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Theoretical conformational analysis of poly(val-pro-gly-gly) with *cis* peptide bond at val-pro portion

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SUMMARY

Theoretical conformational analysis was carried out for Ac-(Val-Pro-Gly-Gly)$_6$-NHMe with cis peptide bond at Val-Pro portion. A right-handed $\beta^{11.6}$-helix was found as the lowest-energy helical conformation for this polypeptide with cis peptide bond. The energy difference between $\beta^{11.6}$-helix and γ-helix, which is the lowest-energy helical conformation with trans peptide bond, is 3.08 kcal/mol per repeating unit(Val-Pro-Gly-Gly sequence). This value almost corresponds to the energy difference between the most stable cis and trans conformations of Ac-Val-Pro-Gly-Gly-NHMe(2.75 kcal/mol). Obtained results indicate that γ-helix is the most stable helical conformation of poly(Val-Pro-Gly-Gly) which is a model polypeptide of elastin, and also that relative stabilities of trans and cis conformations of polypeptide are essentially estimated by short-range interactions.

INTRODUCTION

As a conformational model of elastin, γ-helix was proposed by Oka et al.[1] by the theoretical conformational analysis on poly(Val-Pro-Gly-Gly). γ-Helix has two kinds of characteristic stripes. One of them is composed of the hydrophobic residues such as Val and Pro, and another one is composed of the Gly residue having relatively hydrophilic properties compared with the Val and Pro residues. It was also shown that γ-helix transforms to the other conformations which have more than twice length of γ-helix along helical axis by passing through the low-energy pathway connecting two energy minimum points corresponding to γ-helix and the extended helix in the conformational space[2]. The distribution of hydrophobic and hydrophilic residues of these extended conformations are completely different from that of γ-helix. Above results indicate that γ-helix is a helical conformation which can explain the characteristic properties of elastin. On the above theoretical analysis of poly(Val-Pro-Gly-Gly), all peptide bonds at Val-Pro portion were fixed to trans position(i.e., ω= 180°). Our previous theoretical work[3] on the tetrapeptide Ac-Val-Pro-Gly-Gly-NHMe indicates that the energy difference between the most stable cis and trans conformations($\Delta E_{cis-trans}$) is 2.75 kcal/mol. This energy difference shown that trans conformation of Ac-Val-Pro-Gly-Gly-NHMe is also more favorable than cis conformation as shown for proline containing oligopeptides[4,5]. However, it is not clear that trans conformation is also energetically more favorable than cis conformation in polypeptides containing many proline residues such as poly(Val-Pro-Gly-Gly).

From the viewpoints of molecular design, it is very important to analize the relative roles of intra-residue, short-, mediun-, and long-

range interactions in polypeptides, and also to know how helical conformations of polypeptides are stabilized. In the previous theoretical works on poly(L-Ala-D-Ala)[6], poly(Ala-Gly)[7], and poly(Val-Pro-Gly-Gly) with trans peptide bond at Val-Pro portion[1,2], it was shown that helical conformations stabilized by short-range interactions are also stable ones for the case of considering long-range interactions, and also that their relative stabilities were not drastically changed by the extension of the interaction range. It means that stable helical conformations of polypeptides with the repeated amino-acid sequence can be basically predicted by the theoretical conformational analysis based on the short-range interactions. In this work, theoretical conformational analysis on the helical conformation of poly(Val-Pro-Gly-Gly) with cis peptide bond at Val-Pro portion were tried for analyzing the roles of short- and long-range interactions on stabilizing trans and cis conformations at Val-Pro portion.

