Theoretical Conformational Analysis on Elastin Model Tetrapeptide Ac-Val-Pro-Gly-Gly-NHMe

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ABSTRACT: To investigate the short-range interactions in elastin, conformational energy calculations using ECEPP were carried out for elastin model tetrapeptide, Ac-Val-Pro-Gly-Gly-NHMe. The DCC* conformation is the most favorable one for Val-Pro-Gly portion in the model tetrapeptide. The bend in *trans* Ac-Val-Pro-Gly-Gly-NHMe are recognized at Pro-Gly and Gly-Gly portions with higher probability. Favorable bend type at Pro-Gly is type II and type V which is a distorted type II β -bend, and the summation of these bend probabilities is 0.60 which corresponds to the tendency of forming a type II β -bend at Pro-Gly supposed by experimental works in solution and crystal structure.

KEY WORDS Conformational Energy Calculation / ECEPP / Ac-Val-Pro-Gly-Gly-NHMe / Elastin / β -Bend /

Elastin is a protein which represents characteristic elasticities. Weis-Fogh and Anderson¹ have shown that these characteristic behaviours are not caused by the normal rubber elasticity usually observed for cross-linked random-coiled polymers, but explained as energetical change caused by the change of backbone conformation and entropical change caused by structural change of immanent water molecules through the stretching of backbone conformation. These facts indicate that the origin of the elasticity of elastin exists in its amino acid sequences, and elastin molecule has several stable ordered structures whose stabilities depend on the external conditions.

In sequence studies of water soluble elastin, Gray *et al.*^{2,3} recognized two distinct regions of elastin, Lys and Ala-rich region and Gly, Pro, and Val-rich region, and the latter region

consists of three typical repeated sequences, Val-Pro-Gly-Gly, Val-Pro-Gly-Val-Gly, and Ala-Pro-Gly-Val-Gly-Val. They supposed that the characteristic elasticity of elastin would be concerned with these typical repeated sequences, and also proposed the "oiled coil" structure² which is the loosed spiral containing β -bend conformation. In this model, Gly residues occupy the exterior positions exposed to solvent, while Pro, Val, and Ala residues are buried, and most of the amide protons and carbonyl oxygens were hydrated. While other models were proposed by Urry *et al.*^{4,5} based on the experimental results, which are the temperature dependence of amide proton chemical shift, solvent-component dependence of carbonyl chemical shift for several solvents and NOE (Nuclear Overhauser Enhancement) measurements in NMR, and CD measurements for model

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polypeptides,⁴ poly(Val-Pro-Gly-Gly), poly(Val-Pro-Gly-Val-Gly), and poly(Ala-Pro-Gly-Val-Gly-Val), and model peptides.^{6,7} The basic structure of Urry's model is β -bend conformation at Pro-Gly, while that of Gray's model is β -bend conformation at Gly-Gly.

In this paper, as the first step of the conformational analysis of elastin model polypeptide, poly(Val-Pro-Gly-Gly), conformational energies of Ac-Val-Pro-Gly-Gly-NHMe were calculated with the conformational energy functions of ECEPP (Empirical Conformational Energy Program for Peptides).⁸ Stable conformations of Val-Pro-Gly-Gly sequence of poly(Val-Pro-Gly-Gly) are also analyzed theoretically considering only short-range interactions. Conformational analysis of poly(Val-Pro-Gly-Gly) considering long-range interactions will be followed (manuscript in preparation).

THEORETICAL

The nomenclature and conventions adopted are those recommended by an IUPAC-IUB nomenclature commission.⁹ Conformational energy calculations of the Ac-Val-Pro-Gly-Gly-NHMe tetrapeptide were carried out with ECEPP.⁸

Minimization was carried out with the Powell algorithm¹⁰ until the conformational energy did not change by more than 0.001 kcal mol⁻¹ between successive iterations. During minimization, all ϕ , ψ , and χ for Val and Gly residues and ψ for Pro residue were allowed to vary. All other backbone dihedral angles were fixed at 180°, except for *cis* peptide bond at Val-Pro ($\omega=0^\circ$). All combinations of single-residue minima¹¹ were used as starting conformations. The following conformations were also used as single-residue minima, *i.e.*, $(\phi, \psi) = (-75^\circ, 140^\circ)$ and $(75^\circ, -140^\circ)$ for Gly residue, $\psi = 23^\circ$ for Pro. While $(\phi, \psi) = (-76^\circ, -26^\circ)$, $(-87^\circ, -26^\circ)$, and $(-142^\circ, 29^\circ)$ for Val residue were excluded because they situate in high energy regions for Val-Pro sequence.

