Intermacromolecular Complex Formation between Helix Structural Polypeptides through Hydrogen Bonding

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수소 결합을 통한 Helix 폴리 펩타이드사이의 복합체 형성

조 병기, 김 창규, 이 충남, 이 옥섭
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Abstract

Polypeptide has been used broadly as an active ingredient in cosmetics. We thought it is very important to investigate the adsorption behavior of polypeptide in order to pre-estimate the effect of these polypeptides. For the study of polypeptide adsorption, we have investigated complex formation of basic homopolypeptides, poly(L-proline) Form I [PLP(I)], Form II [PLP(II)] and poly(4-hydroxy-L-proline) (PHLP) with acidic homopolypeptides, poly(L-glutamic acid) (PLGA), poly(D-glutamic acid) (PDGA) and poly(L-aspartic acid) (PLAA) through hydrogen bonding in a hydroalcoholic medium with viscometer, light scatter, pH meter and circular dicroism (CD). The polypeptides used in this study have helical structure in some conditions. The result exhibited that all the complexes were formed as the composition of basic/acidic homopolypeptide = 1:2 irrespective of the complex systems used. A more favorable complex is formed in the PLP(II)-PLGA system than PHLP-PLGA because PLP(II) has a more flexible helical conformation, whereas PHLP has a more rigid helical conformation. The right-handed helix PLGA formed the complex favorably and quickly with the left-handed helix PLP(II), whereas the left-handed helix PDGA formed the complex favorably with the right-handed helix PLP(I). The effect of

side chain of the acidic homopolypeptides on the complexation was also studied. The result showed that more favorable condition for the complexation was PLGA-PLP(II) system which has longer side chain at acidic homopolypeptide than PLAA - PLP(II). All the above facts were well supported by CD measurement for the complex systems. By the CD spectra for the complexes we could deduce the conformational change of each homopolypeptide in the complexes. On the basis of the above results, we performed the adsorption test of PLP(I, II) and PHLP on the hair having a left-handed helix. The adsorption amount of each polypeptide was analyzed by HPLC. The result showed that PLP(I) was adsorbed more than PLP(II), PLP(II) was adsorbed more than PHLP on the hair. On adsorbing polypeptides having a helical structure on the hair through hydrogen bonding, it could be concluded that the helical polypeptides having the opposite directional structure to the hair are adsorbed more than those having the same directional structure with the hair and also the polypeptides having a flexible conformation are adsorbed more than those having a rigid conformation.

Introduction

Polymers in the solution can interact each other to make an interpolymer complex through various secondary binding forces, *i.e.*, Coulomb force, hydrogen bond, van der Waals force, and hydrophobic interaction(1,2) It is well known the fact that many interpolymer complexes governed through hydrogen bonds occur in biological systems, *e.g.*, the formation and stabilization of double helix structure of DNA and higher structure of several proteins. As models of these biological systems, many studies(3-15) have been done on the interpolymer complexes formed through hydrogen bonds. By the way the complex formation is considered to cause the conformational changes of polymer chains. As typical examples, the conformational changes

by complexation of various polymers with specific conformations, *e.g.*, α-helix, β-sheet, random coil, and double-stranded helical structure, etc. have been studied in polyelectrolyte complex system. The purpose of the present study is to investigate whether the hydrogen bonding interactions +between the acidic homopolypeptides, poly(glutamic acid)(PGA), poly (aspartic acid)(PAA) and the basic homopolypeptides, poly (L-proline) (PLP), poly(4-hydroxy-L-proline)(PHLP) occur or not in a solution. We have studied the stoichiometry of complexes, conformational change, and selectivity on interpolymer complexation by circular dichroism(CD), light scatter, viscometer, pH meter, and polarimeter. Particularly, PLP and PHLP used in the present study are the major components of collagen which is a fibrous protein constituting the main structural element in connective tissues. PLP is a very interesting and unique biopolymer having a helical structure due to the steric restriction of the pyrrolidine ring without intramolecular hydrogen bonds. PLP has so far been characterized in two conformational form, Form I and II, which are reversibly transformed each other in a proper solvent system(20-22). Form II has a extended left-handed helical structure with all the peptide bonds in the *trans* conformation, and Form I is a compact right-handed one with all the peptide bonds in the *cis* conformation(20). It has been known that the conformation of PHLP is similar to that of Form II PLP, but in PHLP all possible inter or intramolecular hydrogen bonds are formed between the hydroxyl groups and neighboring carboxyl oxygen atoms(23,24). PGA exists as a helical conformation in the pH range where the charge density on the polypeptide chain is low, but assumes a random coil when its ionizable group is charged at higher range than pH 7(16-20,25,26). This phenomenon shows that the mutual repulsion of ionized groups attached to polypeptide will tend to disrupt the helical structures(20). Although PAA has also the similar property with PGA, the pH range of PAA which has a helical conformation is much lower than that of PGA(16,17).

Interpolymer complexes are divided into three classes on the basis of the main interaction forces, *i.e.*, polyelectrolyte complex, hydrogen bonding complex, and stereocomplex. Polyelectrolyte complexes are formed by mixing oppositely charged polyelectrolytes, *i.e.*, polyanions and polycations, due to Coulomb forces. Hydrogen bonding complexes are formed by combination of polymers bearing proton-accepting units and proton-donating units. Stereocomplexes are generated by combination of isotactic with syndiotactic poly (methyl methacrylate) (PMMA), mainly through van der Waals forces.

Many kinds of polypeptides have been used in cosmetics. Most of polypeptides being used in cosmetics have a helical structure and the fibrous protein (collagen,keratin) which is the main component of human body also has a helical structure. The purpose of this study, when we use polypeptides in cosmetics, is to pre-estimate that which directional helical structural polypeptide is the most effective to the human body and how we enhance the effectiveness of polypeptide from the helical structural point of view.

Experimental

Complexation Test of Polypeptides

Materials

Poly(L-glutamic acid) (PLGA), poly(D-glutamic acid)(PDGA), poly(L-aspartic acid) (PLAA), poly(DL-aspartic acid) (PDLAA), poly(L-proline)(PLP), and poly(4-hydroxy- L-proline)(PHLP) were purchased from Sigma chemical company. The molecular weight (Mw) of these polypeptides is as follows : PLGA (sodium salt), 54,600 ; PDGA(sodium salt), 45,300 ; PLAA (sodium salt), 50,300 and 8,220 ; PDLAA (sodium salt), 5,600 ; PLP, 19,000 ; and PHLP, 13,100, respectively.

Solvents

Solvents used in the present study were distilled water, methanol(99.8%), and *n*-propanol(99.5%).

Sample Preparation

The polypeptides with sodium salt were dialyzed against acidic aqueous solution to remove sodium salt. The 0.5-1.0 %(w/v) aqueous solutions of these polypeptides were put into the cellulose dialysis sack and then were stirred in water adjusted to the pH 3.3 using HCl for two weeks. Thereafter, the dialyzed polypeptides were freeze-dried to obtain pure solid state. PLP and PHLP were used without further purification. Form II PLP was transformed to Form I in water-propanol(1:9 v/v) solvent. Separate dilute solutions of each polypeptide(1.0-2.0 X 10^{-3} unit mole/l) were prepared in a cosolvent of water-methanol (1:2 v/v) and water-propanol (1:9 v/v) as needed.

Measurements

All measurements were conducted at least more than twice and the results were highly reproducible.

Viscometry

Viscosities of mixed solutions of acidic homopolypeptides (PGA, PAA) and basic homopolypeptides (PLP, PHLP) at various unit mole ratios were measured at 20 ± 0.02℃ by Ubbelohde-type viscometer. An inner dilution capillary was used for viscosity measurement. The polypeptide concentration for the viscosity measurement was 1.94 X 10^{-3} unit mole/l.

