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Interactions between Oppositely Charged Polypeptides

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ABSTRACT: Conformation-directing interactions between poly(L- or poly(D-glutamic acid)) (PLG or PDG) and poly-L-lysine (PLL) in aqueous media were investigated as functions of pH by using the optical rotatory dispersion and circular dichroism spectroscopies. The results indicate that at pH <4 PLL molecules surround the core of the right-handed α-helix of PLG, and at pH about 7, the polyelectrolyte complex formed may be in a scramble-salt form, whereas at pH >8, complexes of PLG—PLL are in a form close to β-structure. On the other hand, with PDG—PLL systems, in the low pH region, the left-handed α-helical structure of PDG is markedly disrupted by the presence of PLL, and in the high pH region, the complexes may be in a form of a scramble-salt aggregate composed of β-structures.

KEY WORDS Polyelectrolyte Complex / Polypeptide / Poly(L-glutamic acid) / Poly(D-glutamic acid) / Poly-L-Lysine / Conformations / Optical Rotatory Dispersion / Circular Dischroism /

Interactions between polyelectrolytes carrying opposite charges in aqueous media lead to the formation of polyelectrolyte complexes. The study of such complexes may be traced to the work of Bungenberg de Jong and coworkers,[1] who interpreted the ionic interactions in water between naturally occuring weak polyelectrolytes, gelatin and gum arabic, under changes of ionic strength, temperature, pH, etc., as a kind of phase separation referred to the complex coacervation.

In previous works, we have investigated the complex coacervation with synthetic polyelectrolytes, *i.e.*, partly aminoacetalyzed poly(vinyl alcohol) and partly sulfated poly(vinyl alcohol),[2,3] and further discussed the effects of chain conformations of component polymers on the complex formation elsewhere.[4,5]

Polypeptides are characteristic in what chain conformations, such as α-helix, β-structure, and random coil, they can take in solution, in accordance with the surrounding conditions. Further, the α-helix can be in a left-or right-handed screw sense depending on the configurations of the backbone chains and the nature of the side chains. Accordingly, when an ionized polypeptide is reacted with an oppositely charged polyelectrolyte, the conformational change in the component polymers may be accompanied by the complex formation depending upon circumstances. Such conformation-directing interactions of polypeptides have been investigated by using polysaccharides[6-9] and vinyl polymers[10,11] as the partner polyelectrolytes.

Conformations of ionizable polypeptides in solutions have been extensively investigated. However, very little has been reported[12,13] about the interactions between oppositely charged polypeptides. Noguchi, *et al.*,[12] examined the X-ray diagrams and solubilities of L—L and D—L salts between poly(glutamic acid) and polylysine, and suggested that the L—L salt might have a helical structure with salt-bridges between two oppositely charged molecules in water, whereas the D—L salt might assume a less regular structure having salt-bridges among many molecules. On the other hand, Hammes and Schullery[13] suggested that poly(L-glutamic acid) and poly-L-lysine formed a β-pleated sheet structure with 1 : 1 stoichiometry at pH 4 and 7 in aqueous 0.01*M* NaF, while no appreciable interaction was detected at pH 11.

The purpose of this work is to elucidate the conformation-directing interactions between

poly(glutamic acid) and polylysine in aqueous media as a function of pH by using the optical rotatory dispersion and circular dichroism spectroscopies. The systems investigated are poly(L-glutamic acid)—poly-L-lysine, and poly(D-glutamic acid)—poly-L-lysine systems. A right-handed α-helix is known to be stable for both poly(L-glutamic acid) and poly-L-lysine, whereas the left-handed α-helix is stable for poly(D-glutamic acid).

