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Phase separation in the mixture of schizophyllan and poly(ethylene oxide) in aqueous solution driven by a specific interaction between the glucose side chain and poly(ethylene oxide)

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Abstract

We found that the mixture of schizophyllan and poly(ethylene oxide) in aqueous solution underwent phase separation at around 3–4 °C, and this temperature was independent of both polymer concentration and the difference in poly(ethylene oxide) molecular weight (M_w 6000 and 70,000). The phase-separation took place at the same temperature at which the optical rotation changed. Since the optical rotation change is ascribed to the difference in the nature of hydrogen bonding between the schizophyllan side chain and water, the phase separation is also considered to be due to an interaction between poly(ethylene oxide) and schizophyllan. The phase-separation temperature increased on changing H_2O to D_2O in accordance with a change in the optical rotation, confirming the specific interaction essential for the phase separation. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Schizophyllan; Poly(ethylene oxide); Phase separation; Optical rotation; Specific interaction

1. Introduction

Schizophyllan is a cell-wall polysaccharide produced by the fungus *Schizophyllan commune*. The main chain consists of a triple helix of β-(1 → 3)-D-glucans in which every third glucose residue in the main chain has one β-(1 → 6) D-glucosyl side chain [1]. Since schizophyllan exhibits antitumor activity [1], the possible relationship between the biological activity and the chemical structure has attracted a wide range of attention [2,3]. Yanaki et al. [2] demonstrated the triple-helical conformation essential for the antitumor activity against sarcoma 180. Recently, however, Young and Jacobs [3] claimed that both the triple helix and single coil of schizophyllan show biological activity against such as a lectin of *Limulus amebocyte* lysate. Otherwise, there exists little fundamental information that addresses the interaction between schizophyllan and other biomaterials.

The above-mentioned situation led us to study the interaction between schizophyllan and a model polymer for biological materials. The model polymer has to be simple, well characterized, and well known for its biochemical properties. Since poly(ethylene oxide) (PEG) is one of those, many studies have thus been carried out to apply PEG to drug-

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delivery systems and materials for synthetic organs [4]. In this paper, we report a new exploration of a specific interaction between schizophyllan and PEG.

2. Materials and methods

The schizophyllan sample used in this work was prepared by Taito Co. The crude and native material produced by the fungus *S. commune* was sonicated and then fractionated to yield the purified products. We used one of the fractions provided by Taito Co. and designated it as SPG. The molecular weight of the sample was estimated to be 6.0×10^5 Da by viscosity measurement [5]. Four poly(ethylene oxide) samples, PEG0.2K, PEG6K, PEG70K, and PEG500K with molecular weights of 200, 6000, 7.0×10^4, and 5.0×10^5 Da, respectively, were purchased from Wako Chemicals and used without further purification. We used their trade names as the sample codes in this work

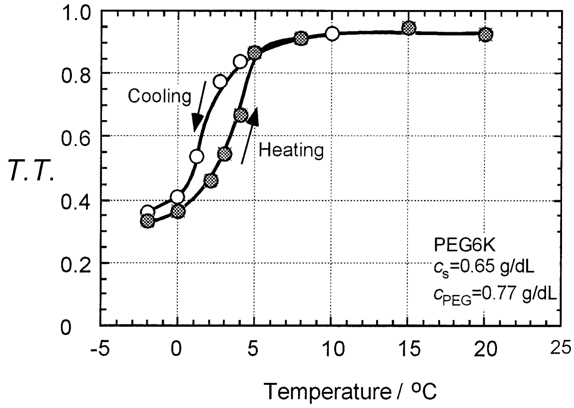

Fig. 1. Temperature dependence of the total transmittance (TT) on heating and cooling for a mixture of SPG and PEG6K with $C_{PEG} = 0.77$ and $C_{SPG} = 0.65$ g/dL.

