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Theoretical conformational analysis of tetrapeptide Ac-Cys-Pro-D-Ala-Cys-NHMe with Disulfide Linkage

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Summary

Theoretical conformational analysis was carried out for the acyclic and cyclic tetrapeptides Ac-Cys-Pro-D-Ala-Cys-NHMe using ECEPP and optimization procedure for investigating the conformational preference of peptides having disulfide linkage. Calculated results indicate that cyclic Ac-Cys-Pro-D-Ala-Cys-NHMe forms compactly fold conformations with type II β-bend at the Pro-D-Ala portion, and also show fairly good agreement with experimental results of the NMR spectroscopy for the tetrapeptides having Cys-Pro-D-Ala-Cys sequence.

Introduction

For the purpose to create new artificial proteins, it is very important to design them through an *a priori* method based on the principle of relations among three basic attributes of molecules, i.e., structural formula, conformation, and function. In nature, proteins have been playing lots of important roles in all phenomena related to life based on such clear relations that the molecular function of a protein is governed by the molecular conformation which is governed by the amino-acid sequence of the protein(1). This principle in nature suggests that the most rational method is such *a priori* method. As one basic process of the research following the *a priori* method, it is very important to make clear the relations between amino-acid sequences and molecular conformations.

As a method investigating such relations, it is well known that experimental methods are basic and important ones. However, it seems that the important roles of the theoretical methods are also growing up year by year from the following reasons. That is, experimental works essentially need plenty of experimental instruments and reagents supported by ample funds for their progress, however, theoretical works can produce excellent progress based only on the idea and intelligence without such economical restriction. Moreover, from the viewpoint of environmental disruption, the methodology controling the use of chemical reagents in research works has recently become of interest.

It is axiomatic that the conclusions obtained in the theoretical works should be always confirmed by the reliable experimental results, and also that theoretical results

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without the experimental proof are lack in reliability. While, it seems meaningless to try theoretical analysis on the systems which were already analyzed experimentally. However, it is very important to try theoretical analysis on such obvious systems in the meaning of confirming the general validity of the methodology in theoretical works, e.g., the validity of the parameters of molecular force-field used in analysis. Then, on the basis of such general validity of the methodology, further theoretical works can be tried to the systems which have not been experimentally elucidated and to those which are difficult to be experimentally analyzed. Moreover, the molecular information of peptides obtained by experimental works is averaged property for the whole possible conformations in the system. It is difficult to make clear the individual conformational character of each possible conformation by the information obtained by experimental works. However, theoretical conformational analysis can make clear the individual conformational character of each stable conformation with the value of conformational energy presenting its relative stability(i.e., occurring probability) in the ensemble of all stable local minimum conformations, and also can estimate the averaged properties of the system by taking statistical average for all local minima obtained by the optimization of their conformational energies.

From such point of view, we have tried theoretical conformational analysis based on the molecular mechanics calculations for searching all stable local minima in the whole conformational space of peptides and polypeptides which are model molecules having the key sequences in native proteins, and also showed that the lowest-energy conformations or the ensembles of the low-energy conformations of such molecules have reasonable structural characters which can explain molecular functions of native proteins. Conformational characters of the peptides(2-5) and polypeptides(6-13) proposed theoretically by the molecular mechanics calculations were also basically supported by the experimental results(14-17).

Disulfide linkage formed between two Cys residues is an important factor for stabilizing three dimensional structures of proteins as well as non-covalent bonded interactions such as hydrogen bonds, hydrophobic interactions, and salt bridges between oppositely charged residues. Especially, topological restriction by forming a disulfide linkage is very important structural factor for stabilizing local conformations at the turn portions of proteins.