EXPERIMENTAL

Materials

Poly(L-glutamic acid) (PLG) and poly(D-glutamic acid) (PDG), and poly-L-lysine (PLL), respectively, were prepared from poly(γ-benzyl-L-glutamate) (PBLG) and poly(γ-benzyl-D-glutamate(PBDG) by debenzylation, and from poly (carbobenzyloxy-L-lysine) (PCBLL) by decarbobenzyloxylation. Thus PBLG, PBDG, and PCBLL were first synthesized by the NCA method. The polymerization was carried out at 25°C in a 1 : 1 (v/v) mixture of dioxane and methylene dichloride in the presence of triethylamine (TEA) as an initiator. The number-average molecular weight, M_n, was determined in dimethylformamide at 30°C with an Electronic High-Speed Membrane Osmometer (Knauer), in which a regenerated cellulose membrane SD (Sartorius Membran-filter GmbH) was used as the semi-permeable membrane.

In Table I, the number-average degree of polymerization, P_n, and the limiting viscosity number $[\eta]$ in dichloroacetic acid (DCA) are given together with the polymerization conditions, the concentration of the NCA, and the mole ratio [M]/[I] of the NCA-monomer to the initiator. Debenzylation of PBLG and PBDG was performed at an initial polymer concentration of 0.5 g/d*l* in benzene by blowing hydrogen bromide gas at room temperature. PCBLL dissolved in a 1 : 1 (v/v) mixture of dioxane—chloroform was decarbobenzyloxylated by blowing hydrogen bromide at room temperature. The products obtained by the debenzylation and decarbobenzyloxylation were identified as the expected materials, *i.e.*, PLG, PDG, and PLL, from the elemental analyses and ultraviolet absorption spectra. From the limiting viscosity data of PLG, PDG, and PLL in 0.1-*N* NaCl aqueous solution, it was concluded that no remarkable chain scission occured during the debenzylation or decarbobenzyloxylation.

Table I. Preparative data of polypeptides

Sample	NCA Concn, %	[M]/[I]	$[\eta]$, d*l*/g	M_n
PBLG	3.0	250	2.02	209,000
PBDG	3.0	50	1.22	146,000
PCBLL	3.0	100	2.20	238,000

The degrees of dissociation α of PLG and PLL in the absence of microsalt were determined by potentiometric titrations. The initial concentrations of PLG and PLL were 0.0033*M* (base-mole) and 0.0073*M*, respectively.

Preparation of Mixtures

Separate dilute aqueous solutions were prepared for PLG, PDG, and PLL. The PLL solution was adjusted at pH 12 by adding 1.0-*N* NaOH. The concentrations of these solutions were denoted in terms of the base-moles of the amino acid residues. Mixtures were prepared by slow dropwise addition of an aqueous solution of PLG or PDG at pH *ca.* 7 to the PLL solution at pH 12, and then the pH of the mixtures was brought to the desired values by adding concentrated HCl. The relative proportions of the two components were quoted as the base-mole ratios.

Circular Dichroism and Optical Rotatory Dispersion

The circular dichroism (CD) and the optical rotatory dispersion (ORD) spectra were measured at 25±0.5°C in the range of wavelength 200—280 nm, using a JASCO J-20 CD/ORD Spectropolarimeter equipped with a quartz cell of path length 0.5 or 1 mm. The total polymer concentrations in aqueous solutions were from 0.002 to 0.003*M* with respect to amino acid residues. The concentrations were determined by conductometric titration and/or gravimetry. The residue ellipticity $[\theta]$ and the residue rotation [R] for complex systems were calculated based on the base-mole concentration of the amino acid residue which is capable of existing in α-helix conformation at the given pH, *i.e.*, L- or D-glutamic acid at pH less than 7, and L-lysine at pH larger than 7.

pH Measurements

The pH meter used was a Hitachi-Horiba pH-Meter Model F-7_{ss} equipped with a combination pH electrode 6028-10T, the sensitivity of which was 0.005 pH. The meter was calibrated with the standard buffer solutions before and after the CD and ORD measurements. The experimental error of pH values was within 0.02 pH units.

