

ESR Study of Gel–Sol Transitions of Poly(L-leucine)–Benzene Solution by Spin-Labeling Technique

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ABSTRACT: An ESR study of poly(L-leucine)–benzene solution ranging in concentration from 0.1 to 6.0 wt% was carried out by spin-labeling technique in the temperature range from room temperature to 90°C. The ESR spectra of these samples showed that radicals undergo restricted motion in this temperature range. It was found that the line separation of the ESR spectrum changes remarkably around 50 and 70°C. These temperatures correspond to the temperatures of the gel–sol transitions, suggesting the existence of two kinds of gel states in these samples. Heats of dissociation of these gels were estimated at 160 and 42 kcal for a mol of network junctions, respectively, for the poly(L-leucine) with a number-average molecular weight of 10.⁴

KEY WORDS Poly(L-leucine) / Spin-Labeling Technique / ESR Spectra / Anisotropic Motion / Gel–Sol Transition /

A spin-labeling technique was developed to study the molecular structure and dynamics in a particular site of a molecule to which a radical is directly attached.^{1–3} This technique was applied to polypeptides containing poly(L-lysine),⁴ poly(L-glutamic acid)⁵ and poly(γ -benzyl L-glutamate),^{6,7} all labeled by a nitroxide radical at the polymer end, in order to investigate the molecular dynamics in the solution. In this work such a study was made on poly(L-leucine).

It has been known that poly(L-leucine)–benzene solution changes its state from gel to sol around 50°C.^{8,9} In this paper, we investigate such gel–sol transition by electron spin resonance (ESR) measurements using spin labeled poly(L-leucine) (SL-PLL). For this purpose, the temperature dependence of ESR

spectra for benzene solutions of SL-PLL over a wide concentration range is investigated in the temperature range from room temperature to 90°C.

EXPERIMENTAL

SL-PLL, labeled at the polymer end with a nitroxide radical, was obtained by polymerizing the *N*-carboxyanhydride (NCA) with 2,2,6,6-tetramethyl-4-aminopiperidine-1-oxyl (TEMPAMINE) as an initiator in benzene at 60°C. The chemical structure of SL-PLL is shown in Figure 1. The number-average molecular weight was estimated at 10⁴ from comparing the ESR spectral intensity of the SL-PLL and TEMPAMINE in benzene.

(SL-PLL)–benzene solutions with concen-

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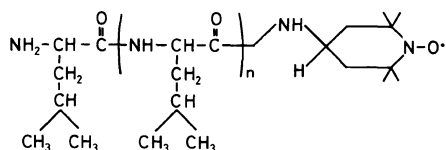


Figure 1. Structural formula of spin-labeled poly(L-leucine).

trations from 0.1 to 6.0 wt% were prepared at 90°C in a pressure bottle. The solutions were injected into ESR sample tubes (quartz 4 mm) and then the tubes were sealed. The solutions in the sealed tubes were annealed at 90°C for at least 1 hour and quenched to 10°C before ESR measurements.

ESR spectra were measured with a JEOL JES-FE1X-type spectrometer (rf. 9.4 GHz) in the temperature range from room temperature to 90°C. The microwave power and the amplitude of the 100 kHz field modulation were 1 mW and 0.8 G, respectively, which were the conditions without saturation effects or modulation broadening. Temperature was controlled with a DP1110-type digital programmable regulator from CHINO WORKS Ltd.

RESULTS AND DISCUSSION

Figure 2 shows the temperature dependence of ESR spectra for the solution with a concentration of 3.4 wt%. The spectral-line shape is highly asymmetric at each temperature, indicating that the motion of the spin-label is highly restricted. The spectral-line shape is, moreover, very similar to that for a spin-labeled poly(γ -benzyl-L-glutamate), PBLG, in organic solvents.^{6,7} The spectral-line shape for PBLG which takes an α -helical conformation was well explained by the axial motion of a rod-like molecule. It has been reported that the conformation of PLL in benzene is the α -helix.⁸ We may, therefore, assume the motion similar to that of a PBLG molecule as the motion of PLL molecule.

In order to express temperature variation of line shape, the line splitting between two outer

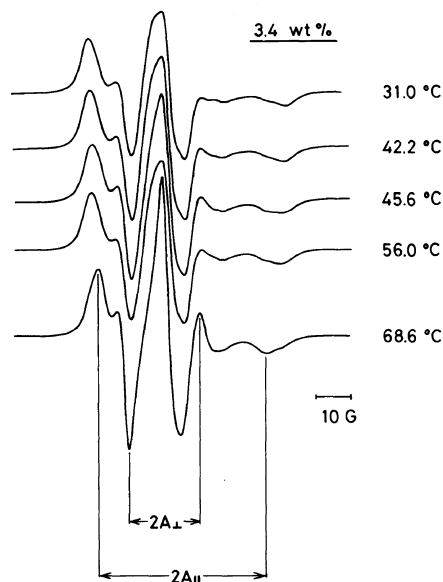


Figure 2. ESR spectra of spin-labeled poly(L-leucine) in benzene solution (3.4 wt%) at the indicated temperatures.

lines of ESR spectrum (the extrema separation), ΔW , was estimated from the observed spectra and the results are shown in Figure 3.