Circular Dichroism

Circular dichroism (CD) was measured at 25 ± 0.5℃ in the range of wave length 190 - 250 nm, using JASCO J-20 CD/ORD spectropolarimeter equipped

with a quartz cell of path length 1 mm. The concentration of each polypeptide was 1.0 X 10^{-3} unit mole/l in water-methanol(1:2 v/v) and water-propanol (1:9 v/v).

Light Scattering

Light scattering provides very useful information for the phenomena of many biocompounds in solution owing to rapid and accurate measurement(30). Aminco-Bowman spectrophotofluorometer was employed to determine the degree of polypeptide aggregation by measuring the intensity of the light scattered at 500 nm and exciting mode of monochrometer was also set at 500 nm.

Polarimetry

The degree of the transition of PLP(II) to PLP(I) was determined by optical rotation measurement by using Rudolph Automatic Polarimeter (Autopol III) equipped with a sodium D line source.

Adsorption Test on the hair

Materials

2g bleached hair

The virginal hair was immersed in the 1:1 mixing solution (500ml) of 2.8% NH_4OH and 5.6% H_2O_2 for 30 min and then air-dried after washing it with running water twice. We repeated the above bleaching operation three times.

Polypeptide adsorption

The PLP(I), PLP(II), and PHLP were adsorbed to the bleached hair by shaking at 30℃ for 2 hrs in 0.15 %(w/v) hydroalcoholic solution (water : propanol = 1 : 7 $^{v}/_{v}$) of each polypeptide, then we air-dried hair after washing it with running water twice and used it as the adsorbed hair.

Protein dissolution from the hair and protein hydrolysis

Adsorbed hair was soaked in 45ml distilled water adjusted to pH 9.0 at 45℃ for 24 hrs. After dissolving proteins from the adsorbed hair(27,28), the filtrate was concentrated to the protein residue under reduced pressure using a rotary evaporator. We hydrolyzed the protein residue in 10ml 6N c-HCl at 110℃ for 24 hrs under reflux.

Amino acid analysis

We analyzed the corresponding amino acids of the above hydrolysates by HPLC(29). The analytical conditions of HPLC were as follows:

- ◆ Mode : Hitachi L 5000
- ◆ Column : Cationic ion exchange resin
- ◆ Column temp : 58℃
- ◆ Flow rate : 0.4ml/min.
- ◆ Reactive reagent : Ninhydrin
- ◆ Detector : Hitachi L 4200
- ◆ Mobile phase : Sodium citrate buffer solution

Results and Discussion

General Aspect on the Complex Formation between Basic Polypeptide and Acidic Polypeptide

The 1:2 complex formation of PLP(II) (PHLP)-PLGA

Figure 1 shows the viscosity changes of PLP(II)-PLGA and PHLP-PLGA complex systems as a function of unit mole fraction of PLGA. The viscosities exhibit the maximum (PHLP-PLGA system) and the minimum (PLP(II)-PLGA system) at 1:2 unit mole ratio of PLP(II)-PLGA and PHLP-PLGA mixtures. This shows that the interpolymer complex is made as the composition of

Ⅲ. Results and Discussion

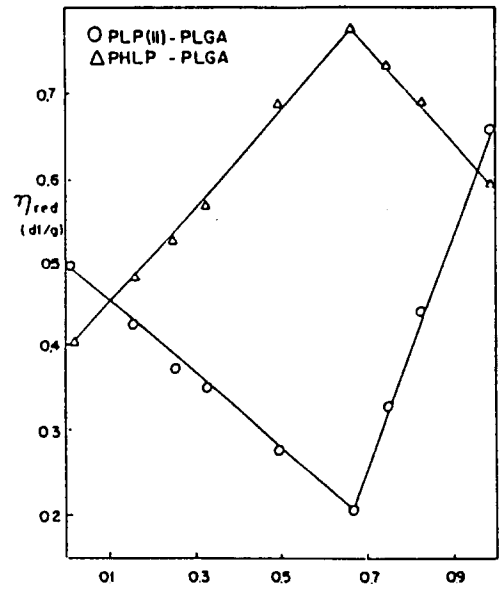

Figure 1. η_{red} of PLP(II)(PHLP)-PLGA complexes in water - methanol vs. unit mole fraction of PLGA. The pHs of PLP(II), PHLP, and PLGA are 6.43, 6.48, and 5.00, respectively.

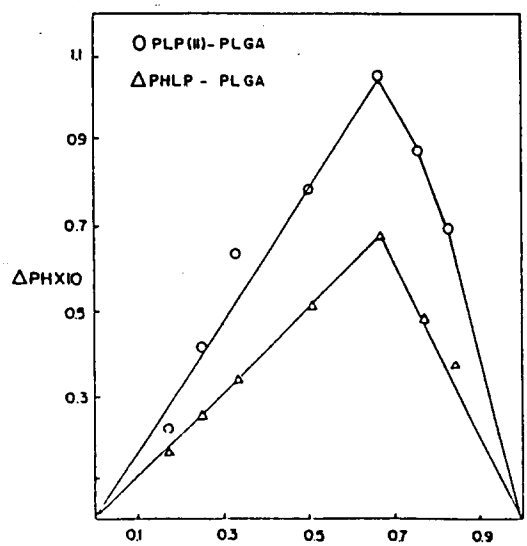

Figure 2. ΔpH of PLP(II)(PHLP)-PLGA complexes in water - methanol vs. unit mole fraction of PLGA. The pHs of PLP(II), PHLP, and PLGA are 6.43, 6.48, and 5.00, respectively

PLP(II)(PHLP)/PLGA = 1:2. The same result was also observed at ΔpH measurement as shown in Figure 2 , *i.e.*, the maximum ΔpH value is shown at PLP(II)(PHLP)/PLGA = 1:2. If the complex is made through hydrogen bonding on complexation, the protons of mixed solutions are captured by hydrogen bonding, thus the concentration of protons is reduced. Therefore, the pH of mixed complex solutions is increased. From the ΔpH measurement, we can deduce that the complex formation is being occured through hydrogen bonding. In Figure 1, the aspects of viscosity changes of PLP(II)-PLGA and PHLP-PLGA complexes are different from each other. This results from the difference of structure of PLP(II) and PHLP, *i.e.*, in the structural point of view, the PHLP has a more rigid conformation than that of PLP(II) due to the existence of intramolecular hydrogen bonding resulting from γ-hydroxy group attached to pyrrolidine ring of PHLP (23,24). Thus, a strong and compact complex exists in the PLP(II)-PLGA system, yielding the viscosity-decrease, whereas a weak complex exists in the PHLP-PLGA system, yielding the viscosity-increase owing to a larger hydrodynamic volume compared to each complementary polymer. The viscosity-decrease in the PLP(II)-PLGA complex is also affected by the PLGA conformation. That is, PLGA is coexisted as helical and random conformation at pH 5.0(16-19). As shown in Figure 3, the complex systems of which all the polypeptide solutions were adjusted to pH 3.2 showed different viscosity behavior from that of Figure 1. The two complex systems represent the increase of viscosity, which is to be attributed to the increase of the hydrodynamic volume in the complex since all the polypeptides have helical conformation at this pH, *i.e.*, in the structural point of view , it is order-order complex formation system. We also performed CD measurement at the various unit mole ratios of PLP(II)-PLGA mixtures to identify the PLP(II)/PLGA = 1:2 complex and to understand the conformational change of complexes. In Figure 4, the curves, c, d, e, and f exhibit the CD spectra for PLP(II)-PLGA at various compositions of the complex and these spectra were

