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Conformational analysis of periodic polypeptides

II. Helical conformations of poly(ala-pro)

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Summary

Theoretical conformational analysis was carried out for a periodic polypeptide composed of the repetitive Ala-Pro sequence, i.e., poly(Ala-Pro) using ECEPP and the conformational minimization procedure. Calculated results showed that a γ-helix is the most stable helical conformation of poly(Ala-Pro), and also that most of the stable helical conformations are other types γ-helices and β-helices with large value of the rise per residue. Obtained conformational preference of poly(Ala-Pro) indicates that the repetitive Ala-Pro sequence is a suitable amino-acid sequence for designing the rod-like backbone conformations of artificial proteins.

Introduction

As mentioned in the previous paper (1), periodic polypeptides are very interesting molecules from the viewpoint for designing the artificial proteins. It has been shown that periodic polypeptides take various helical conformations beyond α-helix or β-strand which are usually favorable for the polypeptides composed of the alanine type residues(i.e., all amino acid residues except for the Pro and Gly residues in 20 naturally occurring residues) and Gly residues. That is, β-helices such as $\beta^{4.6}$-, $\beta^{6.2}$-, and $\beta^{6.8}$-helices were found as stable ones for poly(Ala-D-Ala) (2), poly(Ala-Gly) (3) and poly(Gly-Pro) (1), and a γ-helix is also found as stable one for poly(Val-Pro-Gly-Gly) (4) which is a model poly(tetrapeptide) for the repetitive portion of elastin.

The amino-acid sequence composed of the repetitive Ala-Pro sequence were found in several native proteins, ex., skeletal muscle myosin light chain 1 (5,6), bovine β-crystallin (7) and outer membrane protein A precursor of *E.coli* (8), and those of the repetitive Xaa-Pro sequence were also found in native proteins and peptides, ex., early 32K, 26K and 13K proteins of adenovirus 2 and 5, gene *tonB* protein of *E.coli* (8), bactenecin (9), β-casein (10,11) and morphiceptin (12). It is already known that some of them have biological functions such as antimicrobial or opioid activity, and it is also supposed that the repetitive Ala-Pro portion of myosin light chain 1 has a role as a functional linkage in the thin-filament-based regulatory mechanism (13). Above functionalities based on the repetitive amino-acid sequence, Ala-Pro or Xaa-Pro, suggest the importance of investigating the conformational preference for the periodic polypeptides composed of the repetitive sequence, Ala-Pro or Xaa-Pro.

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In this work, as one of the step for searching interesting helical structures for molecular design of functional polypeptides, theoretical conformational analysis based on the molecular mechanics was tried for the periodic polypeptide with the repetitive Ala-Pro sequence, i.e., poly(Ala-Pro).

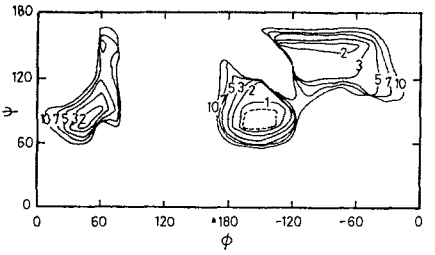
Theoretical

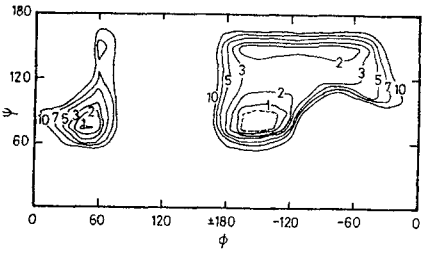
All conformational energy calculations were carried out with the energy functions of ECEPP (14). During minimizations, all (ϕ, ψ, χ^1) of Ala and ϕ of Pro were allowed to vary. All other backbone dihedral angles were fixed to 180° except for ϕ_{Pro}=-75° . Optimization of the helical structure was tried by two methods, i.e., the three-steps method and the grid method, as already tried in the previous optimization of poly(Gly-Pro) (1), poly(Ala-D-Ala) (2) and poly(Ala-Gly) (3). The three-steps method was carried out by the following three steps of minimizations. The first step was the minimization for the dipeptide, Ac-Ala-Pro-NHMe. All combinations of the single residue minima (15) of Ala and Pro residues(i.e., 9 and 4, respectively) were used as starting conformations. The second step was the minimization for the tetrapeptides having two repetitive sequences of Ala-Pro, i.e., Ac-(Ala-Pro)$_2$-NHMe. All minima in the first step were used as starting conformations of the second step. The third step was the minimization for the tetracicosapeptide having twelve repetitive sequences of Ala-Pro, i.e., Ac-(Ala-Pro)$_{12}$-NHMe(abbreviated as poly(Ala-Pro)). All minima found in the second step were also used as starting conformations of the third step. During the 2nd and 3rd minimization step, the condition of helical conformation was used in a similar manner as the previous optimization works (1-4). The grid method was tried as shown in the previous works (1-3). Conformational energy of poly(Ala-Pro) was calculated by changing ϕ_{Ala} and ψ_{Ala} at 15° intervals and fixing ϕ_{Pro} to the energy minima of Ac-Pro-NHMe(i.e., ϕ_{Pro} =-48° , 79° , 159°). Then all local minima found in (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}) space were used as starting conformations for energy-minimization of poly(Ala-Pro).