The temperature dependence of ΔW shown in Figure 3 was measured with increasing temperature at a constant rate of 0.25 K min⁻¹. ΔW decreases remarkably around 50°C and then gradually above that temperature for the solutions with low concentrations, 0.1–1.0 wt% (Figure 3a). We denote this transition temperature as T_L . For solutions with concentrations from 1.0 to 3.0 wt%, the ΔW vs. temperature curve shows a plateau region from 50 to 70°C (Figure 3b). On the other hand, for high concentrations, 3.0–6.0 wt%, ΔW again gets large values between 60 to 70°C as soon as the first decrease occurred at T_L (Figure 3c). The second remarkable decrease in ΔW was observed around 70°C. We denote this temperature as T_H . T_L agrees with the temperature of a gel–sol transition which has been found by some authors^{8,9} for the solution below 1.0 wt%. Therefore, the change in ΔW at T_L relates to the change in the motional state

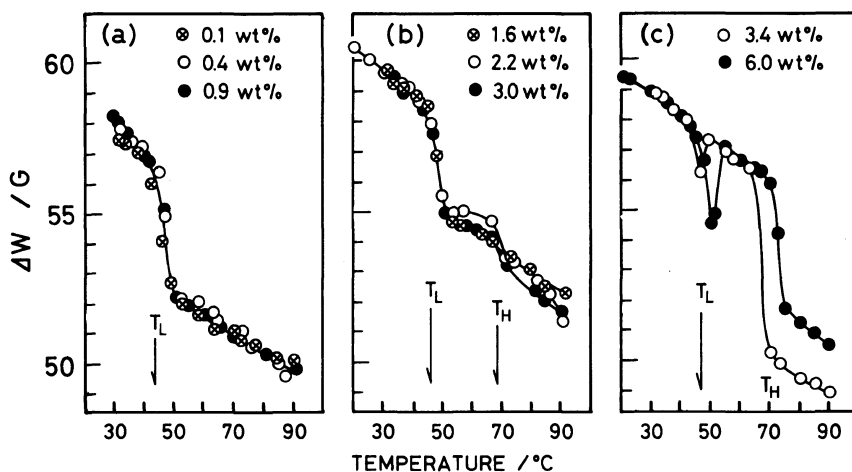


Figure 3. Temperature dependence of the extrema separation for poly(L-leucine)-benzene solutions ranging in concentration from 0.1 to 6.0 wt% measured with increasing temperature. The heating rate is 0.25 K min^{-1} .

of the label due to the gel-sol transition. On the other hand, for solutions with high concentration, the unusual change in ΔW , which increases abruptly with increasing temperature around T_L , suggests that a "sol-gel" transition which has not been reported occurs.

It has been known that the Eldridge-Ferry's equation¹⁰ shown below can be applied to the relation between the melting temperature of gel (T_m) and the concentration of the solution (c) expressed in g l^{-1} :

$$\log c = \Delta H / 2.303RT_m + \text{constant} \quad (1)$$

Here, ΔH is the heat of dissociation of a mole of network junctions in the gel. Figure 4 shows plots of $\log c$ vs. reciprocal of absolute temperature for T_L and T_H . T_L (or T_H) was obtained from the point of intersection of the straight lines drawn on the curves of the gradual decrease just before the transition and the abrupt one due to the transition. It is seen that the plots in Figure 4 give straight lines, indicating that a relation similar to eq 1 holds for each transition. We attempted, therefore, to estimate the heat of dissociation for the processes around T_L and T_H using eq 1 and obtained $160 \text{ kcal mol}^{-1}$ and 42 kcal mol^{-1}

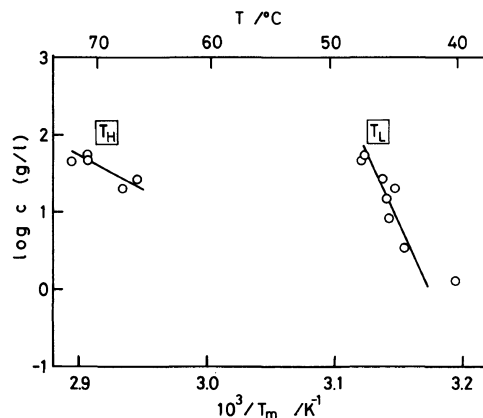


Figure 4. Relation between concentration and reciprocal of the absolute temperature of dissociation for poly(L-leucine)-benzene solution.

for the lower and higher temperature processes, respectively.

