin D by Molecular Dynamics and Monte Carlo Simulations[1)]

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Restrained molecular dynamics calculation *in vacuo* using AMBER* force field implemented in MacroModel/Batchmin showed a major solution conformation in dimethyl sulfoxide-d_6 of pseudostellarin D, *cyclo*(-Gly–Tyr–Gly–Pro–Leu–Ile–Leu-). This is a cyclic heptapeptide isolated from *Pseudostellaria heterophylla* possessing characteristics of a β-bulge motif with three intramolecular hydrogen bonds, two β-turns (one type I at Pro^4 and Leu^5 residues, and one type II at Leu^7 and Gly^1 residues) and all *trans* amide bonds. The solution form of pseudostellarin D, which was homologous to that observed in the solid state, was also supported by Monte Carlo simulation study.

Key words pseudostellarin D; conformation; molecular dynamics; Monte Carlo; β-bulge; *Pseudostellaria heterophylla*

Since the chemical and biological activities of cyclic peptides are intimately related with the conformation, it is essential to establish the factors which determine the dominating conformation. Many systematic conformational examinations of cyclic peptides, such as cyclic penta-,[2)] hexa-[3)] and octapeptides,[4)] have been reported apart from those of cyclic heptapeptides.

The crystal form of a tyrosinase inhibitory cyclic heptapeptide, pseudostellarin D, which was isolated from the roots of *Pseudostellaria heterophylla*, was analyzed by X-ray diffraction study[5)] and formed a classical β-bulge unit[6)] in the crystal. It is desirable to analyze the conformational preferences of cyclic heptapeptides in solution as well as in crystal. NMR spectroscopy is a useful method for conformational analysis of cyclic peptides in solution. In our previous paper,[5)] the solution form of pseudostellarin D in dimethyl sulfoxide-d_6 (DMSO-d_6) was analyzed using NH temperature dependence[7)] and by comparing ϕ angles calculated from NH–Hα coupling constants using a Karplus-type equation with those in crystal structure. The temperature coefficients of amide protons indicated Tyr^2–, Gly^3– and Ile^6–NH protons were involved in intramolecular hydrogen bonds as in the crystal form. One of the calculated ϕ angles was also well consistent with those in the crystal form.

The solution conformation can be determined simultaneously using the combined application of NMR with computational chemical methods, for example, nuclear Overhauser effect (NOE) restrained molecular dynamics (MD) simulations and Monte Carlo (MC) simulations. To verify the solution conformation of pseudostellarin D in DMSO-d_6, conformational analysis using two different computational procedures, NOE restrained MD and MC simulations was examined in this paper.

Results and Discussion

Rotating Frame Nuclear Overhauser Effect (ROE) Restraint MD Simulation Reliable structural information of pseudostellarin D in the solution state was obtained by conducting MD simulations using ROE relationship from coordinates of X-ray structure of pseudostellarin D. Interproton distances (27 constraints) extracted from the volume of the cross peaks in a phase sensitive ROESY spectrum[8)] in DMSO-d_6 were classified into three ranges, 1.8—2.5, 1.8—3.3 and 3.0—5.0 Å, corresponding to strong, medium and weak ROEs, respectively (Fig. 2). No hydrogen bonding or dihedral angle restraints were taken into consideration. The restrained molecular dynamics (rMD) simulation was equilibrated for a duration of 100 ps with a thermal bath at 500 K *in vacuo*. One thousand sampled conformers were finally minimized by the molecular mechanics (MM) calculation of AMBER* force field *in vacuo*. Non hydrogen-atom structures were compared to eliminated possible duplicate conformations and the maximum atomic deviation allowed for identical conformation was set to 0.25 Å. The 1000 conformers obtained by rMD simulation was reduced to 66 in MM calculation. The total energy of the global minimum structure was 6.13 kcal/mol. Ten conformers (231 times) appeared within a range of 2 kcal/mol from the global minimum structure.

These 10 conformers were classified into conformers A and B. The average of the sum of the restraint violations in the 10 conformers was 1.66 Å. Comparison of the backbone atoms in conformer A (7 conformers appeared 196 times) gave an rms deviation (RMSD) of 0.041 Å.

