© 1997 The Chemical Society of Japan
Bull. Chem. Soc. Jpn., 70, 1451—1458 (1997)

Effect of Proline Residue in Polypeptides on the Interactions between Polypeptides and DNA[1)]

Chieko Uchida,† Kaori Wakamatsu, and Masanao Oya*

Department of Biochemistry and Engineering, Faculty of Engineering, Gunma University, Kiryu, Gunma 376

†Yabuzaka-honmachi Medical Center, Yabuzuka-honmachi, Nitta, Gunma 379-23

(Received December 3, 1996)

The interaction between high molecular-weight synthetic polypeptides containing proline residue and salmon DNA were studied by circular dichroism spectra (CD) in aqueous solution, ^{1}H NMR in D_2O solution, and ^{13}C NMR spectra in solid state. The interactions were compared with those of polypeptides containing lysine residue and DNA. The complexes of poly(Pro) and poly(Pro-co-Gly) with DNA were made by lyophilization after dialysis of the mixed solution of the corresponding solutions. These complexes were soluble in water and insoluble in 1 M NaCl, in contrast to the poly(Lys)–DNA complex, which was insoluble in water and soluble in 1 M (mol dm^{-3}) NaCl. The polypeptides containing proline residue (poly(Pro-co-X)) interacted with DNA in the solution, which differed from the interaction of poly(Lys) with DNA. In addition, the interactions of poly(Pro) and poly(Pro-co-Gly) with DNA differed from each other.

Proline (**3**) is unique among amino acids in that the end of its side-chain is covalently bonded to the preceding imino group nitrogen. Proline is rich in collagen which is a structural protein, and is a major component of connective tissue and bones. In a previous paper we reported on the interaction between water soluble diverse synthetic polypeptides in an aqueous solution. The results indicated that polypeptides containing proline residues interacted with poly(Asp-co-Ala) containing acidic aspartic acid.[2)] In this paper we report on the interaction between poly(Pro) (**5**) and poly(Pro-co-X) (**6a** or **b**) and DNA, having an acidic phosphoric group and basic nucleic acids. To date, there have been many studies concerning the interaction with DNA. The interactions between polypeptides containing basic amino acids such as lysine (**1c**) or arginine and DNA have been investigated previously.[3—5)] The interactions between antitumors such as distamycine binding pyrrole residue, with amide bond and DNA have been studied in recent years in order to understand how to recognize DNA.[6,7)] Various amino acids in the native proteins interact in a wide variety of ways with DNA.[8)] The interaction between DNA and Tus (Acetyl-PQNAKLKIKRPVKVQPI-ARRVY-Amide which is a 309 amino acid peptide and arrests replication in *Escherichia coli*) and TPPI (Tus proline peptide **I** which is a 22 amino acid peptide and has a similar sequence to the segment from Tus), both having proline-repeat segment residues, has been reported in more recent studies.[9,10)] However, very little is known about the interaction between high molecular-weight poly(Pro) or proline rich polypeptides. The factors contributing to the interaction between native proteins and DNA are so complicated that studies use synthetic simplified polypeptides containing one or two amino acid residues which are more useful for the analysis of the interactions with native proteins. This study focuses on two points of the polypeptides, including proline residue. First, whether or not the polymer of proline (which has a specific prolyl conformation with the exception of α-helix and β-sheet in solution) interacts with DNA. Second, whether or not the polymer of proline as a model collagen, which is a hard and stable fiber protein, can interact with DNA.

Experimental

Materials. Water soluble poly(pro), poly(pro-co-X),[11)] and the other polypeptides (**7**) used in this study were synthesized by *N*-carboxy amino acid anhydride (**2** and **4**) (Scheme 1).[12)] The poly(Lys) was an acetic acid salt and has higher molecular weight than that purchased commercially. The component ratios in the polypeptides were taken by ^{1}H NMR spectra. DNA and DNA sodium salt purchased from Wako Pure Chemical Industries, Ltd. stem from salmon sperm for biochemistry.

Viscosity Measurement. The viscosities of the polypeptides were measured in a solution of dichloroacetic acid or trifluoroacetic acid, using an Ubbelohde or Ostwald viscometer at 25 °C:

$$\eta_{rel} - 1 = \eta_{sp} \quad (1)$$

$$[\eta] = \lim[\eta_{sp}/c] \quad (2)$$

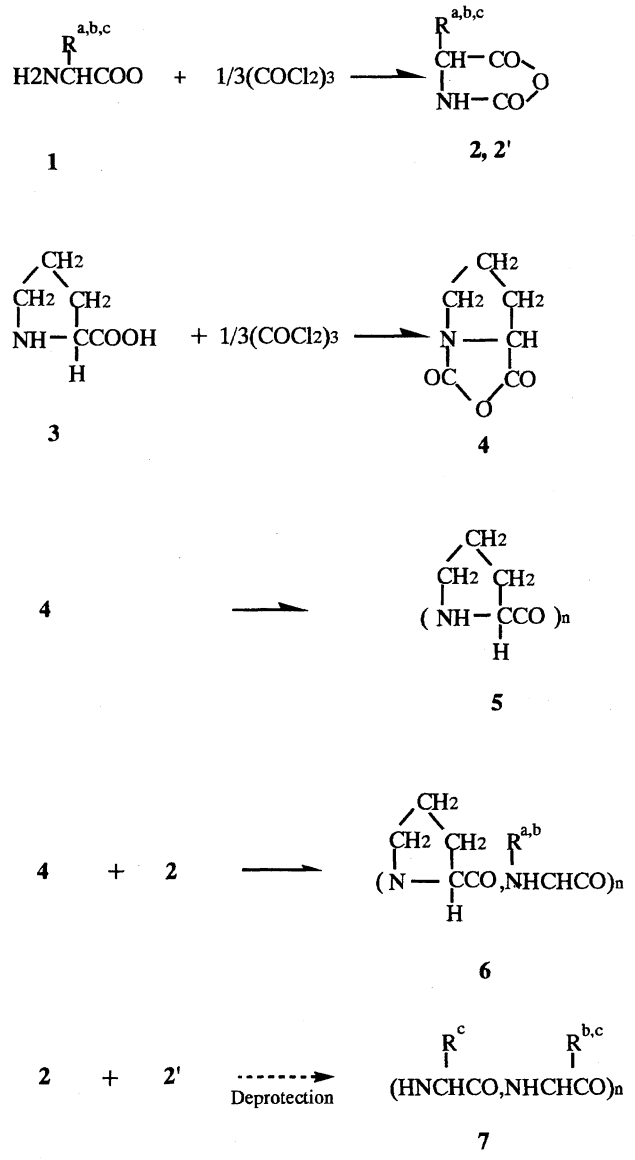
where η_{rel}, η_{sp}, and $[\eta]$ are relative viscosity, specific viscosity, and intrinsic viscosity of the polypeptide solution, respectively. Concentration c is 0.5 g ml^{-1}.

Molecular Weight (M). The molecular weights of the polypeptides were determined by Eqs. 3 and 4. Equation 3 shows that containing lysine. Equation 4 shows that containing other amino acid residues.

$$[\eta] = 2.24 \times 10^{-7} \times M^{1.26} \quad (3)$$

$$[\eta] = 2.78 \times 10^{-5} \times M^{0.87} \quad (4)$$

Circular Dichroism (CD) Measurement. CD were recorded on a JASCO J-20A equipped with DATA PROCESSOR J-DPZ. The



a, H; b, CH_3; c, $H_2N(CH_2)_4$

Scheme 1.

measurements were carried out at room temperature with a 0.1 mm cell from 310 to 190 nm. The polypeptide concentrations were 2 mg ml^{-1} in water or 0.015 M (mol dm^{-3}) sodium citrate. DNA and DNA sodium salt concentrations were 6 mg ml^{-1} in water or 0.015 M sodium citrate. The solution of DNA, DNA sodium salt, and polypeptides was measured for each CD spectrum. On a separate occasion, a polypeptide solution was mixed with equal amounts of DNA or DNA sodium salt solution, and its CD spectrum was measured. The interaction was determined based on the significant difference between the mean ellipticity of each sample and that of the mixed solution in terms of the minimum or maximum peaks of its CD spectra:

$$[\theta] = \theta/CL \quad (5)$$

where $[\theta]$, θ, C, and L are the reduced mean residue ellipticity, observed ellipticity in degrees, polymer concentration mol dm^{-3}, and optical path length in cm.

^{1}H NMR Measurement. ^{1}H NMR spectra were recorded at 400 MHz by a Bruker ARX 400 in the solution of D_2O at the same concentration used for CD measurements.

^{13}C NMR Measurement. ^{13}C solid state NMR spectra were recorded at 100 MHz by a JEOL ALPHA 400 equipped with a CP-MAS accessory. The solid complexes of poly(Pro)–DNA and poly-(Pro-co-Gly)–DNA were made by dialysis using cut 1000 cellulose, and lyophilization of the mixed solutions for CD spectra.

Results and Discussion

CD Spectra. Table 1 shows the properties and ellipticity of the minimum and maximum peaks of the polypeptides used.

The effects of temperature or metal ions on the CD spectra of DNA from various sources have already been reported.[13,14] The salmon sperm DNA for this study was hard in water and soluble in a solution containing a salt such as sodium citrate, whereas the DNA sodium salt was soluble in water. Figure 1 shows the effect on the CD spectra of the DNA and DNA sodium salt in solution. Both had the same spectra pattern and had positive peaks at 216—218 nm and 276—278 nm, and negative peaks at 206 nm and 248 nm.

The interactions of poly(Pro) and poly(Pro-co-X) with DNA were studied. The mixed solutions of the poly(Pro) and the poly(Pro-co-X) with the DNA were clear and stable for a long time. (Mixed solution of the poly(Lys) and poly(Lys-co-X) were cloudy and unstable, and precipitation appeared quickly.) It is well known that poly(Pro) has two types of conformation.[15,16] The conformation of type II has a strong negative peak at 206 nm. The polypeptides of Entries 1 and 2, which have a rich proline residue, take type II conformation in solution. The other polypeptides having poor proline

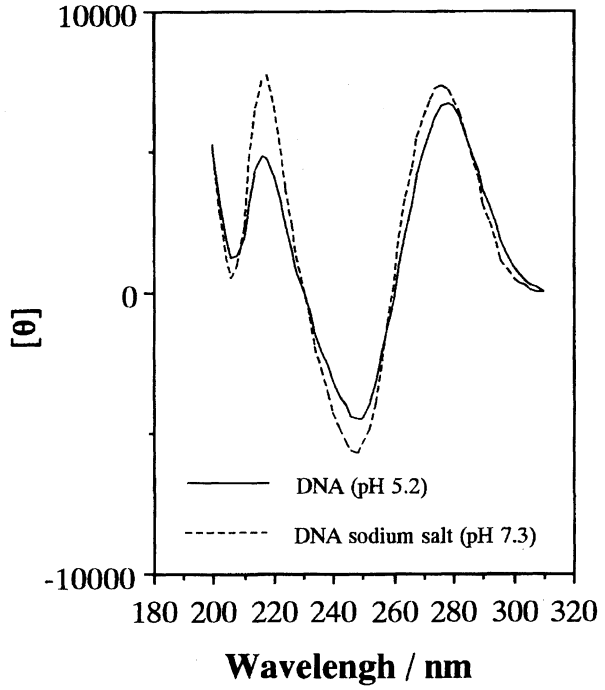


Fig. 1. CD spectra of DNA in sodium citrate solution and DNA sodium salt in water: —, DNA in sodium citrate solution at pH 5.4; - - -, DNA sodium salt in water at pH 7.3.