In the previous works(18,19), theoretical conformational analysis was carried out for the cyclic tetrapeptides Ac-Cys-Pro-Gly-Cys-NHMe and Ac-Cys-Pro-Ala-Cys-NHMe and their related tetrapeptides using ECEPP(20) for designing the suitable amino-acid sequences for the loop portions of artificial functional proteins. Calculated results indicate that cyclic Ac-Cys-Pro-Gly-Cys-NHMe and Ac-Cys-Pro-Ala-Cys-NHMe form compactly fold conformations with type II β-bend at the Pro-Gly portion and those with type III-III double bend at the Pro-Ala-Cys portion, respectively, and also show good agreement with experimental results of the NMR spectroscopy for the tetrapeptides having Cys-Pro-Gly-Cys and Cys-Pro-Ala-Cys sequence. It means that the bend type at the -Cys-Pro-Xaa-Cys- sequence could be controlled by selecting the amino-acid residue Xaa. In this work, as the further step for investigating the conformational preference of peptides with disulfide linkage, theoretical conformational analysis was carried our for the acyclic and cyclic tetrapeptides Ac-Cys-Pro-D-Ala-Cys-NHMe based on the molecular mechanics and optimization procedure to make more clear such bend-type dependency on the amino-acid residue Xaa.

Theoretical

All conformational energy calculations were carried out with the energy functions of ECEPP. During minimizations, all ϕ of Pro,(ϕ, ψ, χ^1) of Ala and D-Ala, and (ϕ, ψ, χ^1, χ^2) of cystein (abbreviate as CyH), were allowed to vary. ϕ of Pro was fixed to -75°. All other backbone dihedral angles were fixed to 180°. Conformational energy of tetrapeptide Ac-Ala-Pro-D-Ala-Ala-NHMe, which is a model peptide of acyclic Ac-CyH-Pro-D-Ala-CyH-NHMe based on the Ala-residue approximation, was minimized using all combinations of the single-residue minima of Ala, D-Ala and Pro residues(9, 9, and 4, respectively). As the first step of minimization of cyclic tetrapeptide Ac-Cys-Pro-D-Ala-Cys-NHMe with disulfide bridge, conformational energy of acyclic tetrapeptides, Ac-CyH-Pro-D-Ala-CyH-NHMe, was minimized using all combinations of the minima of Ac-CyH-Pro-NHMe(obtained in the previous paper(18)) and the single-residue minima of D-Ala and CyH residues(9 and 47, respectively) as starting conformations. As the final step, conformational energy of cyclic tetrapeptide Ac-Cys-Pro-D-Ala-Cys-NHMe with disulfide bridge was minimized using stable minima of acyclic Ac-CyH-Pro-D-Ala-CyH-NHMe.

A bend (occurring at i +1 and i +2th residues) is defined as a conformation in which $R \leqq 7$ Å (R is the distance between i th C^α and i +3th C^α atoms.) and also classified into eleven types given in Table I of ref 21. A polar hydrogen atom and oxygen or nitrogen atom with an interatomic distance of less than 2.3 Å are regarded to be hydrogen-bonded. Vicinal NH-C^αH coupling constants $^3J_{NH\text{-}C^\alpha H}$ of Cys and D-Ala residues for Ac-Cys-Pro-D-Ala-Cys-NHMe were computed using the equation derived by Bystrov *et al*(22) and normalized Boltzmann factor(v) for all minima with ΔE<3kcal/mol. Conformational space is divided into 16 regions with the conformational letter codes shown in Figure 2 of ref 23.

