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## Conformational Transition of Form II to Form I Poly(L-proline) and the Aggregation of Form I in the Transition: Water-Propanol Solvent System

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The conformational transition of poly(L-proline) (PLP), Form II  $\rightarrow$  Form I and the intermolecular aggregation of the product, Form I, during and after the transition in water-propanol (1:7, 1:9, 1:15.7, and 1:29 v/v) were studied. For the study, the viscosity change and excess light scattering intensity were measured in the course of the transition which was determined by the Form I fraction,  $f_i$  of the sample solution. For the PLP sample of molecular weight  $M_w=31,000$  the experimental results show that the reaction course is roughly divided into three regions: in the first region [ $f_i=0.27$  to  $0.40$  ( $-\alpha_D=400$  to  $330$ )], the conformational change of Form II  $\rightarrow$  Form I occurs with decrease of viscosity, in the second region [ $f_i=0.40$  to  $0.80$  ( $-\alpha_D=330$  to  $120$ )], a partial side-by-side (p-S-S) type aggregation in which Form I blocks interact with each other, which induces the increase of viscosity, starts to occur, and in the third region [ $f_i=0.80$  to  $1.00$  ( $-\alpha_D=120$  to  $15$ )], a side-by-side type (raft like) aggregation of Form I or an end-to-end (E-E) type aggregation occurs according to the solvent situation, i.e., in a water-rich medium [water-propanol (1:9 or 1:7 v/v)], the (S-S) type aggregation with a gross decrease in viscosity occurs while in a water-poor medium [water-propanol (1:29 or 1:15.7 v/v)], the (E-E) type aggregation with a large increase in viscosity occurs. The (S-S) type aggregation was promoted at high temperatures. Based on the structure of PLP, a reasonable mechanism for the (p-S-S) and (S-S) aggregation which occurs with the transition of Form II  $\rightarrow$  Form I is considered. The suggested mechanism was also supported by the result of chain length effect of PLP for the aggregation.

### Introduction

As well known, poly(L-proline) (PLP) is a polyimino acid polypeptide which, unlike poly- $\alpha$ -amino acid polypeptides, having no amide hydrogen (-NH), cannot form intermolecular hydrogen bonds and its compound distinctly existed as two helical forms, Form I and Form II, not only in the solution,<sup>1-12</sup> but also in the solid state.<sup>13-15</sup> Form I is a right-handed helix (RHH) (3.3 peptides per turn,  $10_3$ ) with all the peptide bonds in the *cis* conformation, and the Form II is a left-handed helix (LHH) (three peptide per turn,  $3_1$ ) with all the peptide bonds in the *trans* conformation. Structurally, Form I is more compact and rigid while Form II is comparatively extended. A reversible transition of Form I  $\rightleftharpoons$  (RHH) Form II (LHH) is induced by appropriate changes of the solvent conditions.<sup>1-12</sup> Transition Form I  $\rightarrow$  Form II occurs in water and aliphatic acid, and its contrary transition occurs by the dilution of the water and aliphatic acid by 1-propanol (or 1-butanol) [e.g., water (acetic acid)-propanol (1:9 v/v, etc.)].

Particularly, Form II of PLP has been noted because of its similarity to fibrous protein, collagen which has proline as its second most abundant amino acid.

Even though many studies for the PLP compound, as mentioned in the above, have been conducted, no one has studied in detail on the aggregation phenomenon during the transition Form II  $\rightarrow$  Form I. Although it was true that the PLP aggregation phenomenon has been already noted for pure Form I,<sup>16</sup> which is end product of the transition in acetic acid-propanol (1:3 v/v) and for pure Form II (in pure water),<sup>17-19</sup> but it was not clarified the detailed mechanism (or structure) and the reason and also, they have not noted for the aggregation phenomenon which occurs during the transition of Form II  $\rightarrow$  Form I.

Many studies for intermolecular aggregation of helical polypeptides have been conducted.<sup>20-27</sup> Two types of aggregation have been recognized: end-to-end (E-E) type and side-by-side (S-S) antiparallel type. The former type aggregation is formed through the end group interaction between amino and carbonyl residues at each of the helical ends,

and the latter type aggregation is formed by mainly electrostatic (*i.e.*, dipole-dipole) between the surfaces of the helices.

In the previous report<sup>28</sup> of the present series, we observed that the end-to-end (E-E) type aggregation occurred during and after the conformational transition of Form II (LHH) → Form I (RHH) of PLP ( $M_n=31,000$ ) in acetic acid-propanol (1:9 v/v) solvent.

