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폴리알릴아민과 복합체형성에 의한 폴리 D-글루탐산의 구조변화에 관하여

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Conformational Changes of Poly(D-glutamic acid) by the Formation of Complex with Poly(allyl amine)

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요 약 : 수용액중에서 폴리글루탐산(PGA)과 폴리알릴아민(PAA)의 고분자간 전해질복합체 형성에 있어서 pH, 몰비 그리고 온도에 따른 2차구조의 변화를 원편광이색성을 이용하여 관찰하였다. 또한, 전해질복합체 PGA/PAA/메틸오렌지(MO)에 대한 유발원편광이색성에 대해서도 검토되었다. 그 결과, PGA와 PAA는 강한 고분자간 복합체가 형성되었고, PGA 단독에서는 pH 7이상에서 완전 random coil의 구조이나 PAA와 복합체형성으로 인하여 pH 10.5에서도 α-helix구조를 나타내었다. 복합체의 PGA α-helix 구조는 온도가 60℃에서도 유지되었다. PGA/PAA/MO 복합체의 325nm에서의 정-타원율과 450nm에서의 부-타원율은 PGA의 α-helix로 인하여 MO도 α-helix배열을 하는 것으로 나타났다.

Abstract : Ionic polymer-polymer interaction was studied in aqueous solution for poly(D-glutamic acid) (PGA) and poly(allyl amine)(PAA) as functions of pH, the mole ratio of funtional unit of the two polymers and temperature by means of circular dichroism. Induced circular dichroism was also studied on PGA/PAA/methyl orange(MO) complexes. Strong interpolymer complexes were found to be formed. Although PGA itself is well known to take the random coil conformation at such a pH above 7, the PGA conformation in the PGA/PAA complexes took the α-helical conformation at such a high pH as ca. 10.5. The α-helical conformation of PGA in the complex did not change as the temperature increased up to 60℃. A positive ellipticity at 325nm and a negative ellipticity at 450nm indicated a left-handed super helical arrangement of MO molecules about the left-handed α-helix of PGA, where PAA chains were intercalated.

INTRODUCTION

It is of great importance to study the conformational change of synthetic polypeptides, when complexes between polypeptide and other polymers are formed either by ionic or hydrogen bonding, to elucidate and simulate the conformations of proteins.[1~3]

In the previous studies,[4~6] Cho et al. studied conformational changes of poly(L-lysine)(PLL) by the formation of complex with sulfated polymers as models for complexes between polysaccharides and proteins, which constitute biological connective tissues. It has been found that the PLL conformation in PLL/sulfated polymer complexes does not depend on pH, but on the degree of sulfation, temperature, the residue mole ratio of the two polymers, and the rigidity of sulfated polymers.

In this study, we have investigated the interaction between poly(D-glutamic acid)(PGA) and poly (allyl amine)(PAA), the former having an anion group and the latter having a cation group. We examined the conformation of PGA in the PGA/PAA complexes formed as functions of pH, composition in PAA, temperature and the conformation of PGA(without any other polymer) in aqueous solution by means of circular dichroism spectroscopy. Furthermore, in order to clarify the arrangement of PAA chains about the α-helical PGA in the complexes of PGA/PAA, induced circular dichroism was studied on PGA/PAA/methyl orange(Mo) complexes in aqueous solution.

EXPERIMENTAL

Materials

PGA with molecular weight of 100,000(average degree of polymerization : 700) was obtained as sodium poly(D-glutamate) from poly(γ-methyl D-glutamate) which had been synthesized from the corresponding amino acid N-carboxy anhydride in dioxane at 25℃ using triethylamine as initiator.[7]

PAA with molecular weight of 60,000 was supplied from Nittobo Co.(Japan) was used without further purification.

Preparation of the Mixed Solutions of PGA/PAA

Equal volumes of aqueous solutions of 6.6×10^{-4} unit mole/l of PGA and 6.6×10^{-4} unit mole of PAA, which had been adjusted by HCL and NaOH to have the same pH, were mixed by dropwise addition of various volumn of PAA solution into the PGA solution with stirring to adjust [AA]/[GA] ratio.

Circular Dichroism(CD) Measurements

CD spectra of the mixed solutions of PGA/PAA in a thermostated quartz cell were recorded with a path length of 1.0mm and 10.0mm using a JASCO J-500A Recording Spectropolarimeter.