Results and Discussion

There were 669 energy minima for Ac-Ala-Pro-D-Ala-Ala-NHMe with ΔE<10.0kcal/mol, and 10 of them are shown in Table I. The lowest-energy conformation is a compactly fold conformation(DCC*C conformation) which takes type V-V' double bend at Pro-D-Ala-Ala portion stabilized by the hydrogen bonds (Ala_1)CO•••HN(D-Ala) and (Pro)CO•••HN(Ala_4). The 2nd low-energy conformation(ΔE=0.01kcal/mol) is DCC*F one which takes type V β-bend at Pro-D-Ala portion, and is also a compactly fold conformation stabilized by the (Ala_1)CO•••HN(D-Ala) and (Pro)CO•••HN(Ala_4). As the atom-pair distance of (Pro)C^α•••C(NHMe) (7.3 Å) is very close to the critical value of 7.0 Å of defining β-bend structures, this conformation is also regarded as a conformation analogous to the double-bend structures. All of 10 conformations with ΔE<1.0kcal/mol are also compactly fold conformations with β-bend at Pro-D-Ala portion or with double bend at Pro-D-Ala-Ala portion. These compactly fold conformational characters including their bend types almost correspond to those for Ac-Ala-Pro-Gly-Ala-NHMe in the previous work(18) in spite of the difference in their relative stabilities. However, bend types for Ac-Ala-Pro-D-Ala-Ala-NHMe are significantly different from those for Ac-Ala-Pro-Ala-Ala-NHMe in the previous work(19) in spite of their common conformational characters that both of them take commonly compact conformations. That is, stable conformations of Ac-Ala-Pro-D-Ala-Ala-NHMe take the type V or II β-bend at

Table I. Calculated Minimum Energy Conformations[a] of Ac-Ala-Pro-D-Ala-Ala-NHMe

Conformational Letter Code	ΔE^b (kcal/mole)	v^c	Bend Type[d]	ϕ Ala1	ψ Ala1	ϕ Pro	ψ D-Ala	ϕ D-Ala	ψ Ala4	ϕ Ala4
DCC*C	0.00	0.150	V V'	-152	80	75	79	-85	-87	77
DCC*F	0.01	0.146	V -	-153	80	81	86	-71	-79	148
DCD*A	0.30	0.091	IV III	-152	80	85	155	-58	-79	-40
DCB*E	0.45	0.071	II -	-154	79	78	105	-50	-151	157
DCC*D	0.51	0.063	V IV	-151	80	84	88	-72	-149	76
DCD*C	0.61	0.054	IV IV	-151	80	78	152	-62	-87	74
DCA*D	0.75	0.042	II -	-152	79	79	71	42	-152	63
DFC*A	0.76	0.042	II III	-152	80	158	86	-68	-68	-48
DFC*C	0.86	0.036	II V'	-152	80	143	82	-78	-86	77
DCC*D	0.95	0.030	V IV	-151	80	78	80	-81	-154	51

[a]All minima with $\Delta E < 1.00$ kcal/mole.
[b]E_0 =-8.24 kcal/mole, $\Delta E = E - E_0$
[c]Normalized Boltzmann factor at 300K.
[d]Bend type for Pro-D-Ala and D-Ala-Ala.

Table II. Calculated Minimum Energy Conformations[a] of Ac-CyH-Pro-D-Ala-CyH-NHMe

Conformational Letter Code	ΔE^b (kcal/mole)	v^c	Bend Type[d]	ϕ CyH1	ψ CyH1	ϕ Pro	ψ D-Ala	ϕ D-Ala	ψ CyH4	ϕ CyH4
DCC*F	0.00	0.017	V -	-155	81	73	81	-77	-84	151
DCC*F	0.04	0.016	V -	-155	81	73	81	-77	-85	151
DCC*C	0.08	0.015	V V'	-155	80	71	77	-85	-87	97
DCC*C	0.10	0.014	V V'	-155	80	71	77	-85	-87	98
DCC*C	0.17	0.013	V V'	-155	80	71	76	-86	-88	91
DCC*C	0.22	0.012	V V'	-155	80	71	76	-86	-88	91
DCC*A	0.25	0.011	V II'	-153	83	83	79	-104	-79	-50
DCC*A	0.27	0.011	V II'	-153	83	83	79	-104	-78	-49
DCC*F	0.35	0.009	V -	-155	81	72	80	-77	-78	155
DCC*F	0.36	0.009	V -	-154	83	72	80	-77	-85	152