THEORETICAL

All conformational energy calculations of poly(Val-Pro-Gly-Gly) were carried out with the energy function of ECEPP[8]. During minimization, all (ϕ,ψ,χ^1,$\chi^{2'1}$,$\chi^{2'2}$) of Val, (ϕ,ψ) of Gly, and ψ of Pro were allowed to vary. All other backbone dihedral angles were fixed to 180° except for ω_{Val}=0°(cis peptide bond at Val-Pro) and ϕ_{Pro}=-75°. Minimization procedure was followed by the three steps method[1,2,6,7]. The first step of minimization was carried out for Ac-Val-Pro-Gly-Gly-NHMe using all combinations of the single-residue minima of Val, Gly, and Pro residues as starting conformations as shown in Table III of ref. 3. The second step of minimization was carried out for the octapeptide having two repeating units of Val-Pro-Gly-Gly, i.e., Ac-(Val-Pro-Gly-Gly)$_2$-NHMe. During minimizations, the condition of helical conformation[1,2,6,7] was used. All minima with the relative energy ΔE<5 kcal/mol found in the first step were used as starting conformations. The final step of minimization was carried out for the polypeptide having six repeating units, i.e., Ac-(Val-Pro-Gly-Gly)$_6$-NHMe(abbreviated as poly(Val-Pro-Gly-Gly)). All minima with the relative energy ΔE<10 kcal/mol found in the second step were used as starting conformations. As additional analysis, conformational energy minimizations of Ac-Val-Pro-NHMe with trans and cis peptide bonds at Val-Pro were also tried. All combinations of the single-residue minima of Val and Pro residues were used as strating conformations of minimization.

A bend(occuring at i+1 and i+2 th residues) is defined as a conformation in which R$\leq$7Å(R is the distance between i th C^α and i+3 th C^α atoms.) and also classified into eleven types given in Table I of ref. 4. A polar hydrogen atom and an oxygen or nitrogen atom with an interatomic distance of less than 2.3Å are regarded to be hydrogen-bonded. Conformational space is divided into 16 regions with the conformational letter codes as shown in Figure 1 of ref. 9. The conformational energy per whole molecule, ΔE, is defined by $\Delta E=E-E_o$, where E_o is the value of E at the global minimum on the potential energy surface of the particular molecules, and ΔE_{res} is defined by $\Delta E_{res}=\Delta E/m$, where m is number of residues of a molecules. ΔE_{cis} is 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 molecules with cis peptide bond at Val-Pro portion. $\Delta E_{cis,res}$ is defined by $\Delta E_{cis,res}=\Delta E_{cis}/m$. $\Delta E_{cis-trans}$, which is the energy difference between the lowest-energies of the trans and cis conformations, is defined by $\Delta E_{cis-trans}=(E_{cis,min}-E_o)/m_{Pro}$, where m_{Pro} is number of the Pro residues of a molecule. The β^x_{Pro}-helix is defined as a helix which has a spiral structure with x residues per turn.

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

Conformational Letter Code	ΔE[b] (kcal/mol)	ϕ_{Val}	ψ_{Val}	χ^{1}_{Val}	$\chi^{2,1}_{Val}$	$\chi^{2,2}_{Val}$	ψ_{Pro}
D C	0.00	-132	86	-178	57	68	79
D F	0.68	-132	86	-178	57	68	161
A*C	0.99	55	79	-175	58	68	79
D A	1.25	-132	86	-178	57	68	-47
D A	1.32	-132	86	-178	57	68	-27
A*F	1.83	55	79	-174	59	68	159
D C	1.96	-139	80	-81	64	48	79
E C	2.24	-137	154	-68	66	53	79
D F	2.62	-139	80	-81	64	48	160
E F	2.80	-136	154	-69	66	53	161
A*A	2.91	55	79	-174	59	68	-47
A*A	2.94	56	79	-174	59	68	-25
D A	3.19	-139	80	-81	64	48	-48
E A	3.54	-137	154	-69	66	53	-47
D A	4.44	-153	84	48	50	41	-47
D A	4.92	-156	82	61	88	58	-47

[a] All minima with $\Delta E<5$ kcal/mol are shown.

[b] $\Delta E=E-E_o$, $E_o=-10.81$ kcal/mol.

RESULTS AND DISCUSSION

Stable Conformations of Trans Ac-Val-Pro-NHMe and Cis Ac-Val-Pro-NHMe

All 16 stable conformations of trans Ac-Val-Pro-NHMe with $\Delta E<5$ kcal/mol were shown in Table I. Bend conformations are not favorable for trans Val-Pro, i.e., all of them are non-bend conformations. These results correspond to the previous results for Ala-Pro[4], Asn-Pro[4], Phe-Pro[5] and Tyr-Pro[5]. Table I also indicates that DC, DF, A*C and DA conformations are favorable. Overall conformational preference of Val-Pro dipeptide almost corresponds to those of X-Pro dipeptides[4,5], however, relative stability of each conformation of Val-Pro is slightly different from that of X-Pro caused by the difference of side-chain/backbone and side-chain/side-chain interactions.