The objective of present study is to elucidate in detail the aggregation phenomena of PLP during the transition of Form II → Form I and after the transition in water-propanol (1:9 v/v) solvent. In order to understand the dependence of water content on the aggregation we also used various compositions of water-propanol, 1:7, 1:15.7, and 1:29 v/v as well as 1:9 v/v. The study was made by the measurements of viscosity and excess light scattering intensity during the transition of Form II → Form I as a function of  $f_I$  calculated by the  $-\alpha_D$ , the specific optical rotation of D-light for the sample solution in the course of the transition.

## Experimental

### Material and Sample Preparation

All PLP (pure Form II) samples ( $M_n$  are 54,000, 31,000, 19,000 and 7,600) were purchase from the Sigma Chem. Co.. We also identified whether the sample is a pure Form II or not. The infrared spectra of samples by the FT-IR measurement were identical with the known Form II spectrum, and we never observed the typical absorption bands of Form I, 960 and 1,355  $\text{cm}^{-1}$ .<sup>29</sup> The specific optical rotation in acetic acid (or water) exhibit  $-\alpha_D=540$ , the characteristic value for Form II.<sup>1,4-6</sup> The solvent propanol (Merk) was highly purified, and water was triply distilled.

The conformational transition of PLP from Form II to Form I was initiated by the one volume of solution of PLP (Form II) in water by adding the required volumes of propanol.

### Measurement

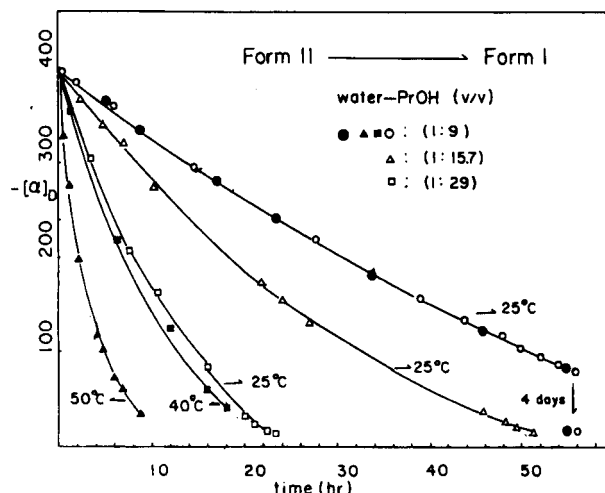
**Viscometry.** Viscosities were measured in an automatic Ubbelohde-type viscometer. Temperature was controlled within  $\pm 0.02$  °C.

**Light Scattering Measurement.** Light scattering measurements were carried out using a Brookhaven instrument (model BI-2030). A He-Ne laser was employed in this study as a light source. The measurement of excess scattering intensity of the sample was conducted using  $\lambda_0=632.8$  nm at  $\theta=90$ . Before the study of the transition Form II → Form I, all solutions were filtered more than three times through a millipore filter of 0.45  $\mu$ .

**Polarimetric Measurement.** Optical rotation was measured by using a Rudolph Automatic Polarimeter (AUTOPOL III) equipped with a sodium D line source. We calculate the Form I fraction (cis fraction) of the sample PLP in water-propanol solvent by:<sup>6,7</sup>

$$f_I = \frac{[\alpha]_{II} - [\alpha]}{[\alpha]_{II} - [\alpha]_I} \quad (1)$$

where  $[\alpha]_{II} = -540$  for the pure Form II,<sup>1,4-6</sup> and  $[\alpha]_I = -15$  for the pure Form I with sodium D line in our experimental, and  $[\alpha]$  is the observed specific optical rotation of the sam-



**Figure 1.** The specific optical rotation  $-\alpha_D$  of PLP vs. time during the transition Form II → Form I for the sample  $M_n=31,000$ . The initial concentration of Form II,  $C_0$  (Form II)=0.5 mg/mL [black circle:  $C_0$  (Form II)=2.0 mg/mL].

ple.

