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Theoretical conformational analysis of disulfide-linked tetrapeptides Ac-Cys-Pro-D-Xaa-Cys-NHMe having hydrophobic D-Xaa amino-acid residues

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Summary

Theoretical conformational analysis was carried out for four disulfide-linked tetrapeptides Ac-Cys-Pro-D-Xaa-Cys-NHMe(D-Xaa= D-Val, D-Phe, D-Leu, and D-norleucine) using ECEPP and optimization procedure for investigating how the stabilities of the β-bend conformation at D-Xaa-Pro portion are affected by branching and bulkiness of hydrophobic side-chain groups of D-Xaa residue. Calculated results indicate that cyclic Ac-Cys-Pro-D-Xaa-Cys-NHMe commonly have a dominant character taking type II β-bend at the Pro-D-Xaa portion and also show fairly good agreement with experimental results of the NMR spectroscopy for a tetrapeptide Ac-Cys-Pro-D-Val-Cys-NH_2. It is shown that the Ala-residue approximation is also a reasonable method to investigate the basic local conformational character of the Cys-Pro-D-Xaa-Cys sequence as well as that of the Cys-Pro-Xaa-Cys one. Moreover, it is suggested that the disulfide-linked tetrapeptides Ac-Cys-Pro-D-Xaa-Cys-NHMe are good candidates of the simple standard molecules for investigating the spectroscopic characters related to type II β-bend conformations.

Introduction

In the previous works(1-3), we theoretically analyzed the disulfide-linked tetrapeptides Ac-Cys-Pro-Gly-Cys-NHMe(1), Ac-Cys-Pro-Ala-Cys-NHMe(2), and Ac-Cys-Pro-D-Ala-Cys-NHMe(3) using ECEPP(4) for the purpose of investigating the suitable sequences for constructing the specific local structure in artificial proteins. It was shown that cyclic Ac-Cys-Pro-Gly-Cys-NHMe and Ac-Cys-Pro-D-Ala-Cys-NHMe form type II β-bend at the Pro-Gly and Pro-D-Ala portions, respectively, and also that cyclic Ac-Cys-Pro-Ala-Cys-NHMe forms type III β-bend at the Pro-Ala portion. These results mean that stable bend type at the Cys-Pro-Xaa-Cys sequence could be controlled by selecting the amino-acid residue Xaa. In these work, the Ala and D-Ala residues were used as the most simplified model residues for general amino-acid residues having various side-chain groups by applying the Ala-residue approximation(5).

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Then, we also tried theoretical works for the disulfide-linked tetrapeptides Ac-Cys-Pro-Xaa-Cys-NHMe(Xaa=Val, Phe, Leu, and norleucine abbreviated as Nle) whose Xaa residues have non-polar side-chain groups, ant it was shown that the type III β-bend is essentially stable local conformations for the Pro-Xaa portion in these four tetrapeptides(6). And, conformational characters of these disulfide-linked tetrapeptides are supported by the experimental results of X-Ray crystallography for cyclic Ac-Cys-Pro-Val-Cys-NHMe and Boc-Cys-Pro-Leu-Cys-NHMe(7,8), and also by those of NMR measurements for cyclic Boc-Cys-Pro-Xaa-Cys-NHMe(Xaa= Val, Phe and Leu) in $(CD_3)_2SO$ or $CDCl_3$ solution(9-12). These results indicate that the disulfide-linked Cys-Pro-Xaa-Cys sequences have common conformational characters forming type III β-bend at the Pro-Xaa portion for the case that Xaa residue has no favorable side-chain/side-chain or side-chain/backbone interaction. That is, they also indicate that the Ala-residue approximation is a reasonable method to investigate the basic local conformational character of the amino-acid sequence in the initial step for designing backbone structures of artificial proteins.

In this work, as a further step for investigating the effects of the side-chain groups on the conformational preference of the disulfide-linked tetrapeptides having Cys-Pro-D-Xaa-Cys sequence, i.e., for elucidating whether they, as well as the Cys-Pro-D-Ala-Cys sequence in the Ala-residue approximation, have propensities forming type II β -bend at the Pro-D-Xaa portion or not, theoretical conformational analysis was carried out for four cyclic tetrapeptides Ac-Cys-Pro-D-Xaa-Cys-NHMe(D-Xaa=D-Val, D-Phe, D-Leu, and D-Nle) using ECEPP(4) and optimization procedure(13).