All 5 stable conformations of cis Ac-Val-Pro-NHMe with $\Delta E_{cis}<6$ kcal/mol are shown in Table II. Bend conformations are favorable for cis Val-Pro, i.e., all of them are bend conformations (type VI). Table II also indicates that EF and EA conformations are favorable. These results are also correspond to the previous results for cis dipeptides[4,5].

$\Delta E_{cis-trans}$ is 3.78 kcal/mol showing that trans conformation of Ac-Val-Pro-NHMe is more favorable than cis conformation as shown for X-Pro (X=Gly, Ala, Asn, Phe, and Tyr)[4,5]. The value of 3.78 kcal/mol is slightly larger than those of other X-Pro dipeptides such as Ala-Pro (2.48), Asn-Pro(2.48), Phe-Pro(1.95), and Tyr-Pro(2.97).

Stable Conformations of Cis Ac-(Val-Pro-Gly-Gly)$_2$-NHMe

A total of 66 energy minima of cis Ac-(Val-Pro-Gly-Gly)$_2$-NHMe were found in $\Delta E_{cis}<10$ kcal/mol. The lowest-energy conformation is EACD* con-

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

Conformational Letter Code	ΔE^{b}_{cis} (kcal/mol)	ϕ_{Val}	ψ_{Val}	$^{1}\chi_{Val}$	$^{2,1}\chi_{Val}$	$^{2,2}\chi_{Val}$	ψ_{Pro}
E F	0.00	-137	149	-71	65	52	159
E A	0.63	-135	149	-71	65	52	-29
E F	4.43	-150	130	67	70	65	161
E A	5.37	-151	131	67	70	65	-26
A*F	5.65	56	79	-174	59	69	151

[a]All minima with ΔE_{cis}<6 kcal/mol are shown.
[b]$\Delta E_{cis}=E-E_{cis,min}$, $E_{cis,min}$=-7.03 kcal/mol.

formation with (ϕ_{Val}, ψ_{Val}, ϕ_{Pro}, ψ_{Pro}, ϕ_{Gly3}, ψ_{Gly3}, ϕ_{Gly4}, ψ_{Gly4})= (-140°,146°,-75°,-29°,-83°,117°,149°,-74°). This conformation was obtained from the 5th and 8th low-energy conformations of cis Ac-Val-Pro-Gly-Gly-NHMe, that is, EACD*(-141°,149°,-75°,-27°,-71°,103°,143°,-75°) with ΔE_{cis}=0.75 kcal/mol and EAFC*(-139°,149°,-75°,-41°,-75°,159°,103°,-72°) with ΔE_{cis}=1.40 kcal/mol, respectively. The second low-energy conformation of cis Ac-(Val-Pro-Gly-Gly)$_2$-NHMe is EFD*A*(-135°,149°,-75°,152°,139°, -54°,85°,69°) with ΔE_{cis}=0.72 kcal/mol, and it is obtained from the 2nd low-energy conformation of cis Ac-Val-Pro-Gly-Gly-NHMe, EFD*A*(-137°,149°,-75°, 152°,139°,-53°,93°,56°) with ΔE_{cis}=0.39 kcal/mol. The most stable conformation of cis Ac-Val-Pro-Gly-Gly-NHMe, EFD*G(-133°,148°,-75°,145°,153°, -63°,-169°,-78°) is destabilized to the 24th conformation of cis Ac-(Val-Pro-Gly-Gly)$_2$-NHMe, EFD*G(-132°,149°,-75°,142°,179°,-64°,-179°,-57°) with ΔE_{cis}=5.69 kcal/mol. These results indicate that low-energy conformations stabilized by the short-range interactions slightly change their conformations by the additional further-range interactions and also change their relative stabilities as shown in the theoretical conformational analysis of poly(L-Ala-D-Ala)[6], poly(Ala-Gly)[7], and trans poly(Val-Pro-Gly-Gly)[1,2].