Preparation of PGA/PAA/MO Complexes

The PGA/PAA/MO complexes were prepared by mixing the first two polymer solutions and then adding MO solution after the pH of the solution had been adjusted to 6.5.

RESULTS AND DISCUSSION

pH Dependence of PGA Conformation in Mixture

Fig. 1(b) shows the CD spectra of the PGA/PAA mixture with equal moles of the D-glutamic acid group(GA) and the allyl amine group (AA)(i. e., [GA]/[AA] of 1) in aqueous solutions of various pHs at 25℃. The CD spectra of PGA itself is shown in Fig. 1(a). As is well known, PGA takes α-helical conformations and random coil at pHs below and above ca. 5.7, respectively. The helix-coil transition of PGA in the PGA/PAA mixture [Fig. 1(b)] occurs at ca. pH 9.5, while that of PGA itself at about 5.7. This may indicate that a strong complex formation between the carboxyl group and amino group supported the formation of the α-helix of PGA even at pH as high as 10.5. Ellipticity at 222nm $[\theta]_{222}$ is plotted against pH for various samples in Fig. 2. $[\theta]_{222}$ is frequently used to estimate the α-helical content, $[\theta]_{222}$ is nearly zero for the random coil and 4×10^4 for the α-helical conformation.[8] As seen from Fig. 2, $[\theta]_{222}$ is dependent on the pH. The pH dependence of PGA conformation in the mix-

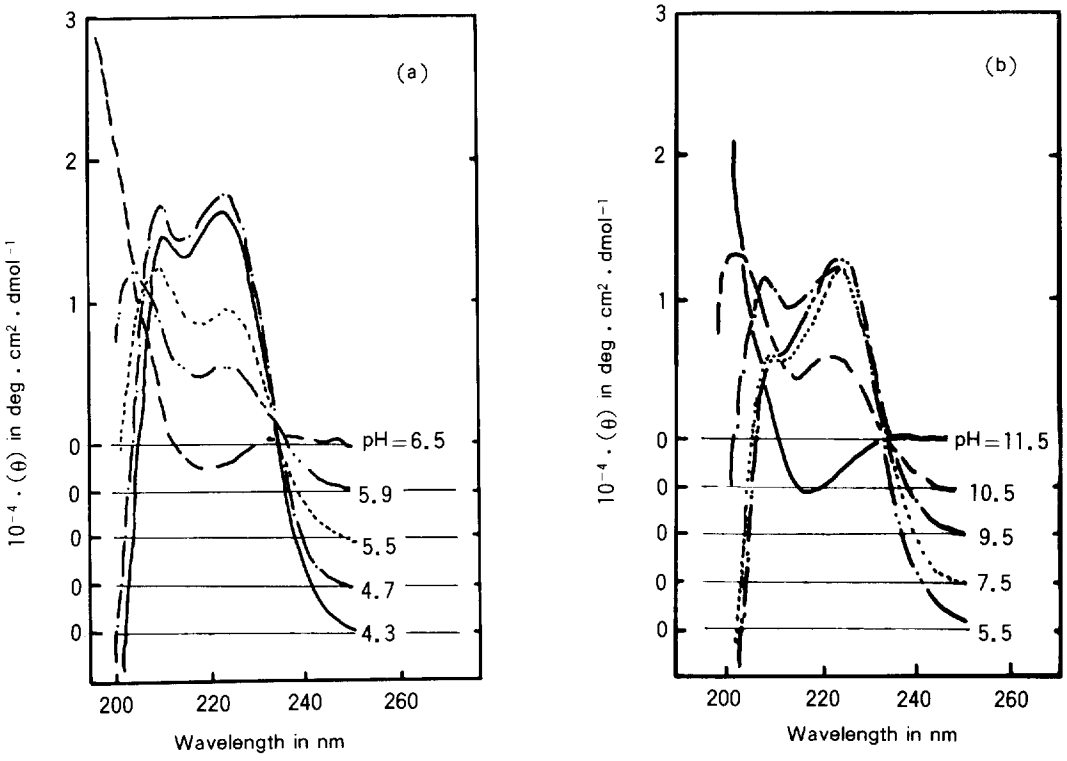

Fig. 1. CD spectra of PGA(a) and PGA/PAA complex(b) as a function of pH in aqueous solution at 25℃.

