Molecular Conformation of Tetragastrin in Aqueous Solution by the Monte-Carlo Simulation

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A conformational analysis of tetragastrin in an aqueous solution was carried out by a Monte-Carlo simulation, assuming a molecular model in a continuous media. The hydration energy of the peptide was evaluated from a calculations of the peptide–water accessible surface area. The tetrapeptide conformers in the low-energy state take an extended form, in which the two aromatic rings on both termini of the peptide are apart. The extended conformation in which the hydrophilic portions are exposed to solvent water is quite different from that in DMSO.

Gastrin is a 17 amino acid peptide; the C-terminal tetrapeptide, tetragastrin, possesses the same biological activity as that of the full sequence of gastrin.[1] This C-terminal tetragastrin, Trp–Met–Asp–Phe–NH_2 (Fig. 1), has the same sequence as that of chorecystokinin (CCK), which is a brain-gut-peptide producing gallbladder contraction and pancreatic secretion.[2] On the basis of many experimental and theoretical investigations about the conformations of the tetrapeptide, it has been revealed that the tetrapeptide shows a random-coil nature, and takes a large number of conformations in solution.[3—15] The conformations could be characterized as a whole by the displacement of the two aromatic rings of Trp^1 and Phe^4. A fluorescence transfer experiment has shown that the distance between the aromatic rings depends on the kinds of solutions or pH of the aqueous solution.[8] Theoretical studies by the molecular-orbital, molecular-mechanics, and Monte-Carlo methods have predicted that two different kinds of the dominant conformations with long and short distances exist in random conformations.[11—15] In our previous Monte-Carlo simulation, which took account of the solvent effects of DMSO, we obtained the result that a compact conformation with a short distance between the aromatic rings was in the lowest energy state in DMSO.[16,17] In the present study we carried out a Monte-Carlo simulation of tetragastrin in an aqueous solution in order to compare the conformation with that in DMSO.

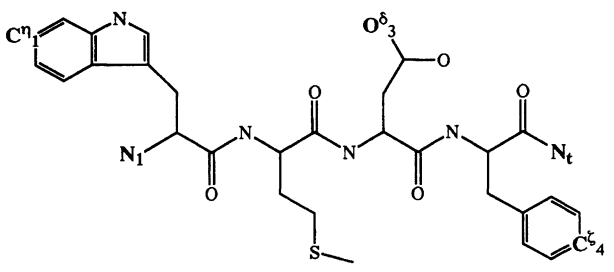

Fig. 1. Structure of tetragastrin with labeling of the specific atoms.

Method and Procedure

A Monte-Carlo simulation of the conformations of tetragastrin in an aqueous solution was undertaken using a modified version of the program used for the previous simulation for a DMSO solution.[16—18] The hydration energy in the aqueous solution was evaluated from the peptide–water accessible surface area, which was calculated by assuming atomic spheres of the 1.4 Å plus the van der Waals radius for each kind of atom. The surface area, calculated by a method developed by Perrot et al.,[19] was multiplied by the characteristic hydration coefficients, which had been evaluated by Ooi et al.[20] The hydration energy was added to the usual conformation energy, which included the nonbonding, electrostatic, bond-torsional, and hydrogen-bonding energies. The Lennard–Jones 6—12 potential function for the nonbonding energy, and the 10—12 type function for the hydrogen-bonding energy, were calculated by assuming modified ECEPP force field parameters.[21] The dielectric constant in the Coulombic electrostatic function was set to 80 for the solvent. This MC method must be able to evaluate fairly reasonable hydration energies of the molecule by relatively fast calculations. In comparison with the MC or MD methods assuming the solute model in a water assembly, this method permits calculations of a large number of different conformations of the molecule within a limited cpu time; also, it is possible to give efficiently reliable statistic profiles of the molecule in the aqueous circumstance. The results must be practically compared with those of the DMSO solution obtained from the similar MC simulation performed previously. We thus adopted this method to the present conformational analysis.