A bend (occurring at *i+1* and *i+2* th residues) is defined as a conformation in which $R \leqq 7$ Å (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 16. 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. Conformational space is divided into 16 regions with the conformational letter codes shown in Figure 1 of ref 15. The conformational energy per whole molecule, ΔE is defined by $\Delta E = E - E_0$, where E_0 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 the molecule. β^X-helix is defined as a helix which has a spiral structure with X residue per turn. γ-Helix is defined as helical conformation which cannot be categorized to the α-helix and β^X-helix. Two helical parameters, n and h, are the number of residues per turn and rise per residue, respectively. All molecular diagrams are drown by the molecular graphic program PEPCON (17,18).

Results

Local Minima in (ϕ_{Ala}, ψ_{Ala}) Maps of Poly(Ala-Pro) for the Specified Backbone Conformation of Pro Residue





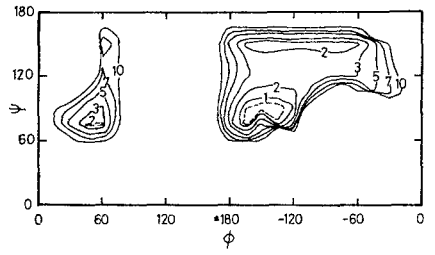


Figure 1. Energy contour (ϕ , ψ)maps of the Ala residue of poly(Ala-Pro) for the specified (ϕ , ψ)of the Pro residue; (a) (-75° ,79°), (b) (-75° ,159°), (c) (-75° ,-48°).The contour lines are labeled as energy in kcal/mol per residue above the minimum energy point of each energy map. The dashed lines indicate the 0.5 kcal/mole energy contour lines.

Three (ϕ , ψ) maps of Ala residue of poly(Ala-Pro) with the specified value of(ϕ_{Pro}, ψ_{Pro})=(-75° ,79°), (-75° ,159°), and (-75° ,-48°) under the condition of helical conformation are shown in Figures 1a, 1b, and 1c, respectively. The region with $0° \leqq \phi \leqq 180°$ are only shown in Figure 1, because no energetically favorable regions were found in the region with $-180° \leqq \phi \leqq 0°$. Contour lines of Ala residue are represented by the energy difference from the lowest-energy in each (ϕ, ψ) map, and energy difference is also designated as the value per residue. For the case of (ϕ_{Pro}, ψ_{Pro})=(-75° ,79°), i.e., the C conformational region (15), the region around (ϕ_{Ala}, ψ_{Ala}) =(-160° ,140°) is destabilized by the interactions within the four repetitive units. However, for (ϕ_{Pro}, ψ_{Pro})=(-75° ,159°) and (-75° ,-48°), the energetically favorable regions in the (ϕ_{Ala}, ψ_{Ala}) space of poly(Ala-Pro) almost correspond to those of Ac-Ala-Pro-NHMe. It means that the interresidue interactions within Ala-Pro dipeptide unit have very important roles for stabilizing the helical conformations of poly(Ala-Pro), and also that the medium-range interactions are not so important except for the case whose (ϕ_{Pro}, ψ_{Pro}) belong to the C conformational region.