## Results and Discussion

### General Features of the Transition Form II (LHH) → Form I (RHH)

#### Transition Form II → Form I monitored by $-\alpha_D$

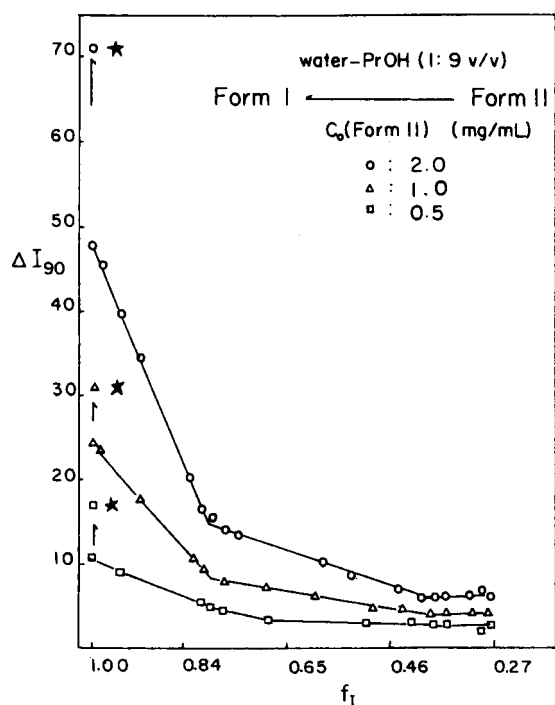
Figure 1 shows the change of the  $-\alpha_D$  of the transition system Form II → Form I of PLP with time in three water-propanol cosolvent systems, water-propanol (=W-PrOH) (1:29, 1:15.7 and 1:9 v/v). One notes from Figure 1, (1) the rate of transition Form II Form I decreases with an increase of the water fraction in the solvent, (2) the rate of transition is independent of the initial concentration of Form II, and (3) the rate increases with raising temperature, in agreement with Steinberg *et al.*<sup>6</sup> and the results in acetic acid-propanol cosolvent system.<sup>28</sup>

When a solution of PLP Form II in water is diluted ten-fold with 1-propanol, the initial decrease in specific optical rotation from  $-\alpha_D=540$  (pure Form II) to  $-\alpha_D=400$  occurs instantaneously. This phenomenon was also already observed by Steinberg *et al.*<sup>6</sup> and it is not elucidated for the reason even up to the present.

Note: In the next all figures, we will use the Form I Fraction  $f_I$  (which calculated by the Eq. (1)) as a method to show the progress of the transition Form II → Form I, instead of  $-\alpha_D$  value.

**The change  $\Delta I_{90}$  and  $\eta_{sp}/C$  vs.  $f_I$  during the transition Form II → Form I.** Typical curves of  $\Delta I_{90}$  and  $\eta_{sp}/C$  vs.  $f_I$  at 25 °C during the transition Form II → Form I in water-propanol (1:9 v/v) solvent are shown in Figures 2 and 3 respectively, where the effects  $C_0$  of Form II are also shown. [The  $f_I$  is 0.27 ( $-\alpha_D=400$ ) at  $t=0$  in water-propanol (1:9 v/v) solvent, and it is 1.00 ( $-\alpha_D=15$ ) at the end of the transition, thus  $f_I$  is used as a time-scale, as well as to show the progress of the transition.]

### Occurrence of Molecular Aggregation during and After the Transition Form II → Form I

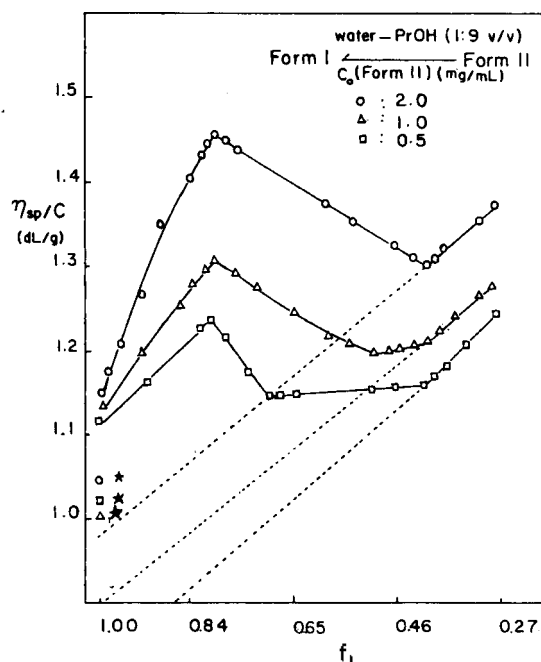


**Figure 2.** Excess scattering intensity  $\Delta I_{90}$  ( $=I_{90}(\text{soln})-I_{90}(\text{solv})$ ) at  $\lambda_{90}=632.8$  nm at 25 °C of the system during and after the transition Form II  $\rightarrow$  Form I in water-propanol (1:9 v/v). The molecular weight of PLP is 31,000. The  $C_0$  (Form II) are shown in the figure. [Note: The asterisked mark indicates the last value after aggregation ended completely. The same mark will also be used in the next figures.]