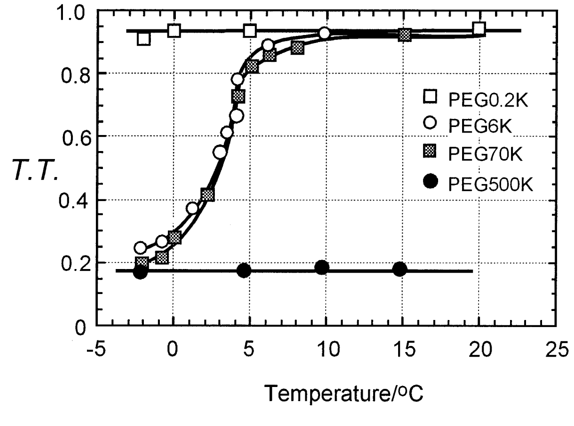

Fig. 2. TT vs. temperature curves for the four mixtures with different molecular weight PEG. For all samples, C_{PEG} and C_{SPG} are about same (1.0 g/dL).

The temperature dependence of the transmittance of ultraviolet and visible light (200–600 nm) was measured in the UV mode with a Jasco J-720 CD spectrometer. We examined turbidity in the concentration range of 0.15–1 g/dL for the individual solute with a 1-cm cell. The total transmittance (TT) was calculated by dividing the integrated transmittance of the sample by that of water. The optical rotatory dispersion (ORD) measurements were made with a 10-cm cell on a Jasco J-720 VII.

3. Results and discussion

Phase-separation behavior for schizophyllan–PEG aqueous solutions.—When a SPG + PEG6K solution with $C_{SPG} = 0.65$ g/dL and $C_{PEG}{}^1 = 0.77$ g/dL was cooled from 20 to − 2 °C, we found that the solution became turbid. When we heated it again to 10 °C, it again became homogeneous. Fig. 1 presents the temperature dependence of TT for this system. In the temperature range 20–5 °C, the solution was transparent and no substantial change was observed in TT; however, with the lowering of the temperature from 5 to 0 °C, the transmittance decreased drastically. At 2 °C we found that the solution was visibly opaque; therefore, the decreasing transmittance is ascribed to the fact that some phase separation takes place in the solution. Since we confirmed that no turbidity is observed for both the SPG–water and PEG–water binary solutions with the concentration of 0.5–1.5 g/dL in the temperature range 0–20 °C, this phase separation is distinctive for the mixture of SPG and PEG aqueous solutions.

Fig. 2 compares the heating curves of TT for four PEG samples with different molecular weights. For PEG500K (the highest M_w), a white opaque precipitate was formed for all temperatures and the values of TT ranged

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[1] C_{SPG} and C_{PEG} are the mass concentrations of SPG and PEG, respectively.

Table 1
Phase-separation behavior and the cloud temperature for aqueous solutions of schizophyllan and poly(ethylene oxide) mixtures with equal concentration [a]

Sample code for PEG	Concentration (g/dL)				
	0.15	0.33	0.5	0.66	1
PEG500K	PS	PS	PS	PS	PS
PEG70K	H	3.2	3	3	4
PEG6K	H		3	4	3.5
PEG0.2K	H	H	H	H	H

[a] PS, phase-separated; T, homogenous.

around 0.2. On the other hand, the mixture with PEG0.2K (the lowest M_w) showed no phase separation and was homogeneous at all temperatures. For the other two PEG samples with intermediate molecular weights, TT was lowered to around 3 °C. Although the results are not shown, when we changed the polymer concentrations to between 0.6 and 1.5 g/dL, the phase separation always occurred at 3 °C for PEG6K and PEG70K. We defined the cloud temperature as the one where TT becomes the middle value between the transparent and opaque solution. The results as well as the absence or presence of phase separation are summarized in Table 1.