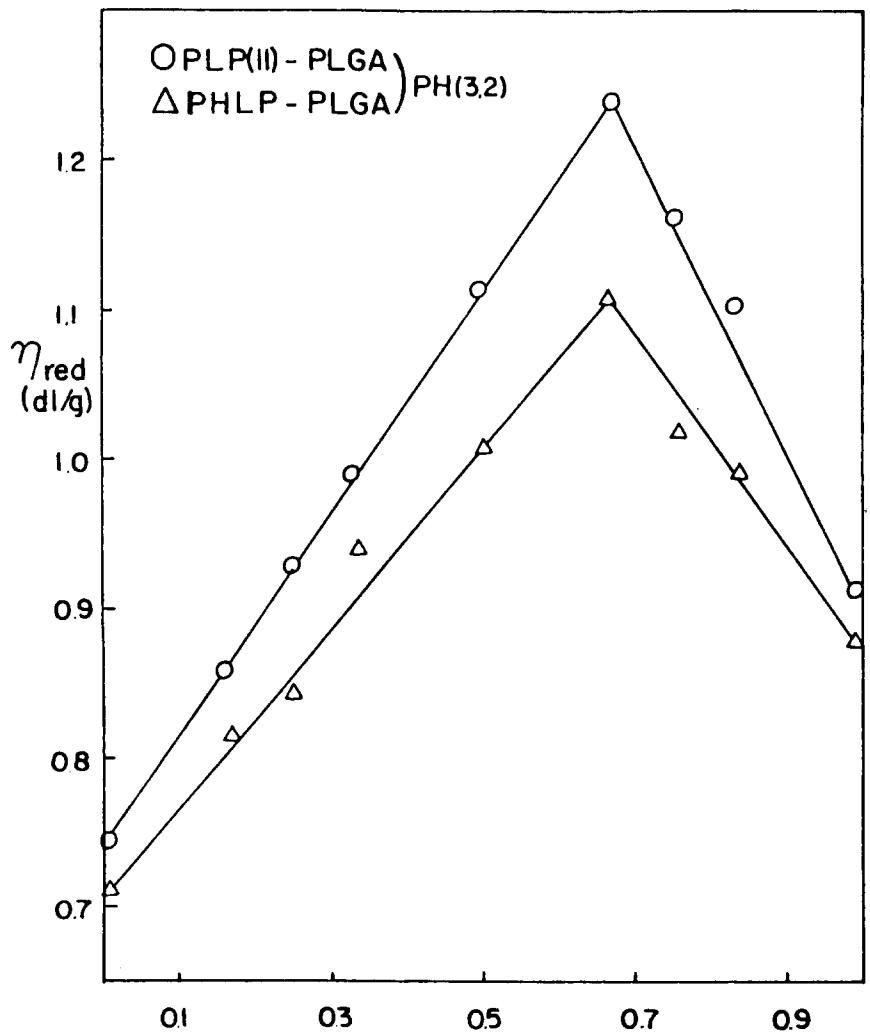

Figure 3. η_{red} of PLP(II)(PHLP)-PLGA complexes in water - methanol vs. unit mole fraction of PLGA. The pH of each polypeptide solution is 3.2 in all.

also calculated quantitatively from those of each pure polypeptide assuming that the complex does not occur between PLP(II)-PLGA at all, and curves, C, D, E, and F exhibit the actual spectra of the same mixtures as the above. The perturbations of actual spectra from the ideal curves, *i.e.*, c, d, e, and f are the largest at PLP(II)/PLGA = 1:2 mixture of all the mixtures. This result is decisive evidence of PLP(II)/PLGA = 1:2 complex, and also exhibits that the conformational change occurs the most strongly at the 1:2 mixture.

Side Chain Length Effect of Acidic Polypeptide on the Complexation

To invesitigate the side chain effect on the complexation, we compared the result of PHLP-PLAA system with that of PHLP-PLGA. Figure 5 shows the intensity of the light scattered for PHLP-PLGA(PLAA) complexes as a function of PLGA(PLAA). The ΔI value of PHLP-PLGA system is higher than that of PHLP-PLAA. This result indicates that a strong complexation occurs in PHLP-PLGA system compared to PHLP-PLAA because the ΔI value is proportional to the degree of complexation. This is to be attributed to the difference of side chain length of PLGA and PLAA, that is, PLGA with a longer side chain forms the complex favorably. In Figure 6 and 7, the viscosity was measured kinetically to invesitigate the aspect of complex. In the PHLP-PLGA complex system, the viscosity is increased with the progress of complexation leading to the maximun value at 1:2 unit mole ratio, whereas in the PHLP-PLAA complex system, the viscosity is decreased with the progress of complexation leading to the minimum at 1:2 unit mole ratio. The other result for viscosity behavior seems to be due to the difference of conformation of PLGA and PLAA. At pH 5.0, the PLGA nearly attains to a helical structure, but the PLAA attains to a random coil structure. Therefore, structurally, the complex between PHLP and PLGA is order-order complex formation, yielding a larger hydrodynamic volume, thus induce the viscosity increase, while the complex between PHLP-PLAA is

order-disorder complex formation, yielding a smaller hydrodynamic volume compared to the no complex state, *i.e.*, each polypeptide, thus induce the viscosity decrease in the complex solutions.

Selective Complex Formation between Basic Polypeptide and Acidic Polypeptide

Light Scattering Intensity Measurement

Figure 8 shows the intensities of the light scattered for various complex systems as a function of unit mole fraction of PLGA(PLAA). The increase of ΔI is caused by the interpolymer complex of PLP(PHLP) and PLGA(PLAA). The observation of the maximum at 0.67 unit mole fraction of PLGA(PLAA) of all complex systems is also indicative of PLP(PHLP)/PLGA(PLAA) = 1/2 complex system. However, the degree of increase of ΔI is different from each other and the values of ΔI increase in the order of the following: PLP(II)-PLAA < PHLP-PLGA < PLP(I)-PLGA < PLP(II)-PLGA, *i.e.*, this order is parallel to the ability of complex formation between polypeptides. Thus, from the above result we could know the following facts : firstly, the complex is formed more favorably between left-handed helix PLP(II) and right-handed helix PLGA, secondly, the PHLP with a more rigid conformation forms the complex less favorably with PLGA compared to PLP(II) with a flexible conformation, thirdly, polyacid with longer side chain forms the complex favorably with polybase, *i.e.*, PLP(II)-PLGA > PLP(II)-PLAA. To confirm more surely the selective complexation mentioned in the above, we also investigated the time-dependent complex formation. In Figure 9, the ΔI values in PLGA-PLP(II) system increase more rapidly with the lapse of time than PDGA-PLP(II) system, indicating formation of a more favorable complex at PLGA-PLP(II). The same result was also observed for PHLP having a guasi PLP(II) conformation (3_1 left-handed helix) as shown in Figure 10. These results show that the complex formation is highly dependent on the

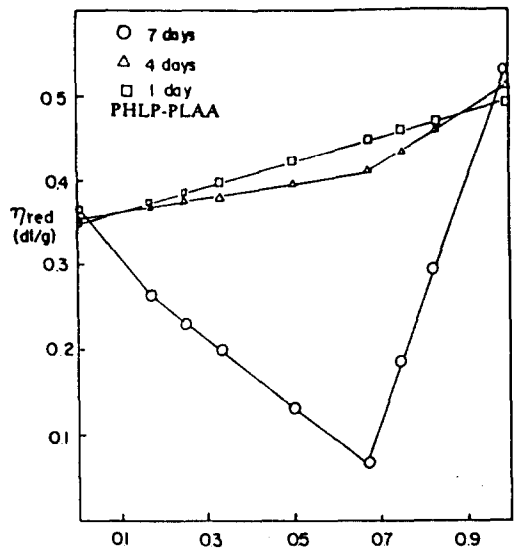

Figure 7. η_{red} of PHLP - PLAA complex in water - methanol vs. unit mole fraction of PLAA as a function of time.