RESULTS AND DISCUSSION

Conformations of Poly(L-glutamic acid), Poly(D-glutamic acid), and Poly-L-Lysine as Functions of pH

The circular dichroism data, expressed as the residue ellipticity $[\theta]$, of PLG, PLL, and PDG at typical pH values are shown in Figures 1, 2, and 3, respectively. As revealed by Holzwarth and Doty,[14] the CD spectrum of the α-helix conformation is characterized with a negative 222 nm band assigned to the $n \rightarrow \pi^*$ transition,

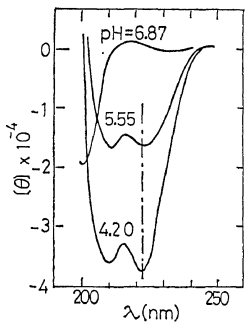

Figure 1. CD spectra of PLG in aqueous solutions at 25°C and various pH values.

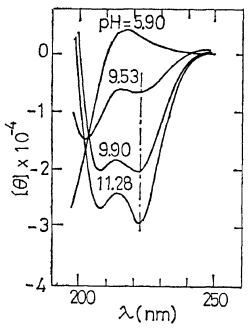

Figure 2. CD spectra of PLL in aqueous solutions at 25°C and various pH values.

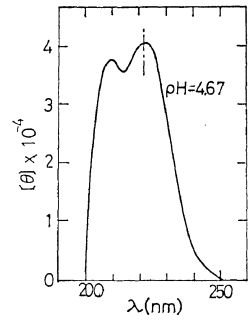

Figure 3. CD spectra of PDG in aqueous solutions at 25°C and pH 4.67.

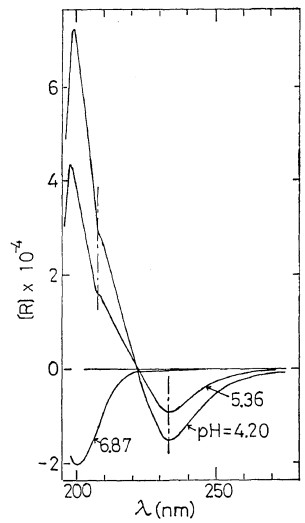

Figure 4. ORD spectra of PLG in aqueous solutions at 25°C and various pH values.

and a positive 190 nm and a negative 208 nm band, both assigned to the $\pi \rightarrow \pi^*$ transition; the random coil conformation is characterized by a negative 200 nm Cotton effect. The β-structure was characterized[15] by a negative 217 nm band ($[\theta] = -19300$) and a positive 195 nm ($[\theta] = +28000$) using PLL. The ORD data for PLG and PLL shown in Figures 4 and 5 are in accord with the results of Holzwarth, *et al.*,[14,16] and Blout, *et al.*,[17] and moreover are consistent with the results given in Figures 1 and 2. One can estimate the helical content from $[\theta]_{222}$ at 222 nm or $[R]_{232}$ at 232 nm, because the 222 nm band of the CD spectra corresponds to the 232 nm band of the ORD spectra.

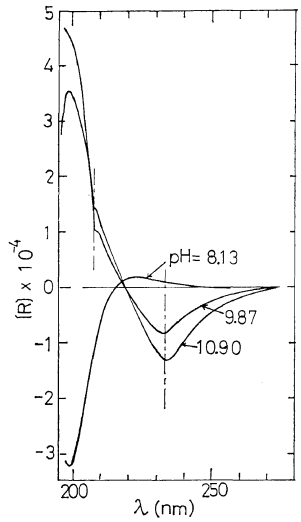

Figure 5. ORD spectra of PLL in aqueous solutions at 25°C and various pH values.

As is obvious from Figures 1 and 2, the 222 nm trough appears at pH=4.20~5.66 for PLG and at pH=9.53~11.28 for PLL; the depth of the trough depends on pH. The CD curve of PDG, as shown in Figure 3, is symmetrical to the corresponding curve of PLG with respect to the *abscissa*. These facts show that PLG and PLL can exist in the right-handed α-helix, whereas PDG is in the left-handed α-helix. The maximum values of $-[\theta]_{222}$ obtained for PLG, PDG, and PLL are 37500, −40400, and 30000, respectively, the value of PLL being considerably smaller than 40000 reported[18] for a perfect α-helix of PLG. It is not known whether the value 30000 obtained for PLL is characteristic of PLL, or whether it originates from the possibility that PLL can not form a perfect α-helix. With PLL, the maximum value of $[R]_{232}$ is also smaller than the value assigned for a perfect α-helix. The weak positive maximum which appeared at 218 nm for PLL at pH 5.90 may be understandable, if we assume an extended structure of charged coils[19] for PLL at this pH.