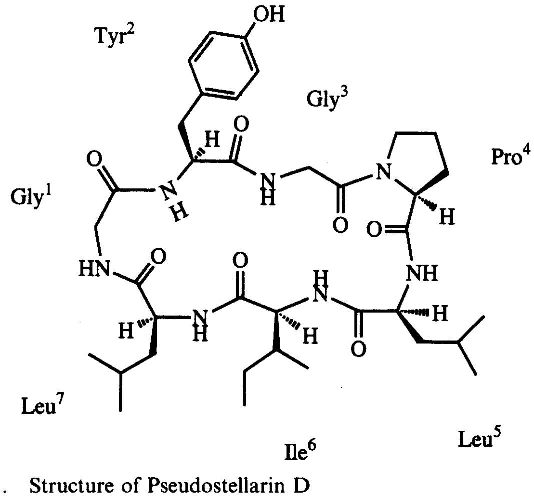

Fig. 1. Structure of Pseudostellarin D

One of the Gly residues was provisionally numbered as a first amino acid.

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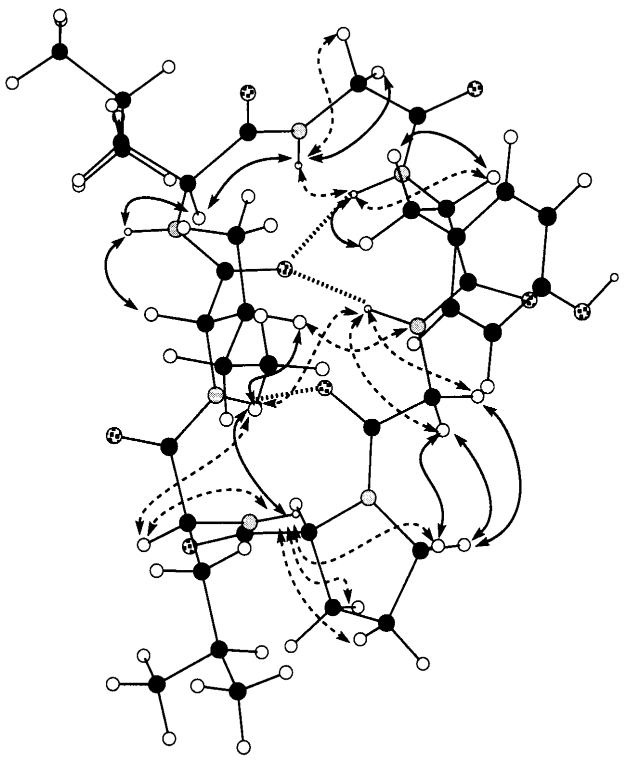


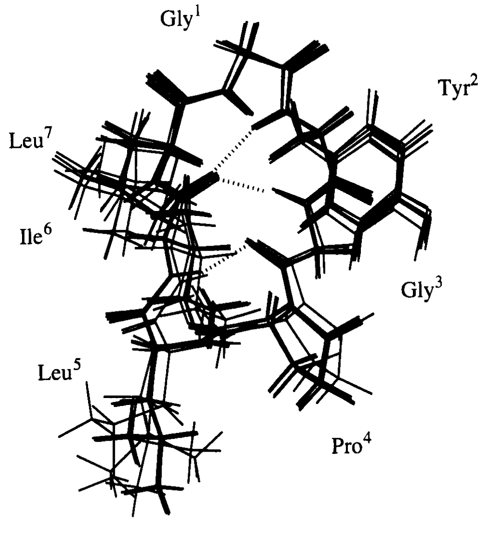
Fig. 2. Proposed Conformation of Pseudostellarin D in Solution

Arrows show strong ROE relationship and dashed arrows show medium and weak ROE relationship. Three wide dashed lines show intramolecular hydrogen bonds.