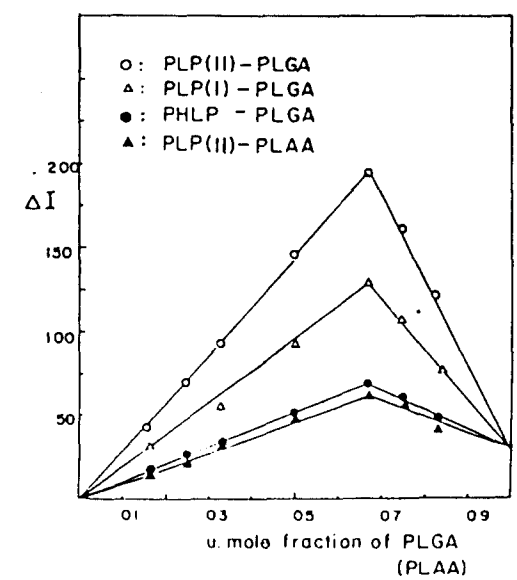

Figure 8. ΔI of various complex systems, PLP(II) - PLGA, PLP(I) - PLGA, PHLP - PLGA, and PLP(II) - PLAA vs. unit mole fraction of PLGA (PLAA).

conformation of complementary polypeptides, indicating the stereocomplex formation in this system. It could be concluded that more favorable complex system is formed between left-handed helix PLP(II) and right-handed helix PLGA, but unfavorable complex system is formed between left-handed helix PLP(II) and left-handed helix PDGA. Figure 11 shows the complex formation with the lapse of time for the system of PLGA(PDGA)-PLP(I). The result shows that the complex is formed more favorably between right-handed helix PLP(I) and PDGA, yielding a quick increase in the ΔI value at PDGA-PLP(I) than PLGA-PLP(I). This is also caused by the difference of conformation of PLP(I) and PLGA(PDGA) in forming the complex. Lastly, we measured the light scattering intensity to investigate the conformation effect and molecular weight effect of the acidic homopolypeptides, *e.g.*, PLAA, PDLAA. As shown in Table 1, the ΔI value of PDLAA-PLP(II) is higher than that of PLAA-PLP(II) and at the PLAA-PLP(II) complex, the higher ΔI value is obtained in the higher molecular weight of PLAA. From this result, we could deduce that the polypeptides with the random conformation make the complex more favorably and the bigger the molecular weight of polypeptides is, the more favorably the complex is made.

Table 1. Comparison of the light scattering intensity(ΔI) in the various complex systems

Complex Systems	ΔI
PDLAA - PLP (II) (Mw : 5,600)	34.0
PLAA - PLP (II) (Mw : 50,300)	30.0
PLAA - PLP (II) (Mw : 8,220)	17.0

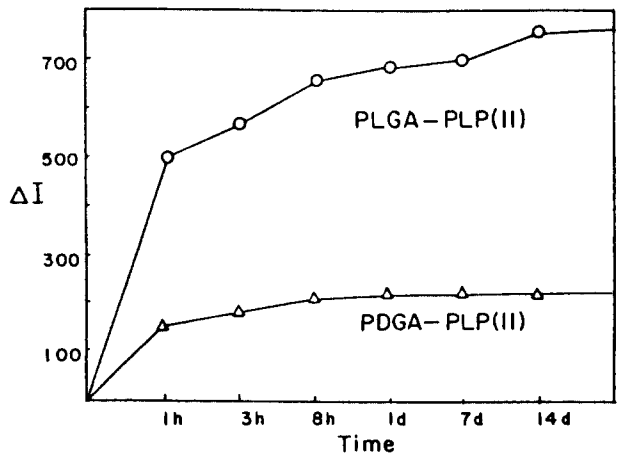

Figure 9. ΔI of PLGA (PDGA) - PLP(II) complexes in water - methanol vs. time.

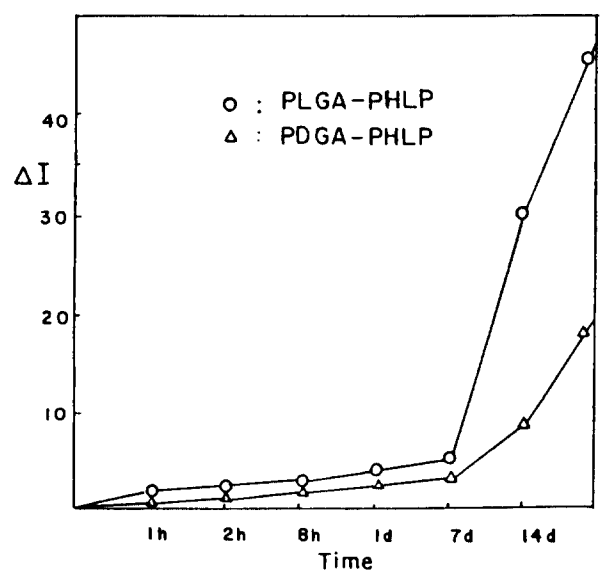

Figure 10. ΔI of PLGA (PDGA) - PHLP complexes in water - methanol vs. time.

CD Measurement

CD spectra of the pure basic polypeptides [PHLP, PLP(I,II)] (31) and the pure acidic polypeptides(PLGA, PDGA, PLAA) (19,31,32)

It was well known that circular dicroism (CD) (16,17,32) has usually been used in investigating a specific conformation such as bioproducts, *i.e.*, polynucleotides, antibiotics, and polypeptides. We measured CD in order to investigate conformational change of polypeptides and also selectivity on the complex formation between basic polypeptides (PLP,PHLP) and acidic polypeptides (PGA, PAA). Figure 12 shows the typical CD spectra for three kinds of polypeptide conformations, *i.e.*, α-helix, β-sheet, and random coil structure. By these spectra we could distinguish the conformation of the polypeptides at a certain condition. Figure 13 shows the typical CD spectra of basic polypeptides, PLP(I),PLP(II), and PHLP used in this study. These spectra are well consistent with the spectra reported by the other author (31) and their conformations shown in these spectra are as follows : PLP(II) is a 3_1 left-handed helix, PLP(I) is 10_3 right-han
ded helix, and PHLP is 3_1 left-handed helix , *i.e.*, a quasi PLP(II) structure. Figure 14 also shows the CD spectra at pH 3.2 of acidic polypeptides, PLGA (right-handed helix), PDGA (left-handed helix), and PLAA (random coil). This exhibits that the ellipticity of CD spectra for PLGA and PDGA is the same value, but the ellipticity sign is opposite each other, *i.e.*, the PLGA is a negative ellipticity, the PDGA is a positive.