Table 1. CD Spectra of Polypeptides Used for Interaction

Entry	Polypeptides	$[\eta]$ in DCA	pH	Maximum peak nm	$[\theta]$	M
(1a)	Poly(Pro)	0.36	7.41	206	−54600	53600
				228	3400	
(2a)	Poly(Pro : 43, Ala : 57)	0.23	7.19	206	−13400	32000
(3a)	Poly(Pro : 31, Ala : 69)	0.34	6.72	200	−16400	50200
(4a)	Poly(Pro : 29, Gly : 71)	0.17	7.12	200	−8400	22600
				226	500	
(5a)	Poly(Pro : 25, Gly : 75)		7.08	200	−8000	22600
				228	500	
(6b)	Poly(Lys)	4.20d	7.43	196	−38700	550000
				218	4700	
(7c)	Poly(Lys)		7.99	194	−29700	
				214—220	1900	
(8b)	Poly(Lys : 59, Ala : 41)	0.83	7.52	196	−30100	155000
				216—218	1200	
(9c)	Poly(Lys : 59, Ala : 41)		8.61	204—206	−19100	
				218—222	−17300	
(10b)	Poly(Lys : 85, DL-Ala : 15)	0.40d	7.07	196	−21400	86800
				218	2100	
(11b)	Poly(Lys : 46.5, DL-Ala : 53.5)	0.30	6.94	196	−15400	69200
				216—220	900	
(12b)	Poly(Lys : 26, DL-Ala : 74)	0.29d	7.46	196—194	−4900	67400

a) 0.015 M Sodium citrate solution. b) Water solution. c) 1 M Sodium salt. d) Ubbelohde viscometer C=0.48 g dm^{-3}.

residue (Entries 3, 4, and 5) take random conformation in solution. The $[\theta]_{206}$ of a mixed solution of poly(Pro) and DNA was less than that of free poly(Pro), and was greater than that of free DNA. It was less than the average, and the difference between the mixture and the average value was 8000 (Fig. 2a). Furthermore, the first peak of the mixed solution (which was at 276 nm) was less than that of free DNA, and was greater than that of the average of free poly(Pro) and DNA. The difference between the mixture and the average was 1200. The peak shifted from 278 to 276 nm. These results indicate that poly(Pro) interacts with DNA.

Poly(Pro : 43-co-Ala : 57) also had a proline II conformation in the solution. The $[\theta]_{206}$ of the mixed solution of this peptide and DNA was greater than the average (Table 2, Entries 11 and 12). The difference was 1800. But the first peak of the mixture (which was at 278 nm) was similar to the average. At this point, the interaction of this peptide with DNA was distinct from that of poly(Pro).

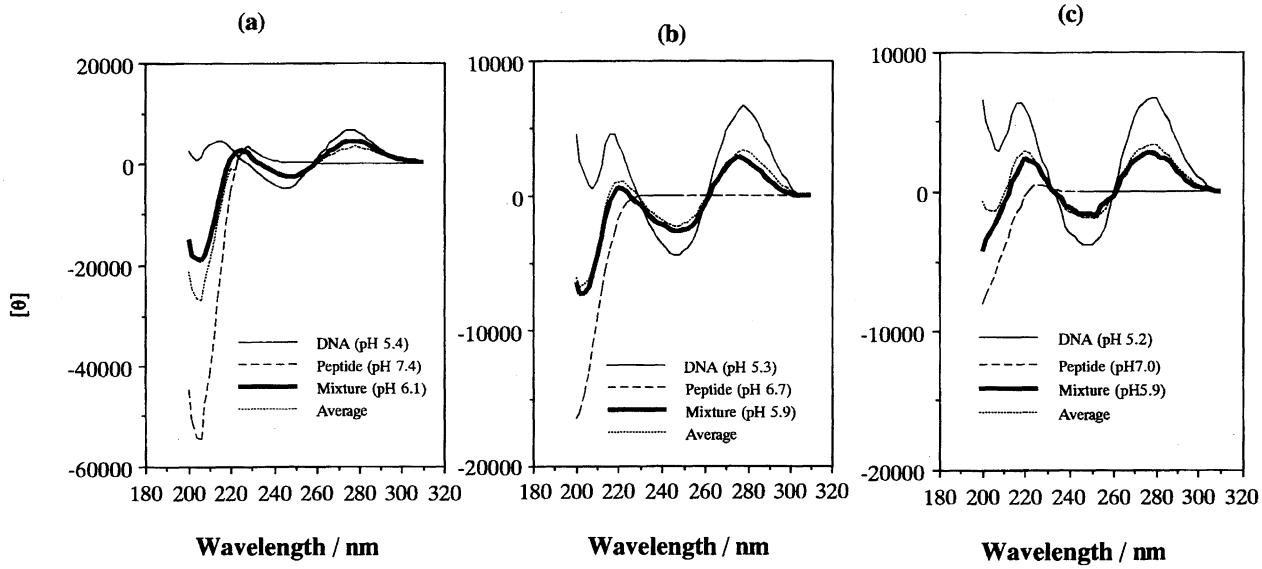


Fig. 2. CD spectra of polypeptides containing proline residue, DNA, the mixed solution, and the average: —, DNA; - - -, polypeptide; —, the mixed solution; ····, the average. (a) poly(Pro), etc., (b) poly(Pro : 31-co-Ala : 69), etc., and (c) poly(Pro : 25-co-Gly : 75), etc.