Stable Helical Conformations of Poly(Ala-Pro)

A total of 14 energy minima of poly(Ala-Pro) was found in $\Delta E_{res} < 3$ kcal mole^{-1}, and 10 of them are shown in Table I. The lowest-energy conformation is a γ -helix(DA conformation) with (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}, ψ_{Pro})=(-150° ,80° ,-75° ,-27°) (Figure 2). The 4th low-energy conformation is a similar type γ -helix(DA conformation) with ΔE_{res}=0.19 kcal mole^{-1} and (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}, ψ_{Pro})=(-158° ,77° ,-75° ,-56°). Both of these γ -helices take type VII bend structure(i.e., an distorted type I bend) at Pro-Ala portion. The 6th and 7th low-energy minima are other types γ -helices (A*C conformation) with ΔE_{res}=0.72 and 0.76 kcal mole^{-1}, respectively. They

Table I. Calculated Minimum Energy Conformations[a] of Poly(Ala-Pro)

Conformational Letter Code	ΔE_{res}[b] (kcal mole^{-1})	Helix[c] Type	h[d]	ϕ Ala	ϕ Ala	ϕ Pro
DA	0.00	γ	2.26	-150	80	-27
EC	0.04	$\beta^{8.5}$(R)	0.68	-155	158	85
DC	0.10	$\beta^{10.4}$(L)	2.07	-153	79	77
DA	0.19	γ	2.25	-158	77	-56
DF	0.53	$\beta^{32.8}$(R)	2.74	-151	80	163
A*C	0.72	γ	2.30	56	80	96
A*C	0.76	γ	2.27	49	75	71
FC	1.57	$\beta^{8.5}$(R)	2.46	-76	153	79
EA	1.59	γ	2.24	-153	153	-25
EF	1.75	$\beta^{8.6}$(R)	3.16	-155	154	162

[a]All 10 minimum-energy conformnations with $\Delta E_{res} < 1.75$ kcal mol^{-1}.

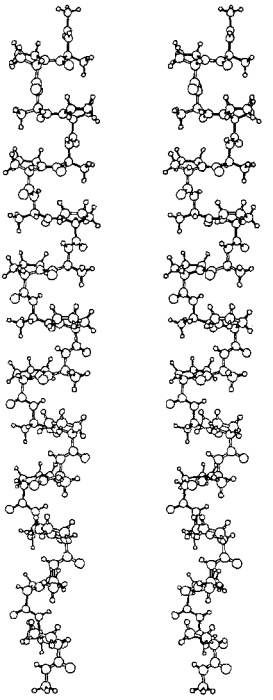
[b]E_o=-90.77 kcal mol^{-1}, $\Delta E_{res}=(E-E_o)/24$.

[c]Helix sense is abbreviated as R or L for right- or left-handded, respectively.

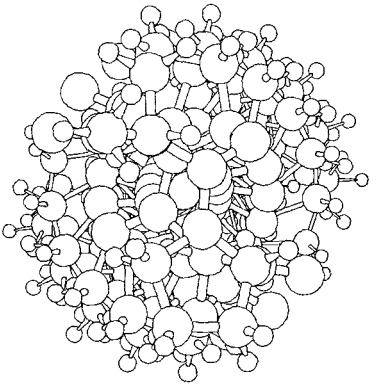
[d]Rise per residue.

take type II bend structure at Pro-Ala portion with the favorable interaction between the carbonyl oxygen of the Ala residue in the *i* th repetitive unit and the amido hydrogen of the Ala residue in the *i+1* th repetitive unit. The 9th low-energy minimum is also γ-helix(EA conformation) with ΔE_{res}=1.59 kcal mole^{-1} and(ϕ Ala, ϕ Ala, ϕ Pro, ϕ Pro)=(-153° ,153° ,-75° ,-25°) taking type VII bend structure at Pro-Ala portion. For the case of Ac-Ala-Pro-NHMe, DA, A*C and EA conformations were found as the 3rd, 4th and 10th low-energy minima with ΔE_{res}=0.62, 1.09 and 1.90 kcal mole^{-1}, and these conformations are stabilized to ΔE_{res}=0.38, 0.97 and 1.73 kcal mole^{-1} for the case of Ac-(Ala-Pro)$_2$-NHMe, respectively. These results indicate that the short-range interactions are important for stabilizing γ-helices.

