A Cooperative Order-Disorder Transition of Carboxylated Schizophyllan in Water-Dimethylsulfoxide Mixtures

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Abstract

Carboxylated schizophyllan ('sclerox') is a chemically modified polysaccharide obtained by partial periodate oxidation and subsequent chlorite oxidation of schizophyllan, a water-soluble neutral polysaccharide having a β-1,3-linked glucan backbone and a β-1,6-linked D-glucose residue side chain at every third residue of the main chain. The triple helix of schizophyllan in water has a cooperative order-disorder transition associated with the side chains. The transition is strongly affected by the presence (mole fraction) of dimethylsulfoxide (DMSO). In the present study, the solvent effects on the order-disorder transition of sclerox with different degrees of carboxylation (DS) in water-DMSO mixtures were investigated with differential scanning calorimetry and optical rotation. The transition temperature (T_r) and transition enthalpy (ΔH_r) strongly depended on the mole fraction of DMSO (x_D). Data were further analyzed with the statistical theory for the linear cooperative transition, taking into account the solvent effect, where DMSO molecules are selectively associated with the unmodified side chains. The modified side chain does not contribute to the transition, hence ΔH_r decreases with increasing DS. The dependence of T_r on the DMSO content becomes weaker than that for unmodified schizophyllan. The theoretical analyses indicated that the number of sites binding with

the DMSO molecule and the successive ordered sequence of the ordered unit of the triple helix are changed by carboxylation.

1. Introduction

Schizophyllan is an extracellular polysaccharide consisting of three β-1,3-glucoses and one branched β-1,6-glucose of the repeating unit produced by the fungus *Schizophyllum commune*.[1,2] Scleroglucan is an analogue of schizophyllan elaborated by *Sclerotium rolfsii*.[3] Norisuye *et al.* showed that schizophyllan dissolves in water as a triple helix, which has a quite rigid structure modeled by the wormlike chain with the persistence length over $q=150$ nm.[4-6] The triple helix of these polysaccharides has two kinds of the order-disorder transition in aqueous solution, namely the cooperative order-disorder transition of the side chains at low temperatures,[7-11] and the dissociation of the triple helix at high temperatures.[4,12,13] The cooperative order-disorder transition, or the triple helix I- triple helix II transition, is one of the unique properties of the triple helical conformation of schizophyllan. This transition is thermally reversible, and the transition temperature and the transition enthalpy depend strongly on the molar mass and the solvent. The molar mass dependence is an important feature of this transition, which is different from the dissociation of the triple helix. Itou *et al.*[8,9] analyzed the molar mass dependence of the heat capacity and optical rotation of the transition with the statistical thermodynamic theory for the linear cooperative transition. They concluded for the molecular mechanism of this transition that the ordered triple helix (triple helix I) is formed between the side chain and solvent molecules at low temperature, and the structure is destroyed cooperatively into the disordered triple helix (triple helix II) through the partially ordered triple helix. Therefore, the polysaccharide chains maintain the triple helical conformation in this transition region. The solvent effect is also remarkable; the transition temperature is shifted to higher temperatures by changing the solvent from H_2O to D_2O,[9] or their mixtures with DMSO.[14,15] Hirao *et al.*[15] analyzed the heat capacity and optical rotation data of schizophyllan in water-DMSO and D_2O-DMSO mixtures and concluded that DMSO molecules are selectively associated to the side chains, and that the ordered structure is stabilized by the association with DMSO molecules. Kitamura *et al.*[16] showed that the degree of branching of β-1,6-D-glucose affects the transition temperature and transition enthalpy. However, this effect has not been further

elucidated, because there was no theoretical approach enough to express the effects of the side chain structure on the transition.

Chemical modification may provide another insight to understand the molecular mechanism for this transition. Oxidation is a useful reaction to synthesize chemically modified derivatives of schizophyllan. Periodate oxidation is one of the important reactions in polysaccharide chemistry, which have been used for the determination of the primary structure of polysaccharides and preliminary reactions to synthesize their derivatives.[17] In the case of schizophyllan, the branched β-1,6-D-glucoses can be selectively oxidized into the ring-opened dialdehyde side chains because of the resistance of the main chain to periodate oxidation.[18-20] Furthermore, subsequent chlorite oxidation can convert the dialdehyde side chain into the corresponding dicarboxylates. [21-28] Carboxylated schizophyllan ('sclerox') is an ionic polysaccharide showing different solution properties from parent schizophyllan.

In previous studies on sclerox solutions, it was shown that sclerox at low degree of carboxylation (*DS*) retains a triplex conformation in aqueous solution.[27] However, the conformation of the triplex differs from the parent schizophyllan as the chain stiffness decreases and the helical pitch becomes larger. On the other hand, the modification of the side chains strongly affects the order-disorder transition.[28] The major differences of the transition behavior between the parent and modified samples may be summarized as follows: The transition temperature is shifted to lower temperatures, while the enthalpy of transition decreases with increasing *DS*. The differences are ascribed to the fact that the modified side chains cannot adopt the ordered structure.

