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Theoretical conformational analysis on elastin analogue tetrapeptide Ac-Ala-Pro-Gly-Gly-NHMe

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SUMMARY

To investigate the role of the Val residue on stabilizing the γ-helix which is a proposed model conformation of elastin, conformational energy calculations using ECEPP were carried out for Ac-Ala-Pro-Gly-Gly-NHMe which is an analogous tetrapeptide for the sequence Val-Pro-Gly-Gly of elastin. The lowest-energy conformation is changed by the amino-acid substitution from Val to Ala residues, however, overall conformational characters in the ensemble of energy-minima of tetrapeptides are fundamentally maintained. The double-bend structure at Pro-Gly-Gly portion of Ac-Ala-Pro-Gly-Gly-NHMe is as favorable as that of Ac-Val-Pro-Gly-Gly-NHMe.

INTRODUCTION

To analyze the relations between amino-acid sequence and three dimensional structure of elastin, helical conformations of elstin-model polypeptide with repeated Val-Pro-Gly-Gly sequence were theoretically analyzed by Oka et al.[1,2] with the procedures of molecular force field method and three-steps method for the optimization of conformational energy. A new type helix, γ-helix, was found as the most stable helical conformation of poly(Val-Pro-Gly-Gly)[2]. γ-Helix is composed of the local turn structure formed by the 12 consecutive backbone atoms from the carbonyl carbon of Val residue to the α-carbon of Val residue in the next Val-Pro-Gly-Gly unit and its overall structure is not a spiral structure having a hole along helical axis such as α- and β-helices. γ-Helix has two kinds of the characteristic stripes along helical axis. One of them is a hydrophobic region composed of non-polar side-chain groups of Val and Pro residues, and another one is a hydrophilic region composed of polar groups such as NH and CO. These characteristic structures of γ-helix suggest that elastin could interact with imannent water molecules and then forms higher-ordered structure which induces the characteristic elasticity of elastin. Calculated results indicate that the side-chain group of Val residue strongly interacts with that of Pro residue, and these facts were also supported by the experimental results[3] that signal enhancements of Val γ-CH_3 protons of poly(Val-Pro-Gly-Gly) were caused by irradiating Pro δ-CH_2 protons in D_2O at the temperatures of 20°C to 40°C. Above results suggest the importance of the inter-residue interactions between Val and Pro residues for stabilizing the γ-helical structure.

Conformational preference of backbone conformations of the Val residue is different from that of the Ala residue within intra-residue interactions as clearly shown in the (ϕ,ψ) contour maps of Ala and Val residues

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(See Figure 2 of ref.4 and Figure 3 of ref.5). That is, energetically stable regions in the C, D, E and F conformational regions[6] of the Val residue are more restricted to small regions than those of Ala residue by the effects of the favorable side-chain/backbone interactions. However, these C, D, E and F conformations are still stable ones for the Val residue. So, it is very interesting to investigate the roles of the Val residue to stabilize the γ-helix and to analyze the effects of amino-acid substitution of Val to Ala residues on the relative stability of γ-helix. In this work, as the first step of analyzing the role of the Val residue on stabilizing the γ-helix, theoretical conformational analysis was tried for Ac-Ala-Pro-Gly-Gly-NHMe as an analogous tetrapeptide for the sequence Val-Pro-Gly-Gly in elastin molecule.

THEORETICAL

All conformational energy calculations were carried out with the energy function of ECEPP[7]. During minimizations, all (ϕ,ψ,χ^1) of Ala, (ϕ,ψ) of Gly, and ψ of Pro were allowed to vary. All other backbone dihedral angles were fixed to 180° except for ϕ_{Pro}=-75° and cis peptide bond at Val-Pro(ω_{Ala}=0°). All combinations of single residue minima were used as starting conformations. The following conformations were also used as additional starting conformations, i.e., (ϕ,ψ)=(-75°,140°) for Ala, (ϕ,ψ)=(-75°,140°) and (75°,-140°) for Gly, and ψ=-23° for Pro.