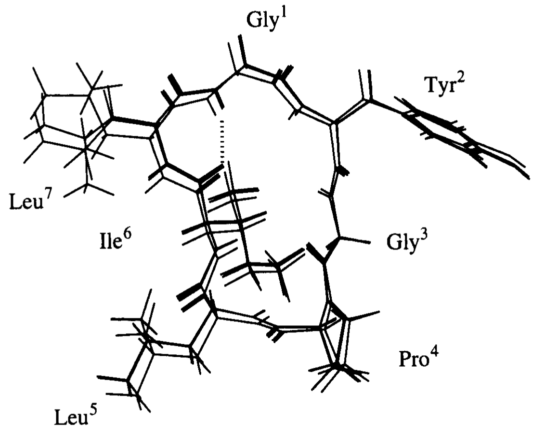
Table 1. Average Interatomic Distances and Bond Angles Involved in Hydrogen Bonds during MD Calculations

Acceptor	Donor	Distance (Å)[a]	NH...O angle (°)[a]	%[b]
Ile^6 C=O	Tyr^2-NH	2.60 (0.75)	120.4 (28.9)	48.8
Ile^6 C=O	Gly^3-NH	2.34 (0.58)	140.7 (23.6)	67.2
Gly^3 C=O	Ile^6-NH	2.32 (0.45)	154.4 (14.5)	68.7

a) The values were averaged distance and angle. *b)* The percentage was calculated from the following criteria: Maximum NH...O distance 2.5 Å; minimum NH...O angle 120°.



Conformer A



Conformer B

Fig. 3. Superposition of Conformers A (7 Conformers) and B (3 Conformers) from MD/MM Simulation

Wide dashed lines show intramolecular hydrogen bonds. All backbone atoms are superimposed to a best fit.

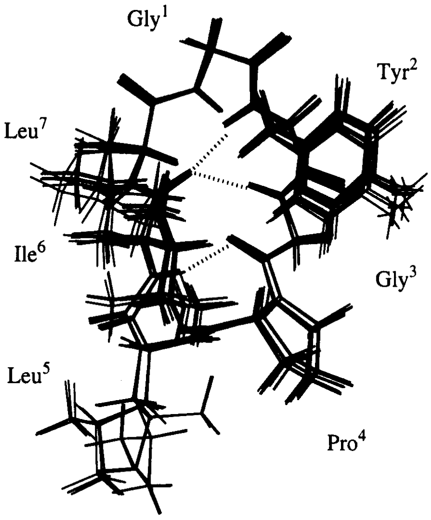
Conformer A contains two β-turn structures between Pro^4 and Leu^5 (type I), and between Leu^7 and Gly^1 (type II) as in the crystal structure. The geometry of all peptide bonds was *trans*.

During the MD simulation, three intramolecular hydrogen bonds between Tyr^2-NH...Ile^6-C=O, between Gly^3-NH...Ile^6-C=O, and between Ile^6-NH...Gly^3-C=O were detected as shown in Table 1. In conformer A, the averaged structure suggests hydrogen bonding between Ile^6-NH and Gly^3-C=O, which is present during 68.7% of the rMD simulation. This is consistent with the low temperature gradient of the Ile^6-NH (−0.7 ppb/K). In addition, two other intramolecular hydrogen bonds between Tyr^2-NH and Ile^6-C=O, and between Gly^3-NH and Ile^6-C=O, involving a β-bulge structure (Table 1), were also consistent with the low temperature effects on the Tyr^2 and Gly^3 amide protons (Tyr^2-NH 3.0; Gly^3-NH 2.3 ppb/K). Conformer A also possessed the above three intramolecular hydrogen bonds (the average value of conformer A: Tyr^2-NH...Ile^6-C=O, 2.716 Å, 157.5°; Gly^3-NH...Ile^6-C=O, 2.763 Å, 156.0°; Ile^6-NH...Gly^3-C=O 2.857 Å, 157.9°).

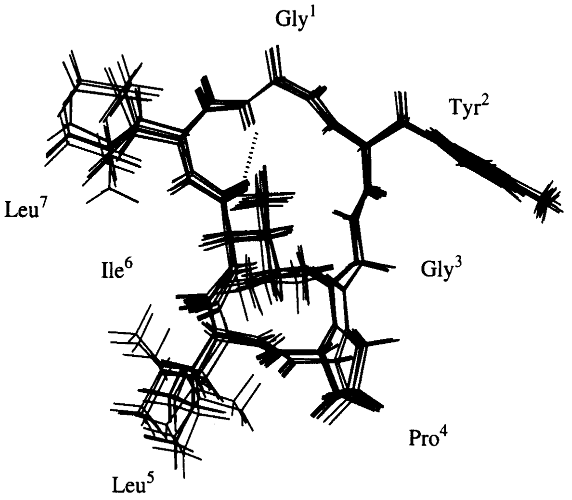
The side chain conformation of Tyr^2, Ile^6 and Leu^7 was shown to be *trans*, *gauch*⁻, and *gauch*⁻, respectively, whereas in that of Leu^5 six of the seven conformers took *gauch*⁻ conformation and one *trans*. The pyrolidine conformation of Pro^4 took C_2-$C\gamma$ *endo* form as in the crystal.[9)]

Conformer B (Fig. 3) was not in agreement with the experimental data such as that on intramolecular hydrogen bonds.