CD Spectra of Complex Systems

PLGA(PDGA)-PLP(II) Complex System

Figure 15 shows the CD spectra for the PLGA-PLP(II) and PDGA-PLP(II) complex systems at the mixture of PLGA(PDGA)/PLP(II) = 2:1 (unit mole ratio) that the maximum complex occurs, which was already well

characterized in the previous ΔI (Fig.8) and the viscosity measurement(Fig.1,3). In Figure 15, the dotted line, (1) exhibits the ideal CD curve for PDGA-PLP(II) and the other dotted line, (2), PLGA-PLP(II) assuming that any appreciable interaction does not occur. These spectra were obtained by the addition of each pure spectrum of basic polypeptides (Fig. 13) and acidic polypeptides(Fig. 14). By comparing the solid curve indicating actual spectrum with dotted curve, We could know easily that the conformational change occurs on the complex formation between PLGA(PDGA) and PLP(II) because the CD spectra of the complex are deviated from the dotted line. Particularly, the deviation is larger in the PLGA-PLP(II) than PDGA-PLP(II). This shows that the conformational change occurs greatly in the PLGA-PLP(II) system due to the strong complex formation between PLGA-PLP(II). The PLP(II) having a left-handed helix conformation forms the complex more easily through hydrogen bonding with PLGA having a right-handed helix than PDGA having a left-handed helix. This is also consistent with the fact observed in the ΔI and the viscosity measurement. Thus, we could elucidate the selectivity on complexation by the CD measurement. The above result (Fig. 15) is more supported by the CD measurement for the ternary systems of PDGA-PLGA-PLP(II), as shown in Figure 16. The dotted line,(a) indicates ideal spectrum adding up simply the pure PDGA spectrum (Fig. 14) and the spectrum of PLGA-PLP(II) complex (Fig. 15) on the assumption that no interaction occurs each other and the other dotted line ,(b), the pure PLGA spectrum (Fig. 14) and the spectrum of PDGA-PLP(II) complex (Fig. 15). The solid line exhibits the CD spectrum of the mixed solution of PDGA, PLGA, and PLP(II). It is observed that the spectrum of the mixed solution is similar to the spectrum (a), indicating that PLGA-PLP(II) system is in the more favorable condition for the complex than PDGA-PLP(II). At any rate, we could also prove effectively the selective complexation phenomenon between PLGA-PLP(II) by the CD measurement for the ternary system of complexation.

PLGA(PDGA)-PLP(I) Complex System

The selectivity on complexation of PLP(I) having a compact right-handed helix with PLGA and PDGA was also studied in Figure 17. The dotted lines also indicate the ideal spectra. Both spectra of PDGA-PLP(II) and PLGA-PLP(II) complexes were deviated from the ideal spectra. This shows that any conformational changes occur in the complexes. We also knew that the deviation from the dotted line as well as from the isodichroic point(31) which is marked by black circle in Figure 17, is larger in PDGA-PLP(I) than PLGA-PLP(I). Therefore, we could deduce that the conformational change occurs more greatly in the PDGA-PLP(I) due to the strong complexation. This result is contrary to that of the system of PDGA(PLGA)-PLP(II) complex and is due to the difference of the conformation of basic polypeptide as well as that of acidic polypeptide, *i.e.*, the PLP(I) having a right-handed helix forms the complex favorably with PDGA having a left-handed helix, whereas the PLP(II) having a left-handed helix forms the complex favorably with PLGA having a right-handed helix. Figure 18 shows the CD spectra of the mixed solutions for the ternary system of PDGA, PLGA, and PLP(I) (the solid line). The dotted line, (a) is acquired by adding up simply the spectrum of pure PDGA (Fig. 14) and the spectrum of PLGA-PLP(I) complex (Fig. 17) and the other dotted line, (b) is acquired by summing up simply the spectrum of pure PLGA (Fig. 14) and the spectrum of PDGA-PLP(I) complex (Fig. 17). The spectrum for ternary system is smilar to the spectrum, (b), *i.e.*, that of PDGA-PLP(I) + PLGA system. This result also supports that of Figure 17 for the selective complexation of PLP(I) on PDGA.

PLGA (PDGA)-PHLP Complex System

In Figure 19 , the CD measurement was performed to invesitigate the selectivity and kinetic effect on the complexation of PHLP for PLGA and PDGA. The deviation from the ideal spectrum (the dotted line) according to

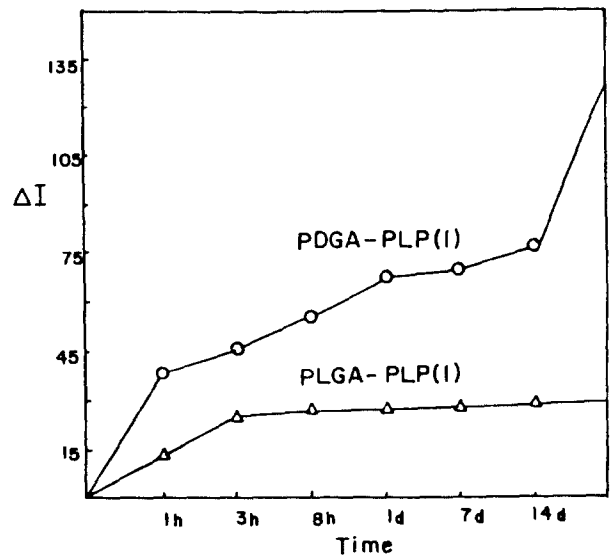

Figure 11. ΔI of PLGA (PDGA) - PLP(I) complexes in water - propanol vs. time.

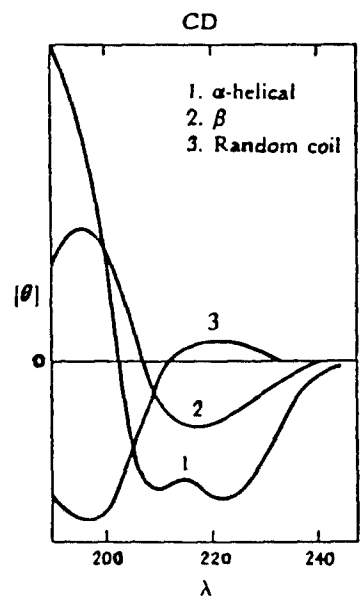

Figure 12. CD spectra for PLGA in the α-helical, β, and random coil conformations.

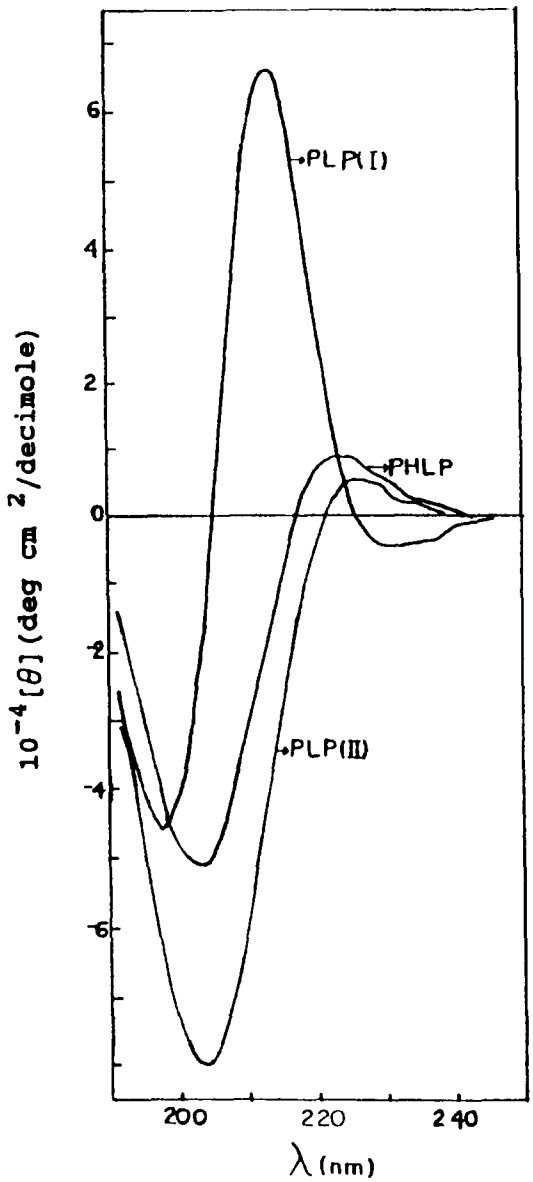

Figure 13. CD spectra of pure basic polypeptides, PLP(I), PLP(II), and PHLP in water - propanol.