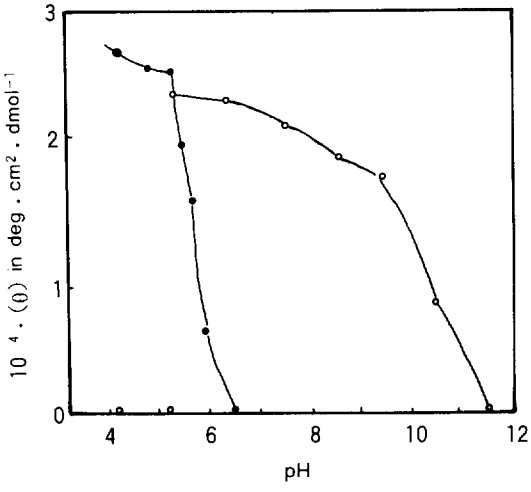

Fig. 2. Ellipticity at 222nm, $[\theta]_{222}$, plottd against pH for PGA(●) and PGA/PAA(○) in aqueous solution.

ture may come from the weak electrolytes nature of PGA and PAA. Although the formation of complexes occurs below pH 5.7, the estimation of $[\theta]_{222}$ was not possible because precipitation took place to some extent. This result may be due to the occurrence of aggregation of α-helix[9] during the formation of the complex with PAA. $[\theta]_{222}$ of PGA in the mixture decreases rapidly above pH ca. 9. This result may be due to the deprotonation of the amino groups of PAA as a weak electrolyte above this pH value.

Dependence of PGA Conformation on [AA]/[GA] Ratio

Fig. 3 shows plots of $[\theta]_{222}$ vs. [AA]/[GA] measured at pH 6.5 for the PGA/PAA complex. The ellipticity linearly increases with increasing [AA]/[GA] ratio. This result indicates that the complex formation between PGA and PAA took place stoichiometrically.

Fig. 4 shows CD spectra of PGA as a function of unit mole ratio higher than 1. The ellipticity decreases with increasing mole ratio of the repeating

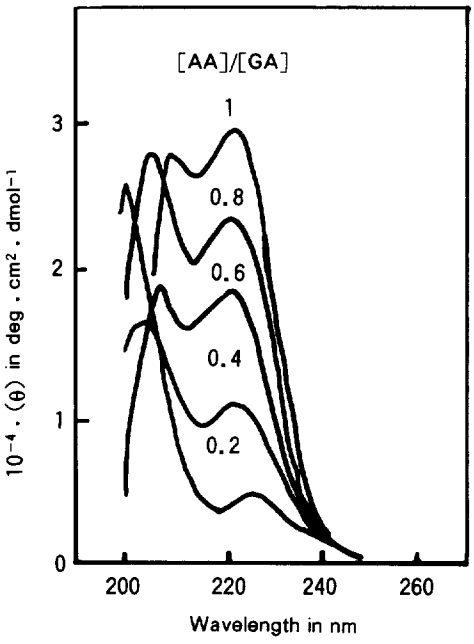

Fig. 3. CD spectra of PGA as a function of unit mole ratio for PGA/PAA complexes at pH=6.5 in aqueous solution. [GA]=3.3×10^{-4} unit mole/l, [AA]/[GA]≤1.

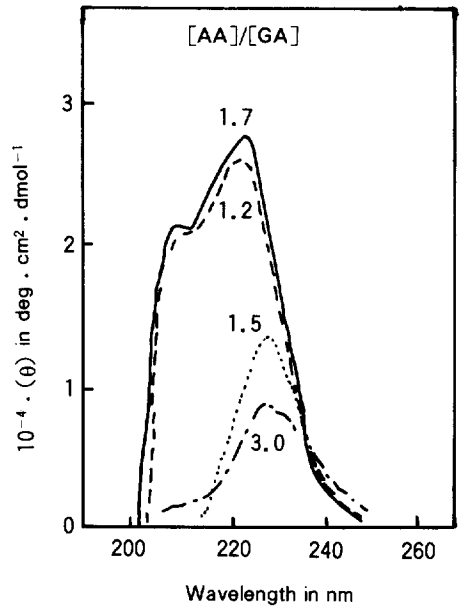

Fig. 4. CD spectra of PGA as a function of unit mole ratio for PGA/PAA complexes at pH=6.5 in aqueous solution. [GA]=3.3×10^{-4} unit mole/l, [AA]/[GA]>1.

units of the polymers, [AA]/[GA]. Also, the maximum wavelength was shifted to longer one 228nm for [AA]/[GA] of 3. This result may be due to the occurrence of turbidity and precipitates. It may be expected that the helix content of PGA remains unchanged for [AA]/[GA] higher than 1. But, this result may indicate aggregation of α-helix[9] in the PGA.