In the present study, we studied the solvent effects on the order-disorder transition of sclerox in water-dimethylsulfoxide (DMSO) mixtures using differential scanning calorimetry and optical rotation. It is shown that the transition temperature (T_r) and transition enthalpy (ΔH_r) both depend drastically not only on *DS*, but also on the DMSO content of the (aqueous) solvent. The DSC and optical rotation data were analyzed with the statistical thermodynamic theory for the linear cooperative transition theory. The solvent association between the side chains and DMSO molecules was introduced into the theory to express the reduction of the number of sites binding with the DMSO molecule after the carboxylation of schizophyllan.

2. Experimental Section
2.1. Schizophyllan and Carboxylated Schizophyllan

A stock schizophyllan sample supplied from Taito Co. (now Mitsui Sugar Co. Ltd., Tokyo, Japan) was dissolved in water, and the 1.0 % (w/v) solution was sonicated with Branson Sonifier 250 (Branson Ultrasonics Co., Danbury, USA). The sonication was performed in ice bath for 6 hour and 14 hour to obtain two schizophyllan samples with different degrees of polymerization. The sonicated samples were further purified by the fractional precipitation in the aqueous solution with ethanol as a precipitant to obtain the middle fractions (SPG-a and SPG-b). The schizophyllan samples were freeze-dried, and dried *in vacuo* overnight before use. The fractionated sample was oxidized with sodium periodate (Wako Chemical Co., Osaka, Japan) and subsequent oxidized with sodium chlorite (Kanto Chemical Co., Tokyo, Japan) to obtain the carboxylated derivative ('sclerox'). The detailed sample preparation was described in our previous study on sclerox.[23,27,28] The sclerox samples were designated as SC-a-*X* and SC-b-*X* from the corresponding parent samples, SPG-a and SPG-b, where *X* represents the molar ratio of periodate to the side chain of schizophyllan in the oxidation. The degree of substitution of the samples was determined from potentiometric titration with 0.01 M NaOH containing 0.1 M NaCl after the deionization immersed with Amberlite 120-B (Organo Co., Japan).

2.2. Size Exclusion Chromatography Combined with a Multi-Angle Light Scattering Detection (SEC-MALS)

Size exclusion chromatography combined with a multi-angle light scattering detection (SEC-MALS) was performed to determine the number average molar mass (M_n) and weight average molar mass (M_w) for schizophyllan and sclerox samples. Three SEC columns, TSKgel PWH (precolumn), TSKgel G6000PW$_{XL}$ and G5000PW$_{XL}$ (Tosoh Co., Tokyo, Japan), were serially connected in front of DAWN HELEOS II MALS detector (Wyatt technology Co., USA). 0.15 M NaNO$_3$ was used as an eluent. Optilab-TrEX (Wyatt technology Co., USA) was used for the differential refractive index (RI) detector. The column temperature was kept at room temperature and the flow rate was fixed at 0.8 ml min^{-1}. The sample solution (0.5 mg ml^{-1}) in 0.15M NaNO$_3$ with 0.01 M NaOH was filtered with MILEX disposable filter (Millipore Co., US; pore size, 0.80 μm), and injected to SEC system (loop volume, 100 μl). The elution curve obtained from MALS and RI detectors was used to calculate M_n and M_w with ASTRA version 6.0 software (Wyatt technology Co., USA). The refractive index increment, $(\partial n/\partial c)_\mu$ =0.141 cm^{3}g^{-1}, was used to calculate M_n and M_w. Table I summarizes M_n and M_w determined by SEC-MALS. The degree of

carboxylation (*DS*) determined from the potentiometric titration is included in Table 1.

2.3. Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was performed on a Pyris 1 differential scanning calorimeter (Perkin Elmer Co., USA). The freeze-dried sample was put into a large volume stainless steel pan (Perkin Elmer Co., USA) and solvent was added into the pan. The measurements were performed at the scanning rate of 2.0 K min^{-1} with the solvent as a reference at a nitrogen gas flow of 20 ml min^{-1}. The temperature scale of the calorimeter was calibrated with the melting temperature of cyclohexane and indium and the heat flow was corrected with the enthalpy of fusion of indium. The measurement was operated by a Pyris$^{®}$ software (Perkin Elmer Co., Massachusetts, USA).

2.3. Optical Rotation

Optical rotation measurements were performed with a P-1000 digital polarimeter (JASCO Co., Tokyo, Japan) at the wavelength of 589 nm. The freeze-dried sample was dissolved in water-DMSO mixture at 1.00 *wt*%. The solution was filled in a cylindrical cell of the optical pass length of 5.0 cm with a water jacket. The cell was thermo-stated by circulating water and the temperature was recorded by thermocouples attached on the cell wall. The density of solution was calculated from the density of DMSO-H_2O mixture[29] and partial specific volume of schizophyllan (v_{sp}=0.619 cm^{3}g^{-1})[4].