**Light Scattering Intensity Measurement.** From the results of light scattering measurements (Figure 2) and viscosity measurements (Figure 3), we divide the region of  $f_1$  in abscissa into three parts: from 0.27 to 0.40, 0.40 to 0.80, and 0.80 to 1.00. In Figure 2, in the first region  $\Delta I_{90}$  is about constant; in the second region,  $\Delta I_{90}$  increases slowly; and in the third region  $\Delta I_{90}$  increases strongly. After reaching the final value of  $\Delta I_{90}$  at  $f_1=1.00$  it increases further to the asterisked points.

In a water-propanol cosolvent system, the preferential absorption of solvents which depends on the conformation of Form II and Form I of PLP will also affect the light scattering intensities. However, we neglect this effect by the reason that the solvent system is propanol-excess (90%), i.e., quasi one component system, thus the contribution by this effect to the light scattering intensity is very small. The claim on this small effect is also enough proved by the result of sample  $\bar{M}_n=7,600$  in Figure 6, where the aggregation does not occur, because the  $\Delta I_{90}$  is about constant over the range of the transition.

Since the  $\Delta I_{90}$  is proportional to the molecular weight of PLP we can conceive that intermolecular aggregation with any type occurs during the transition. It is noted that the aggregation depends on Form I units of PLP (i.e.,  $f_1$ ) which were produced by the transition. That is, the aggregation starts to occur from  $f_1=0.40$  ( $-\alpha]_D=330$ ) and occur slightly with the progress of the transition until  $f_1=0.80$  ( $-\alpha]_D=120$ ). However, the aggregation occurs strongly after  $f_1=0.80$  to the final asterisked value. After the completion of the tran-



**Figure 3.**  $\eta_{sp}/C$  vs.  $f_1$  at 25 °C for the system during the transition Form II  $\rightarrow$  Form I in water-propanol (1:9 v/v) for various initial concentration of Form II PLP ( $\bar{M}_n=31,000$ ).

sition Form II  $\rightarrow$  Form I ( $f_1=1.00$ ), the aggregation continues increasing the  $\Delta I_{90}$  to the asterisked value. It is possible to be thought that the above phenomenon is only the time dependent aggregation. But this possibility is nearly excluded by the result of concentration effect in Figures 2 and 3. The characteristic points  $f_1=0.40$  and 0.80, were not affected by the initial concentration of Form II,  $C_0$  (Form II).

**Viscosity measurement.** In order to acquire the information for the aggregation type we measured the viscosity over the full range of the transition as shown in Figure 3. The dotted lines in this figure exhibit the viscosity change in assuming intermolecular aggregation does not occur. These lines which exhibit monotonous decrease in viscosity change can be easily obtained by the next two results: (1) Steinberg *et al.*'s result<sup>6</sup> for the viscosity during the transition Form I  $\rightarrow$  Form II in solvent of aggregation-free, acetic acid, (2) the result of viscosity during the transition Form I  $\rightarrow$  Form II for the sample  $\bar{M}_n=7,600$  in Figure 5, where the aggregation does not occur. The dotted lines can be also deduced by comparing the structure of Form I and Form II of PLP because the chain length, the latter attains more short than the former (see Figure 4).

From the result of viscosity we can divide the region of  $f_1$  into three part by the same reason as shown in the light scattering measurement (Figure 2). In the first region ( $f_1=0.27$  to 0.40), the decrease of  $\eta_{sp}/C$  is due to the conformational transition of Form II (LHH) to Form I (RHH) which is more compact since  $\Delta I_{90}$  is constant. In the second region ( $f_1=0.40$  to 0.80),  $\eta_{sp}/C$  increases to reach a maximum, due to intermolecular aggregation which make induce the increase of molecular chain length compared to the single chain of PLP.

In the third region ( $f_1=0.80$  to 1.00),  $\eta_{sp}/C$  decreases. After reaching the final value of  $\eta_{sp}/C$  at  $f_1=1.00$  it decreases

further to the asterisked point. Thus, in this region, a side-by-side (S-S) type (or raft-like) aggregation occurs by which the molecule becomes more compact decreasing  $\eta_{sp}/C$ . After the completion of the transition of Form II  $\rightarrow$  Form I, the (S-S) type aggregation continues decreasing the  $\eta_{sp}/C$  to the asterisked value.

From the results of a rapid increase in  $\Delta M_w$  (Figure 2) and decrease in  $\eta_{sp}/C$  (Figure 3) after  $f_I=0.80$  it is reasonable to conclude that the (S-S) type aggregation are promoted by the existence of above 80% of Form I units within helical PLP chain. The  $C_0$  (Form II) promotes the (S-S) type aggregation after  $f_I=0.80$  because the decreasing degree in viscosity and the increasing degree in  $\Delta M_w$  is larger as  $C_0$  (Form II) increases.