Table 2. CD Spectra of the Mixture of a Polypeptide with DNA or DNANa in Solution and That of the Average of a Polypeptide and DNA

Entry	Polypeptide	DNA	pH	nm	$[\theta]$
(11)	Mixture of Poly(Pro : 43, Ala : 57)	with DNA	5.89	206	−7700
				222	450
				246—250	−2300
				276—278	2900
(12)	Average of Poly(Pro : 43, Ala : 57)	and DNA		206	−5900
				222	900
				244—250	−2000
				278	3300
(13)	Mixture of Poly(Pro : 29, Gly : 71)	with DNA	5.90	204	−4200
				218—220	2000
				250—252	−2300
				274—276	2600
(14)	Average of Poly(Pro : 29, Gly : 71)	and DNA		206	−2700
				220	2200
				250	−2600
				276—278	3300
(15)	Mixture of Poly(Lys : 59, Ala : 41)	with DNANa	7.68	208	1800
				216	2900
				242—244	−2000
				276—280	2700
(16)	Average of Poly(Lys : 59, Ala : 41)	and DNANa		198	−10800
				216	4000
				246—248	−2600
				274—278	3200
(17)	Mixture of Poly(Lys : 85, DL-Ala : 15)	with DNANa	8.80	206—208	700
				218—220	2600
				248—252	−2300
				280	2500
(18)	Average of Poly(Lys : 85, DL-Ala : 15)	and DNANa		200	−5400
				218	4700
				248	−3200
				276	3800
(19)	Mixture of Poly(Lys : 46.5, DL-Ala : 53.5)	with DNANa	7.42	204—208	0
				218—220	2000
				248—250	−2000
				276—280	2200
(20)	Average of Poly(Lys : 46.5, DL-Ala : 53.5)	and DNANa		200	−4400
				216—218	3800
				244—246	−2800
				276—278	3400

Poly(Pro : 31-co-Ala : 69) had a random conformation in the solution. This peptide did not interact with DNA because the CD spectrum of the mixed solution between the peptide and DNA had roughly the same spectrum as the average (Fig. 2b). These facts prove that proline-richer copolypeptides interact with DNA very well.

Poly(Pro : 29-co-Gly : 71) is a copolypeptide which has converted glycine residue from the alanine residue of previous copeptides, and has less proline residue content than the previous two peptides. The conformation was a random structure. The mixed solution with DNA had a trough at 204 nm, and had a blue shift from the average at 206 nm. The difference was 1500 (Table 2, Entries 13 and 14, and Deposit Fig. 1 (The figure is deposited as Document No. 70015 at the Office of the Editor of Bull. Chem. Soc. Jpn.)).

Ply(Pro : 25-co-Gly : 75) was similar to the CD spectrum of poly(Pro : 29-co-Gly : 71) in the solution. The mixed solution with DNA had a trough at 200 nm, and had a blue shift from the average at 206 nm (Fig. 2c). The difference was 2800. In the case of poly(Pro-co-Gly), the two results indicate that the copolypeptides, consisting of lesser amounts of proline residue, are more likely to interact with DNA. The reason is thought to be the size of glycine. Because it is small, this amino acid contributed to greater flexibility in the copolypeptide structure. Therefore, poly(Pro-co-Gly) facilitated an interaction with DNA through its high flexibility due to the small size of glycine.

Next, the polypeptides containing lysine residue (which definitely interacted with DNA) were studied for comparison with proline residue. The mixed solution of polypeptides containing lysine residue and DNA sodium salt became white turbid and precipitated upon long standing. Therefore, these CD were measured immediately. This fact differs from polypeptides containing proline residue. All of the polypep-

tides containing lysine had a random conformation in water (Table 2, Entries 6, 7, 8, 9, and 10). The CD spectrum of the mixed solution of poly(Lys) and DNA sodium salt red-shifted in comparison with the average (Fig. 3a). The differences of each $[\theta]$ were 4100 at the shift from 218 to 218—220 nm, 2400 at the shift from 276 to 280—282 nm, 15000 at the shift from 198 to 208 nm, and 1500 at the shift from 246 to 250 nm. The differences of $[\theta]$ among all the peaks were above 1000. Definite interaction was observed. The interactions of copolypeptides containing lysine residue and DNA sodium salt were studied as well. As for poly(Lys : 59-co-Ala : 41), the second trough of the CD spectrum moved to the red side significantly, and a difference of $[\theta]$ of about 12600 existed (Table 2, Entries 15 and 16). The copolypeptides of lysine and DL-Ala were studied for their interaction with DNA sodium salt. The copolypeptides containing rich lysine residue (Entries 8 and 9) interacted with DNA sodium salt (Table 2, Entries 17, 18, 19, and 20). But the spectrum of the mixed solution of poly(Lys : 26-co-DL-Ala : 74) (which had poor lysine residue) and DNA sodium salt agreed with the average (Fig. 3b).

This CD study demonstrates that proline polymers or lysine interacted with DNA.