3. Results

Figure 1A shows the DSC curves of schizophyllan (SPG-a, red) and SC-a-0.4 (blue) and SC-a-1.0 (green) samples in water-DMSO mixtures, where x_D represents the molar fraction of DMSO in the solvent. A well-defined peak was found in the DSC curves, corresponding to the order-disorder transition of schizophyllan.[14,15] The transition temperature (T_r) was taken as the peak of the DSC curve. The baselines were determined from the linear portions of the DSC curves at the low and high temperature sides of the peak to calculate the transition enthalpy (ΔH_r). With increasing x_D, T_r is shifted to higher temperature, and ΔH_r becomes larger. However, the sclerox samples have lower transition temperature than those of the parent schizophyllan. The DSC curve became broader with increasing *DS*. These effects of carboxylation are consistent with previous results on D_2O solution of sclerox.[28]

Figure 1B shows DSC results for SPG-b (red) and SC-b-0.4 (blue) in DMSO-water mixtures. T_r for SPG-a and SPG-b at x_D=0.05 was 295.0 K and 293.2 K, respectively. Slightly lower T_r of SPG-b is ascribed to the molar mass dependency of this transition. SC-b-0.4 has broader DSC curves due to the effect of carboxylation, as mentioned above. These DSC curves were reproduced by repeated runs in all the samples, which indicate that the transition is thermally reversible, as reported in our previous study on D_2O solution of sclerox.[28]

Table 1. The number average molar mass (M_n), the weight average molar mass (M_w), and degree of carboxylation (DS) of schizophyllan and carboxylated schizophyllan (sclerox) samples

Sample	M_n / g mol^{-1}	M_w / g mol^{-1}	DS
SPG-a	203,000	247,000	0
SC-a-0.4	199,000	249,000	0.25
SC-a-1.0	155,000	217,000	0.32
SPG-b	104,000	152,000	0
SC-b-0.4	96,000	143,000	0.21
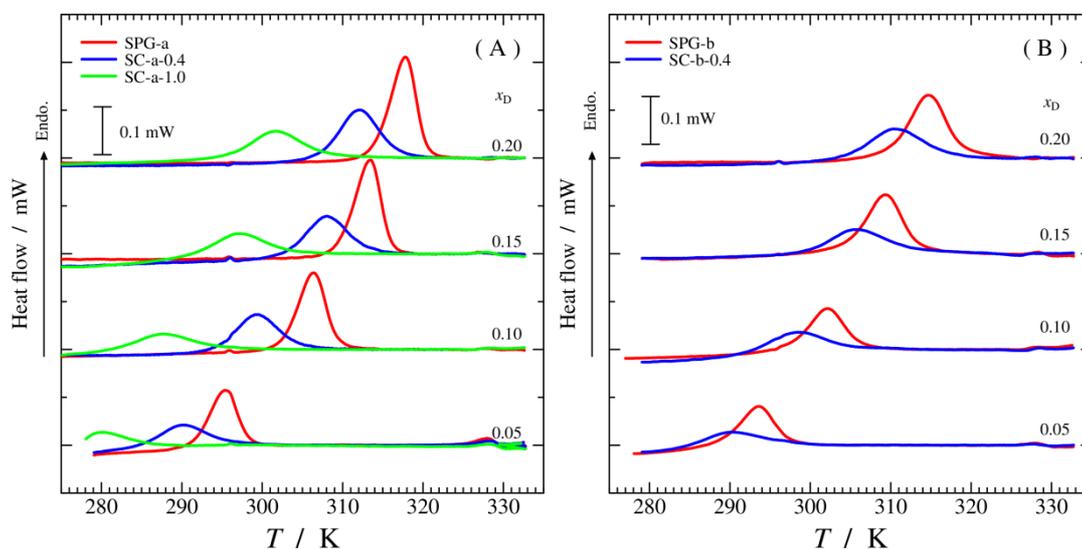
Figure 1. DSC curves for schizophyllan and sclerox in water + dimethylsulfoxide (DMSO) mixtures at indicated mole fractions, x_D, of DMSO (10 *wt*%): A, SPG-a, SC-a-0.4 and SC-a-1.0; B, SPG-b and SC-b-0.4. DSC curves are vertically shifted for clarity.

Table 2. The transition temperatures (T_r) and transition enthalpies (ΔH_r) of the order-disorder transition of schizophyllan (SPG-a, SPG-b) and sclerox (SC-a-*X* and SC-b-*X*) in DMSO-H$_2$O mixtures.