[a]All minima with $\Delta E < 0.37$ kcal/mole.
[b]E_0 =-9.99 kcal/mole, $\Delta E = E - E_0$
[c]Normalized Boltzmann factor at 300K.
[d]Bend type for Pro-D-Ala and D-Ala-CyH.

the Pro-D-Ala portion, but those of Ac-Ala-Pro-Ala-Ala-NHMe take the type III β-bends at the Pro-Ala portion. These results caused by the different characters of Pro-Xaa, i,e., conformational preference of Pro-D-Ala is resemble to that of Pro-Gly, but it is completely different from that of Pro-Ala(21,24).

There were 12014 energy minima for Ac-CyH-Pro-D-Ala-CyH-NHMe with ΔE<6.9kcal/mol, and 10 of them are shown in Table II. The lowest-energy conformation is a compactly fold conformation(DCC*F conformation) which takes type V β-bend at Pro-D-Ala portion stabilized by the hydrogen bonds (CyH$_1$)CO•••HN(D-Ala) and (Pro)CO•••HN(CyH$_4$) as shown in Figure 1. As the atom-pair distance of (Pro)C$^\alpha$••• C(NHMe) (7.02Å) is just out of the critical value of 7.0Å of defining β-bend structures, this conformation is also regarded as a conformation analogous to the double-bend structures. The 2nd low-energy conformation(ΔE=0.04kcal/mol) is also DCC*F one. Conformational difference between them is only found in χ^2 of CyH$_4$, i.e., χ^2=

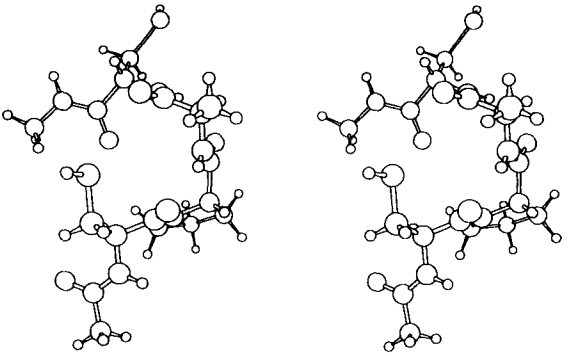

Fig. 1. The lowest-energy conformation(DCC*F) of Ac-CyH-Pro-D-Ala-CyH-NHMe.

180° and -65° for the lowest- and 2nd low-energy conformations, respectively. The 3rd, 4th, 5th and 6th low-energy conformations(ΔE=0.08, 0.10, 0.17 and 0.22kcal/mol, respectively) are DCC*C ones which take type V-V' double bend at Pro-D-Ala-CyH portion stabilized by the hydrogen bonds (CyH_1)CO•••HN(D-Ala) and (Pro)CO •••HN(CyH_4). Their overall backbone conformations are very resemble to those of the DCC*F ones. All of 28 stable conformations with ΔE<0.5kcal/mol are one of the following 6 conformation-types whose conformational letter codes are DCC*F(type V β-bend), DCC*C (type V-V' double bend), DCC*A(type V-II' double bend), DCC*D(type V-IV double bend), DFC*A(type II-II' double bend), and DCC*E(type V-IV double bend). These results indicate that overall conformational feature of Ac-CyH-Pro-D-Ala-CyH-NHMe is compactly fold conformation with double-bend or double-bend like structure taking type V(which is a modified bend of type II) or type II β-bends at Pro-D-Ala portion. These conformational characters Ac-CyH-Pro-Ala-CyH-NHMe including the bend type almost correspond to those of Ac-CyH-Pro-Gly-CyH-NHMe(18) in spite of the slight difference in their relative stabilities. However, there are significant difference in bend types between stable conformations of Ac-CyH-Pro-D-Ala-CyH-NHMe and those of Ac-CyH-Pro-Ala-CyH-NHMe(19) caused by the difference in the conformational characters between D-Ala and Ala residues. The conformational feature of Ac-CyH-Pro-D-Ala-CyH-NHMe is very resemble to that of Ac-Ala-Pro-D-Ala-Ala-NHMe. That is, all above 6 types of backbone conformations of Ac-CyH-Pro-D-Ala-CyH-NHMe are also found as the stable conformations of Ac-Ala-Pro-D-Ala-Ala-NHMe in spite of the change in relative stability of each conformation. It suggests that the SH group of the cystein residue has not so important roles for stabilizing the backbone conformation of Ac-CyH-Pro-D-Ala-CyH-NHMe as a whole, as already shown in the previous works for Ac-CyH-Pro-Gly-CyH-NHMe and Ac-CyH-Pro-Ala-CyH-NHMe(18,19).