The peptide in the aqueous solution was assumed to take a zwitterionic form with the ionized N-terminal group ($-NH_3^+$) and the deprotonated carboxyl group of the side chain of Asp^3 ($-COO^-$), to compare that in DMSO on the same

condition.[17] The atomic coordinates of the initial molecular model were fixed in the standard geometry for each amino acid residue. Seventeen dihedral angles of ϕ and ψ for the main chain and χ for the side chain were varied in the model. All of the ω angles were set to 180°, and the rotation of the methyl group of Met[2] was fixed. In the calculations of the hydration energy, since the coefficients for the ionized groups were not defined, the coefficients for the unionized groups were substituted for those of the ionized groups.

The MC sampling of the conformations of the peptide chain was performed in a number of separate series of MC iterations. Each series included three stages of annealing, energy minimizing and sampling. In the annealing stage, the acceptance of the new conformation was artificially increased by assuming a small dumping factor (0.1) for the energy difference between the present conformation and the new one. In the energy-minimizing stage the system was led into the local energy minimum. In the sampling stage, the first 500 steps before the energetic equilibrium of the system were rejected; the following 1000 steps were adopted into the statistical calculations. A total of 3000 series of MC iterations were calculated. From the 3×10^6 generated conformers, the representative conformations were extracted through the local energy-minimum search routine.[17] The program, written by FORTRAN language, was prepared, and all of the computations were performed on an IBM RS/6000.

Results and Discussion

Figure 2 (a) illustrates a stereographic view of the lowest-energy conformer of tetragastrin in an aqueous solution. The torsion angles about the peptide bonds in this conformer are listed in Table 1. The conformation **b** in Fig. 2 is the lowest-energy conformation, which was obtained in our previous work for the zwitterion in DMSO.[17] In comparing both conformations, they are quite different from each other in the overall views of the molecules. Conformation **a** in the aqueous solution has an extended form, whereas conformation **b** in DMSO has a folded form. Common structural features are partially seen in the portions of the main chain and of the methionine side chain. The most obvious difference between the two structures is, however, found in the relative position of the two aromatic rings on both termini of the peptide. The two rings of conformer **a** are largely separated, whereas those of conformer **b** are close to each other.

Although a large number of the local energy-minimum conformers were extracted through the present calculations for the aqueous solution, most of them took the type-**a** conformation. Although other types of conformations, such as a folded conformation **b**, were also found in the aqueous solution, their energy levels were higher than the lowest one by more than ca. 63 kJ. Thus, their existing probabilities were very low, and conformer **a** is representative of the major conformers existing in an aqueous solution. The major conformers, however, have vast structural variations within a certain tolerance. The distribution of the total conformers including the minor groups is probably broadened and shifted in geometric space. The statistical distributions of the conformation number vs. the interatomic distance between the selected atoms in the molecule are shown in Fig. 3, where the distributions for the DMSO solvent, which were calculated in the previous work, are also shown for a comparison; the solid lines indicate that in the aqueous solution, and the dotted lines indicate that in DMSO. Figure 3 (i) shows the distributions vs. the distance between the two nitrogen atoms of the N-terminus and C-terminal amide group. Both distributions for the different solvents are similar to each other. The peaks of the distribution curves are located at about 11 Å for both cases. The N···N distances in conformers **a** and **b**, indicated in the figure, are 12.6 and 12.2 Å, respectively. Thus, the main chains in both solutions tend to prefer the extended conformation. The distributions vs. the distance between the nitrogen atom of Trp[1] and the oxygen atom of the Asp[3] side group are shown in Fig. 3 (ii). The distributions