	SPG-a		SC-a-0.4		SC-a-1.0	
x_D	T_r / K	ΔH_r / Jg^{-1}	T_r / K	ΔH_r / Jg^{-1}	T_r / K	ΔH_r / Jg^{-1}
0.050	295.0	6.8	289.6	4.8	279.5[a]	
0.100	305.9	8.7	298.6	6.3	287.1	4.7
0.150	313.1	9.8	306.8	6.8	296.1	5.8
0.201	317.6	10.4	311.3	7.4	300.6	6.2
0.100 (OR)	302.0		298.1		287.9	

|	SPG-b		SC-b-0.4			
x_D	T_r / K	ΔH_r / Jg^{-1}	T_r / K	ΔH_r / Jg^{-1}		
0.050	293.2	6.3	289.9	3.8		
0.100	301.7	7.2	298.0	4.8		
0.150	309.1	9.4	305.1	6.3		
0.201	314.2	10.1	310.0	6.7		

a, T_r only determined

Figure 2 shows the temperature dependences of the specific optical rotation, ($[\alpha]_{589}$) for SPG-a, SC-a-0.4 and SC-a-1.0 in water-DMSO mixtures at x_D=0.1. SPG-a has an abrupt change of $[\alpha]_{589}$ around 302 K, which is attributed to the order-disorder transition. The linear portions above and below the transition zone define the baselines for the ordered state ($[\alpha]_{ord}$) and the disordered state ($[\alpha]_{dis}$), respectively. In previous studies on schizophyllan solutions,[8-10] $[\alpha]_{dis}$ was determined by least square fitting, whereas $[\alpha]_{ord}$ was taken to be parallel to $[\alpha]_{dis}$. The fraction of the ordered units (f_N) was subsequently calculated according to Eq. 1.

$$f_N = \frac{1}{1-DS}\left(\frac{[\alpha]_{589}-[\alpha]_{dis}}{[\alpha]_{ord}-[\alpha]_{dis}}\right) \qquad (1)$$

The transition (changes in $[\alpha]_{589}$) for SC-a-0.4 and SC-a-1.0 shifted to lower temperatures, and the $[\alpha]_{589}$ curves became broader, with increasing *DS*. The

transition temperature was defined as the temperature corresponding to 50% disorder (midpoint).[30,31] Values were consistent with those obtained by the DSC (Table 2)
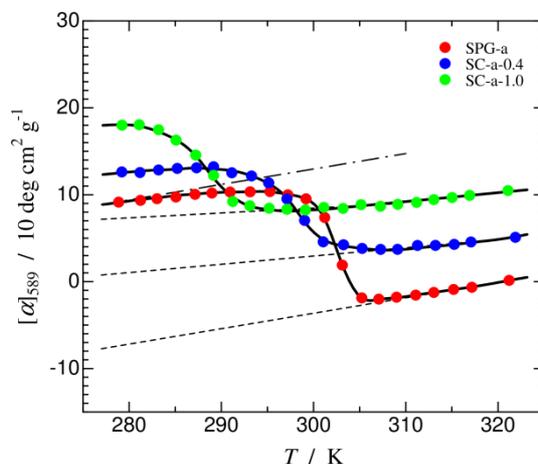
Figure 2. Optical rotation data for SPG-a, SC-a-0.4 and SC-a-1.0 (1.00 *wt*%) at x_D=0.10. The dashed lines are the baselines for the disordered state, and the dash-dotted line is the baseline for the ordered state. The solid curves represent guides to the eye.

4. Discussion

4-1. Statistical Thermodynamic Theory for the Linear Cooperative Transition in the Mixed Solvent System

The order-disorder transition of the schizophyllan solution is a typical cooperative phenomenon, which has a characteristic molar mass dependency. This feature has been described by the linear cooperative transition (LCT) theory,[32] where the transition can be characterized by the degree of polymerization (*N*), the transition temperature at infinite *N* (T_r^{∞}), the transition enthalpy at infinite *N* (ΔH_r^{∞}) and the cooperativity parameter ($\sigma^{1/2}$). For schizophyllan in water-DMSO mixtures, T_r^{∞} and ΔH_r^{∞} depend strongly on the DMSO composition in the mixed solvent.[14] The solvent effect on LCT was first formulated by Schellman[33] and Peller[34] for the helix-coil transition of synthetic polypeptides. Hirao *et al.*[15] further analyzed the stabilizing effect of DMSO on the order-disorder transition of schizophyllan. Here, DMSO molecules were assumed to interact favorably with the side chains of schizophyllan to stabilize the ordered structure.

For sclerox, the interaction between the side chain and DMSO may be altered by

8

periodate and subsequent chlorite oxidations. The chemical modification into dicarboxy residues may reduce the number of sites binding with the DMSO molecule on the triple helix. Therefore, the LCT theory should be extended to introduce the effect of the chemical modification on the order-disorder transition of sclerox in water-DMSO mixtures.