There were 20 energy minima for Ac-Cys-Pro-D-Ala-Cys-NHMe with ΔE<3.0kcal/mol, and 10 of them are shown in Table III. The lowest-energy conformation is a DCA*F conformation(Figure 2) taking type II bend at Pro-D-Ala portion and non-bend structure at D-Ala-Cys portion. As shown in Figure 2, the atom-atom pair (Pro)C^{α}H•••HN(D-Ala) presents very close contact, i.e., its distance is 2.4 Å. These short interatomic distances show fairly good agreements with NOE measurements that an

Table III. Calculated Minimum Energy Conformations[a] of Ac-Cys-Pro-D-Ala-Cys-NHMe

Conformational Letter Code	ΔE[b] (kcal/mole)	v[c]	Bend Type[d]	ϕ Cys1	ψ Cys1	ϕ Pro	ϕ D-Ala	ψ D-Ala	ϕ Cys4	ψ Cys4
DCA*F	0.00	0.350	II -	-155	76	75	70	29	-74	153
DFA*E	0.24	0.236	II -	-153	84	131	72	49	-128	145
DFA*D	0.43	0.171	II -	-153	83	132	70	47	-125	82
DCD*C	0.95	0.071	II -	-154	92	87	152	-31	-57	130
DCD*A	1.24	0.044	II III	-154	93	87	153	-31	-58	-51
A*CA*E	1.60	0.024	II -	61	82	128	74	49	-126	146
A*FA*D	1.76	0.018	II -	60	81	131	71	47	-124	79
DFA*G	1.77	0.018	II -	-153	84	133	70	47	-125	-59
DCG*E	2.24	0.008	IV -	-154	85	69	142	57	-113	138
A*CA*F	2.29	0.008	II -	54	75	75	71	29	-75	153

[a]All minima with $\Delta E < 2.31$ kcal/mole.
[b]E_0 =-5.07 kcal/mole, $\Delta E = E - E_0$
[c]Normalized Boltzmann factor at 300K.
[d]Bend type for Pro-D-Ala and D-Ala-Cys.

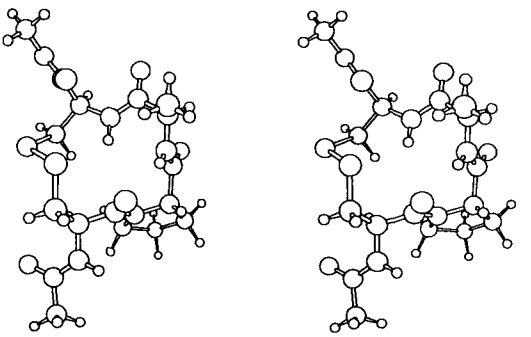

Fig. 2. The lowest-energy conformation(DCA*F) of Ac-Cys-Pro-D-Ala-Cys-NHMe.