The second low-energy conformation is a right-handed $\beta^{8.5}$-helix(EC conformation) with ΔE_{res}=0.04 kcal mole^{-1} and(ϕ Ala, ϕ Ala, ϕ Pro, ϕ Pro)=(-155° ,158° ,-75° ,85°) . This conformation has hydrogen bonds, (Ala$_i$)CO···HN(Ala$_{i+1}$) and (Ala$_i$)NH···OC(Ala$_i$), and takes non-bend structure at both of the Ala-Pro and Pro-Ala portions. For the case of Ac-Ala-Pro-NHMe, EC conformation is the 5th low-energy minimum with ΔE_{res}=1.76 kcal mole^{-1} , indicating that medium-range interactions are also important for stabilizing the $\beta^{8.5}$-helix. All other 4 minima(3rd, 5th, 8th and 10th) are also β-helices, i.e., a left-handed $\beta^{10.4}$-helix(DC conformation), a right-handed $\beta^{32.8}$-helix(DF conformation), a right-handed $\beta^{8.5}$-helix(FC conformation) and a right-handed $\beta^{8.6}$-helix(EF conformation) with ΔE_{res} =0.10, 0.53, 1.57 and 1.75 kcal mole^{-1} , respectively. They are constructed by the continual non-bend structures as the 2nd low-energy conformation, a right-handed $\beta^{8.5}$-helix. However, *h* (rise per residue) of them are significantly larger than that of the second one (0.68), and they almost correspond to



(a)



(b)

Figure 2.
A γ -helix(DA conformation) of poly(Ala-Pro) with the lowest-energy.
(a) Side-view, (b) Top-view.

those of γ -helices. These results indicate that extended helical conformations are favorable for poly(Ala-Pro) in spite of the existence of several energy minima with extended conformational features and an exceptional folded helix (the 2nd low-energy minimum).

Discussion

The conformational preference of poly(Ala-Pro) are significantly different from those of poly(Ala-D-Ala) (2) and poly(Ala-Gly) (3) obtained by the optimization with the energy function of ECEPP. A right- and left-handed α -helices, which are energetically favorable conformations for poly(Ala-D-Ala) and poly(Ala-Gly), are not stable ones for poly(Ala-Pro). For the case of Ac-Ala-Pro-NHMe, a local minimum with (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}, ψ_{Pro})=(-60° ,-55° ,-75° ,-32°), which has the closest dihedral angles to those of the α -helical region in the (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}, ψ_{Pro}) space, is found at ΔE_{res}=8.84 kcal mole^{-1}, indicating that α -helical conformation is essentially unstable with the interactions in dipeptide, Ac-Ala-Pro-NHMe. This minimum is shifted to the local minimum with (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}, ψ_{Pro})=(-86° ,-63° ,-75° ,9°) which is the outside of the α -helical region in the (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}, ψ_{Pro}) space and ΔE_{res} increases to 20.97 kcal mole^{-1}, by the short-range interactions within the tetrapeptide Ac-(Ala-Pro)$_2$-NHMe, and further shifted to the local minimum with (ϕ_{Ala}, ψ_{Ala}, ϕ_{Pro}, ψ_{Pro})=(-92 ° ,-65° ,-75° ,13°) and ΔE_{res}=23.70kcal mole^{-1} by the medium-range interactions within the tetracicosapeptide Ac-(Ala-Pro)$_{12}$-NHMe. These results indicate that α -helix is destabilized by the interresidue interactions between the Ala and Pro residues in Ala-

Pro portion. γ-Helices are energetically favorable conformations for poly(Ala-Pro), i.e., they are found as the lowest-energy and the 4th, 6th and 7th low-energy minima with ΔE_{res}=0.00, 0.19, 0.72 and 0.76 kcal mole^{-1}, respectively. The former two minima are DA conformations and the latter two ones are A*C conformations. These two types of γ-helices were not found as the stable helical conformations of poly(Ala-D-Ala) and poly(Ala-Gly). The CA* conformation with (ϕ_{Ala}, ϕ_{Ala}, ϕ_{Pro}, ϕ_{Pro})=(-68°,105°,76°,48°) is found at ΔE_{res}=1.93 kcal mole^{-1} for poly(Ala-D-Ala), and the CA*(-69°,103°,75°,42°), A*C(55°,56°,-78°,82°), and DA(-142°,55°,-65°,-42°) conformations are found at ΔE_{res}=2.52, 3.54 and 3.33 kcal mole^{-1} for poly(Ala-Gly), respectively. A γ-helix, which has a different structure from those of poly(Ala-Pro), is also found as a stable conformation of poly(Val-Pro-Gly-Gly) theoretically analyzed as a model periodic polypeptide of elastin. These results indicate that periodic polypeptides composed of the Pro residue indicate the characteristic conformational properties which are not usually found in the polypeptides composed of alanine type and Gly residues.