For the solutions containing two different polymer solutes, the entropy of mixing decreases with increasing molecular weight. Therefore, the higher-molecular-weight polymers tend to phase separate at the lower temperature, and the cloud point, defined by a phase boundary in the temperature versus composition diagram, usually strongly depends on the molecular weight [6]. This general law can rationalize the presence and absence of phase separation for PEG500K and PEG0.2K, respectively. However, it is not consistent with the fact that the cloud temperature is independent of both the molecular weight of PEG and the concentration of the solutes. This feature suggests that other mechanisms may be involved in the phase separation for the PEG6K and PEG70K systems.

Teramoto [7] found that the ORD of the schizophyllan aqueous solution shows a drastic change at the same temperature where the above-mentioned phase separation occurs. The molecular mechanism of this change in ORD is not yet well understood; however, the dielectric dispersion measurement [7] shows that the change is associated with the nature of the hydrogen bonding between water and the glucose side chain. According to Teramoto, at the lower-temperature region ($<$ 2 °C for our SPG sample) the water molecules surrounding schizophyllan become less mobile than those in bulk water, due to the tight hydrogen bonding. On the other hand, at the higher-temperature region ($>$ 6 °C), the mobility becomes indistinguishable between the surrounding and bulk water. Since PEG is hydrophilic, adding PEG to a SPG aqueous solution should perturb the interaction between water and the schizophyllan side chain.

Change in optical rotatory dispersion by adding PEG to schizophyllan aqueous solutions.—Fig. 3 demonstrates typical examples of the ORD curves at three different temperatures; the phase-separated (0.5 °C), transition (4 °C), and homogeneous (7 °C) states for a SPG solution, as well as the three mixtures with PEG70K with different PEG concentration. At 7 °C, all samples show the same ORD curves, indicating that addition of PEG causes no perturbation. At 4 °C, the curve for $C_{\mathrm{PEG}} = 0.90$ g/dL shows an abnormal divergence in the lower-wavelength region. Since the solution became visibly opaque and the photodetector voltage exceeded the dynamic range, this divergence is ascribed not to real optical rotation but to scattering due to the turbidity[2]. For the other ORD curves of $C_{\mathrm{PEG}} = 0.008$ and 0.04 g/dL at this temperature, the data appreciably deviate downward from the schizophyllan curve in the 240–310 nm range. The deviation becomes more prominent at 0.5 °C for both the solution mixtures. This feature indicates that added PEG alters the nature of the water molecules bonded to schizophyllan. The temperature dependence of the ORD curves is consistent with that of the cloud points described in the previous section. Therefore, we can conclude that the same origin that leads to the ORD change is associated with the phase-separation behavior, which cannot be rationalized only by the mixing entropy.

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[2] Jasco J-720 VII manual, 1985.