Stable Conformations of Cis Poly(Val-Pro-Gly-Gly)

All 8 stable helical conformations of cis poly(Val-Pro-Gly-Gly) with $\Delta E_{cis,res}$<0.5 kcal/mol are shown in Table III. The lowest-energy conformation, EFD*C(-139°,148°,-75°,146°,154°,-61°,-82°,74°), is a right-handed $\beta^{11.6}$-Helix(Figure 1a). This conformation has two types of hydrogen bonds, $(Gly4_i)CO\cdots HN(Gly4_{i+1})$ and $(Gly3_i)CO\cdots HN(Val_{i+1})$, and forms double-bend conformation(type VI-IV) at Val-Pro-Gly portion and non-bend conformation at Gly-Gly-Val portion. The EFD*C conformation is found as the 3rd low-energy conformation of cis Ac-Val-Pro-Gly-Gly-NHMe with (-135°,149°,-75°,149°,144°,-68°,-85°,75°) and $\Delta E_{cis,res}$=0.16 kcal/mol, and as the 4th low-energy conformation of cis Ac-(Val-Pro-Gly-Gly)$_2$-NHMe with (-140°,148°,-75°,145°,154°,-59°,-82°,71°) and $\Delta E_{cis,res}$=0.18 kcal/mol. These results indicate that the lowest-energy $\beta^{11.6}$-helix is essentially stabilized by the short-range interactions(i.e., intra-unit interactions), and also that the further-range interactions are also important for stabilizing $\beta^{11.6}$-helix. This helix has three hydrophobic and hydrophilic stripes along helical axis. The former stripes are composed of the Val and Pro residues, and the latter ones are composed of the

Table III. Calculated Minimum Energy Conformations[a] of Cis Poly(Val-Pro-Gly-Gly)

Conformational Letter Code	$\Delta E^{b}_{cis,res}$ (kcal/mol)	Helix[c] Type	h[d]	ϕ_{Val}	ψ_{Val}	ψ_{Pro}	ϕ_{Gly3}	ψ_{Gly3}	ϕ_{Gly4}	ψ_{Gly4}
E F D*C	0.00	$\beta^{11.6}$(R)	0.69	-139	148	146	154	-61	-82	74
E A D E	0.17	$\beta^{6.5}$(L)	0.88	-135	152	-43	-149	56	-178	-171
E A F E	0.23	$\beta^{8.4}$(R)	0.89	-135	148	-40	-82	140	-179	174
E A E E	0.26	$\beta^{8.7}$(R)	0.66	-136	145	-19	-111	146	-167	155
E F D*C*	0.28	$\beta^{10.6}$(R)	0.71	-133	149	145	150	-60	90	-66
E A C D*	0.31	γ	1.06	-141	144	-27	-83	117	150	-73
E F D*F	0.38	$\beta^{7.5}$(R)	0.99	-137	148	150	144	-63	-73	143
E F C D*	0.48	$\beta^{10.3}$(R)	0.84	-137	149	170	-69	97	158	-39

[a]All minimum energy conformations with $\Delta E_{cis,res}$<0.5 kcal/mol are shown.
[b]$\Delta E_{cis,res}=(E-E_{cis,min})/24$, $E_{cis,min}$=-25.19 kcal/mol
[c]Helix sence is abbreviated as R or L for right- or left-handed, respectively.
[d]Rise per residue.