MC Simulation In our next approach, to avoid the dependence on the initial X-ray structure, an exhaustive conformational search was conducted using systematic pseudo MC technique[10)] in MacroModel/Batchmin[11)] (Ver. 4.5). The pseudosystematic MC procedure of Goodman and Still[10)] was used by changing the torsion angles of 20 ϕ, ψ, χ bonds in the backbone ring in the range of 0—180°. A total of 8000 MC steps were performed to produce 3848 conformers. After the MC con-



Conformer A



Conformer B

Fig. 4. Superposition of Conformers A (20 Conformers) and B (13 Conformers) from MC/MM Simulation

Wide dashed lines show intramolecular hydrogen bonds. All backbone atoms are superimposed to a best fit.

formational search, each of the resulting conformations was subjected to energy-minimization calculation using AMBER* force field to reduce the gradient rms to less than 0.001 kcal/Å mol. Those conformers whose energy level was less than 25 kJ/mol above the global minimum-energy conformation were chosen. To eliminate possible duplicate conformations, only the heavy atoms were compared. Among the resulting 2740 conformers, 33 appeared within a range of 2 kcal/mol. These low energy conformers were found to fall into two families: conformers A and B (Fig. 4).

Conformer A (20 conformers, 59 structures) possessed the average restraint violation of 1.65 Å (0.02), and was almost identical with conformer A obtained by MD simulation. The backbone RMSD of these 20 conformers is 0.060 Å and conformer A is characteristic of two β-turns between Pro^4 and Leu^5 (type I), and between Leu^7 and Gly^1 (type II), and a β-bulge constructed by Tyr^2–Gly^3 sequence, as in X-ray structure. The pattern and potency of three intramolecular H-bonds (the average value of conformer A: Tyr^2–NH...Ile^6–C=O, 2.722 Å, 157.2°; Gly^3–NH...Ile^6–C=O, 2.761 Å, 156.5°; Ile^6–NH...Gly^3–C=O 2.855 Å, 159.0°) was also identical with that of solid state and those obtained by MD simulation. The calculated conformer A is in good agreement with detailed experimental observations. The conformation of side chains and pyrolidine ring was also identical with the results from MD calculation.

In contrast, conformer B (13 conformers, 33 structures) shown in Fig. 4, which was almost identical with conformer B produced by MD simulation, took a different backbone conformation. Conformer B showed a γ-turn centered around the Leu^7 residue and a loose β-turn with Pro^4–Leu^5 at the $i+1$ and $i+2$ residues. A hydrogen bond between Gly^1–NH and Ile^6–C=O, taking part in construction of a γ-turn from Ile^6 to Gly^1, was only observed in conformer B and was not in agreement with the temperature coefficients measured in DMSO-d_6.

The backbone dihedrals of conformers A and B obtained from the above calculations and from ^3J coupling constants using Bystrov equation[12)] were compared with those of the X-ray structure of pseudostellarin D (Table 2). The backbone conformation of conformer A is very similar and showed homogeneity between the solution form in DMSO-d_6 and the crystal form of pseudostellarin D.

Experimental

All NMR data were recorded on Bruker AM400, AM500 and Varian Unity 400 spectrometers. Ten mg sample of pseudostellarin D in a 5 mm tube (0.5 ml DMSO-d_6, degassed) was used for the homonuclear measurements. The spectra were recorded at 300 K. A phase sensitive ROESY experiment was made with a mixing time of 90 ms and was used to evaluate a secondary structure. Temperature effect on NH chemical shifts was measured to assess the solvent accessibilities to the amide protons at 10 °C intervals over the range of 300—330 K, using a linear regression analysis.