### The Aggregation Mechanism in the Transition of Form II $\rightarrow$ Form I

**Structure of PLP.** To elucidate the mechanism of aggregation which occurs during the transition Form II  $\rightarrow$  Form I, first of all, it is necessary to look over the structure of PLP as well as transition mechanism of Form II  $\rightarrow$  Form I.

As already mentioned in the INTRODUCTION, Form I of PLP is compact helix while Form II is extended helix, and that in the former, the carbonyl oxygens are partly shielded, and the hydrophobic pyrrolidone rings are exposed to the solvent while in the latter the carbonyl oxygens are exposed directly to the solvent sides. Thus one may easily understand that Form I exhibits smaller viscosity and larger hydrophobicity than Form II. [The detailed structure about Form I and Form II of PLP refers to the reference (3)].

According to the end-mechanism proposed by a several authors,<sup>30-32</sup> the transition Form II  $\rightarrow$  Form I begins to occur from one terminal of PLP chain, and proceeds by a step-by-step mechanism, and reaches to the other chain end where the transition is completed. Thus we can deduce the intermediate conformation which is produced by the transition. The conformation should be the state where the cis residues (Form I residues) are not randomly distributed, but orderly

making a block of the cis fraction which is connected to the trans fraction belonging to the reactant Form II as shown in Figure 4.

**A partial side-by-side (p-S-S) type and side-by-side (S-S) type aggregation.** By considering the intermediate structure of PLP deduced by the transition mechanism as mentioned in the above we propose a reasonable mechanism for aggregation, as shown in Figure 4. In this mechanism the Form II helix is represented by a wavy line showing the extended helix and Form I helix is represented by roops, as depicted by Holzwarth and Backman<sup>33</sup> and residue repeat of Form II is 3.12 Å and Form I is 1.85 Å<sup>34</sup>. The arrow lines in this loops exhibit an effective dipole moment arising from the peptide group-N<sup>+</sup>=C-O<sup>-</sup>.

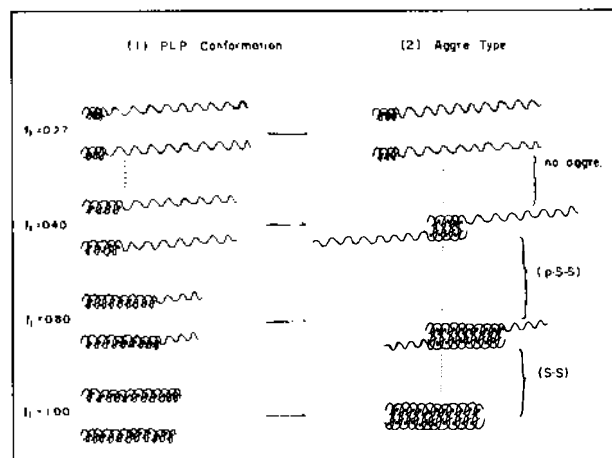
In Figure 4, it is suggested that the aggregation occurs between Form I blocks (which are produced by the transition) by the side-by-side antiparallel type and the main forces which act on between aggregations are considered as the hydrophobic interaction of Form I blocks and a dipole-dipole interaction since the direction of dipole is antiparallel. By this mechanism, we can explain effectively the result of viscosity change in Figure 3 as follow: in the second region ( $f_I=0.40$  to 0.80), a partial side-by-side (p-S-S) type aggregations occur, including viscosity-increase because of the increased chain length compared to a single PLP molecule. However, by the (S-S) type (or raft-like) aggregation in the third region ( $f_I=0.80$  to 1.00), the increase of chain length is small, rather the molecule becomes more compact inducing viscosity decrease. The decrease is explained by the Ree-Eyring theory of viscosity.<sup>35</sup>

According to this theory  $\eta=\beta/\alpha$  where  $\beta$  and  $\alpha^{-1}$  are the quantities proportional to the relaxation time and the shear modulus of the flow unit, respectively.  $\beta$  is small for a compact unit than an extended one. Thus by the (S-S) aggregation, the viscosity becomes small since by this type aggregation the flow unit becomes compact. The reason that the aggregation occurs only after  $f_I=0.40$  is understood by the next fact that in order to occur aggregation between Form I blocks by the dipole-dipole and hydrophobic interaction, the Form I fraction must be exist within PLP chain, at minimum,  $f_I=0.40$  [the number of Form I monomer residues [NMR (Form I)=320 (DP of  $\bar{M}_n=31,000) \times 0.40=128$ ], by the transition. The interactions between Form I blocks seem to be maximized when  $f_I>0.80$  [NMR (Form I)=320  $\times$  0.80=256] because  $\Delta M_w$  highly increases (Figure 2) and  $\eta_{sp}/C$  decreases (Figure 3) from this point. Thus the (S-S) type (raft-like) aggregation is promoted from  $f_I=0.80$  to the final asterisked value.