Temperature Dependence of PGA Conformation in the Complex

PGA undergoes a conformational transition from the α-helix to the β-form above 50℃ in aqueous solution.[10] It is worth to examine the temperature dependence of the conformation of PGA in the complex solutions. The CD spectra taken for pH 6.3 at various temperatures are shown in Fig. 5(a). It shows a typical conformational transition of PGA in the absence of PAA from the α-helix(partially α-helix at pH 6.3) to the β-form with increasing temperature. In the case of the PGA/PAA complex(Fig. 5(b)), the shape of the CD curve did not change but $[\theta]_{222}$ decreased from 2.8×10^4 to 1.75×10^4 with increasing temperature, the apparent helicity being calculated as 70 and 44 mole-%, resp. This result indicated that the very strong polymer-polymer interaction between PGA and PAA took place, but some decrease in the helicity may be due to possible dissociation of the complex during heating.

Effect of the Residue Mole Ratio, [AA]/[GA] on the Ellipticity of the PGA/PAA/MO Complexes

When MO molecules are added to a complex solution of the PGA/PAA where amino groups of the PAA exist unbound to the carboxylic groups of PGA, the MO molecules should be bound to the free amino groups because of the amino groups and the MO molecules being completely ionized with opposite charges before mixing in the pH of 6.5. On this viewpoint, the CD spectra were taken as a function of [AA]/[GA] ratio for the PGA/PAA/MO complexes, as is shown in Fig. 6 together with the absorption spectra. The complex with [AA]/[GA] of 0.5 gave the same absorption spec-

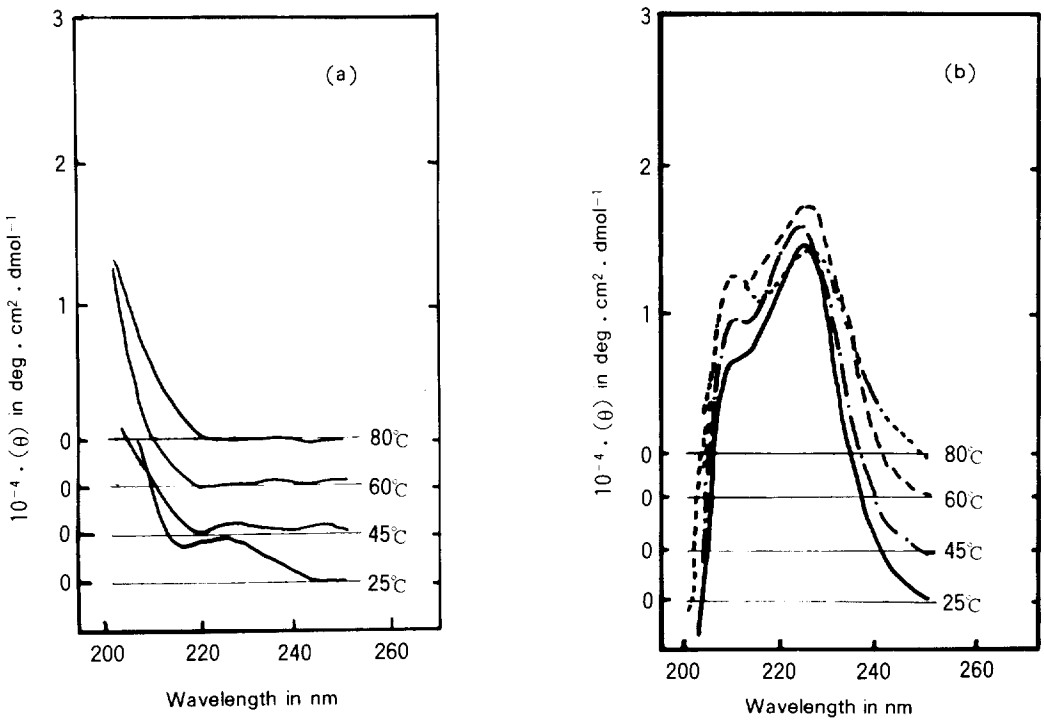

Fig. 5. CD spectra of PGA(a) and PGA/PAA complex(b) as a function of temperature in aqueous solution at pH=6.3. Unit mole ratio [AA]/[GA] was 1.