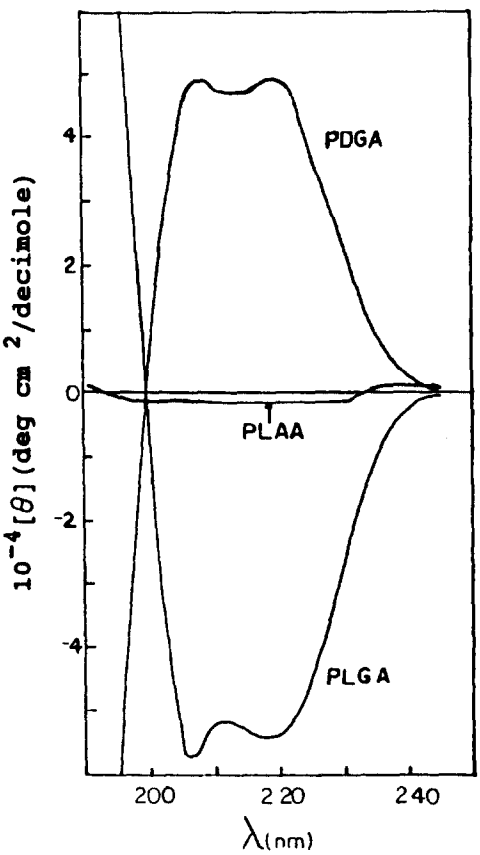

Figure 14. CD spectra of pure acidic polypeptides, PLGA, PDGA, and PLAA in water - methanol.

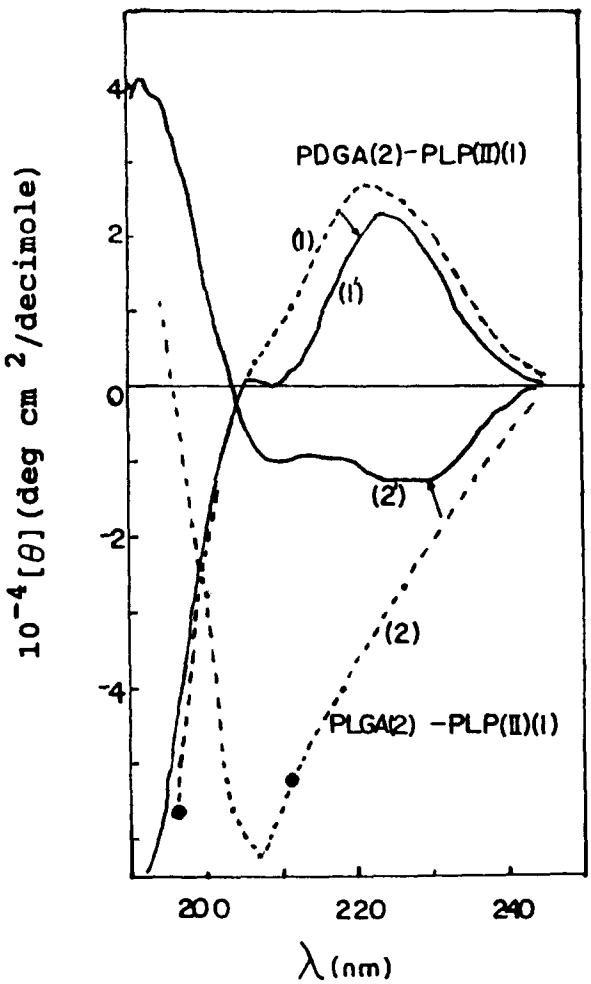

Figure 15. CD spectra of PDGA (PLGA) - PLP(II) complexes. Dotted lines, (1), (2) represent the ideal spectra which no interaction does occur each other, and solid lines, (1'), (2') represent the actual spectra. All the dotted and solid lines represent the same meaning in all next figures. The black circle represents isodichroic point.

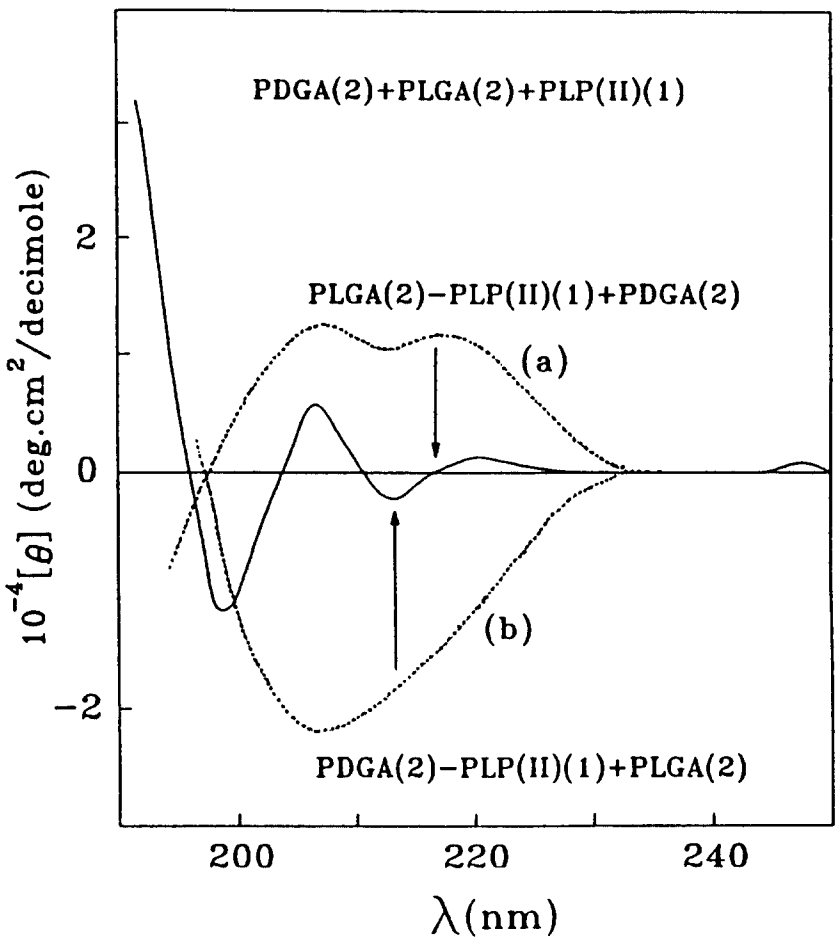

Figure 16. CD spectra of the mixed solution for the ternary system of PDGA, PLGA, and PLP(II). (a) represents ideal spectrum summed up the spectra of PLGA - PLP(II) and PDGA, and (b), PDGA - PLP(II) and PLGA.