The conformational preference of poly(Ala-Pro) is significantly different from that of poly(Gly-Pro) investigated in the previous work (1). That is, relatively folded helical conformations with small value of h are stable ones for poly(Gly-Pro), however, relatively extended helical conformations with large value of h are stable ones for poly(Ala-Pro). It caused by the following reasons. That is, 1) both of E*C and F*C conformations, which are stable ones for poly(Gly-Pro), are destabilized by the interresidue interactions between Ala and Pro residues for the case of poly(Ala-Pro), and 2) the hydrogen bond, (Gly$_i$)CO···HN(Gly$_{i+1}$), which stabilizes a right-handed $\beta^{6.8}$-helix(EC conformation) of poly(Gly-Pro), is stronger than the hydrogen bond (Ala$_i$)CO···HN(Ala$_{i+1}$), which stabilizes a right-handed $\beta^{8.5}$-helix(EC conformation) of poly(Ala-Pro) because of the shorter CO···HN distance of poly(Gly-Pro) than that of poly(Ala-Pro) (Their energy difference is almost 0.8 kcal mole^{-1} per one hydrogen bond.), i.e., EC conformation of poly(Gly-Pro) is relatively more stabilized than that of poly(Ala-Pro), then the extended helical conformations such as DA and DC conformations are relatively destabilized although they can exist as the local minima with $2 < \Delta E_{res} < 3$ kcal mole^{-1} for the case of poly(Gly-Pro). These results indicate that the short-range interactions are dominant for characterizing the difference on the conformational preference between poly(Ala-Pro) and poly(Gly-Pro).

By the measurements of NOE and spin lattice relaxation time, T_1, for the proline-rich N-terminal of the LC1 alkali light chain of rabbit skeletal muscle myosin, Bhandari et al., adopted the extended rod-like conformation, cylinder 4 nm long and of radius 0.35 nm, for the (Ala-Pro)$_7$ segment in the above peptides (13). Moreover, the behavior of these peptides on polyacrylamide gels and that for gel filtration (19) also suggest that the extended helical conformation is reasonable for above peptides. These results correspond to the conformational features of γ-helices and β-helices with large h shown in Table I. Such γ-helices and β-helices with large h are stabilized by the short-range interactions, however, for the case of the right-handed $\beta^{8.5}$-helix(the 2nd low-energy conformation in Table I), the medium-range interactions between the residue in the i th repetitive unit and those in the $i \pm 4$ th ones are also important for stabilizing the folded helical conformations. As the peptide, (Ala-Pro)$_7$, has not enough number of residues for stabilizing the right-handed $\beta^{8.5}$-helix, conformational features based on the folded helical conformation such as $\beta^{8.5}$-helix have minor contribution on the detected experimental results in the above experimental conditions. So, it is supposed that (Ala-Pro)$_7$ takes one or several of the γ-helices and β-helices with large h shown in Table I. The CD spectra of poly(Ala-Pro) by Mattice and Mandelkern[20] present that poly(Ala-Pro) exhibits a negative band at 201 nm and positive band at 224 nm at -42℃

in ethylene glycol-water(2 : 1, v/v), and the negative band decreased in magnitude and shifted to 206 nm with increasing temperature from -42℃ to 75℃. Moreover, intrinsic viscosity[η] of poly(Ala-Pro) in water is 0.13 dl/g at 5℃ and it drastically decreases to 0.08 dl/g at 70℃ with increasing temperature[20]. These results suggest that poly(Ala-Pro) takes certain ordered conformations(at low temperature) which may be changed to disordered conformations with increasing temperature. However, it is difficult to specify which helical conformations presented in Table I corresponds to the above ordered conformations speculated by experimental results.

Optimized results indicate that rod-like helical structures such as γ -helices or β -helices with large h value are stable for poly(Ala-Pro). These conformational characters are significantly different from those of poly(Ala-D-Ala) and poly(Ala-Gly) in which α -helix or β -helices with small h value are stable helical conformations. That is, the repetitive Ala-Pro sequence is a very interesting amino-acid sequence for designing the rod-like backbone conformations of artificial proteins.

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