The normalized Boltzmann factor v_i is given by Eq(1) of ref.1. The statistical average of the conformation-dependent quantity for the ensemble is defined by Eq(3) of ref 1. A bend(occuring at i+1 and i+2 th residues) is defined as a conformation in which $R \leq 7A$(R is the distance between i th C^α and i+3 th C^α atoms.) and classified into eleven types given in Table I of ref 8. A polar hydrogen atom and an oxygen or nitrogen atom with an interatomic distance of less than 2.3A are regarded to be hydrogen-bonded[8]. Conformational space is divided into 16 regions with the conformational letter codes shown in Figure 1 of ref 6. Vicinal NH-$C^\alpha H$ coupling constants $^3J_{NH-C^\alpha H}$ of Val residue for each conformation were computed with the following expression of Bystrov et al[9].

$$^3J_{NH-C^\alpha H} = 9.4\cos^2\theta - 1.1\cos\theta + 0.4 \qquad (1)$$

where $\theta=\phi-60°$, and those of Gly residue were computed with the following expression of Bystrov et al[9].

$$^3J_{NH-C^\alpha H} = -9.4\cos^2\theta - 1.1\cos\theta + 14.9 \qquad (2)$$

The conformational energy per whole molecule, ΔE, is defined by $\Delta E=E-E_o$, where E_o is the value of E at global minimum on the potential energy surface of the particular molecules. ΔE_{cis} is also defined by $\Delta E_{cis}=E-E_{cis,min}$, where $E_{cis,min}$ is the value of E of the lowest-energy conformation of the particular molecule with cis peptide bond at X-Pro portion.

RESULTS AND DISCUSSION

There are 75 low-energy minima for Ac-Ala-Pro-Gly-Gly-NHMe with trans Ala-Pro peptide bond having $\Delta E<3$ kcal/mol, indicating that this peptide system is also represented by the ensemble composed of many stable conformations as shown in other linear oligopeptides containing Pro residues[1,8,10]. Backbone conformations, bend types and conforma-

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

Conformational Letter Code	ΔE^b (kcal mol^{-1})	ϕ_{Ala}	ψ_{Ala}	ψ_{Pro}	ϕ_{Gly3}	ψ_{Gly3}	ϕ_{Gly4}	ψ_{Gly4}	Bend Type	
									Pro-Gly	Gly-Gly
D C A*C*	0.00	-151	80	85	74	52	90	-71	II	I'
D C C*C	0.15	-153	79	76	80	-78	-86	76	V	V'
D C C*A	0.73	-150	80	77	81	-77	-71	-67	V	II'
D F C*C	0.90	-152	80	139	80	-77	-86	73	II	V'
D C D*A	0.95	-152	80	78	153	-50	-80	-37	IV	I
D C C*F	0.98	-152	80	83	86	-70	-83	154	V	
D C C*D	0.99	-151	80	86	87	-68	-155	67	V	IV
D F C*A	1.03	-152	80	158	84	-68	-69	-51	II	IV
D A C D*	1.06	-152	80	-51	-85	68	166	-52	I	IV
D C A*A*	1.08	-152	80	93	78	37	69	46	II	III'
D C A*D*	1.18	-151	80	74	63	49	167	-83	II	VII
D C A*F*	1.26	-153	80	84	78	48	90	-153	II	
D C C*D	1.28	-151	80	81	82	-72	-163	53	V	IV
D C D*C	1.32	-151	80	76	153	-66	-88	72	IV	IV
D C B*E	1.38	-153	79	80	106	-43	-164	170	II	
D F A*G	1.48	-153	80	138	68	46	-179	-57	II	VII
D A A A	1.49	-151	80	-43	-68	-39	-70	-51	III	III

[a] All minima with $\Delta E<1.5$ kcal mol^{-1}.

[b] $E_o=-10.82$ kcal mol^{-1}.

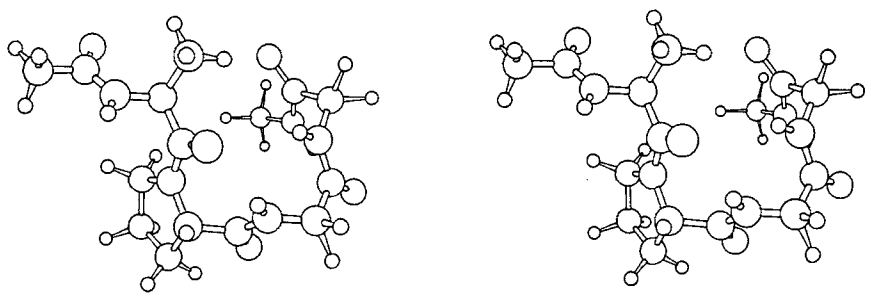

Figure 1. The lowest-energy DCA*C* conformation of Ac-Ala-Pro-Gly-Gly-NHMe with trans Ala-Pro peptide bond.