It has been proposed that the detailed molecular structure of poly(Lys)–DNA is a complex with the relationship of NH_2 : P=1 : 1.[3] That of the poly(Pro)–DNA must be different from that of poly(Lys)–DNA. As mentioned above, the two interactions had a different chemistry. This is because the mixed solution of poly(Pro) and DNA was a clear solution but that of poly(Lys) and DNA was cloudy and precipitated upon long standing. The complexes poly(Lys)–DNA and poly(Lys : 59-co-Ala : 41) were soluble in 1 M NaCl, as well as in many other nuclear proteins. In this solution, the helical CD spectra were observed on free poly(Lys), free poly(Lys : 59-co-Ala : 41), and on their complexes (Fig. 4). But the complexes poly(Pro) or poly(Pro-co-Gly) with DNA were insoluble in the same solution.

^{1}H NMR Measurement in the Solution. In order to further investigate this phenomenon, ^{1}H NMR of poly-

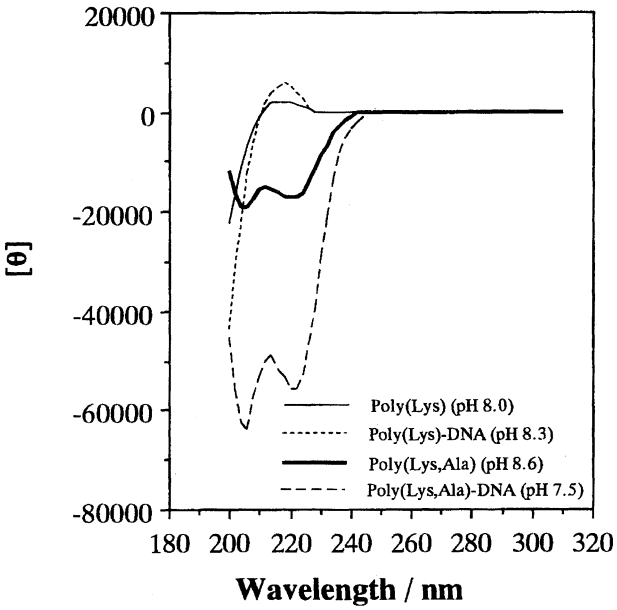


Fig. 4. CD spectra of poly(Lys), the complex with DNA sodium salt, poly(Lys : 59-co-Ala : 41), and the complex with DNA sodium salt in 1 M NaCl solution: —, poly(Lys); ····, poly(Lys)–DNANa; —, poly(Lys, Ala); - - -, poly(Lys, Ala)–DNANa.

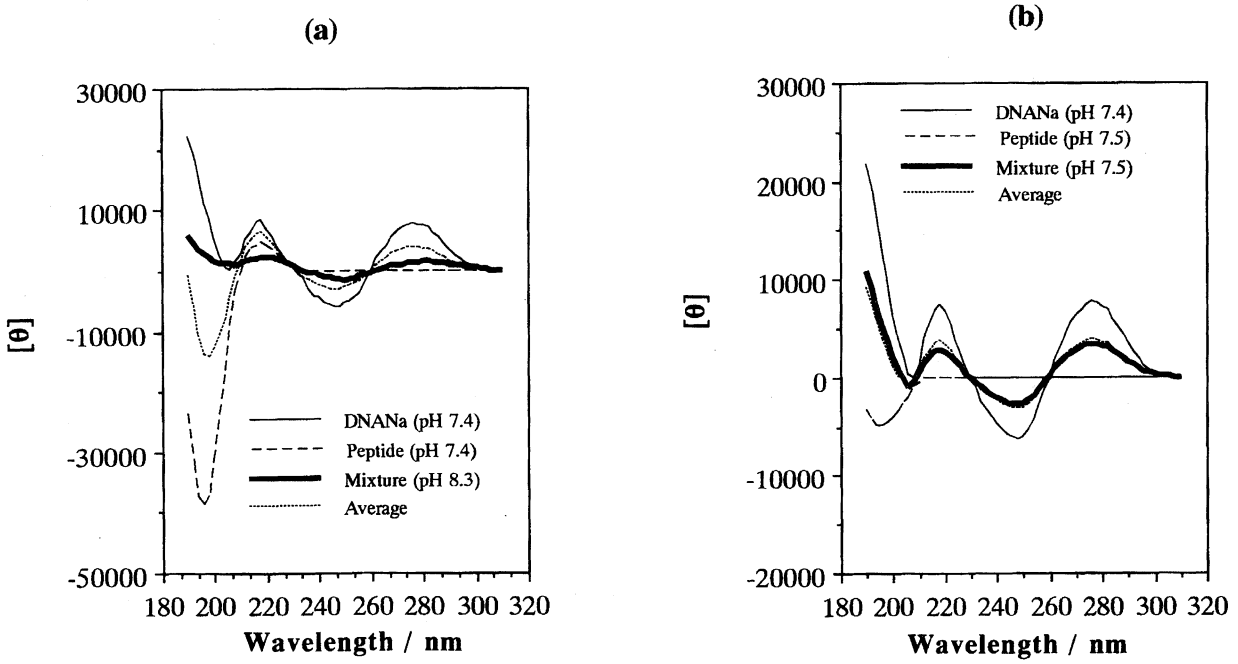


Fig. 3. CD spectra of polypeptides containing lysine residue, DNA sodium salt, the mixed solution, and the average: —, DNA sodium salt; - - -, polypeptide; —, the mixed solution; ····, the average. (a) poly(Lys), etc., and (b) poly(Lys : 26-co-DL-Ala : 74), etc.