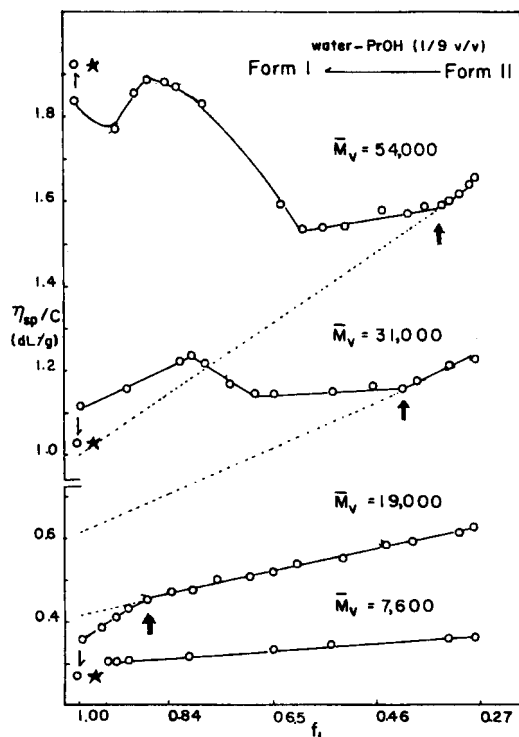
### The Chain Length Effect on the Aggregation

To more confirm the above fact for the aggregation, i.e., a constant Form I units are required to occur the aggregation between Form I blocks by side-by-side manner, we try to investigate the chain length effect of PLP on the aggregation.

From the viscosity measurement (Figure 5) and excess scattering intensity measurement (Figure 6), it is observed that the initial points for the aggregation during transition Form II  $\rightarrow$  Form I is  $f_I=0.34$ , 0.40 and 0.86 for the  $\bar{M}_n=54,000$  (DP=557), 31,000 (DP=320) and 19,000 (DP=196) respectively (The points show as the arrow mark in Figure



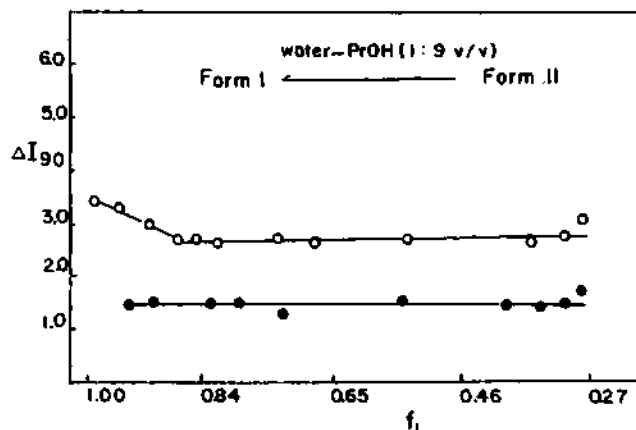
**Figure 4.** A reasonable mechanism for the aggregation in the transition Form II  $\rightarrow$  Form I; column (1): the structures of PLP (pure Form II, pure Form I, and intermediate conformations which were produced by the transition Form II  $\rightarrow$  Form I), column (2): (p-S-S) type and (S-S) type aggregation.



**Figure 5.**  $\eta_{sp}/C$  vs.  $f_i$  at 25 °C during the transition Form II  $\rightarrow$  Form I in water-propanol (1:9 v/v) for the various samples of PLP. The initial concentration of Form II,  $C_0$  (Form II)=0.5 mg/mL.

5). That is, higher value in  $f_i$  is shown as the molecular weight decreases. It is also noted that in the case of the smallest molecular weight used in the present study,  $M_v=7,600$ , aggregation does not occur over the full range of the transition since  $\Delta I_{90}$  is about constant (see Figure 6).

The number of Form I monomer residues [NMR(Form I)] for the aggregation at the various molecular weight are listed in Table 1. The NMR (Form I) values show some difference according to molecular weight. The reason for this difference is not certain in the present, but it seems to be



**Figure 6.**  $\Delta I_{90}$  vs.  $f_i$  at 25 °C during the transition Form II  $\rightarrow$  Form I in water-propanol (1:9 v/v) for the two PLP samples; open circle:  $M_v=19,000$ , black circle:  $M_v=7,600$ . The initial concentration of Form II,  $C_0$  (Form II)=0.5 mg/mL.