Theoretical

All conformational energy calculations were carried out for four disulfide-linked oligopeptides Ac-Cys-Pro-D-Xaa-Cys-NHMe(D-Xaa=D-Val, D-Phe, D-Leu, and D-Nle) with the energy functions of ECEPP(4). During minimizations using the Powell Algorithm(13), all ϕ of Pro, (ϕ, ϕ, χ^1, $\chi^{2,1}$, $\chi^{2,2}$) of D-Val, (ϕ, ϕ, χ^1, χ^2) D-Phe, (ϕ, ϕ, χ^1, χ^2, $\chi^{3,1}$, $\chi^{3,2}$) of D-Leu (ϕ, ϕ, χ^1, χ^2, χ^3, χ^4) of D-Nle, and (ϕ, ϕ, χ^1) of cystine were allowed to vary. ϕ of Pro was fixed to -75°. All other backbone dihedral angles were fixed to 180°. All combination of single residue minima of Cys, Pro, and D-Xaa residues were used as starting conformations of minimization. Selected number of all stable single-residue minima were 21, 4, 10, 28, 15, and 60 for Cys, Pro, D-Val, D-Phe, D-Leu, and D-Nle, respectively. As additional calculations, conformational energy of Ac-Pro-D-Xaa-NHMe(D-Xaa=D-Val, D-Phe, D-Leu, and D-Nle) was also minimized in a similar manner as tetrapeptides.

A bend (occurring at i +1 and i +2th residues) is defined as a conformation in which $R \leq 7$ 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 14. 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. The conformational energy per whole molecule, ΔE is defied by $\Delta E = E - Eo$, Eo is the value of E at the global minima on the potential energy surface of the particular molecules. Vicinal NH-C^αH coupling constants $^3J_{NH\text{-}C^\alpha H}$ of Cys and D-Xaa residues for Ac-Cys-Pro-D-Xaa-Cys-NHMe were computed using the equation derived by Bystrov et al.(15) and normalized Boltzmann factor(v) for all minima with Δ

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

Conformational Letter Code	ΔE^b (kcal/mole)	v^c	Bend Type[d]	ϕ Cys1	ψ Cys1	ϕ Pro	ϕ D-Val	ψ D-Val	ϕ Cys4	ψ Cys4
DCA*E	0.00	0.282	II -	-153	83	125	78	50	-131	146
DCA*F	0.07	0.250	II -	-155	75	74	72	30	-75	153
DCA*D	0.13	0.229	II -	-153	82	127	75	49	-128	78
DCA*F	1.09	0.045	II -	-155	76	75	71	25	-70	144
A*CA*E	1.29	0.032	II -	61	81	122	80	51	-130	147
A*CA*D	1.38	0.028	II -	60	80	125	77	49	-127	176
DCA*G	1.58	0.020	II -	-153	83	127	76	48	-128	-59
DCG*F	1.64	0.018	II -	-154	83	78	125	59	-99	136
DCA*F	1.70	0.016	II -	-153	86	100	95	53	-83	136
ECA*D	2.17	0.008	II -	-160	153	127	79	43	-126	79

[a]All minima with $\Delta E < 2.17$ kcal/mole.
[b]E_0 =-4.10 kcal/mole, $\Delta E = E - E_0$
[c]Normalized Boltzmann factor at 300K.
[d]Bend type for Pro-D-Val and D-Val-Cys.

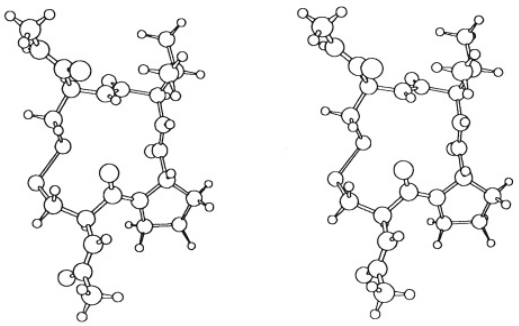

Fig. 1. *The lowest-energy conformation(DCA*E) of Ac-Cys-Pro-D-Val-Cys-NHMe.*

E<3kcal/mol. Conformational space is divided into 16 regions with the conformational letter codes shown in Figure 2 of ref 16. All molecular diagrams are described by the modified PEPCON program which is a new version of the original PEPCON(17) and NAMOD(18) programs.