The normalized Boltzmann factor v_i is given by

$$v_i = Q^{-1} \exp(-\Delta E_i/RT) \quad (1)$$

where

$$Q = \sum_{i=1}^n \exp(-\Delta E_i/RT) \quad (2)$$

ΔE_i is the conformational energy difference of the i th minimum from that of global minimum, RT is the gas constant times the temperature, n is the number of low-energy minima. The statistical average of the conformation-dependent quantity A for the ensemble is defined by

$$A = \sum_{i=1}^n A_i v_i \quad (3)$$

A bend (occurring at $i+1$ and $i+2$ th residues) is defined as a conformation in which $R \leq 7 \text{ \AA}$ (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 12. A polar hydrogen atom and an oxygen or nitrogen atom with an interatomic distance of less than 2.3 Å are regarded to be hydrogen-bonded. Vicinal NH-C α H coupling constants $^3J_{\text{NH-C}\alpha\text{H}}$ of Val residue for each conformation were computed with the following expression of Bystrov *et al.*¹³

$$^3J_{\text{NH-C}\alpha\text{H}} = 9.4 \cos^2 \theta - 1.1 \cos \theta + 0.4 \quad (4)$$

where $\theta = \phi - 60^\circ$, and those of Gly residue were computed with the following expression of Bystrov *et al.*¹³

$$^3J_{\text{NH-C}\alpha\text{H}} = -9.4 \cos^2 \theta - 1.1 \cos \theta + 14.9 \quad (5)$$

Conformational space is divided into 16 regions with the conformational letter codes shown in Figure 2 of ref 11.

RESULTS

There are 116 low-energy minima for Ac-Val-Pro-Gly-Gly-NHMe with *trans* Val-Pro peptide bond having $\Delta E < 3 \text{ kcal mol}^{-1}$, indicating that this peptide system is represented

Table I. Calculated minimum energy conformations^a of *trans* Ac-Val-Pro-Gly-Gly-NHMe

Conformational letter code	ΔE^b kcal mol ⁻¹	ϕ_{Val}	ψ_{Val}	ψ_{Pro}	ϕ_{Gly3}	ψ_{Gly3}	ϕ_{Gly4}	ψ_{Gly4}	Bend type	
									Pro-Gly	Gly-Gly
DCC*A	0.00	-132	85	77	80	-81	-86	-66	V	II'
DCC*D	0.34	-132	86	84	86	-70	-165	55	V	IV
DACD*	0.49	-131	87	-45	-83	69	163	-49	I	IV
DCC*C	0.60	-133	85	74	73	-97	-94	69	V	V'
DFC*A	0.66	-132	86	156	84	-67	-69	-50	II	III
DCC*F	0.72	-132	85	75	80	-77	-86	162	V	
DFC*C	0.98	-132	87	156	81	-82	-90	68	II	V'
DCD*C	1.08	-132	86	78	164	-89	-88	69	IV	IV
DACC*	1.09	-131	88	-21	-81	80	86	-76	I	V
DFA*G	1.10	-132	87	140	68	45	-179	-56	II	VII
A*FA*G	1.10	56	81	139	72	44	-180	-55	II	VII
DCC*E	1.11	-132	86	81	92	-61	-154	176	V	
DACA*	1.12	-131	87	-29	-84	79	78	69	I	IV
DCA*C*	1.14	-133	82	76	59	74	84	-71	IV	IV
A*CC*D	1.16	56	80	84	178	-52	-167	49	IV	VII
DCC*G	1.16	-132	87	82	88	-65	-159	-64	V	IV
A*FC*A	1.23	56	80	154	86	-65	-70	-49	II	III
DCG*C*	1.27	-132	87	75	167	68	86	-75	IV	IV
DCC*C*	1.29	-132	87	80	86	-73	82	-77	V	
DCD*D	1.31	-132	86	84	180	-55	-171	54	IV	VII
DCA*C*	1.40	-139	80	86	76	60	94	-66	IV	III'
A*CC*A	1.50	55	80	78	81	-81	-85	-66	V	II'