Let us first consider the association−dissociation equilibrium of DMSO molecules onto associating sites of the schizophyllan triple helix. Assuming that DMSO molecules associate randomly onto the associating sites in the ordered state, the temperature dependent degree of association $\xi_{ass}(T)$ for the triple helix may be expressed by

$$\xi_{ass}(T) = \frac{K(T)x_D}{1 + K(T)x_D}$$

(2)

where $K(T)$ and x_D are the equilibrium constant for association and the mole fraction of DMSO in the solvent, respectively. The equilibrium constant $K(T)$ may follow the van't Hoff type of the temperature dependence,

$$K(T) = B \exp\left(-\frac{\Delta H_{a-m}}{RT}\right)$$

(3)

where R, T and ΔH_{a-m} represent the gas constant, the absolute temperature, and the sum of the contributions from the enthalpy of association and the partial molar enthalpy of mixing, *i.e.* the enthalpy change in exchanging associating molecules from the solvent medium to the associating sites. The pre-exponential factor B is an arbitrary constant. For the sclerox triple helix, the degree of association may reduce to $(1 - DS)\xi_{ass}(T)$, because oxidized side chains cannot interact with DMSO molecules. (Figure 3)
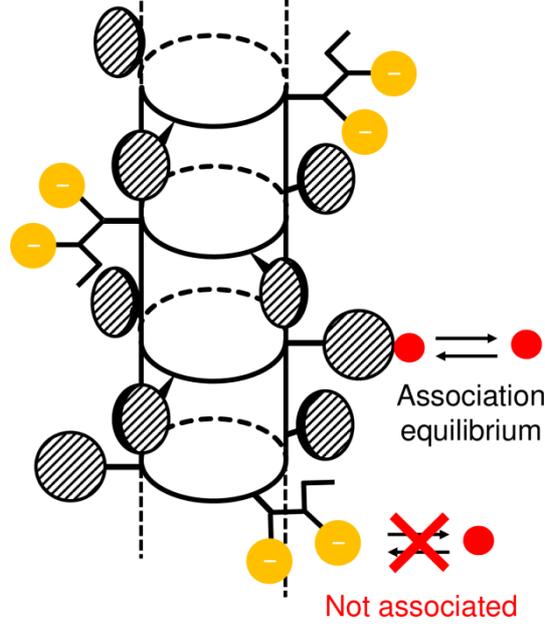
Figure 3. Schematic illustration for the order-disorder transition of carboxylated schizophyllan. The central cylinder represents the triple helical core, and hatched disks are the side chains. The carboxylated side chains do not interact to associating molecules (red circles) in this model.

Sayama *et al.*[35] discussed the helix-coil transition of polypeptides in a mixed solvent, where the transition is accompanied with the association-dissociation equilibrium of one solvent component. Applying their discussion to our system, the enthalpy change ΔH_r^∞ (per transition unit) at the disorder to order transition of the infinitely long sclerox triple helix is given by

$$\Delta H_r^\infty = \Delta H_{r,0}^\infty - (1-DS)\xi_{ass}(T)\Delta H_{a-m}$$

(4)

Here, $\Delta H_{r,0}^\infty$ is ΔH_r^∞ at $DS = 0$.

According to Bixson and Lifson,[36] the statistical weight for the ordered unit in the mixed solvent system can be expressed by

$$s(T,x_D) = \left[\frac{s_0}{1-(1-DS)\xi_{ass}(T)}\right]^3$$

(5)

Here, it is worthwhile to clarify the consistency with our previous study on D_2O solutions of sclerox,[28] that is, s_0 can be written by the statistical weight for the triplex unit, s, related with

$$\ln s(T,0) = \ln s_0^3 = \frac{3\Delta H_{r,0}^\infty}{R}\left(\frac{1}{T} - \frac{1}{T_{r,0}^\infty}\right)$$

(6)

10

where $T_{\mathrm{r},0}{}^{\infty}$ is the transition temperature at $x_{\mathrm{D}} = 0$ for infinite N. For the mixed solvent system, eq. 5 satisfies the following condition at $T_{\mathrm{r}}{}^{\infty}$;

$$\frac{\Delta H_{r,0}^{\infty}}{R}\left(\frac{1}{T_r^{\infty}} - \frac{1}{T_{r,0}^{\infty}}\right) = \ln\left[1 - (1 - DS)\xi_{ass}(T_r^{\infty})\right]$$

(7)

The theoretical parameters at $x_{\mathrm{D}}=0$ are available from our previous study on schizophyllan solution ($T_{\mathrm{r},0}{}^{\infty} = 280.2$ K, $\Delta H_{\mathrm{r},0}{}^{\infty} = 3.5$ kJmol^{-1}, and $\sigma^{1/2} = 0.01$),[37] so that Eq. 7 provides $T_{\mathrm{r}}{}^{\infty}$ and $\Delta H_{\mathrm{r}}{}^{\infty}$ for the mixed solvent system in addition to eq. 4 at the set of B and $\Delta H_{\mathrm{a\text{-}m}}$.