One of the authors (S. Tokura) determined the heat of dissociation of the gel with a PLL content of less than 1 wt% by the well-known "falling-ball" method.⁹ The value of about 50 to 60 kcal mol^{-1} obtained for the PLL of the number average molecular weight 10^4 was one-third of that for the lower temperature process in the present study. Such disagreement seems

attributable to the underestimation of the heat by the "falling-ball" method. At lower concentrations, the value of the melting temperature may be observed smaller than the true one by this method, as constant stress is applied to the gel. Consequently, the smaller value of the heat was obtained. However, such an effect of the stress is weakened if the high-molecular weight PLL is used as the sample. Actually, the heat for the PLL of the average molecular weight 10^5 determined by the "falling-ball" method, $180 \text{ kcal mol}^{-1}$,^{9,*} was in agreement with that obtained by the spin-labeling technique. Such a comparison is allowable if the heat is independent of molecular weight as suggested by Eldridge and Ferry.¹⁰

The present results indicate that two kinds of gel states appear for the higher concentration solution of PLL in the temperature range from room temperature to T_H . We named the first gel state as that between room temperature and T_L Gel I and the second gel state as that between T_L and T_H Gel II.

Consequently, gel-sol transitions of (SL-PLL)-benzene solution are found to be monitored by measurements using the spin-labeling technique. Therefore, some properties of these gels were investigated further using this method.

In Figure 5 are shown ΔW vs. temperature curves for the gels prepared from the solution of 3.4 wt% by different cooling rates. The curve for the gel prepared by rapid cooling rate (a rapidly-cooled gel) is similar to that shown in Figure 3. A difference of minimum values of ΔW around T_L is due to the difference in the increasing rates of measured temperature. ΔW for the sample of the gel prepared by cooling slowly (a slowly-cooled gel) decreases gradually until the abrupt decrease occurs around 70°C and the "depression" of the curve around T_L disappears. The transition temperature of the slowly-cooled gel agrees well with T_H , and

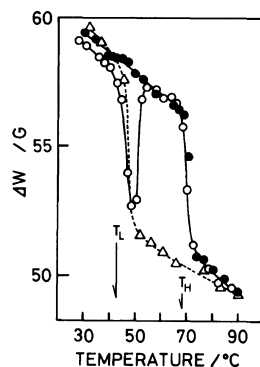


Figure 5. Temperature dependence of the extrema separation for poly(L-leucine)-benzene solution (3.4 wt%). Circles express the extrema separation measured with increasing temperature. Filled circles, for the gel prepared by cooling slowly from sol state; open circles, for the gel prepared by cooling rapidly from sol state. Triangles express the extrema separation measured with decreasing temperature at a rate of 0.5 K min^{-1} .

thus the slowly-cooled gel and Gel II of the rapidly-cooled gel seem to be similar gel states of the (SL-PLL)-benzene solution.

Speculation on the molecular mechanism of the unusual change in the extrema separation was made as described below. If a (SL-PLL)-benzene solution was cooled rapidly, the structure of the solution from the viewpoint of molecular orientation remained unchanged. Such a gel, which corresponds to Gel I, is, therefore, made up of PLL molecules in less mutual orientation. If the sample in Gel I state is heated, rod-like motion occurs and overcomes intermolecular interactions such as hydrophobic interactions suddenly at T_L . At the same time, PLL molecules are given the freedom for molecular orientation. As a result, more ordered molecular aggregates such as lyotropic liquid crystals appear above this temperature. This state corresponds to Gel II and these aggregates melt at T_H . On the other hand, if the solution is cooled slowly, mutual orientation of the molecules progresses much more than that in the sol state

* The heat of dissociation for the PLL of the average molecular weight 10^5 was evaluated incorrectly in ref 9. The correct value is presented here.

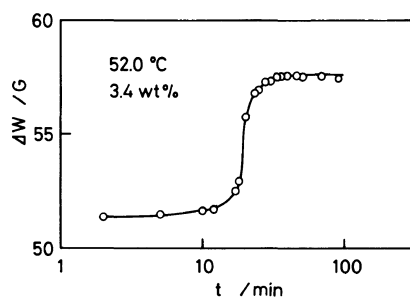


Figure 6. Time dependence of the extrema separation for poly(L-leucine)-benzene solution (3.4 wt%) at 52.0 °C.

and a gel made up of more ordered molecular aggregates containing the solvent is obtained. Such a gel, which is similar to Gel II, does not melt until it is heated to T_H . Therefore, remarkable change in ΔW was not observed at T_L . The change in ΔW measured with decreasing temperature represented by triangles in Figure 5 may suggest that the formation of the ordered state occurs around T_L if the solution was cooled at a rate of 0.5 K.

Furthermore, if the highly concentrated sample is cooled to an arbitrary temperature in the range between T_L and T_H and kept at that temperature, after a certain short time ΔW increases suddenly as shown in Figure 6, and reaches the value for Gel II. Subsequent measurement with decreasing temperature gives similar results along the curve for the slowly-cooled gel shown in Figure 5. The time dependence of ΔW in Figure 6 seems to reflect the gelation process. We may find a molecular mechanism for the gelation, if ΔW vs. time curve is analyzed with the theory for a pro-

posed gelation model¹¹ which explains the time dependence of gel fraction on the basis of a kinetic equation. The investigation of the molecular mechanism on the basis of more precise data will be reported in the near future.

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