The pH dependences of $-[\theta]_{222}$ for PLG and PLL are shown by the broken curves in Figures 11 and 12, respectively. These curves represent the change of helical content against pH, indicating that the PLG exists in α-helix at pH less than 5 and in random coil conformation at pH higher than 7; the PLL exists in α-helix at pH higher than 11 and in random coil conformation at pH less than 9.

Interaction of Poly(L-glutamic acid) with Poly-L-Lysine, and Its Effects on Chain Conformations

When PLG reacts with PLL in an aqueous solution of a suitable pH, a polyelectrolyte complex linked with salt-bridges, as shown in Figure 6, is formed; the number of such salt-bridges is a function of pH.

Figure 7 shows the relations between the degree of dissociation α and the pH for PLG and PLL. These curves are compared with the helix—coil transition curves of PLG and PLL indicated in Figures 11 and 12. Obviously, both PLG and PLL exist in random coil conforma-

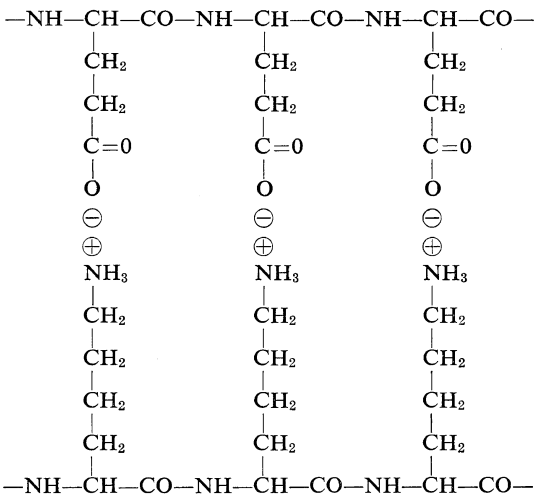

Figure 6. Polyelectrolyte complex formed from poly(glutamic acid) and polylysine.

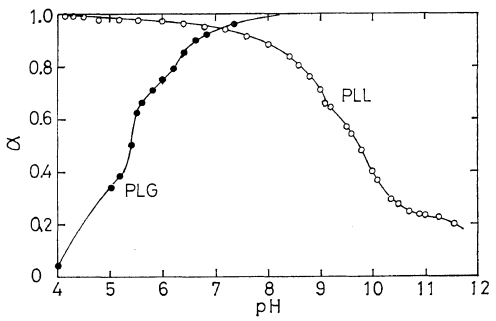

Figure 7. Degree of dissociation α plotted against pH for PLG and PLL.

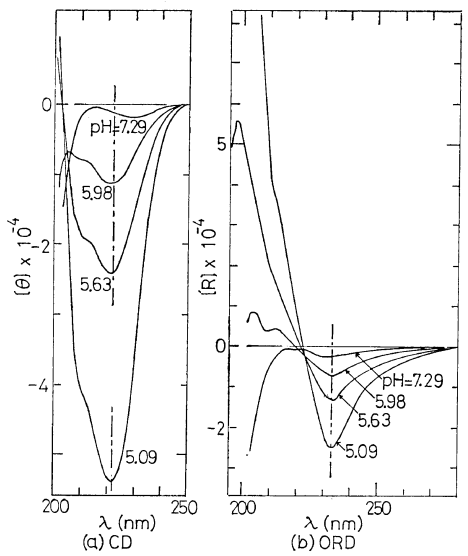

Figure 8. CD (a) and ORD (b) spectra of 74 : 26 mole ratio PLG—PLL mixture at 25°C and various pH values.

tion when α is close to unity, and transform to α-helix conformation with decreasing α.