^a All minima with $\Delta E < 1.5$ kcal mol⁻¹.^b $E_0 = -9.20$ kcal mol⁻¹.

by the ensemble composed with many stable conformations as shown in other linear oligopeptides containing Pro residue.^{12,14} Backbone conformations, bend types and conformational letter codes of all 22 minima with value of $\Delta E < 1.5$ kcal mol⁻¹ are listed in Table I. The lowest-energy conformation is DCC*A conformation with $v=0.138$ (Figure 1a), and the second lowest is DCC*D with $\Delta E=0.34$ kcal mol⁻¹ and $v=0.078$ (Figure 1b). They have common conformation DCC* at Val-Pro-Gly sequence with two hydrogen-bonds (Val)CO...HN(Gly3) and (Pro)CO...HN(Gly4). DCC* conformation is the most favorable conformation for Val-Pro-Gly sequence with 13 conformations in $\Delta E < 3$ kcal mol⁻¹ and $v=0.381$. The stable conformations of Val residue are restricted to only D and A* conformation in $\Delta E < 3$ kcal mol⁻¹ except for 11 conformations with

letter code F existing in $2.43 \leq \Delta E \leq 3.00$ kcal mol⁻¹. Comparing to the results for Ac-Ala-Pro-NHMe,¹² Ac-Phe-Pro-NHMe,¹⁴ and Ac-Tyr-Pro-NHMe,¹⁴ stability of A* conformation is increased. As shown in Table I, all stable conformations in $\Delta E < 1.5$ kcal mol⁻¹ are β -bends at Pro-Gly portion, and also most of the stable conformations in $\Delta E < 1.5$ kcal mol⁻¹ are β -bend at Gly-Gly portion. Bend probabilities are shown in Table II. Type II and type V, which is a distorted type II β -bend, are favorable at Pro-Gly portion. Summation of their probabilities exceeds 0.60. These facts show that Pro-Gly sequence have a tendency to form type II β -bend. Bend probabilities at Pro-Gly portion for Ac-Pro-Gly-NHMe,¹² Ac-Pro-Gly-Gly-NHMe, and Ac-Val-Pro-Gly-Gly-NHMe show that the tendency forming bend at this portion is mainly decided by the intra- and inter-residue

Figure 1. Minimum energy conformations of Ac-Val-Pro-Gly-Gly-NHMe with *trans* Val-Pro peptide bond. a) the lowest-energy DCC*A conformation: $\Delta E=0.00$ kcal mol⁻¹. b) the second lower-energy DCC*D conformation: $\Delta E=0.34$ kcal mol⁻¹.

interactions in Pro-Gly sequence, and influenced by the inter-residue interactions from Val and another Gly residue in some extents. Gly-Gly portion also represents high bend probability, but there are no favorable bend types. Bend probabilities at Gly-Gly portion for Ac-Gly-Gly-NHMe,¹⁵ Ac-Pro-Gly-Gly-NHMe, and Ac-Val-Pro-Gly-Gly-NHMe also show that the tendency to form bend at this portion is mainly 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). While they show that inter-residue interactions from the next-to-nearest-neighbor residue (*i.e.*, Val residue) are

Figure 2. The lowest-energy EFD*G conformation of Ac-Val-Pro-Gly-Gly-NHMe with *cis* Val-Pro peptide bond.

not so important.

There are 34 low-energy minima of Ac-Val-Pro-Gly-Gly-NHMe with *cis* Val-Pro peptide bond having $\Delta E < 3$ kcal mol⁻¹. All 8 minima with values of $\Delta E < 1.5$ kcal mol⁻¹ are listed in Table III. The lowest-energy conformation is EFD*G conformation with $v=0.269$ (Figure 2), and the second lowest-energy conformation is EFD*A* with $\Delta E=0.39$ kcal mol⁻¹ and $v=0.140$. There are many stable conformations and no significantly favorable ones are found in this tetrapeptide system. However, EFD* conformation is the most favorable one for Val-Pro-Gly portion with $v=0.618$ (8 conformations with $\Delta E < 3$ kcal mol⁻¹) of which value is significantly larger than v for the most favorable conformation for Val-Pro-Gly portion with *trans* peptide bond (0.318). Moreover, only EF and EA conformations were found for Val-Pro portion in the *cis* conformation-ensemble with $\Delta E < 3$ kcal mol⁻¹. That is, the conformations with *cis* Val-Pro peptide bond are restricted to more specified conformational space than those with *trans* Val-Pro peptide bond (DC, DA, DF, A*F, A*C, A*A, EC, and EA were allowed for Val-Pro portion with *trans* peptide bond and $\Delta E < 3$ kcal mol⁻¹). These results correspond to the results of conformational analysis for Ac-X-Pro-NHMe^{12,14} with *trans* or *cis* peptide bonds, and show that confor-