**Table 1.** The number of Form I monomer residues [NMR (Form I)] at initial points ( $f_i$ ) for aggregation of PLP in the various PLP molecular weight

$M_v$	$f_i$	NMR (Form I)
54,000(DP=557)	0.34	188
31,000(DP=320)	0.40	128
19,000(DP=196)	0.86	164

due to any specific properties according to the PLP molecular weight, for example, an abnormal increase of chain flexibility in high molecular weight sample.<sup>36,37</sup>

In spite of the difference in NMR (Form I) value, it is apparent that a constant Form I units are required to occur the aggregation by the side-by-side manner because the initial points for the aggregation observed after the transition progressed in some degree at most of all the samples, except for  $M_v=7,600$ . This result is more supported by the fact that the aggregation does not occur nearly at the lowest molecular weight sample  $M_v=7,600$  having very short chain length. Because in this sample enough Form I units required for the occurrence of aggregation are not produced by the transition even though the transition Form II  $\rightarrow$  Form I is completed [i.e., at  $f_i=1.00$ , the value of NMR (Form I)=78]. This value does not far reach even up to 128 (for  $M_v=31,000$ ), which is the smallest value observed in Table 1.

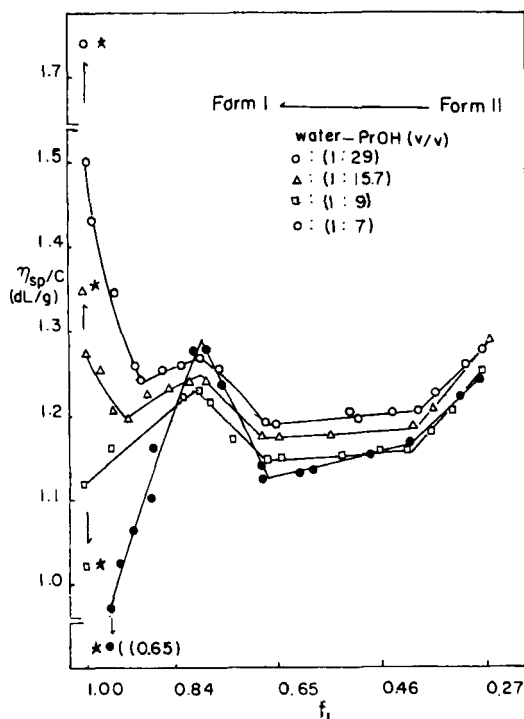
In Figure 5, in the case of the highest molecular weight sample,  $M_v=54,000$ , a small increase of viscosity is observed at the end of the transition. This phenomenon may be caused by the existence of the (E-E) following the (S-S) aggregation.

#### Solvent Effect on the (S-S) and (E-E) Aggregation of PLP

To investigate the aggregation type according to the water content and also, to identify the existence of hydrophobic interaction between aggregates we measured the viscosity during the transition Form II  $\rightarrow$  Form I in the solvents of various water/propanol ratios, i.e., 1:7 (water volume fraction=12.5%), 1:9 (10%), 1:15.7 (6.0%), and 1:29 (3.3%) where the curve for 1:9 (v/v) solvent is reproduced from Figure 3. All the results are shown in Figure 7.

The shape of curves of  $\eta_{sp}/C$  vs.  $f_i$  is not affected greatly by the solvent conditions until  $f_i=0.80$ , but it is affected greatly after  $f_i=0.80$ , i.e., increases to the final values through some minimum in solvents of 1:29 and 1:15.7 (v/v). Furthermore the degree of viscosity-increase after the minimum is more large in water-propanol (1:29 v/v) than (1:15.7 v/v). The increase in  $\eta_{sp}/C$  is larger for the solvent with smaller fraction of water. One also notes that in 1:9(v/v) solvent,  $\eta_{sp}/C$  decreases after  $f_i=0.80$  as previously pointed out. We find that the decrease appears in water-propanol (1:7 v/v) also, i.e., when water constant is large (12.5%) a larger decrease in  $\eta_{sp}/C$  appears than in the 1:9(v/v) solvent.

From the result in Figure 7, we conclude that the (S-S) type aggregation after  $f_i=0.80$  takes place in solvents of water-propanol 1:7 and 1:9(v/v), while it does not occur in solvents water-propanol 1:15.7 and 1:29(v/v), instead the (E-E) type aggregation occurs yielding the viscosity increase. Thus it can be concluded that the ratio water:propanol is a controlling factor for determine the aggregation



**Figure 7.**  $\eta_{sp}/C$  vs.  $-[\alpha]_D$  at 25 °C for the  $\bar{M}_w=31,000$  during the transition Form II  $\rightarrow$  Form I in water-propanol: 1:29, 1:15.7, 1:9, and 1:7 v/v. The initial concentration of Form II,  $C_0=0.5$  mg/mL.