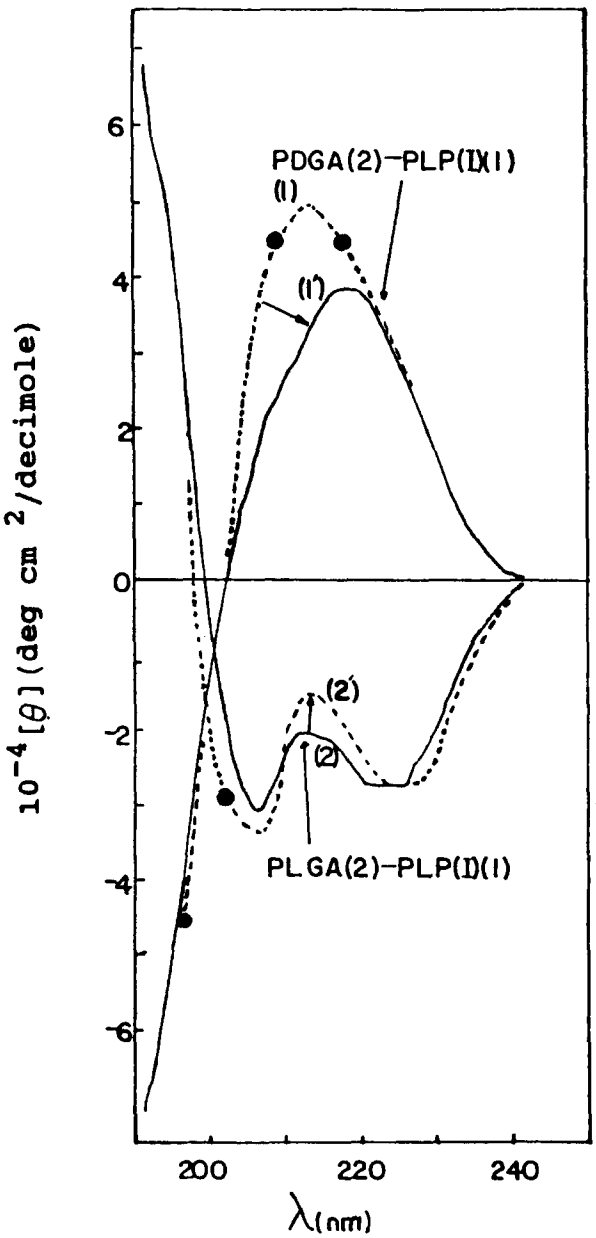

Figure 17. CD spectra of PDGA (PLGA) - PLP(I) complexes in water - propanol.

the time occurs abruptly in the PLGA-PHLP complex and the final CD spectrum after complete complexation is also more deviated from the ideal curve in the PLGA-PHLP than PDGA-PHLP complex. We could conclude that the strong complex is formed in PLGA-PHLP system, inducing a larger conformational transition. The same result was already observed in PLP(II) complex system (Fig. 15,16). Thus, this result could confirm the previous result for the PLGA(PDGA)-PLP(II). Figure 20 shows the result of CD measurement of the mixed solutions for the ternary system of PDGA, PLGA, and PHLP. The result also exhibits that PHLP has more favorable selectivity with PLGA than PDGA on complexation, since the spectrum of the mixed solution is more similar to the spectrum, (b) which sums up simply the spectrum of PLGA-PHLP complex (Fig. 25) and the pure PDGA spectrum.

Comparison of the Adsorption Amount of PLP(I), PLP(II), and PHLP in Adsorption Test on the Hair

Figure 21,22, and 23 show the adsorption amount of each amino acid corresponded to PLP(I), PLP(II) and PHLP adsorbed on the keratin of hair. As shown in the HPLC spectra, PLP(I) with a right-handed helix is adsorbed more on the hair with a left-handed helix than PLP(II) with a left-handed helix and also PLP(II) with a more flexible conformation is adsorbed more on the hair than PHLP with a more rigid conformation because of the existence of intramolecular hydrogen bonding resulting from γ-hydroxy group attached to pyrrolidine ring of PHLP. From the above test results, when we use polypeptides in hair care products, it could be concluded that the helical polypeptides having the opposite directional structure to the hair are adsorbed more than those having the same directional structure with the hair and also the polypeptides having a flexible conformation are adsorbed more than those having a rigid conformation. On the basis of the above test results, we could extend to apply the above result to the skin care . As

known very well, collagen which is the main component of the skin is a right-handed helical structure. So it could be pre-estimated that if we use polypeptides in skin care, those having a left-handed helix, namely, the opposite directional helical structure to the skin are the most effective.

요 약

물-알콜 용액에서 염기성으로 작용하는 폴리펩타이드[PLP(Ⅰ),PLP(Ⅱ),PHLP]와 산성으로 작용하는 폴리펩타이드(PLGA,PDGA,PLAA,PDLAA) 사이에 수소결합을 통한 복합체 형성에 관한 연구를 점도, pH, 빛산란, 원편광이색성(CD), 광회전도 등으로 조사했다. 얻어진 결과는 여러가지 복합체 시스템 모두가 1:2 (염기성/산성폴리펩타이드) 조성으로 복합체 형성을 한다는 것을 알수 있었으며, 우선성 헬릭스를 가지는 폴리펩타이드와 좌선성 헬릭스를 가지는 폴리펩타이드, 즉 반대방향성의 헬릭스 구조를 가지는 폴리펩타이드들 사이에 강한 상호작용을 나타내고, 반면, 같은방향성의 헬릭스 구조를 가지는 폴리펩타이드들 사이에는 상호작용이 약하다는 것을 알수 있었다. 이것은 폴리펩타이드의 형태(conformation)가 복합체 형성에 매우 중요한 역할을 한다는 것을 나타낸다. 즉, 입체선택적 복합체 형성을 보인다. 또한 구조적으로 유연한 구조를 가지는 폴리펩타이드가 강한 상호 작용을 나타낸다. 즉, PHLP보다 PLP(Ⅰ)이, PLP(Ⅰ)보다 PLP(Ⅱ)가, PAA보다 PGA가 더 강한 상호작용을 나타낸다. 이런 상호 복합체 형성이 일어나면 형태전이가 일어난다는 것도 확인할수 있었다.

위의 결과를 근거로 하여, 좌선성 헬릭스 구조의 모발의 케라틴에 PLP(Ⅰ,Ⅱ)와 PHLP를 흡착시킨후, 흡착량을 HPLC로 측정한 결과, PLP(Ⅱ)보다 PLP(Ⅰ)이, PHLP보다 PLP(Ⅱ)가 더 많이 흡착되었다. 결론적으로, 모발에 폴리펩타이드를 사용시, 좌선성헬릭스 구조의 폴리펩타이드 보다 우선성헬릭스 구조의 폴리펩타이드가 더 많이 흡착되고, rigid conformation의 폴리펩타이드보다 flexible conformation의 폴리펩타이드가 더 많이 모발에 흡착되어, 효과가 좋다는것을 알수 있다.

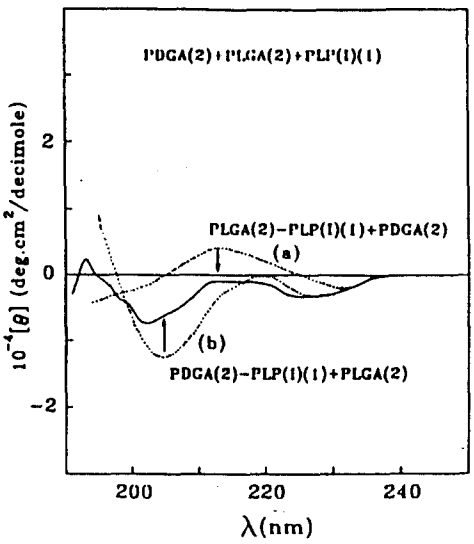

Figure 18. CD spectra of the mixed solution for the ternary system of PDGA, PLGA, and PLP(I).