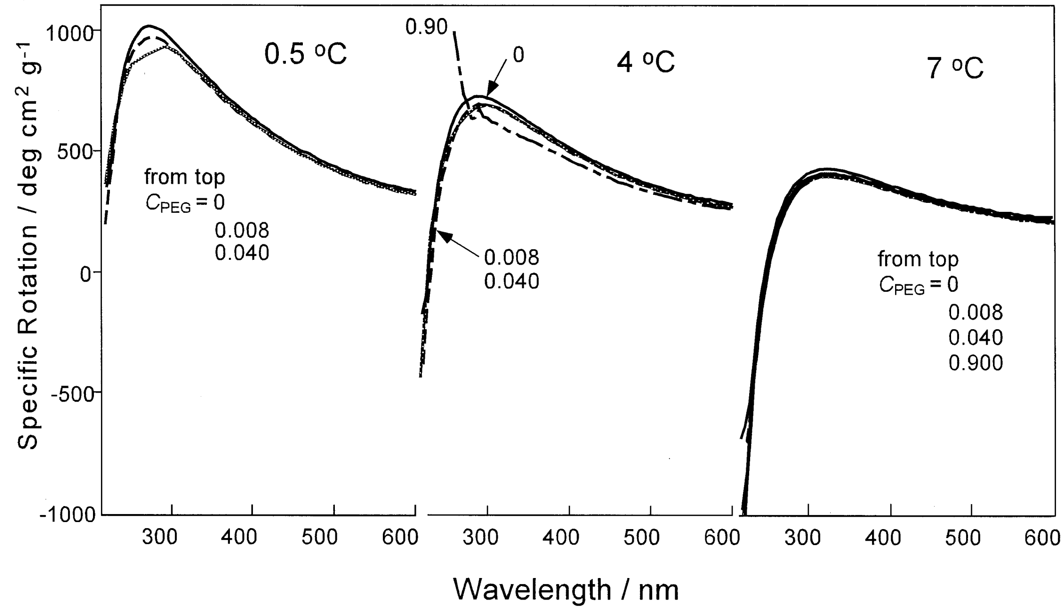

Fig. 3. Change in ORD curves with increasing temperature from the phase-separated state (0.5 °C) to the transition (4 °C) and then to the homogeneous state (7 °C). The curves for a SPG aqueous solution and three mixtures of SPG + PEG70K with $C_{PEG} = 0.008$, 0.040, and 0.90 g/dL are presented.

Solvent effect of the cloud temperature.— Since hydrogen bonds are responsible for the ORD change, the change undergoes solvent effects. The remarkable one is the isotope effect, where the transition temperature increases by about 15 °C on changing the solvent from H_2O to D_2O [8]. Fig. 4 shows the isotope effect on the cloud temperature for a mixture of SPG and PEG70K with $C_{SPG} = 1.01$ and $C_{PEG} = 1.09$ g/dL. In accordance with the ORD transition temperature increasing (lower part of the Figure), the cloud temperature increases by about 10 °C. This feature confirms the above mentioned-conclusion.

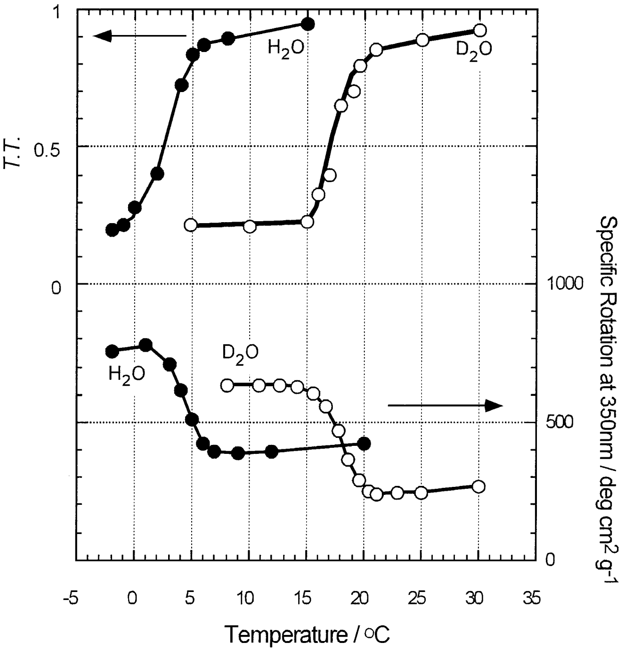

Fig. 4. Isotope effect of the cloud temperature for a SPG + PEG70K mixture with C_{PEG} and C_{SPG} is about 1.0 g/dL. For comparison, the optical rotation at 350 nm is plotted against temperature for SPG D_2O and H_2O solutions.

4. Conclusions

The mixture of schizophyllan and poly(ethylene oxide) in aqueous solution underwent phase separation around 3–4 °C, and this temperature was independent of both the polymer concentration and molecular weight. The phase separation took place at the same temperature at which the ORD changed. Since the change in ORD is ascribed to the difference in the nature of hydrogen bonding between the schizophyllan's side-chain and water, the phase separation is also considered to be associated with the interaction between poly(ethylene oxide) and schizophyllan. The isotope effect observed for the phase separation confirms the specific interaction essential for it to take place.

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