Results and Discussion

There were 350 energy minima for Ac-Cys-Pro-D-Val-Cys-NHMe with ΔE<10.0kcal/mol, and 10 of them(ΔE<2.17kcal/mol) are shown in Table I. The lowest-energy conformation is a DCA*E conformation(D, C, A* and E are conformational letter codes for the Cys1, Pro, D-Val and Cys4 residues, respectively.) taking type II β-bend at the Pro-D-Val portion as shown in Figure 1. Its atom-atom pair (Pro)C^{α}H•••HN(D-Val) presents very close contact. This short interatomic distance shows fairly good

agreement with the experimental results that a strong NOE was observed between the D-Val amido proton and the Pro α-proton of Ac-Cys-Pro-D-Val-Cys-NH_2 in $(CD_3)_2SO$(19). Moreover, Figure 1 also presents that the Cys4 amido proton is buried into the interior of the molecule. This structural character is also supported by the small temperature dependence of the chemical shift of the Cys4 amido proton(0.38 ppb/K) in $(CD_3)_2SO$(19). The 2nd low-energy conformation(ΔE=0.07kcal/mol) is a DCA*F one taking type-II β-bend at the Pro-D-Val portion. On the whole, this conformation is resemble to the lowest-energy one except for the differences in the side-chain conformation of Cys residues and values of ϕ Cys1 and ϕ Cys4. By these conformational changes, the Cys4 amido proton is more moved into the interior of the molecule compared to that of the lowest-energy conformation. The 3rd low-energy conformation(ΔE=0.13kcal/mol) is a DCA*D one. This conformation almost corresponds to the lowest-energy one except for the value of ϕCys4, i.e., the conformational difference between them is only found in the direction of the peptide group at the C-terminal. Furthermore, all of 25 stable conformations with ΔE<3.0kcal/mol also take type II β-bend at the Pro-D-Val portion. Calculated results indicate that the conformations taking type II β-bend at the Pro-D-Val portion are essentially favorable in the whole ensemble of the stable conformations of Ac-Cys-Pro-D-Val-Cys-NHMe. It also corresponds to Garcia-Echeverra et al.'s conclusion(19) that Ac-Cys-Pro-D-Val-Cys-NH_2 takes type II β-bend at the Pro-D-Val portion in $(CD_3)_2SO$. These points are also supported by the good agreement between the calculated vicinal NH-$C^{\alpha}H$ coupling constants $^3J_{NH\text{-}C^{\alpha}H}$(7.9, 6.1, and 8.9 for the Cys1, D-Val, and Cys4 residues, respectively) and the experimentally evaluated ones for Ac-Cys-Pro-D-Val-Cys-NH_2 in $(CD_3)_2SO$ solution(8.4, 7.6, and 7.4 for the Cys1, D-Val, and Cys4 residues, respectively).

There were 495 energy minima for Ac-Cys-Pro-D-Phe-Cys-NHMe with ΔE<10.0kcal/mol, and 10 of them(ΔE<1.66kcal/mol) are shown in Table II. The lowest-energy conformation is a DFA*D conformation taking type II β-bend at the Pro-D-Phe portion as shown in Figure 2. Its conformational feature almost corresponds to the 3rd low-energy conformation of Ac-Cys-Pro-D-Val-Cys-NHMe even though their letter codes of the Pro residue are different. The 2nd and 3rd low-energy conformations are DCA*F(ΔE=0.14kcal/mol) and DFA*E(ΔE=0.50kcal/mol) conformations, and they correspond to the 2nd low- and the lowest-energy ones of Ac-Cys-Pro-D-Val-Cys-NHMe, respectively. Moreover, 34 of 35 stable conformations with ΔE<3.0kcal/mol also take type II or type IV(which is a distorted type of the type II) at the Pro-D-Phe portion and their total occurring probability is 0.997. Only one type VII β-bend conformation was found as the 16th low-energy one(ΔE=2.01kcal/mol). That is, calculated results indicate that the conformations taking type II β-bend at the Pro-D-Phe portion are essentially favorable in the whole ensemble of the stable conformations of Ac-Cys-Pro-D-Phe-Cys-NHMe.