tional letter codes of all 17 minima with value of $\Delta E<1.5$ kcal/mol are listed in Table I. The lowest-energy conformation is DCA*C* conformation with a hydrogen bond (Ala)CO...HN(Gly3) as shown in Figure 1. A similar DCA*C* conformation was found as the 14th one with $\Delta E=1.14$ kcal/mol for Ac-Val-Pro-Gly-Gly-NHMe[1]. One the contrary, DCC*A conformation, which is the lowest-energy one for Ac-Val-Pro-Gly-Gly-NHMe, is found as the 3rd one with $\Delta E=0.73$ kcal/mol. The second low-energy conformation is DCC*C one ($\Delta E=0.15$ kcal/mol) with three hydrogen bonds (Ala)CO...HN(Gly3), (Pro)CO...HN(Gly4), and (Gly3)CO...HN(NHMe). This conformation was found as the 4th one with $\Delta E=0.60$ kcal/mol for Ac-Val-Pro-Gly-Gly-NHMe. Two DCC*D conformations, which correspond to the conformational unit of the γ-helix, exist as the 7 and 13th ones with $\Delta E=0.99$ and 1.28 kcal/mol, respectively, indicating that DCC*D conformation is destabilized by the amino-acid substitution from Val to Ala residues. As shown by the high probability occuring D conformation at the Ala residue ($v=0.976$), D conformation is overwhelmingly favorable for the Ala residue of Ac-Ala-Pro-Gly-Gly-NHMe,

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

Conformational Letter Code	ΔE^{b}_{cis} (kcal mol^{-1})	ϕ_{Ala}	ψ_{Ala}	ψ_{Pro}	ϕ_{Gly3}	ψ_{Gly3}	ϕ_{Gly4}	ψ_{Gly4}	Bend Type[c]	
									Pro-Gly	Gly-Gly
F A D D*	0.00	-74	148	-45	-158	60	168	-50	VII	VII
F A D A*	0.30	-66	149	-47	-153	83	87	41	VII	II
F F C*C	0.61	-81	148	161	98	-68	-84	75	II	V'
E F C*D	0.63	-157	149	150	74	-82	-160	43	II	IV
F A D G*	0.67	-67	148	-44	-161	48	154	60	VII	VII
E A C D*	0.85	-160	148	-25	-77	90	166	-79	I	IV
E F D*A	1.06	-150	147	151	140	-71	-87	-65	IV	IV
E F D*A	1.23	-149	147	170	117	-70	-78	-68	II	III
F F C*F	1.26	-77	148	161	93	-70	-86	-169	II	
F A D C*	1.29	-74	148	-41	-165	67	84	-74	VII	

[a] All minima with ΔE_{cis}<1.5 kcal mol^{-1}.

[b] $E_{cis,min}$=-8.52 kcal mol^{-1}.

[c] All conformations in this Table take type VI bend at Ala-Pro portion.

only 8 non-D conformations(A*, E and F) are found in 75 minima. Comparing with the results of Ac-Ala-Pro-NHMe(v=0.739)[8], it is clear that D conformation of Ala residue of Ac-Ala-Pro-Gly-Gly-NHMe is further stabilized by the inter-residue interactions between Ala and Gly residues. The most favorable conformation of Ala-Pro portion is DC one whose probability(v=0.746) is higher than that of Ac-Ala-Pro-NHMe(v=0.508), indicating that DC conformation is further stabilized by the inter-residue interactions between Ala-Pro and Gly-Gly portions. DCC* and DCA* conformations are favorable for Ala-Pro-Gly portion with v=0.306 and 0.301, respectively. For the case of Ac-Val-Pro-Gly-Gly-NHMe, those of DCC* and DCA* are 0.381 and 0.053, respectively. These results mean that the role of the Ala and Val residue is impotant for stabilizing the DCC* conformation, however, the further precise conformational restriction caused by the Val residue is indispensable to the dominant stabilization of DCC* conformation at X-Pro-Gly sequence.

As shown in Table I, all stable conformations in ΔE<1.5 kcal/mol form bend structure at Pro-Gly portion, and most of their bend types are type II and type V which is a distorted type II bend. These results correspond to the high propensity forming types II and V bend at Pro-Gly portion of Ac-Val-Pro-Gly-Gly-NHMe[1], Ac-Pro-Gly-Gly-NHMe[1], and Ac-Pro-Gly-NHMe[8], showing that the tendency forming bend at Pro-Gly portion is essentially determined by the intra- and inter-residue interactions in Pro-Gly sequence, and influenced by the inter-residue interactions from Ala and another Gly residue in some extents. Table I also presents that 14 of 17 conformations form bend structure at Gly-Gly portion without specific favorable bend types. These results also correspond to those of Ac-Val-Pro-Gly-Gly-NHMe[1]. Comparing to the previous results for Ac-Pro-Gly-Gly-NHMe[1] and Ac-Gly-Gly-NHMe[11], it is clear that the tendency to form bend at Gly-Gly portion is basically governed by the intra- and inter-residue interactions in Gly-Gly sequence and influenced by the inter-residue interactions from the nearest-neighbor residues(i.e., Pro residue), and also that inter-residue interactions from the next-to-nearest-neighbor residue(i.e., Ala residue) are not so important.