(pro), poly(Pro : 25-co-Gly : 75), DNA, and the complexes of the polypeptides and DNA were measured in D_2O solution (Figs. 5 and 6). The H2, H5, H6, and H8 proton chemical shifts of the nucleic acid base (7—9 ppm) in the

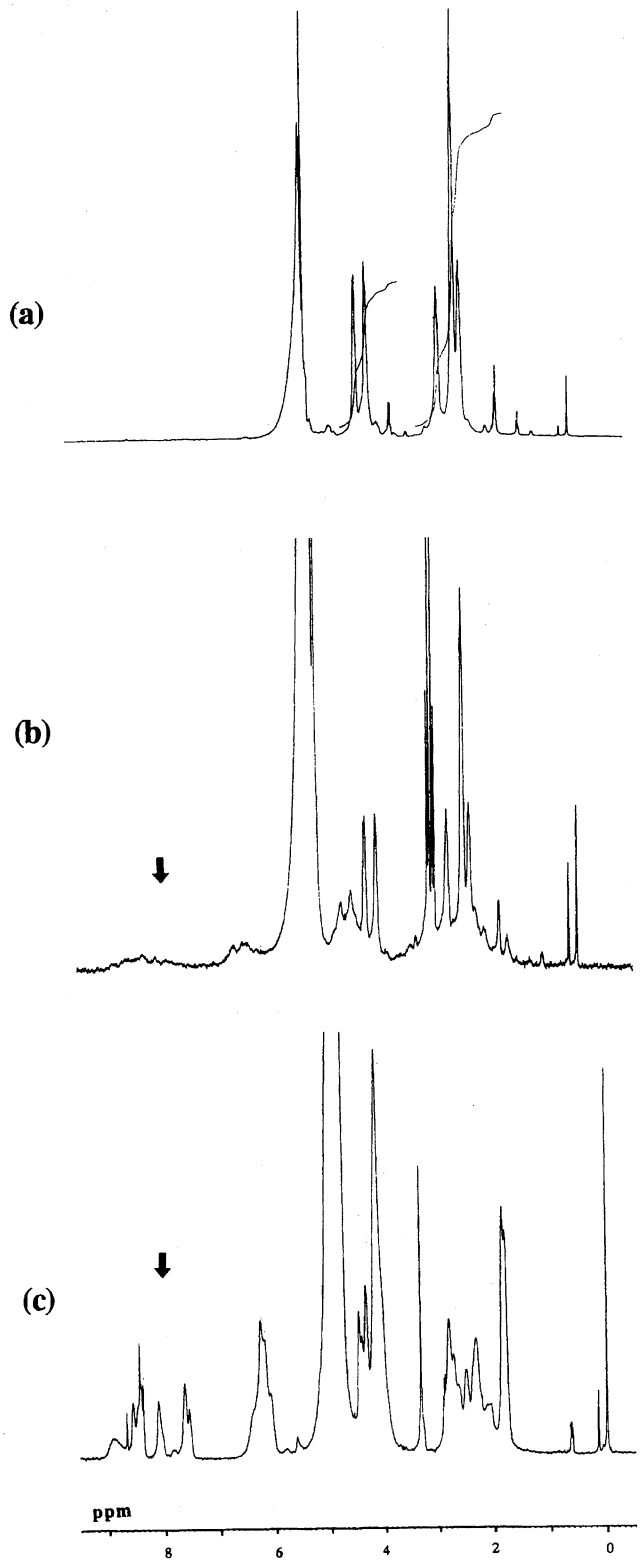


Fig. 5. ^{1}H NMR spectra of poly(pro), DNA, and the complex in D_2O solution: (a), poly(pro); (b), the complex of poly-(Pro) and DNA; (c), DNA.

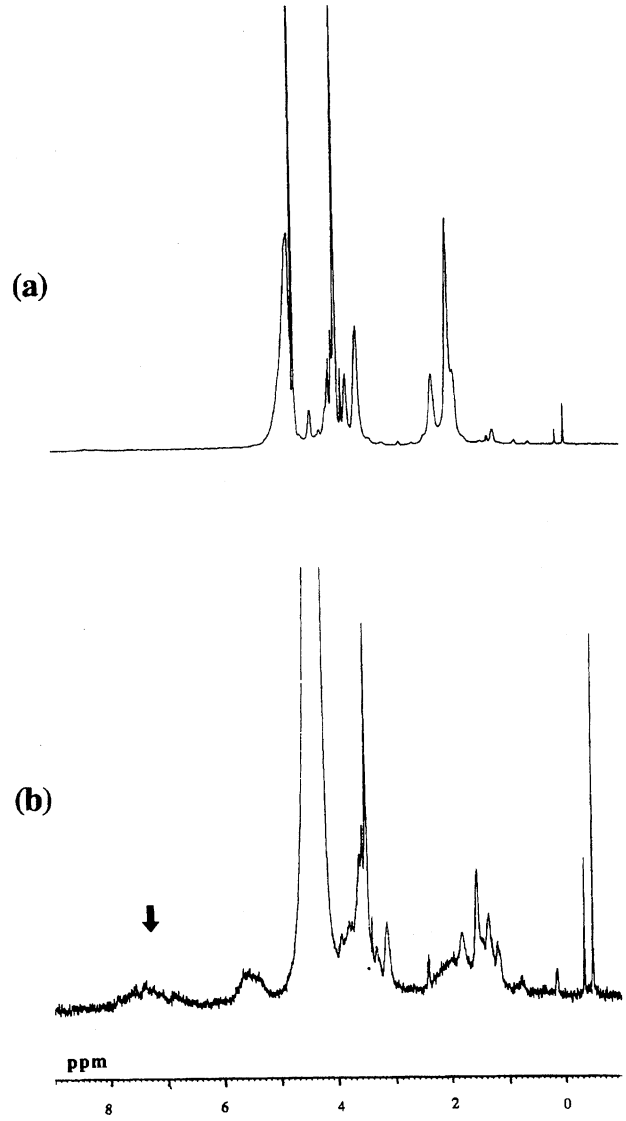


Fig. 6. ^{1}H NMR spectra of poly(Pro : 25-co-Gly : 75) and the complex with DNA in D_2O solution: (a), poly(Pro : 15-co-Gly : 75); (b), the complex of poly(Pro : 15-co-Gly : 75) and DNA.

peptide–DNA complexes were weak and broad in comparison with free DNA. It is suggested that the poly(Pro-co-X) does not interact with the sugar-phosphoric backbone but with the nucleobase.