From $T_{\mathrm{r}}{}^{\infty}$ and $\Delta H_{\mathrm{r}}{}^{\infty}$ in the mixed solvent system, the fraction of the ordered unit, f_N, can be calculated by the LCT theory. According to the LCT theory, the general expression of the partition function for the linear polymer is represented by[32]

$$Z_N = \left(au_{\mathrm{M}} \quad u_{\mathrm{P}}\right)\begin{pmatrix} u_{\mathrm{M}} & vu_{\mathrm{P}} \\ vu_{\mathrm{M}} & u_{\mathrm{P}} \end{pmatrix}^{N-2}\begin{pmatrix} b \\ 1 \end{pmatrix}$$

(8)

where u_{M}, u_{P} are the statistical weights for **M** and **P** state, and v represents the transition probability to turn **M** state to **P** state. The coefficients, a and b characterize the type of the transition. In the case of the order-disorder transition of the aqueous schizophyllan solutions, **M** and **P** states express the ordered and disordered states, respectively, and then the statistical weights, u_{M}, u_{P}, and v are replaced to s, 1, and $\sigma^{1/2}$, respectively. Furthermore, the coefficients a and b can be put as 0 and 1, because the terminal units are restricted at the disordered units throughout the transition from the molecular consideration. The fraction of the ordered unit, f_N, is represented by

$$f_N(T;N) = \frac{1}{N}\frac{\partial \ln Z_N}{\partial \ln s}$$

(9)

The detailed expression for this equation was given by Teramoto,[32] which allows us to compute the numerical value of f_N. The transition heat capacity, ΔC_{s}, can be calculated from

$$\Delta C_s = -(1 - DS)\Delta H_r^{\infty}\left(\frac{\partial f_N}{\partial T}\right)$$

(10)

Therefore, the transition temperature (T_{r}) for finite N can be determined from the peak maximum of ΔC_{s}, so that the peak maximum from DSC curve can be compared to confirm the analysis.

4-2 Analysis of the Order-Disorder Transition of Sclerox in the Mixed Solvents

Kitamura *et al.*[14] showed that T_r of the order-disorder transition of the schizophyllan in DMSO-water mixture increases with the weight fraction of DMSO (w_D) whereas T_r decreases at $0.7 < w_D < 0.87$, and finally T_r converges to the dissociation of the triple helix at $w_D = 0.87$. The same phase diagram was observed in lentinan, another β-1,3-D-glucan with different degree of branching.[38] They considered that the change of the dependence of T_r on w_D result in a specific hydration of DMSO with water molecules and dissociation of the triple helix. In our range of x_D ($0 < x_D < 0.2$) and T, the specific hydration and dissociation of the triple helix are not so important for the analysis of this transition. Since the parameters, B and $\Delta H_{a\text{-}m}$ are already known for schizophyllan solutions in DMSO-water system,[15] a set of T_r^∞ and ΔH_r^∞ at any x_D can be given from eq. 7.

Figure 4 shows T_r determined as the peak temperature in the DSC curves plotted as a function of x_D, where the solid curves represent calculated ones for $B = 0.0008$ and $\Delta H_{a\text{-}m} = -18.9$ kJmol^{-1} with $N = 104$ for SPG-a, $N = 76$ for SC-a-0.4 and $N = 29$ for SC-a-1.0. Here, it was assumed for the present analysis that $\sigma^{1/2}$ is temperature-independent to calculate f_N; $\sigma^{1/2} = 0.01$.[15] N for SPG-a was determined from M_n, whereas N for the others was fitted as an adjustable parameter. This situation is the same as our previous work on the D_2O solutions of sclerox,[28] that is, the transition unit of three carboxylated side chains cannot take the ordered state throughout the transition. The sequence of the consecutive ordered unit is reduced by this unit, the permanent disordered unit, produced in the sclerox trimer. Consequently, N for SC-a-0.4 and SC-a-1.0 becomes smaller than SPG-a. The calculated curves are illustrated by the solid curves, where each curve has slightly different curvatures. These indicate that the reduction of the associating sites after the carboxylation results in the solvent effects of SC-a-0.4 and SC-a-1.0 different from those of parent SPG-a.

Figure 5 shows the transition enthalpy, ΔH_r, for SPG-a, SC-a-0.4 and SC-a-1.0 in water-DMSO mixtures. At low x_D, ΔH_r data abruptly increases, and then the dependence becomes monotonous at higher x_D. The solid curves are the theoretical curves calculated by eq. 4 with $B = 0.0008$ and $\Delta H_{a\text{-}m} = -18.9$ kJmol^{-1}. The deviation between the data and theory becomes large especially at low x_D, where ΔH_r data may be underestimated by a curve tailing at low temperature side of the DSC curve. This error may be corrected by $f_N(275K) \times \Delta H_r$; $f_N(275K)$ is the value of f_N at 275 K, which corresponds to the initial temperature for the integration for ΔH_r. The $f_N(275K)$ can be