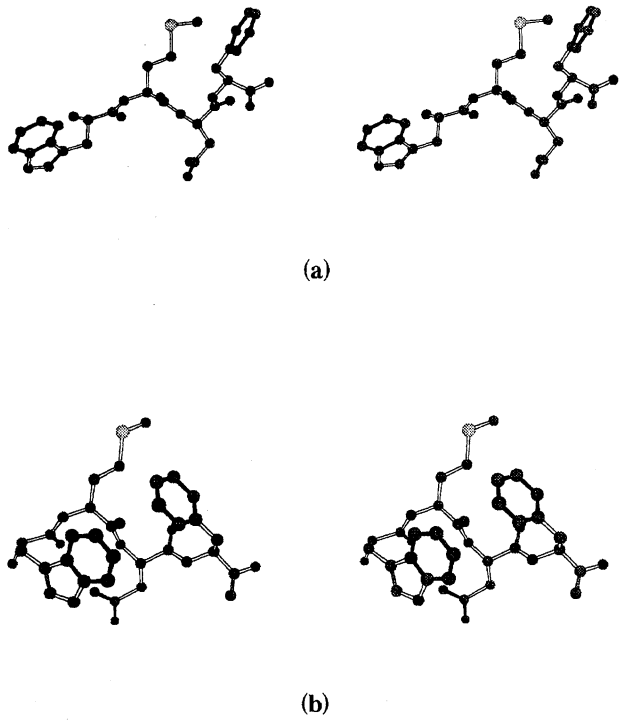

Fig. 2. Stereographic views of the lowest energy conformers, (**a**) in the aqueous solution and (**b**) in DMSO.

Table 1. Dihedral Angles for the Lowest Energy Conformation in Aqueous Solution

Residue	Torsion angle/degree				
	ϕ	ψ	χ^1	χ^2	χ^3
Trp[1]		139	−65	115	
Met[2]	−122	119	−178	−177	−171
Asp[3]	−80	104	−56	98	
Phe[4]	−80	125	−179	61	