^{13}C Solid State NMR Measurement. Furthermore, research on the interaction was tested by ^{13}C solid state NMR (Table 3 or Deposit, Figs. 2 and 3 (The figures are deposited as Document No. 70015 at the Office of the Editor of Bull. Chem. Soc. Jpn.)). The conformations of oligopeptides containing proline residue such as Z–Gly–Pro, Z–Gly–Pro–Leu, Z–Gly–Pro–Gly–Gly, and Z–Gly–Pro–Ala–Ala have been reported.[17] Two conformations of poly(Pro) (I and II) are easily distinguished by their peak positions at C_β and C_γ.[18—24] H. Saito et al.[25] have reported that the difference in the ^{13}C chemical shifts between the C_β and C_γ signals, $\Delta_{\beta\gamma}$, were 2.4 and 9.3 ppm for $(Pro)_n$ II and $(Pro)_n$ I. In this study, there was no conformational transition from II to I when poly(Pro)

Table 3. ^{13}C NMR Chemical Shift of Poly(Pro), Poly(Pro : 25, Gly : 75), Poly(Lys), Poly(Lys : 41, Ala : 59), and the Complexes of Each Polymer with DNA

Sample	Comformation by CD	^{13}C chemical shifts/ppm						
			Pro				Gly	
		Amid C=O	C_α	C_β	C_γ	C_δ	C_α	
Poly(Pro)	Helix(trans)	171.4	60.0	29.2	25.7	44.3		
Complex of Poly(Pro) and DNA		172.9	58.6	27.9	25.7	47.9		
Poly(Pro : 25, Gly : 75)	Random	171.4	58.6	27.1	24.3	42.9[a)]	42.9[a)]	
Complex of Poly(Pro, Gly) and DNA		172.9	60.1	28.6	22.9	41.4	37.9	

Sample	Comformation by CD	^{13}C chemical shifts/ppm							
			Lys					Ala	
		Amid C=O	C_α	C_β	C_γ	C_δ	C_ε	C_α	C_β
Poly(Lys)	Random	180.0	59.1	28.2[b)]	25.4	28.2[b)]	40.0		
Complex of Poly(Lys) and DNANa		178.2	60.0	30.0	28.2	30.0	38.2		
Poly(Lys : 41, Ala : 59)	Random	181.0	59.1	29.1[c)]	24.5	29.1[c)]	40.1	53.6	15.5
Complex of Poly(Lys, Ala) and DNANa		180.0	59.1	30.0[d)]	30.0[d)]	30.0[d)]	40.0	50.9	17.3

a),b),c),d) Overlapped.

interacted with DNA, because the $\Delta_{\beta\gamma}$ of free poly(Pro) and the complex with DNA, respectively, were not significantly different in value. The poly(Pro)–DNA complex did not have a different C_α signal value compared with that of free poly(Pro). The C_δ position of the poly(Pro)–DNA complex moved more than other carbon positions compared with free poly(Pro). (The difference was 3.6 ppm.) By ^{13}C NMR chemical shift, the C=O shift is influenced by the hydrogen bond.[26] This sample had no significant shift. The difference of the ^{13}C chemical shift between free and complex was 1.2—1.4 ppm on each of the carbons except the C_δ position.

The ^{13}C chemical shift of free poly(Pro : 25-co-Gly : 75) closely resembled poly(Pro). The difference of the ^{13}C chemical shift between the C_β and C_γ signals, $\Delta_{\beta\gamma}$, was 2.8 ppm for free poly(Pro-co-Gly) and 5.7 ppm for the complex. There was a greater difference in the value of the $\Delta_{\beta\gamma}$ in complex than in the free. This reflected the mode of change from *trans* to *cis*. These results indicate that the conformational transitions for interaction with DNA were different from poly(Pro) and poly(Pro-co-Gly). This did not contradict the results by CD. The C_α signals of $(Pro)_n$ are not varied by changing from form II to form I.[26] The difference of C_α between free poly(pro-co-Gly) and the complex with DNA was small (1.5 ppm). There was no significant shift of the C_δ position for poly(Pro-co-Gly)–DNA in distinction to poly(Pro)–DNA, and the other carbon shifts were similar to poly(Pro)–DNA. That is to say, the difference of the ^{13}C chemical shifts between free and complex of poly(Pro-co-Gly) was 1.4—1.5 ppm on each of the other carbons.

Poly(Pro), which is a homopolymer, has restricted conformation change, because it has a helix for the mutual repulsion of a pyrrolidine ring. On the other hand, the copolymers containing proline and glycine have a more flexible conformation than the homopolymer of proline, because glycine residue does not have a long side chain, and the mutual pyrrolidine ring of proline residue has minimal influence on the conformation. Accordingly, the C_β and C_γ positions of proline residue in poly(Pro-co-Gly) shift more, and proline residue changes from *trans* to *cis* because of the minimal influence of the pyrrolidine ring by rich glycine.