There were 1017 energy minima for Ac-Cys-Pro-D-Leu-Cys-NHMe with ΔE<10.0kcal/mol, and 10 of them(ΔE<1.62kcal/mol) are shown in Table III. All of three stable conformations with ΔE<1.0 kcal/mol also correspond to those with ΔE<1.0 kcal/mol of Ac-Cys-Pro-D-Val-Cys-NHMe. And 36 of 37 stable conformations with ΔE=3.0kcal/mol also take type II or type IV at the Pro-D-Leu portion and their total occurring probability is 0.998. Only one type VII β-bend conformation was found as

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

Conformational Letter Code	ΔE^b (kcal/mole)	V^c	Bend Type[d]	ϕ Cys1	ϕ Cys1	ϕ Pro	ϕ D-Phe	ϕ D-Phe	ϕ Cys4	ϕ Cys4
DFA*D	0.00	0.251	II -	-153	82	137	65	40	-115	84
DCA*F	0.14	0.197	II -	-156	76	75	73	25	-72	150
DFA*E	0.50	0.109	II -	-153	83	135	67	42	-118	138
DCD*C	0.79	0.067	II -	-154	92	87	150	-25	-60	128
DCD*C	0.87	0.058	II -	-154	93	89	147	-26	-59	129
DCD*A	0.89	0.056	II -	-154	94	85	152	-27	-62	-51
DCA*F	0.94	0.052	II -	-155	76	74	75	24	-70	153
DCD*A	1.18	0.035	II -	-154	94	89	147	-27	-60	-51
A*FA*D	1.27	0.030	II -	60	80	136	66	40	-113	83
DFA*D	1.63	0.016	II -	-153	82	135	72	40	-114	80

[a]All minima with $\Delta E < 1.66$ kcal/mole.
[b]E_0 =-8.91 kcal/mole, $\Delta E = E - E_0$
[c]Normalized Boltzmann factor at 300K.
[d]Bend type for Pro-D-Phe and D-Phe-Cys.

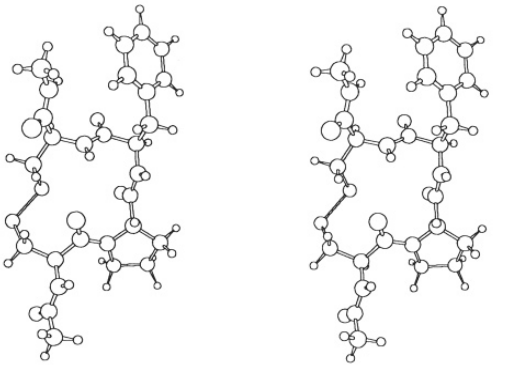

Fig. 2. *The lowest-energy conformation(DFA*D) of Ac-Cys-Pro-D-Phe-Cys-NHMe.*

the 26th low-energy one(ΔE=2.51kcal/mol). It is also shown that Ac-Cys-Pro-D-Leu-Cys-NHMe dominantly takes the type II β-bend at the Pro-D-Leu portion.

There were 1877 energy minima for Ac-Cys-Pro-D-Nle-Cys-NHMe with ΔE<10.0kcal/mol, and 10 of them(ΔE<1.38kcal/mol) are shown in Table IV. All of six stable conformations with ΔE<1.0 kcal/mol also correspond to those with ΔE<1.0 kcal/mol of Ac-Cys-Pro-D-Val-Cys-NHMe. And 67 of 68 stable conformations with ΔE<3.0kcal/mol also take type II or type IV at the Pro-D-Nle portion and their total occurring probability is 0.998. Only one type VII β-bend conformation was found as the 55th low-energy one(ΔE=2.82kcal/mol). It is also shown that Ac-Cys-Pro-D-Nle-Cys-NHMe dominantly takes the type II β-bend at the Pro-D-Nle portion.

As mentioned above, these four cyclic tetrapeptides Ac-Cys-Pro-D-Xaa-Cys-NHMe(D-Xaa=D-Val, D-Phe, D-Leu, and D-Nle) have similar conformational preference in spite of the difference in branching and bulkiness of side-chain groups, and they