trum as that for MO(not shown in Fig.) whthout the polymer, which has only the α-band at 475nm (due to the monomeric MO) indicating no interaction of the MO molecules with the PGA/PAA complex. This can be accounted for by no free groups. i. e., association of all the AA groups with the PGA. The CD spectrum for [AA]/[GA] of 0.5 coincides with the absorption spectrum, revealing the disordered arrangement of the MO molecules. It can be said that the MO molecules, which are not incorporated in the PGA/PAA/MO complex, do not give any induced CD. The absorption apectra and CD spectra for the other [AA]/[GA] values, which are different from those for [AA]/[GA] of 0.5, indicate that the MO molecules in an aggregated form (γ-band) are arranged in a spatially ordered conformation to give a trough at ca. 450nm and a peak at ca. 325nm in the CD curves. The magnitudes of the trough and the peak increase and then decrease with increasing [AA]/[GA] value, having maxima at [AA]/[GA] of 2.5. The induced CD (ICD) for [AA]/[GA] of 1 reveals that all the amino groups are not bound to the side chain carboxylic groups of the α-helical PGA. The increase in the magnitudes of the CD troughs and peak with increasing [AA]/[GA] can be compared with the increase in the amount of the AA groups bound to the carboxylic groups of PGA. The low magnitudes of the CD trough and peak for [AA]/[GA] of 10 can be responsible for the MO molecules bound to the AA groups which are disordered in the PGA/PAA complex.

These results lead to the conclusion that the structures of the complex may be classified two categories : one is the structure in which the PAA molecule binds to the left-handed α-helix of PGA in such a manner as to form a super helix[11~13] surrounding the core : the other is the structure in which both polymer components form random aggregated, as shown in Fig. 7. In the former case,

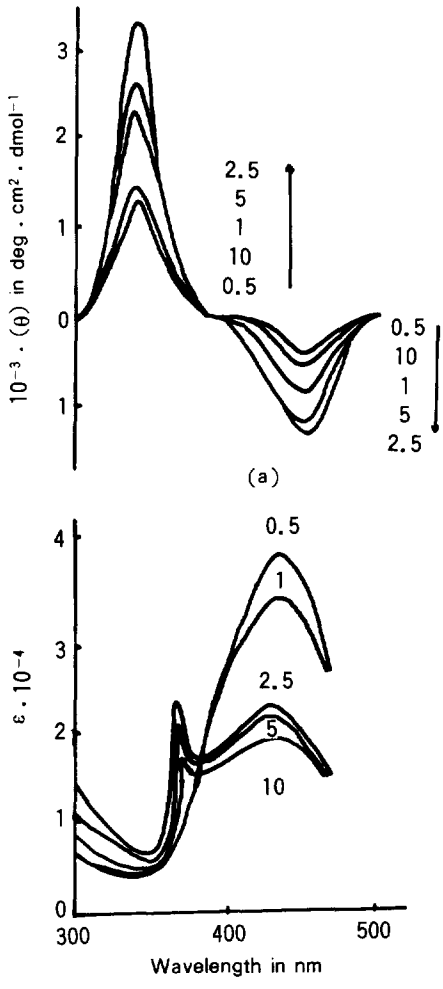

Fig. 6. ICD (a) and UV (b) spectra of PGA/PAA/MO complexes as a function of [AA]/[GA] ratio.

the charged groups would be neutralized almost stoichiometrically by the oppositely charged groups, while in the latter case, many free charged groups would be remaining in the random aggregates due to the inadequate mutual location of oppsitely charged groups.

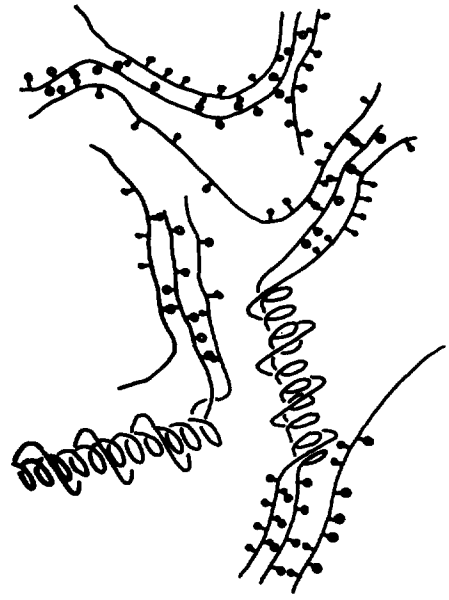

Fig. 7. Schematic models of PGA/PAA complexes.

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