calculated from eq. 9. The dashed curves are the corrected curve of $f_N(275K) \times \Delta H_r$. The corrected curves well express the ΔH_r data. These results indicate that the ordered sequence of the unmodified side chains remains partially intact after oxidation at low *DS*, while the carboxylated side chains cannot participate in the ordered structure of sclerox trimer.
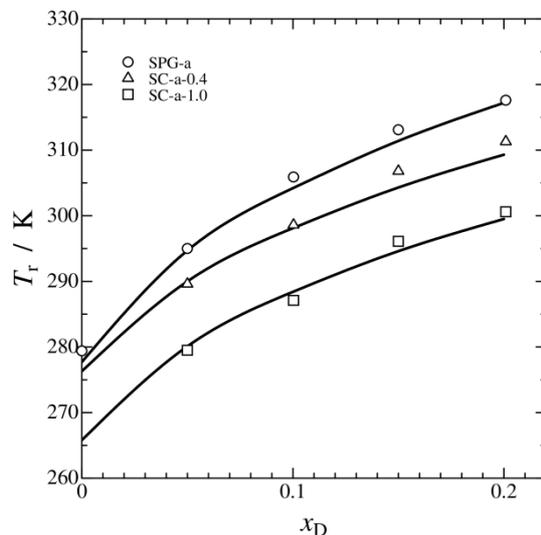
Figure 4. Analysis of T_r for SPG-a, SC-a-0.4 and SC-a-1.0 in water-DMSO mixtures. Solid curves are calculated from LCT theory; N=104 for SPG-a, N=76 for SC-a-0.4 and N=29 for SC-a-1.0. T_r^∞ and ΔH_r^∞ were determined from eq. 4 and eq. 7 with B=0.0008 and ΔH_{a-m} = -18.9 kJ mol^{-1}. T_r at x_D=0 is taken from the reference.[37]
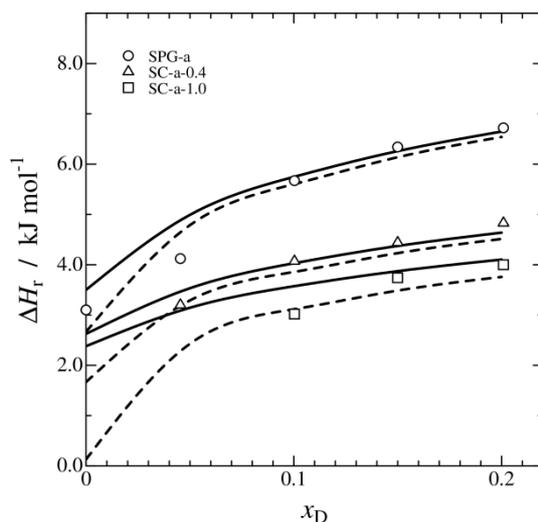

Figure 5. Analysis of ΔH_r for SPG-a, SC-a-0.4 and SC-a-1.0 in the solution containing DMSO. Solid curves were calculated by eq. 4 with B=0.0008 and ΔH_{a-m} = -18.9 kJ mol^{-1}. Dashed curves represent the corrected curves of ΔH_r multiplied with

f_N(275K). ΔH_r at x_D=0 is taken from the reference.[37]

Figure 6 demonstrates the comparison of T_r for their molar mass dependence between schizophyllan samples (SPG-a and SPG-b), and sclerox samples (SC-a-0.4 and SC-b-0.4) in water-DMSO mixtures. These samples have different molar masses, whereas two sclerox samples have almost the same DS. T_r for SPG-a strongly depends on x_D, reflecting the solvent effects, as mentioned above. SPG-b has almost parallel dependency of T_r, but these are slightly lower T_r than SPG-a. The solid curves represent the theoretical curves with the association parameters, B=0.0008 and $\Delta H_{a\text{-}m}$ = -18.9 kJ mol^{-1} for N=104 and 54 (DS=0), which are determined from their M_n. On the other hand, SC-a-0.4 and SC-b-0.4 have weaker dependencies than those for SPG-a and SPG-b. Dashed curves are the theoretical curves for SC-a-0.4 (N=76) and SC-b-0.4 (N=50) with B=0.0008 and $\Delta H_{a\text{-}m}$ = -18.9 kJ mol^{-1}. N is decreased by carboxylation from N=104 to N=76 for SC-a-0.4 and from N=54 to N=50 for SC-b-0.4, which can be considered as the number of sequence of the ordered unit of the triple helix of sclerox. As mentioned in our previous study,[28] the permanent disordered unit is less produced in low molar mass sample, because the numbers of the modified side chains per a trimer is smaller than that of the high molar mass sample. From above analyses, we may point out that B is quite small (B=0.0008). This means that DMSO molecules are selectively associated to the unmodified side chain of the sclerox trimer, because the modified side chains are inactive for both of the transition and association.

The shape of the DSC curves in Figure 1A and 1B were almost independent of x_D. This suggests that the molecular mechanism of the transition is essentially unchanged by x_D. Therefore, we can use a complete set of parameters for the calculation of f_N with eq. 9 from the above analyses. Figure 7 demonstrate comparisons of f_N curves at x_D=0.1 calculated with LCT theory. Here, the solid curves were calculated on eq. 9 and the parameters (T_r^∞, ΔH_r^∞) were determined from eq. 4 and eq. 7, and f_N data were determined from the optical rotation data. With increasing DS, the f_N curve becomes broader like a shorter triple helix of schizophyllan. This effect can be expressed by using smaller N in the above analyses. The theoretical curves well express the change of the f_N curves for the sclerox solutions after the carboxylation. The periodate oxidation alters the side chain glucoses into carbonyl side chains, which is further oxidized at chlorite oxidation into carboxylated ones. If the oxidations are