PLG was reacted with PLL in different mole ratios, and the residue ellipticity $[\theta]$ and the residue rotation [R] of the mixtures were measured at different pH values. Figures 8a and b are the CD and ORD data of the 74 : 26 mole ratio PLG—PLL mixture. Comparison of Figure 8a with Figure 1 leads to the following characteristic differences between the CD spectra of the PLG—PLL mixture and of PLG: (a) No shift of wavelength is observed for the negative Cotton effect at 222 nm at pH's lower than about 6, while the depth of $[\theta]_{222}$ of the PLG—PLL mixture at pH lower than 5 is considerably larger than that of PLG. (b) The negative Cotton effect at 208 nm assigned to the $\pi \rightarrow \pi^*$ transition appears as a shoulder for the PLG—PLL mixture in contrast to the trough which appeared for PLG. The numerical values of $[\theta]_{208}$ for the PLG—PLL mixture at different pH's are almost the same as those of PLG at corresponding pH's. (c) At pH 7.29, however, the negative 222 nm band shifts to a slightly longer wavelength and the shoulder at 208 nm disappears. The $\pi \rightarrow \pi^*$ transition originates mainly from the main chain structure, while the $n \rightarrow \pi^*$ transition originates from the relative conformation of the side chain residue to the peptide plane. The experimental facts (a) and (b) may indicate that the 222 nm band ($n \rightarrow \pi^*$) is important for conformational investigations of polymer complexes formed by the salt-bridges between oppositely charged side chain residues. Comparison of Figure 8b with Figure 4 leads to the same conclusion with respect to the ORD spectra as that for the CD spectra, *i.e.*, the numerical value of $[R]_{232}$ of the PLG—PLL mixture is considerably larger than that of PLG, and the location of this band is unchanged.

Generally speaking, the ORD spectra are sensitive to the turbidity of the solutions, while the CD spectra are less sensitive. The PLG—PLL mixtures examined here are slightly or weakly turbid. But such an extent of turbidity may hardly affect the CD and ORD spectra, because of the fact that the helical content estimated from the ORD data coincides with that from the CD data for the mixtures.

The CD spectra of the 56 : 44 mole ratio PLG—PLL mixture and the 18 : 82 mole ratio PLG—PLL mixture are shown in Figures 9 and 10, respectively, in which $[\theta]$'s at pH's below 7 and above 8 are based on the concentrations of PLG and PLL, respectively. At pH's below 7, the negative Cotton effect assigned to $n \rightarrow \pi^*$ transition appears correctly at 222 nm, but at pH's above 9, a negative band appears at 217 nm for both the 56 : 44 mole ratio PLG—PLL, and the 18 : 82 mole ratio PLG—PLL mixture. Further, the trough or shoulder at 208 nm is

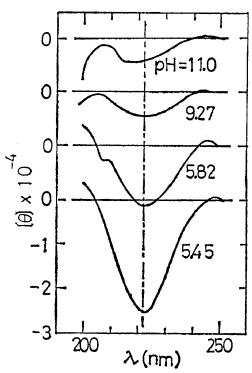

Figure 9. CD spectra of 56 : 44 mole ratio PLG—PLL mixture at 25°C and various pH values.

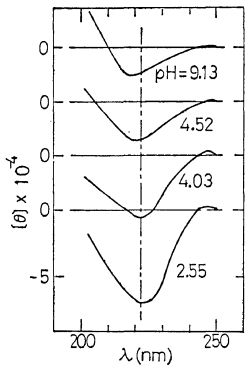

Figure 10. CD spectra of 18 : 82 mole ratio PLG—PLL mixture at 25°C and various pH values.

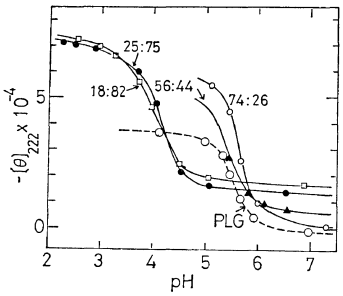

Figure 11. $-[\theta]_{222}$ plotted against pH for 74 : 26 (○), 56 : 44 (▲), 25 : 75 (●), and 18 : 82 (□) mole ratio PLG—PLL mixtures, and for PLG (broken curve) at pH's below 7.5.