Gly residue. These characters of $\beta^{11.6}$-helix are similar to those of the third low-energy one(right-handed $\beta^{11.7}$-helix) of trans poly(Val-Pro-Gly-Gly), however, they are completely different from those of γ-helix which is the lowest-energy one of trans poly(Val-Pro-Gly-Gly)[1,2]. It indicate that the trans conformation at Val-Pro portion is very important for stabilizing γ-helix. The 3rd and 4th low-energy conformations, EAFE (-135°,148°,-75°,-40°,-82°,140°,-179°,174°) and EAEE(-136°,145°,-75°,-19°, -111°,146°,-167°,155°), respectively, are the typical ones for cis poly-(Val-Pro-Gly-Gly). They are clossified into the right-handed $\beta^{8.4}$- and $\beta^{8.7}$-helices, however, their structural characters are clearly different from those of the general β-helices in the meaning that the Gly-Gly-Val portion with extended conformations forms short β-sheet-like structures which right- and left-handly twisted along helical axis, as shown in Figures 1b and 1c, respectively. The 3rd $\beta^{8.4}$-helix(EAFE conformation) is obtained from the 26th low-energy conformation of cis Ac-Val-Pro-Gly-Gly-NHMe, EACE*(-141°,150°,-75°,-47°,-83°,97°,174°,-165°) with $\Delta E_{cis,res}$= 0.66 kcal/mol, also obtained from the 14th low-energy conformation of cis Ac-(Val-Pro-Gly-Gly)$_2$-NHMe, EAFE(-138°,148°,-75°,-34°,-82°,134°,-180°, 174°). The 4th $\beta^{8.7}$-helix is obtained from the 94th low-energy conformation of cis Ac-Val-Pro-Gly-Gly-NHMe, EAEE(-135°,149°,-75°,-24°,-173°,174°, -180°,-178°) with $\Delta E_{cis,res}$=1.25 kcal/mol, and also obtained from the 12th low-energy conformation of cis Ac-(Val-Pro-Gly-Gly)$_2$-NHMe,EAEE*(-134°,146°, -75°,-15°,-136°,171°,178°,-175°) with $\Delta E_{cis,res}$=0.53 kcal/mol. These results indicate that EAFE and EAEE conformations are not so stable ones with short-range interactions, and also that they are stabilized by the long-range interactions. These results correspond to the interaction-mode of proteins that β-sheet structures are stabilized by the long-range interactions[10].

As shown in Table III, stable conformations of cis poly(Val-Pro-Gly-Gly) are obviously different from those of trans poly(Val-Pro-Gly-Gly) in

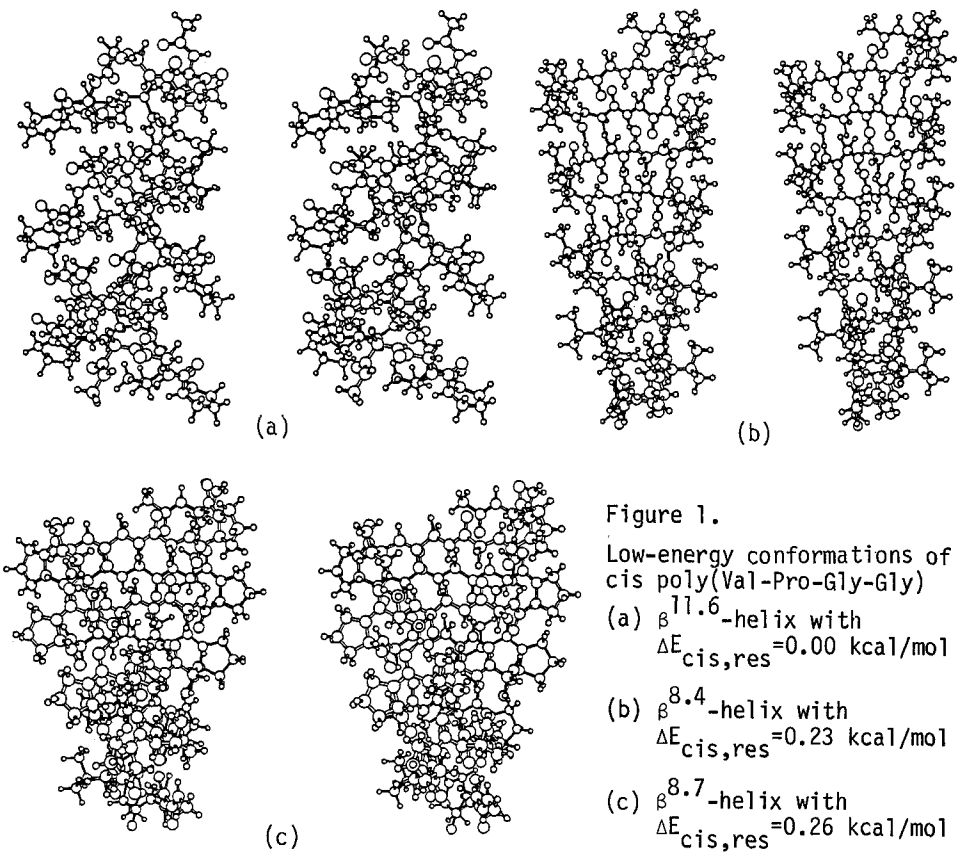

Figure 1.
Low-energy conformations of cis poly(Val-Pro-Gly-Gly)
(a) $\beta^{11.6}$-helix with $\Delta E_{cis,res}$=0.00 kcal/mol
(b) $\beta^{8.4}$-helix with $\Delta E_{cis,res}$=0.23 kcal/mol
(c) $\beta^{8.7}$-helix with $\Delta E_{cis,res}$=0.26 kcal/mol