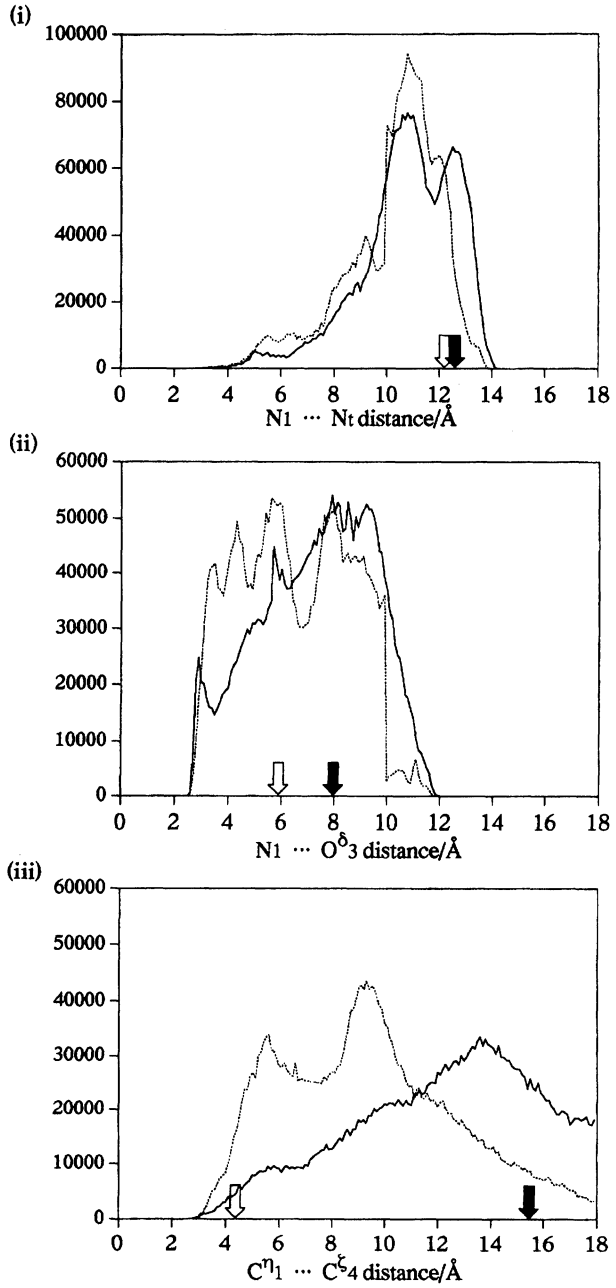

Fig. 3. Distributions of the conformers population vs. the distance (Å) between the two specified atoms, (i) $N_1 \cdots N_t$, (ii) $N_1 \cdots O_3^{\delta}$, and (iii) $C_1^{\eta} \cdots C_4^{\zeta}$. The full lines show the distribution in the aqueous solution and the dotted lines show that in DMSO. The close arrow indicates the distance in the conformer **a**, and the open arrow indicates that in the conformer **b**.

are slightly different between the aqueous and DMSO solutions. Since the nitrogen and oxygen atoms are oppositely charged in the assumed zwitterionic state, the special attractive interactions including hydrogen bonds is possible to act between these atoms. Since such electrostatic interactions might be intrinsically affected by the solvent media, they may result in a distribution that is slightly biased to the long N···O distance in an aqueous solution. Quite different distributions between aqueous and DMSO solutions were found in the distributions vs. the C···C distance between the two aromatic rings of Trp[1] and Phe[4], given in Fig. 3 (iii). The distribution for the aqueous solution is shifted to the long-distance side compared with that for DMSO. The main peak for the aqueous solution is located at about 14 Å, whereas that for DMSO is located at about 9 Å. The C···C distances in conformers **a** and **b** are 15.5 and 4.4 Å, respectively. Thus, the major conformers in the aqueous solution are extended with the two separated aromatic rings. In contrast to this, the conformers in DMSO tend to be folded with the two close aromatic rings. A fluorescence energy transfer study showed that the distance between the two aromatic rings was 15.5 Å in an aqueous solution.[8] Our present simulation for the aqueous solution is in good agreement with this result. If we compare the water-accessible surface areas of the two conformers in the lowest energy, the surface area of conformer **a** is 715.1 $Å^2$, and that of conformer **b** is 702.7 $Å^2$; the area of **a** is slightly larger than that of **b**. When the surface area is divided into hydrophobic and hydrophilic portions, the calculated percentage ratio of the hydrophobic portion vs. the hydrophilic portion of the peptide is 54 : 46 for **a** and 62 : 38 for **b**. The hydrophilic portion of **a** is larger than that of **b**. For the folded conformer **b**, in which the two aromatic rings are close to each other, the hydrophilic atoms on the main chain are hidden by the aromatic rings of the both termini. Such a conformation would thus be favorable in the hydrophobic organic solvent. For the extended conformer **a**, in which the two aromatic rings are apart from each other, on the other hand, the hydrophilic atoms on the main chain are exposed to the solvent more than for the folded conformation. Since the interactions between the hydrophilic atoms and the solvent water molecules might contribute to a decrease in the conformational energy, an extended conformation would be more stable than a folded conformation in an aqueous solution.

In conclusion, although it has been reported that tetragastrin takes random-coil conformations in solutions, the present MC investigation indicates that this tetrapeptide obviously has a tendency to take an extended conformation as the dominant form in an aqueous solution. Comparing the result with that in DMSO, we could say that a favorable conformation in solutions remarkably depends on the kind of solvent, and that the solute–solvent interactions are important for determining the conformation of the peptide. Thus, under actual physiological conditions, the tetrapeptide can possibly change the conformations. The hydrophilic interactions of the exposed main chain in the extended conformation would probably have a certain contribution to the peptide-receptor interactions in the aqueous environment, whereas the hydrophobic interactions of the aromatic groups in the folded conformation would also be important in other circumstances.

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