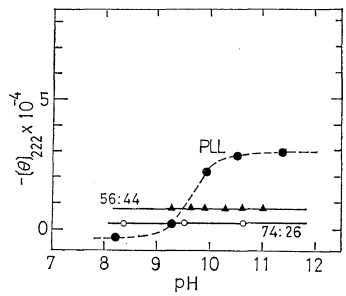

Figure 12. $-[\theta]_{222}$ plotted against pH for 74 : 26 (○) and 56 : 44 (▲) mole ratio PLG—PLL mixtures, and for PLL (broken curve) at pH's above 8.

difficult to distinguish, except for one instance (pH 5.82, Figure 9). The characteristic features of these curves are that the numerical values of $[\theta]_{222}$ of the mixture at pH less than 7 are markedly larger than those of PLG at corresponding pH's, whereas the $[\theta]_{222}$'s at pH's higher than 9 are smaller than those of PLL and, moreover, almost constant independent of pH.

In Figures 11 and 12, $-[\theta]_{222}$ of PLG—PLL mixtures is plotted against pH, in which data not shown in previous figures are also included. If the salt formation between COO^- of PLG and NH_3^+ of PLL proceeds stoichiometrically, then the composition of the polyelectrolyte complexes, given by the base-mole fraction, is obtained from Figure 7 as a function of pH, as shown in Figure 13. As is obvious from Figure 11, at PLG-to-PLL mole ratios higher than 1, the pH's at the midpoint of the transition curves do not significantly differ from that of PLG, while the numerical values of $-[\theta]_{222}$ of the mixtures are higher than those of PLG over the whole pH range. This result may indicate that the helix formation of PLG is not greatly affected, but the helix formation of PLG is supported by the presence of small amount of PLL. With decreasing PLG-to-PLL mole ratios, the pH's at the midpoint of the transition shift toward lower pH values, and, at the same time, $-[\theta]_{222}$ *vs.* pH curves of the mixtures shift upward compared with that of PLG. The $-[\theta]_{222}$ of the mixtures at sufficiently low pH (2~3) are about twice as much as that of PLG, and the $-[\theta]_{222}$ at pH about 7 is about 20000, which is considerably higher than the value (~0) assigned to the random coil conformation. The shift of pH at the midpoint toward lower pH may imply that the presence of a large amount of PLL rela-

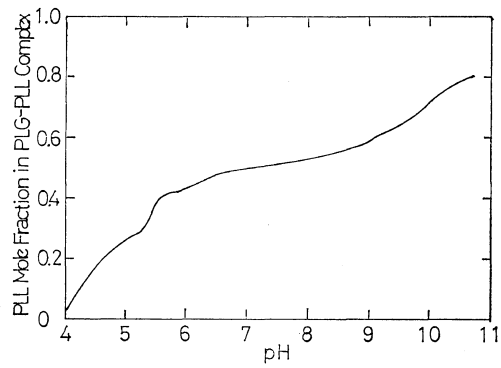

Figure 13. Mole fraction of PLL in stoichiometric PLG—PLL complex as a function of pH.

tive to PLG acts as to suppress helix formation.

As is obvious from Figure 7, at pH's lower than 4, most of the carboxyl groups of PLG are not protonated and the chain is in an α-helix conformation, while PLL is in a random coil conformation at close to unity. The results shown in Figure 11 may also indicate that the PLL molecules surround the core of the right-handed α-helix of PLG in this pH region. This conclusion is different from that of Hammes and Schullery,[13] their experiments were carried out in aqueous 0.01-*M* NaF in contrast to our experiments, which were done in the absence of added microsalt.