Materials Pseudostellarin D used in this experiment was isolated as described.[5)]

Computational Methods Computer modeling experiments were carried out using the MacroModel program (version 4.5) on an IRIS 4D computer. Molecular mechanics and dynamics calculations were performed with the AMBER* force field. The dielectric constant (ε) was assumed to be proportional to the interatomic distances (r) as $\varepsilon=r$. Solvent molecules were not included in the calculations. The extended cutoff distances employed were 8 Å for van der Waals, 20 Å for charge/electrostatics and 10 Å for charge/multipole electrostatics. The ROE relationships shown in Fig. 2 were taken into account in the calculations of the constraint minimizations and dynamics with an extra harmonic term of the form $k(\theta–\theta_0)$ added to the force field ($k=100\,\text{kJ/Å}^2$). The obtained structures were energy minimized using the derivative convergence criteria at a value of 0.001 kJ/Å-mol.

MD Calculation: Initial calculation was derived from the coordinates of the crystal structure of pseudostellarin D analyzed by X-ray crystallography.[5)] After an initial equilibration period of 15 ps at 500 K, MD at 500 K with 1.0-fs time steps for a total of 100 ps was calculated with $\varepsilon=R_{ij}$. Structures were sampled at 0.1-ps intervals. The sampled structures derived from the dynamics trajectories were then energy minimized with the AMBER* all-atom force field.

Pseudo MC Calculation: MC search was carried out using the Pseudo MC routine in MacroModel. The starting structure was chosen from the coordinates of the crystal structure of pseudostellarin D. The closure bond was chosen at C1–C2 with a closure limit of 1—3 Å. Eight thousand MC steps were performed and the produced 3848 conformers, which were obtained within 25 kJ/mol of the lowest energy conformer, were

Table 2. Torsion Angles in Pseudostellarin D Calculated by Vicinal NH–CαH Coupling Constants, MD/MM and MC/MM Simulations, and X-Ray Analysis

Residue	NMR	MD/MM$^{a)}$		MC/MM$^{a)}$		X-Ray
		Conformer A	Conformer B	Conformer A	Conformer B	
Gly^1 ϕ	90	138.7	162.2	138.4	163.0	89.0
ψ		−46.0	−93.5	−46.0	−94.2	−14.0
ω		−174.2	174.8	−172.9	−174.7	−172.0
Tyr^2 ϕ	−108	−122.5	−147.0	−124.2	−146.0	−113.4
ψ		−19.9	108.1	−19.2	100.2	−53.0
ω		179.8	169.7	180.0	−168.5	180.0
χ_1		−178.8	175.8	−178.9	177.2	−172.5
χ_2		75.7, −104.1	73.4, −105.9	73.6, −106.1	72.4, −107.1	81
Gly^3 ϕ		−172.2	173.8	172.1	174.3	−161.7
ψ		176.6	179.9	176.5	173.7	−161.6
ω		−165.4	−170.6	−165.4	−166.0	172.1
Pro^4 ϕ		−64.6	−63.2	−64.4	−62.1	−71.1
ψ		−25.3	−26.7	−23.8	−26.7	−18.0
ω		175.9	168.9	176.0	−170.9	178.8
χ_1		17.6	17.0	17.2	16.4	15
χ_2		−27.0	−26.1	−26.7	−25.8	−23
χ_3		26.5	25.5	26.3	25.7	22
χ_4		−16.4	−15.7	−16.4	−16.4	−11
Leu^5 ϕ	−97	−121.4	−160.3	−123.7	−150.4	−106.0
ψ		16.9	64.9	16.8	52.1	−12.0
ω		179.5	179.1	178.6	−178.5	−172.8
χ_1			−175.1			−67
χ_2			−176.3			−83
Ile^6 ϕ	−146	−141.3	−131.3	−141.1	−127.7	−133.9
ψ		66.7	128.7	69.6	124.7	116.8
ω		−162.3	178.1	−164.5	176.8	−172.9
χ_1		−54.0	−61.1	−54.9	−59.5	−58.5
χ_2			−173.2			172
Leu^7 ϕ	−52	−59.4	−71.1	−58.4	−70.8	−56.0
ψ		106.4	79.4	106.1	80.5	126.2
ω		−167.9	−174.5	−168.6	−174.3	−179.7
χ_1						−171.1
χ_2						62

a) Averaged values of conformers; standard deviation was less than 4.2° except for side chains.

minimized by MM calculation of AMBER* all-atom force field with an extra harmonic term as in MD simulation.

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