Table II. Calculated bend probabilities of Ac-Val-Pro-Gly-Gly-NHMe

Bend type and peptide	<i>trans</i> ^a		<i>cis</i> ^a		
	Pro-Gly	Gly-Gly	Val-Pro	Pro-Gly	Gly-Gly
I	0.131	0.004	—	0.189	—
II	0.171	0.005	—	—	0.090
III	0.004	0.078	—	0.014	—
IV	0.196	0.323	—	0.741	0.225
V	0.429	0.032	—	—	0.004
VI	—	—	1.000	—	—
VII	0.014	0.105	—	0.044	0.033
II'	—	0.155	—	—	0.094
III'	—	0.045	—	—	0.043
V'	—	0.098	—	—	—
Total	0.945	0.844	1.000	0.988	0.429
Ac-Pro-Gly-Gly-NHMe ^b	0.889	0.879			
Ac-Pro-Gly-NHMe ^c	0.61				
Ac-Gly-Gly-NHMe ^d		0.53			

^a *trans* and *cis* peptide bonds at Val-Pro.^b Unpublished data.^c From ref 12.^d From ref 15.**Table III.** Calculated minimum energy conformations^a of *cis* Ac-Val-Pro-Gly-Gly-NHMe

Conformational letter code	ΔE_{cis}^b									Bend type		
	kcal mol ⁻¹	ϕ_{Val}	ψ_{Val}	ψ_{Pro}	ϕ_{Gly3}	ψ_{Gly3}	ϕ_{Gly4}	ψ_{Gly4}		Val-Pro	Pro-Gly	Gly-Gly
EFD*G	0.00	-133	148	146	153	-63	-169	-78		VI	IV	
EFD*A*	0.39	-137	149	152	139	-53	93	56		VI	IV	
EFD*C	0.63	-135	149	149	144	-68	-85	75		VI	IV	IV
EFC*D	0.63	-141	150	155	69	-91	-143	32		VI	IV	II'
EACD*	0.75	-141	149	-27	-71	103	143	-75		VI	I	IV
EFD*G*	0.95	-135	148	146	151	-72	175	88		VI	IV	
EADA*	1.09	-133	151	-49	-114	67	66	56		VI	I	III'
EAFC*	1.40	-139	149	-41	-75	159	103	-72		VI	I	II

^a All minima with $\Delta E_{cis} < 1.5$ kcal mol⁻¹.^b $\Delta E_{cis} = E_i - E_{cis,min}$ and $E_{cis,min} = -6.14$ kcal mol⁻¹.

mation of Val-Pro portion are essentially decided by the intra- and inter-residue interactions within Val and Pro residues and energetically allowed conformations with *cis* peptide bond are more restricted than those with *trans* peptide bond.

$\Delta E_{cis-trans}$, which is the energy difference between the lowest-energies of *trans* and *cis* conformations, is 2.75 kcal mol⁻¹ showing

that *trans* conformation of Ac-Val-Pro-Gly-Gly-NHMe is also more favorable than *cis* conformation as shown for proline containing oligopeptides^{12,14} with intra-molecular interactions. All 34 conformations with *cis* Val-Pro peptide bond ($\Delta E < 3$ kcal mol⁻¹) form type VI β -bend at Val-Pro portion. This result corresponds to the theoretical results for X-Pro dipeptides^{12,14} and X-Pro-Y tripeptides.¹⁴ That