At pH 7, on the other hand, both PLG and PLL by themselves are sufficiently ionized (see Figure 7) and they are in the random coil conformation. The CD spectra in Figure 11 at pH about 7 may indicate that, when the PLG-to-PLL mole ratio is large, chains are nearly in a random coil conformation, while when the mole ratio of PLG is small, the chains are in a less disordered conformation, though a quantitative explanation is not possible. The polyelectrolyte complex (aggregation) formed in this case may be in a scramble-salt form. It is difficult to concluded that the aggregation at pH 7 is in the β-conformation as suggested by Hammes and Schullery.[13]

The CD spectra at pH's higher than 8 are shown in Figure 12. The $-[\theta]_{222}$ values of the 74 : 26 mole ratio PLG—PLL mixture and of the 56 : 44 mole ratio PLG—PLL mixture are respectively independent of pH. Even in the pH region where the PLL by itself exists in an α-helix conformation, the $-[\theta]_{222}$ values of the mixtures are much smaller than those of PLL. This fact may indicate that the presence of PLG acts as to suppress the helix formation of the PLL. In this pH range, PLG is sufficiently dissociated, while PLL dissociates as a function of pH (see Figure 7). As noted in Figures 9 and 10, the CD spectra of PLG—PLL at high pH exhibit a small trough at 217 nm. Presumably the PLG—PLL complexes are in a form close to the β-structure at this pH region. This conclusion also is not in accord with that of Hammes and Schullery,[13] who reported that no interaction was detected in PLG—PLL mixture at pH 11.

Conformation Effect of Poly(D-glutamic acid) to Poly-L-Lysine

The effects of D-polypeptide, which is able to form a left-handed α-helix, on L-polypeptide having an opposite charge were examined with *ca.* 60 : 40 mole ratio PDG—PLL mixtures at typical pH's, *i.e.*, pH 2.5 and pH 12.0, at which PDG and PLL by themselves exist in left- and right-handed α-helices, respectively. The CD spectra obtained are given in Figures 14 and 15. At pH 2.5, a positive peak appears at 222 nm, and the band assigned to the $\pi \rightarrow \pi^*$ transition is observed as a positive shoulder at 208 nm for the PDG—PLL. The general features of the curve are the same as those for the PLG—PLL mixture under corresponding conditions, except that the sign of $[\theta]$ values is reversed, but a marked difference between the PDG—PLL mixture and the corresponding PLG—PLL mixture is that the absolute value of $[\theta]_{222}$ of the PDG—

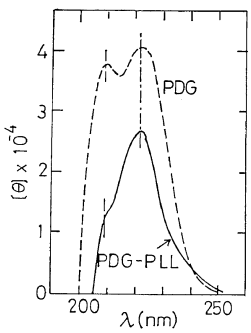

Figure 14. CD spectra of 53 : 47 mole ratio PDG—PLL mixture at pH 2.50 and of PDG (broken curve) at pH 4.76.

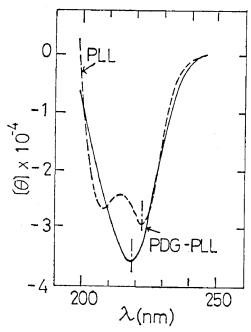

Figure 15. CD spectra of 60 : 40 mole ratio PDG—PLL mixture at pH 12.02 and of PLL (broken curve) at pH 11.00.

PLL mixture is smaller than that of PDG, in contrast to the case of the PLG—PLL mixture, in which the absolute value of $[\theta]_{222}$ was markedly larger than that of PLG. This fact may imply that helix formation of PDG is greatly disturbed by the presence of PLL.

On the other hand, at pH 12 where PLL by itself can form an α-helix, only a negative band is detected at 218 nm for the PDG—PLL mixture. This fact strongly supports the presence of a scramble-salt type aggregate composed of β-structures.

The results obtained in this paper lead to the important conclusion that the interactions between two oppositely charged polypeptides, together with the conformation of complexes formed, are not only influenced by pH but also markedly affected by the combination of helical senses of the partner polymers. An acidic D-polypeptide interacts with a basic L-polypeptide in a quite different way from an acidic L-polypeptide interacting with a basic L-polypeptide.

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