intensity enhancement 6% was observed for Pro $C^{\alpha}H$ resonance on saturating the D-Ala amido proton of Boc-Cys-Pro-D-Ala-Cys-NHMe in $CDCl_3$(25,26). Moreover, Figure 2 also presents that the Cys4 amido proton is buried into inside of the molecule. This structural character is also supported by the small temperature dependence of the chemical shift of the Cys4 amido proton(0.4 ppb/K) in $(CD_3)_2SO$(25,26). The 2nd low-energy conformation(ΔE=0.24kcal/mol) is a DFA*E one. On the whole, this conformation almost corresponds to the lowest-energy one except the difference in the side-chain conformation of Cys residues. All of 20 stable conformations with ΔE<3.0kcal/mol also take type II or type IV(which is a distorted bend resemble to the type II) β-bend at the

Table IV. Vicinal Coupling Constant($^3J_{NH-C^\alpha H}$)of Ac-Cys-Pro-D-Ala-Cys-NHMe

	Cys1	D-Ala	Cys4
Calculated	7.8	5.6	7.9
Experimental[a]	9.0	4.5	9.0

[a]Boc-Cys-Pro-D-Ala-Cys-NHMe in $(CD_3)_2SO$ from ref 9.

Pro-D-Ala portion. Calculated occurring probability indicates that conformations taking type II β-bend at the Pro-D-Ala portion are essentially favorable in the whole ensemble of the stable conformations of Ac-Cys-Pro-D-Ala-Cys-NHMe. It also corresponds to Ravi et al.'s conclusion(25,26) that Ac-Cys-Pro-D-Ala-Cys-NHMe takes type II β-bend at the Pro-D-Ala portion. As shown in Table IV, these points are also supported by the fairly good agreement of the calculated vicinal NH-C$^\alpha$H coupling constants $^3J_{NH-C^\alpha H}$ of Cys and D-Ala residues with experimentally evaluated ones for Boc-Cys-Pro-D-Ala-Cys-NHMe in $(CD_3)_2SO$ solution(26).

Comparing with Tables II and III, it is indicated that forming the S-S linkage stabilizes the type II bend structures and destabilizes the double bend structures(DCC*C and DCC*A conformations) and double bend-like structures(DCC*F conformation). It means that using the conformational constraint with the S-S linkage is a useful method for designing the type II bend structures at the turn portion of artificial proteins.

Conformational preference of Ac-Cys-Pro-D-Ala-Cys-NHMe is very resemble to that of Ac-Cys-Pro-Gly-Cys-NHMe(18) in the meaning that they have remarkable tendency taking the type II β-bend at Pro-Xaa portion, and such character is more remarkable in Ac-Cys-Pro-D-Ala-Cys-NHMe than in Ac-Cys-Pro-Gly-Cys-NHMe. However, Conformational preference of Ac-Cys-Pro-D-Ala-Cys-NHMe is clearly different from that of Ac-Cys-Pro-Ala-Cys-NHMe in the following points that Ac-Cys-Pro-Ala-Cys-NHMe has the tendency taking the type III-III double bend at the Pro-Ala-Cys portion(19).

As already discussed in the previous work(19), an D-Ala residue is used as a model residue for the general alanine-type residues such as D-Val, D-Leu, etc. based on the Ala-residue approximation(27-29). It was already shown that the Ala-residue approximation is a useful method for analyzing the conformational preference of the peptides and polypeptides as the primary procedure in the theoretical conformational analysis(4,6,7). The conclusion obtained in this and the previous works could be generally applicable for the -Cys-Pro-Xaa-Cys-(Xaa is a alanine-type residue) sequences except for the case with significant side-chain/backbone or side-chain/side-chain interactions. These points are experimentally supported by the X-ray diffraction measurements for the single-crystals of Ac-Cys-Pro-Val-Cys-NHMe and Ac-Cys-Pro-Ser-Cys-NHMe(30) and ^{1}H-NMR measurement for Ac-Cys-Pro-D-Val-Cys-NH_2 in $(CD_3)_2SO$(31). That is, it is suggested that different fold structures could be designed as the turn portion of the artificial proteins by selecting the residue Xaa for the -Cys-Pro-Xaa-Cys- sequences.

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