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

Conformational Letter Code	ΔE^b (kcal/mole)	V^c	Bend Type[d]	ϕ Cys1	ψ Cys1	ϕ Pro	ψ D-Leu	ϕ D-Leu	ψ Cys4	ϕ Cys4
DFA*D	0.00	0.233	II -	-153	84	131	68	52	-127	74
DCA*E	0.01	0.230	II -	-153	84	129	71	53	-129	145
DCA*F	0.01	0.228	II -	-156	76	76	70	31	-76	153
DCA*F	1.01	0.043	II -	-155	76	74	72	28	-73	153
DCA*E	1.15	0.034	II -	-153	84	126	76	50	-130	146
A*CA*D	1.31	0.026	II -	61	81	129	70	52	-126	72
A*CA*E	1.37	0.023	II -	61	82	127	73	53	-128	146
DCA*D	1.38	0.023	II -	-153	83	128	74	49	-127	80
DFA*G	1.54	0.018	II -	-153	84	132	69	51	-126	-59
DCG*C	1.61	0.016	IV -	-154	84	69	140	59	-110	103

[a]All minima with $\Delta E < 1.62$ kcal/mole.
[b]E_0 =-6.19 kcal/mole, $\Delta E = E - E_0$
[c]Normalized Boltzmann factor at 300K.
[d]Bend type for Pro-D-Leu and D-Leu-Cys.

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

Conformational Letter Code	ΔE^b (kcal/mole)	V^c	Bend Type[d]	ϕ Cys1	ψ Cys1	ϕ Pro	ψ D-Nle	ϕ D-Nle	ψ Cys4	ϕ Cys4
DCA*E	0.00	0.178	II -	-153	84	129	71	52	-129	145
DFA*D	0.06	0.176	II -	-153	84	131	69	51	-127	74
DCA*F	0.07	0.159	II -	-156	76	76	70	31	-76	153
DCA*F	0.59	0.066	II -	-155	76	74	72	29	-73	153
DCA*E	0.66	0.059	II -	-153	84	127	74	52	-130	146
DCA*D	0.88	0.041	II -	-153	83	129	72	50	-127	81
DFA*D	1.28	0.021	II -	-153	84	131	69	52	-127	69
A*CA*D	1.31	0.020	II -	60	81	129	71	51	-126	72
A*CA*E	1.36	0.018	II -	61	82	127	73	52	-128	146
DCA*F	1.38	0.018	II -	-155	77	75	71	25	-70	144

[a]All minima with $\Delta E < 1.38$ kcal/mole.
[b]E_0 =-6.47 kcal/mole, $\Delta E = E - E_0$
[c]Normalized Boltzmann factor at 300K.
[d]Bend type for Pro-D-Nle and D-Nle-Cys.

dominantly take type II β-bend at the Pro-D-Xaa portions. These characters also correspond to the previous results that Ac-Cys-Pro-D-Ala-Cys-NHMe takes type II β-bend at the Pro-D-Ala portion(3). Therefore, it is shown that the disulfide-linked Ac-Cys-Pro-D-Xaa-Cys-NHMe commonly have a dominant character taking type II β-bend at the Pro-D-Xaa portions regardless of branching and bulkiness of side-chain groups in the case that they have no significant side-chain/backbone interactions such as D-Val, D-Phe, D-Leu, and D-Nle. For dipeptides, calculated bend probabilities of type II β-bend were 0.62, 0.17, 0.51 and 0.47 for D-Xaa=D-Val, D-Phe, D-Leu and D-Nle, respectively. These results clearly indicate that the propensity forming type II β-bend increases by

forming the disulfide-linkage for all four tetrapeptides, i.e., the disulfide-linkage has a great role for stabilizing the type II β-bend at the Pro-D-Xaa portion. Calculated results suggest that the disulfide-linked tetrapeptides Ac-Cys-Pro-D-Xaa-Cys-NHMe are good candidates of the simple standard molecules for investigating the spectroscopic characters related to type II β-bend conformations.

These characters of Ac-Cys-Pro-D-Xaa-Cys-NHMe are remarkably different from those of Ac-Cys-Pro-Xaa-Cys-NHMe, i.e., type III(or type I) is essentially stable bend type for Ac-Cys-Pro-Xaa-Cys-NHMe(2,6). It means that the bend type of the disulfide-linked tetrapeptides can be controlled to either type III or type II by selecting the Pro-Xaa or Pro-D-Xaa sequence as middle portion of tetrapeptide, respectively. Further, it suggests that the tetrapeptide sequences Cys-Pro-Xaa-Cys and Cys-Pro-D-Xaa-Cys are important sequences for introducing type III and II β-bend, respectively, as local conformations in the backbone of artificial proteins. The results obtained in this work indicate that the Ala-residue approximation is a reasonable method to investigate the basic local conformational character of the amino-acid sequence in the initial step for designing backbone structures of artificial proteins having D-amino-acid residues.

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