There are 45 low-energy minima of Ac-Ala-Pro-Gly-Gly-NHMe with cis Ala-Pro peptide bond having ΔE_{cis}<3 kcal/mol. All 10 minima with value

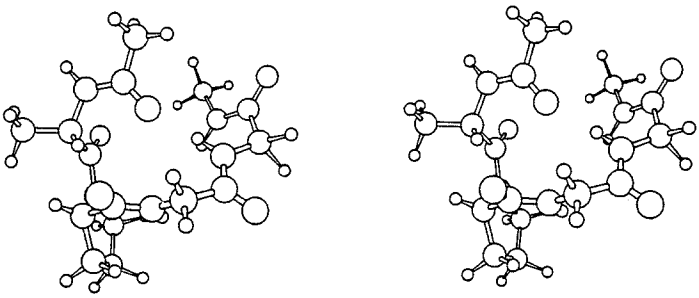

Figure 2. The lowest-energy FADD* conformation of Ac-Ala-Pro-Gly-Gly-NHMe with cis Ala-Pro peptide bond.

of $\Delta E<1.5$ kcal/mol are listed in Table II. The lowest-energy conformation is FADD* conformation with v=0.230. This conformation forms triple-bend structure with one hydrogen bond (Ac)CO...HN(Gly4) as shown in Figure 2. Other 7 conformations in Table II are also triple-bend structure, i.e., triple-bend structures are very favorable for cis Ac-Ala-Pro-Gly-Gly-NHMe. These conformational characters are basically caused by the structural restriction related to the cis peptide bond at Ala-Pro portion. Results presented in Tables I and II indicate that stable conformations of cis Ac-Ala-Pro-Gly-Gly-NHMe are different from those of trans one and that conformational restriction at Ala-Pro peptide bond causes significant effects on the whole conformational preference of this peptide. All 45 low-energy conformations of cis Ac-Ala-Pro-Gly-Gly-NHMe take bend structure at Ala-Pro portion, however, no low-energy conformations take bend structure at Ala-Pro portion among 75 minima of trans Ac-Ala-Pro-Gly-Gly-NHMe. This tendency also corresponds to the previous calculated results for X-Pro-Y[10] and Val-Pro-Gly-Gly[1]. Table II also shows that conformational preference of cis Ac-X-Pro-Gly-Gly-NHMe tetrapeptides are changed by the amino-acid substitution from Val to Ala residues. EF and EA conformations are favorable for X=Val, but FA, FF and EF conformations are favorable for X=Ala. Total probability taking triple-bend of cis Ac-Ala-Pro-Gly-Gly-NHMe (v=0.783 and 21 conformations in $\Delta E<3$ kcal/mol) is higher than that of 0.429 of cis Ac-Val-Pro-Gly-Gly-NHMe, indicating that bend structure at Gly-Gly portion of cis X-Pro-Gly-Gly is more stabilized by the inter-residue interactions between Ala and Gly-Gly portion than by those of Val and Gly-Gly portion.

$\Delta E_{cis-trans}$, which is the energy difference between the lowest-energies of trans and cis conformation, is 2.30 kcal/mol showing that trans conformation of Ac-Ala-Pro-Gly-Gly-NHMe is also more favorable than cis conformations as shown for Pro containing oligopeptides[1,8,10] with intramolecular interactions. This value almost corresponds to those of Ac-Ala-Pro-NHMe[8](2.48 kcal/mol) and Ac-Val-Pro-Gly-Gly-NHMe[1](2.75 kcal/mol). These results indicate that relative stability between cis and trans conformation at X-Pro are essentially determined by the interaction at X-Pro portion and are scarcely affected by the inter-residue interactions from Gly-Gly portion and by the amino-acid substitutions.