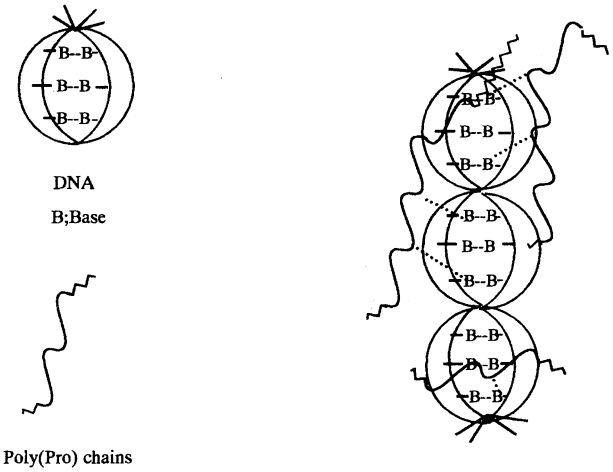


Fig. 7. Illustration of complex formation between poly(pro) and DNA.

The difference of the ^{13}C chemical shift between the free polymer of lysine type (poly(Lys), poly(Lys, Ala)) and the complexes with DNA was dominant on the C_γ signal position (Table 3 and Deposit, Fig. 3).

In this study, proline residue proved to be of great interest in amino acids which interacted with DNA (Fig. 7).

The authors wish to express their thanks to Mr. Yasuyoshi Miyaji of Ajinomoto Company for obtaining some of the ^{13}C NMR spectra.

References

1) A part of this work was presented at "The 5th Symposium of Materials Research Society of Japan," Kawasaki-KSP, Kanagawa, Dec. 1993, Abstr., No. 2-0-2; "The 35th International IUPAC International Symposium on Macromolecules," Acron, Ohio, U.S.A.,

July 1994, Abstr., No. 0-3.2-9t; and "The 68th National Meeting of the Chemical Society of Japan," Nagoya, Oct. 1994, Abstr., No. 3E218.

2) C. Uchida, Y. Iizuka, K. Wakamatsu, and M. Oya, *Nippon Kagaku Kaishi*, **8**, 962 (1993).

3) M. Tsuboi, K. Matsuo, and P. O. P. T'so, *J. Mol. Biol.*, **15**, 256 (1966).

4) H. Jeili, L. Herlands, R. Santella, and P. Epstein, *Biopolymers*, **14**, 2401 (1975).

5) R. Santella and H. Jeili, *Biopolymers*, **16**, 1879 (1977).

6) P. B. Dervan, *Science*, **232**, 464 (1986).

7) M. Mrksich and P. B. Dervan, *J. Am. Chem. Soc.*, **115**, 2572 (1993).

8) Carl O. Pabo, *Ann. Rev., Biochem.*, **61**, 1053 (1992).

9) M. L. Nedved, P. A. Gottlieb, and G. R. Moe, *Nucleic Acids Res.*, **22**, 5024 (1994).

10) D. J. Butcher, M. L. Nedved, T. G. Neiss, and G. R. Mor, *Biochemistry*, **35**, 698 (1996).

11) Y. Iizuka, C. Uchida, K. Wakamatsu, and M. Oya, *Bull. Chem. Soc. Jpn.*, **66**, 1269 (1993).

12) M. Oya and T. Ozaki, "Jikken Kagaku Koza," 4th ed, ed by The Chemical Society of Japan, Maruzen, Tokyo, (1992), No. 28, Polypeptides, p. 287.

13) T. Samejima and J. T. Yang, *J. Biol. Chem.*, **240**, 2094 (1965).

14) P. K. Sarkar, B. Wells, and J. T. Yang, *J. Mol. Biol.*, **25**, 563 (1967).

15) L. Mandelkern, "Poly-α-amino Acids," ed by G. D. Fasman, Marcel Dekker Inc., New York (1967), p. 675.

16) F. A. Bovey and F. P. Hood, *Biopolymers*, **5**, 325 (1967).

17) S. Ando, K. Matsumoto, and I. Ando, *J. Macromol. Struct.*, **212**, 123 (1989).

18) K. Wuthrich, A. Tun-Kyi, and R. Schwyzer, *FEBS Lett.*, **25**, 104 (1972).

19) W. A. Thomas and M. K. Williams, *J. Chem. Soc., Chem. Commun.*, **1972**, 994.

20) F. A. Bogey, "Chemistry and Biology of Peptides," ed by J. Meienhofer, Ann Arbor Sci. Publ., Ann Arbor (1972), p. 3.

21) I. C. P. Smith, R. Deslauries, and R. Walter, "Chemistry and Biology of Peptides," ed by J. Meienhofer, Ann Arbor Sci. Publ., Ann Arbor (1972), p. 29.

22) D. E. Dorman and F. A. Bogey, *J. Org. Chem.*, **38**, 2379 (1973).

23) I. Z. Siemion, T. Wieland, and K. H. Pook, *Angew. Chem.*, **87**, 712 (1975).

24) R. Deslauries and I. C. P. Smith, *Top. Carbon-13 NMR Spectrosc.*, **2**, 1 (1976).

25) H. Saito and R. Tabeta, *Biopolymers*, **23**, 2279 (1984).

26) K. Wuthrich, "NMR in Biological Research: Peptides and Proteins," North-Holland Publishing Company (1976) (Translated by Y. Arata and M. Kainosyou, Tokyo Kagaku Douzin, Tokyo (1979)).