is, type VI β -bend conformation is essentially favorable conformation for X-Pro (except for Gly-Pro) with *cis* peptide bond. Calculated bend probability for Pro-Gly portion is 0.988 and favorable bend types are IV ($P_b=0.741$) and I ($P_b=0.189$). This bend probability is higher than 0.889 for Pro-Gly portion of Ac-Pro-Gly-Gly-NHMe with *trans* peptide bond and 0.945 of Pro-Gly portion of Ac-Val-Pro-Gly-Gly-NHMe with *trans* peptide bond. These results show that *cis* conformation at Val-Pro has higher tendency to stabilize the β -bend conformation for Pro-Gly than *trans* conformation. $P_b=0.429$ for Gly-Gly portion is almost the same as that of Ac-Gly-Gly-NHMe¹⁵ ($P_b=0.53$). That is, conformations of this portion of Ac-Val-Pro-Gly-Gly-NHMe with *cis* peptide bond are stabilized by intra- and inter-residue interactions at Gly-Gly portion. High bend probabilities of Ac-Val-Pro-Gly-Gly-NHMe with *cis* peptide bond for Val-Pro, Pro-Gly, and Gly-Gly portions characterize the favorable conformation of this tetrapeptide as triple bend conformation (11 conformations with $\Delta E < 3 \text{ kcal mol}^{-1}$, $v=0.416$).

DISCUSSION

Model peptides with Val-Pro-Gly-Gly sequence were synthesized and characterized their conformations in solution by Urry *et al.*^{6,7} or crystal by Ashida *et al.*^{16,17} Urry *et al.* proposed the type II β -bend structure at Pro-Gly portion in *t*-Boc-Val-Pro-Gly-Gly-OMe from temperature dependence of NH chemical shift ($d\delta/dT$) in CDCl_3 (with 20% C_6D_6),⁶ and HCO-Val-Pro-Gly-Gly-OMe by the NOE measurements in $\text{DMSO}-d_6$.⁷ As mentioned in Results section, the calculated bend probabilities for Ac-Val-Pro-Gly-Gly-NHMe at Pro-Gly portion are 0.171 for type II and 0.429 for type V which is a distorted type II bend, respectively. Total bend probability of type II and type V exceed 0.60. That is, type II and type V bend conformations are preferable

Table IV. Vicinal coupling constants $^3J_{\text{NH-C}^{\alpha}\text{H}}$ of *trans* Ac-Val-Pro-Gly-Gly-NHMe

	$^3J_{\text{NH-C}^{\alpha}\text{H}}$		
	Val	Gly3	Gly4
Calculated	10.1	13.1	12.0
Experimental ^a	10.4	12.0	11.4

^a *t*-Boc-Val-Pro-Gly-Gly-OMe in CDCl_3 with 20% C_6D_6 at 22°C from ref 6. All experimental values are corrected by the relation $^3J_{\text{NH-C}^{\alpha}\text{H}} = 1.09J_{\text{obs}}$ in ref 13.

conformations in the ensemble of calculated stable conformations. This calculated results corresponds to the above Urry *et al.*'s results.^{6,7} Calculated and experimental $^3J_{\text{NH-C}^{\alpha}\text{H}}$ in CDCl_3 with 20% C_6D_6 ⁶ are presented in Table IV. These results are in fairly good agreements demonstrating that calculated ensemble could represent the system of molecular conformations in non-polar solvents, even if the interactions between oligopeptides and solvents are not considered in conformational energy calculations. Moreover, NOE experiments⁷ for HCO-Val-Pro-Gly-Gly-OMe in $\text{DMSO}-d_6$ showing that NOE of approximately 10% was observed between the $\text{C}^{\alpha}\text{H}$ of Pro and the NH of Gly3 support our calculated results.

Crystal structure was obtained by Ashida *et al.*^{16,17} for Boc-Val-Pro-Gly-Gly-OBzl. Backbone conformation (ϕ_{Val} , ψ_{Val} , ϕ_{Pro} , ψ_{Pro} , ϕ_{Gly3} , ψ_{Gly3} , ϕ_{Gly4} , ψ_{Gly4}) of the crystal is (-139° , 158° , -62° , 136° , 75° , 3° , 74° , 177°) with a type II β -bend at Pro-Gly portion. Result of crystal structure corresponds to our calculated high bend probabilities of types II and V at Pro-Gly portion, but backbone conformations are somewhat different because of favorable hydrogen bond (NHMe)NH $\cdots$ OC(Val) or (NHMe)NH $\cdots$ OC(Gly3) which cannot be formed in Boc-Val-Pro-Gly-Gly-OBzl.

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