The energy function of ECEPP is composed of terms which describe torsional potentials and electrostatic, non-bonded, and hydrogen-bonding interactions. The parameters characterizing these interactions were calibrated by empirical fitting of the crystal structures and rotational barriers of a variety of small molecules[7,12,13]. Conformational energy

Table III. Vicinal Coupling Constants $^3J_{NH-C^{\alpha}H}$ of Ac-Ala-Pro-Gly-Gly-NHMe

	$^3J_{NH-C^{\alpha}H}$		
	Ala	Gly3	Gly4
Calculated	8.1	13.5	12.8
Experimental[a]	8.7	12.6	11.7

[a] HCO-Ala-Pro-Gly-Gly-OMe in $CDCl_3$ from ref. 12. All experimental values are corrected by the relation $^3J_{NH-C^{\alpha}H}=1.09J_{obs}$ in ref. 9.

calculations using ECEPP have been tried for many peptides and polypeptides, and it is shown that the energy function of ECEPP is reliable for the systems composed of peptides[7,10,11,14,15] and polypeptides with random-coiled[4,16] and helical conformations[2,14,17,18] supported by the good agreement between calculated and experimental results. These points are also supported by the following discussion on the calculated and experimental values of $^3J_{NH-C^{\alpha}H}$.

Calculated results indicate that no absolutely favorable conformations are found for Ac-Ala-Pro-Gly-Gly-NHMe within intra-molecular interactions. That is, any conformation-dependent properties should not be estimated from one conformation, but should be discussed as ensemble-averaged values for all stable conformations in the system. As shown in Table III, the ensemble-averaged vicinal $NH-C^{\alpha}H$ coupling constants $\langle^3J_{NH-C^{\alpha}H}\rangle$ of Ala, Gly3, and Gly4 residues for calculated energy minima are 8.1, 13.5 and 12.8. Following three points are shown by Figures 5 and 6 of ref. 9. (1)The vicinal coupling constant $^3J_{NH-C^{\alpha}H}$ indicates explicite dependence on the value of ϕ, and (2)it contains ambiguity in the range of about 1 to 2 Hz for the particular value of ϕ. (3)The value of ϕ also contains ambiguity in the range of about 10 to 20 degrees for the particular value of $^3J_{NH-C^{\alpha}H}$, however, conformation of Ac-Ala-Pro-Gly-Gly-NHMe does not significantly change by the variation of ϕ in the range of 20 degrees. Above feature on the relations between $^3J_{NH-C^{\alpha}H}$ and ϕ means that calculated $\langle^3J_{NH-C^{\alpha}H}\rangle$ of Ala, Gly3, and Gly4 indicate good agreements with the experimental values of them for HCO-Ala-Pro-Gly-Gly-OMe in $CDCl_3$ (8.7, 12.6 and 11.7 for Ala, Gly3 and Gly4) reported by Renugopalakrishnan et al. [19]. Above calculated $\langle^3J_{NH-C^{\alpha}H}\rangle$ of Gly3 and Gly4 residues are almost same as those of Gly3 and Gly4 residues for Ac-Val-Pro-Gly-Gly-NHMe[1], 13.1 and 12.0, respectively, demonstrating that overall effects of amino-acid substitution from Val to Ala residues on the conformational preference of Gly3 and Gly4 residues is not so significant. This point is also supported by the agreement between experimental value of $^3J_{NH-C^{\alpha}H}$ of HCO-Ala-Pro-Gly-Gly-OMe in $CDCl_3$[19] and that of t-Boc-Val-Pro-Gly-Gly-OMe in $CDCl_3$ with 20% C_6D_6[20]. Calculated value of $\langle^3J_{NH-C^{\alpha}H}\rangle$ of Ala in Ac-Ala-Pro-Gly-Gly-NHMe(8.1) is lower than that of Val in Ac-Val-Pro-Gly-Gly-NHMe(10.1)[1]. This difference is caused by the small difference in the value of ϕ, i.e., the most of favorable value of ϕ_{Ala} in the conformational ensemble of Ac-Ala-Pro-Gly-Gly-NHMe is almost -150°, but that

of ϕ_{Val} for Ac-Val-Pro-Gly-Gly-NHMe is almost -130°. Theoretical analysis on the grid research with 15° interval in (ϕ,ψ) space of Ac-Ala-Pro-NHMe and Ac-Val-Pro-NHMe[21] showed that the energetically favorable regions of them are slightly different, i.e., the regions around (-150°,90°) and (-135°,90°), respectively. These results indicate that conformational stabilities of the Ala and Val residues in Ala-Pro and Val-Pro dipeptides are fundamentally maintained in those of X-Pro-Gly-Gly tetrapeptides.

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