the following points. 1) EF and EA conformations are favorable at Val-Pro portion for cis, but DC and DA ones are favorable for trans. These conformations are also found as stable conformations of Ac-Val-Pro-NHMe with trans or cis peptide bonds as shown in Tables I and II, respectively, indicating that conformational restrictions in Val-Pro portion are essentially determined by the rotational states of the peptide bond at Val-Pro portion. 2) Bend structures are formed at Val-Pro-Gly portion for cis, but formed at Pro-Gly-Gly portion for trans. These propencities are also provided by the difference of structural restrictions in trans and cis peptide bonds at Val-Pro portion.

$\Delta E_{cis-trans}$ of poly(Val-Pro-Gly-Gly) is 3.08 kcal/mol. This value almost corresponds to that of Ac-Val-Pro-Gly-Gly-NHMe(2.75 kcal/mol), demonstrating that the relative stability of trans conformation with the short-range interaction is essentially mentained for the case considering the medium- and long-range interactions. $\Delta E_{cis-trans}$=3.78 kcal/mol for Ac-Val-Pro-NHMe shows that trans conformation of poly(Val-Pro-Gly-Gly) is stabilized by the inter-residue interaction at Val-Pro portion, and also that cis conformation is slightly stabilized by the additional inter-residue interactions among whole residues. Above results indicate that trans conformations of poly(Val-Pro-Gly-Gly) are essentially stabler than cis conformations of that within intra-molecular interactions, and also that relative stability between trans and cis conformations of polypeptide

having the repeated amino-acid residues can be estimated by the inter-residue interaction at X-Pro portion and by the short-range interactions. Moreover, $\Delta E_{cis\text{-}trans}$ =3.08 kcal/mol of poly(Val-Pro-Gly-Gly) means ΔE_{res} = 0.77 kcal/mol of the $\beta^{11.6}$-helix(EFD*C, the lowest-energy cis conformation), i.e., $\beta^{11.6}$-helix is the minimum locating between the 17th and 18th minima of trans poly(Val-Pro-Gly-Gly).

Experimental works on NMR[11-14] and CD[15] measurements indicate that poly(Val-Pro-Gly-Gly) takes different conformations depending on solvents such as water, methanol, dimethyl sulfoxide and trifluoroethanol. As already mentioned in the previous paper[1,2], conformational feature of γ-helix, which was theoretically proposed as the lowest-energy conformation of trans poly(Val-Pro-Gly-Gly), presents fundamental agreements with the experimental results for water[11,14] or methanol[11,15]. In dimethyl sulfoxide-d_6, 10-15% cis isomer at Val-Pro was estimated from the presence of a weak peak of cis Pro αCH resonance[13]. Most of stable conformations of cis poly(Val-Pro-Gly-Gly) such as EFD*C, EADE and EAFE conformations have hydrogen bonds related to Gly4 NH. These results correspond to the experimental results[11,13] that Gly4 NH is the aminde proton most shielded from solvent in dimethyl sulfoxide-d_6. It means that cis conformations in Table III may exist in dimethyl sulfoxide-d_6 as one of the stable conformations of poly(Val-Pro-Gly-Gly) or one of the local conformation in poly-(Val-Pro-Gly-Gly).

Based on the theoretical and experimental results mentioned above and reliability of the energy function of ECEPP and optimization procedures supported by the fundamental agreements between theoretical and experimental results for peptides[3,5,10,16,17] and polypeptides[6,7,18,19], it is concluded that γ-helix(DCC*D, the lowest-energy trans conformation) proposed as a model conformation of elstin in the previous work[1] is the most stable helical conformation of poly(Val-Pro-Gly-Gly) within intra-molecular interactions, and it is also suggested that cis conformations shown in Table III may exist as a conformation of poly(Val-Pro-Gly-Gly) in the particular conditions.

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