14

done ideally, these modified side chains are distributed randomly in the trimer, which cannot be associated with DMSO molecules. The permanent disordered unit produced by carboxylation acts as the terminals of the successive ordered sequence.[28] Therefore, the effective N for the transition becomes smaller than parent schizophyllan. On the other hand, the unmodified side chains can take the ordered structure with DMSO molecules, which are strongly associated with the unmodified side chains compared with water molecules in the solvent. Indeed, $\Delta H_{a\text{-}m}$ has a large negative value ($\Delta H_{a\text{-}m}$ = -18.9 kJ mol^{-1}), increasing ΔH_r with x_D according to eq.4. The associated DMSO molecules stabilize the ordered state of the unmodified side chain in the sclerox trimer, so that T_r is shifted to higher temperature than that in water.
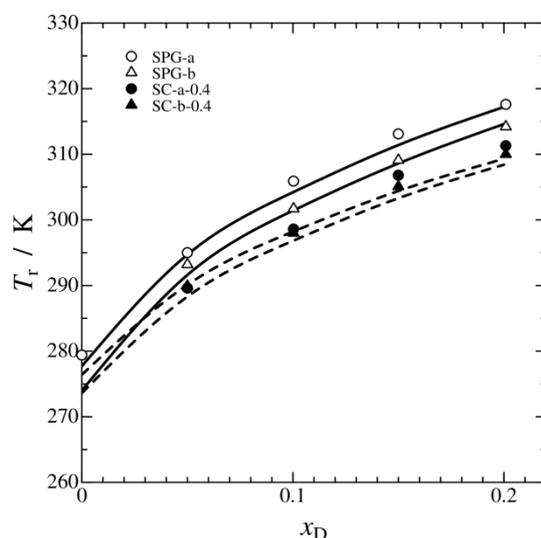
Figure 6. Comparison of T_r for SPG-a, SC-a-0.4, SPG-b, SC-b-0.4 in water-DMSO mixtures with theoretical curves. Solid curves are calculated from LCT theory; N=104 for SPG-a, N=54 for SPG-b. Dashed curves are N=76 for SC-a-0.4, N=50 for SC-b-0.4. T_r^{∞} and ΔH_r^{∞} used for the calculation were determined from eq. 4 and eq. 7 with B=0.0008 and $\Delta H_{a\text{-}m}$ = -18.9 kJ mol^{-1}.
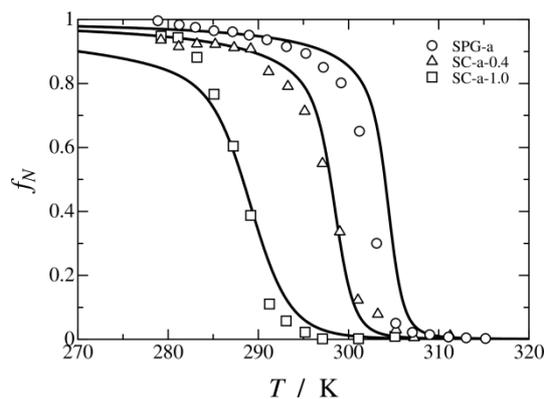
Figure 7. Comparison of f_N curves calculated with LCT theory: circles, SPG-a; triangles, SC-a-0.4; squares, SC-a-1.0. Solid curves are the theoretical curves with T_r^∞=305.96 K, ΔH_r^∞=5.75 kJmol^{-1}, $\sigma^{1/2}$=0.01 N=104 for SPG-a, T_r^∞=301.09 K, ΔH_r^∞=5.37 kJmol^{-1} $\sigma^{1/2}$=0.01 N=76 for SC-a-0.4, and T_r^∞=299.60 K, ΔH_r^∞=5.25 kJmol^{-1} $\sigma^{1/2}$=0.01 N=29 for SC-a-1.0. The values of T_r^∞ and ΔH_r^∞ were given from eq.7 with B=0.0008 and ΔH_{a-m} = -18.9 kJ mol^{-1}.

Conclusions

The effects of carboxylation on the order-disorder transition of a triple helical polysaccharide, schizophyllan, in water-dimethylsulfoxide mixtures were investigated by differential scanning calorimetry and optical rotation. The observed transition temperature and transition enthalpy depend on the degree of carboxylation of carboxylated schizophyllan, sclerox. The reduction of the number of sites binding with DMSO molecules on the sclerox triple helix is introduced into the linear cooperative transition theory to express the effects of the carboxylation on the transition and association for the sclerox solution. The carboxylated side chains are independent of both the transition and association, so that the transition enthalpy decreased with the degree of carboxylation. The transition temperature is dependent on the mole fraction of dimethylsulfoxide as a result of the reduction of the associating sites and the production of the permanent disordered unit of the triple helix after the carboxylation.

Satoka Okamoto for potentiometric titration. The authors declare no competing financial interest.

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TOC Graphic

Carboxylated schizophyllan (Sclerox)

Association equilibrium

Not associated