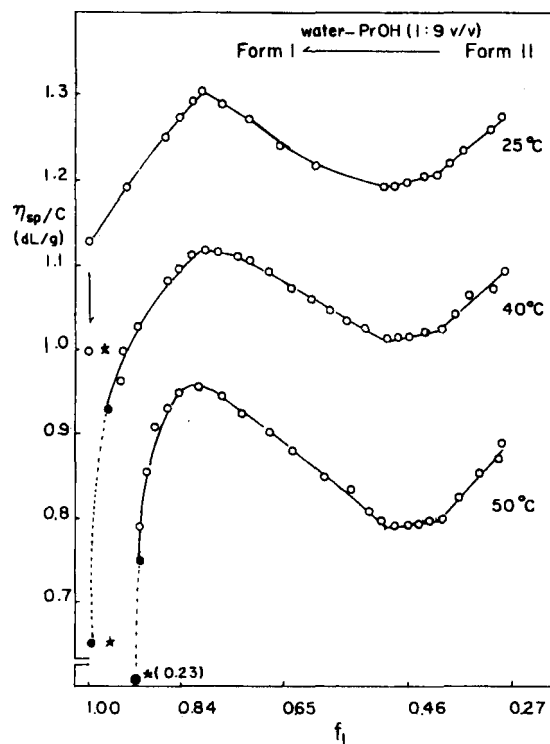
type, *i.e.*, when water content is large, the (S-S) type aggregation occurs favorably because of the increase of hydrophobic interaction between Form I blocks while when it is small the hydrophobic interaction will be reduce, thus the (E-E) aggregation occurs through hydrogen bonding between end groups PLP chain.

#### Temperature Effect on the (S-S) Aggregation of PLP

Figure 8 exhibits the viscosity changes during the transition Form II  $\rightarrow$  Form I in solvent of water-propanol (1:9 v/v) under various temperatures. The shape of curves of  $\eta_{sp}/C$  vs.  $f_I$  is not affected largely by temperature until  $f_I=0.80$ . However, after this point the decreasing degree of viscosity is stronger and more rapid as temperature increases and ultimately, heat precipitation occurs which being a very high aggregation-state phenomenon. [The point at which the heat-precipitation occurs is exhibited by a dotted line between black circles]. The heat-precipitation phenomenon of PLP (Form II) was commonly observed at high temperatures in pure water medium.<sup>38,39</sup>

In conclusion, the occurrence of (S-S) type aggregation of PLP after  $f_I=0.80$  in water-propanol (1:9 v/v) is maximized at high temperature, yielding abrupt decrease in viscosity after  $f_I=0.80$ . The reason for this result may be due to the increase of hydrophobic interaction between Form I blocks at high temperature because the hydrophobic interaction increase at high temperature.

By the result of temperature effect in Figure 8 it can be proved that in the second region ( $f_I=0.40$  to 0.80) the phenomenon of viscosity-increase is not due to the (E-E)



**Figure 8.**  $\eta_{sp}/C$  vs.  $-[\alpha]_D$  for the  $\bar{M}_w=31,000$  during and after the transition Form II  $\rightarrow$  Form I in water-propanol (1:9 v/v) at various temperatures, the initial concentration of Form II,  $C_0=0.5$  mg/mL.

aggregation. Because if the (E-E) type aggregation occurs in this region the aggregation must be reduce at high temperature because of the difficulty of hydrogen bonding between end groups. But rather, on the contrary, in the Figure 8, the viscosity increases slightly at temperature 50 °C in comparing with the temperature 25 °C in the region, because of the increase of (p-S-S) type aggregation at high temperature.

#### Concluding Remarks

In the conformational transition of PLP Form II  $\rightarrow$  Form I in water-propanol (1:9 v/v) solvent, we observed that the (p-S-S) and (S-S) aggregation occur between Form I blocks (which were produced by the transition) and the aggregation highly depends on the Form I fraction. By the assumption of these type of aggregations we were able to explain effectively all the results of light scattering and viscosity measurements during the transition. The present result is contrast to the previous result<sup>26</sup> in acetic acid-propanol (1:9 v/v) solvent system in which occurred the (E-E) type aggregation in the transition Form II  $\rightarrow$  Form I. The opposite result for each other is considered to be due to the difference of solvent, *i.e.*, water and acetic acid.

The main forces which act on between Form I blocks in the (p-S-S) and (S-S) type aggregation are considered as dipole-dipole interaction and hydrophobic interaction. The existence of hydrophobic interaction between Form I blocks was enough proved by the results of the solvent effect and temperature effect in the Results and Discussion.

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