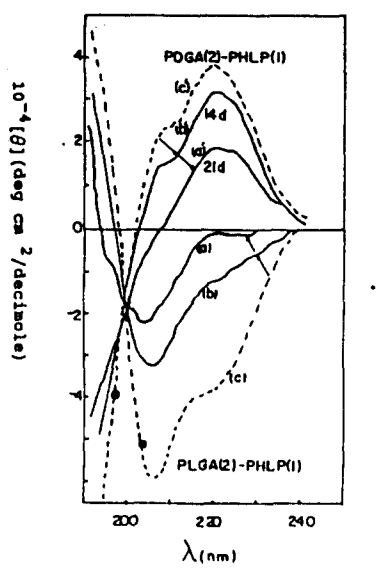

Figure 19. CD spectra of PDGA (PLGA) - PHLP complexes as a function of time.

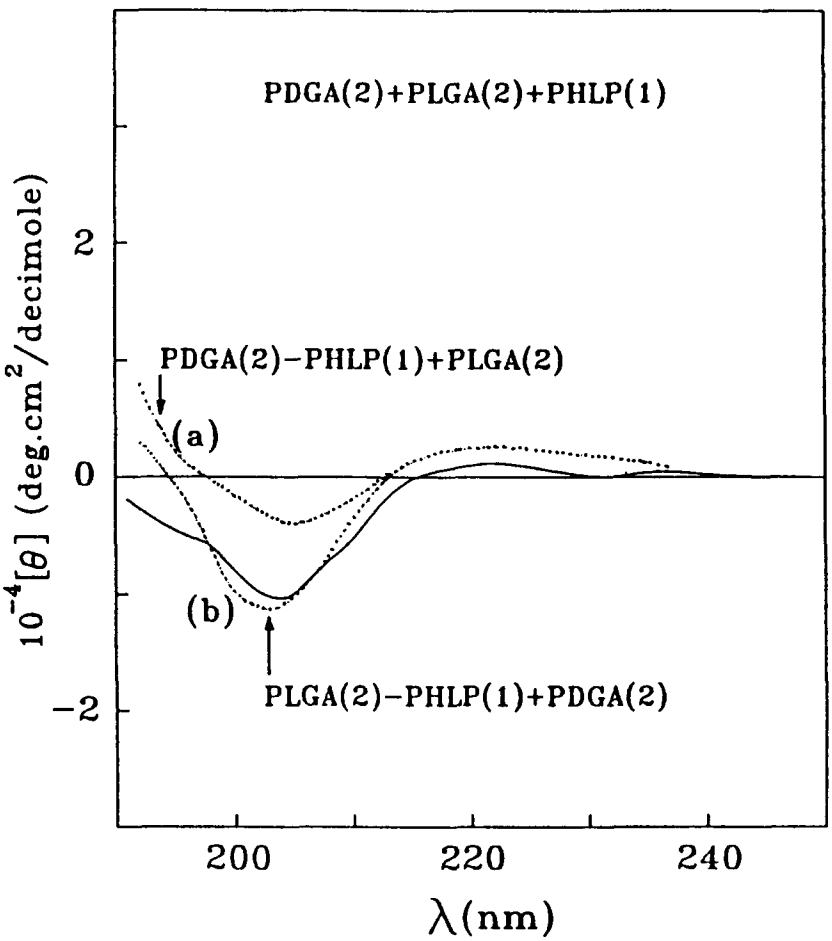

Figure 20. CD spectra of the mixed solution for the ternary system of PDGA, PLGA, and PHLP.

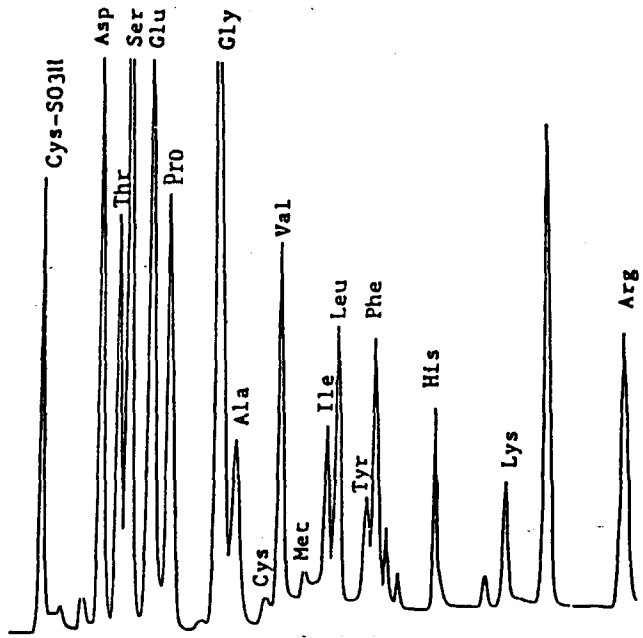

Figure 21. HPLC spectrum of each amino acid of proteins dissolved from the bleached hair adsorbed with PLP(I).

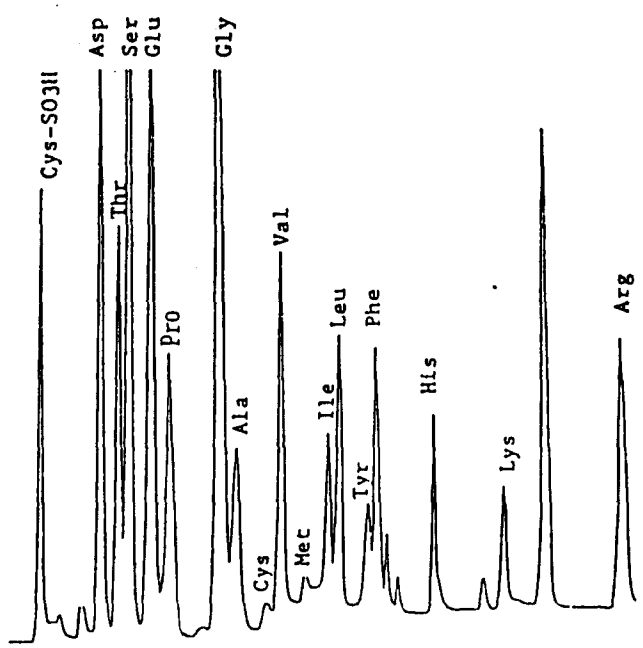

Figure 22. HPLC spectrum of each amino acid of proteins dissolved from the bleached hair adsorbed with PLP(II).

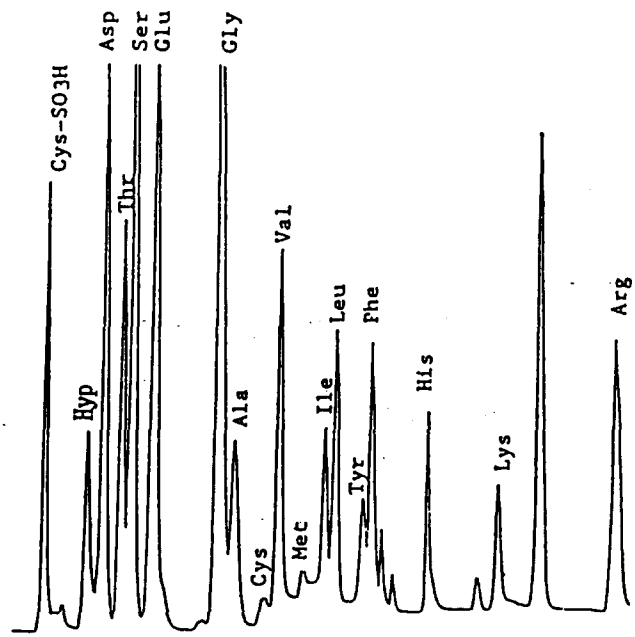

Figure 23. HPLC spectrum of each amino acid of proteins dissolved from the bleached hair adsorbed with PHLP.

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