Effects of carboxylation of the side chains on the order-disorder transition in aqueous solution of schizophyllan, a triple helical polysaccharide

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Abstract

Schizophyllan and scleroglucan are water-soluble polysaccharides having repeating units consisting of three \(\beta-1,3\)-linked glucose residues in the main chain and a single \(\beta-1,6\)-linked glucose residue as the side chain. This polysaccharide dissolves as a triple helix in an aqueous solution and shows a cooperative order-disorder transition between the side chain and solvent molecules while retaining the triple helical conformation. Periodate and subsequent chlorite oxidations selectively modify the side chain glucose to provide the corresponding dicarboxylate units. Optical rotation measurements and differential scanning calorimetry were performed on carboxylated schizophyllan/scleroglucan (sclerox) samples to investigate the effects of the degree of carboxylation on the order-disorder transition in deuterium oxide with 0.1 M NaCl. The transition curves for the sclerox samples are strongly dependent on the degree of carboxylation. The modified side chains cannot take the ordered structure, resulting in a reduction of the transition enthalpy. The transition temperature for carboxylated schizophyllan becomes lowered and the transition curve broadens with increasing the degree of carboxylation. The permanent disordered units are included in a trimer by the carboxylation to inhibit a long sequence of the ordered units.

Keywords: schizophyllan, periodate oxidation, order-disorder transition, differential scanning calorimetry, optical rotation

Introduction

Schizophyllan is a water-soluble polysaccharide produced by Schizophyllum commune, which consists of tetrasaccharide repeating units containing three \(\beta-1,3\)-glucoses and one branched \(\beta-1,6\)-glucose (Kikumoto, Miyajima, Yoshizumi,
Scleroglucan is a structurally similar polysaccharide elaborated by a species of the genus *Sclerotium* (Johnson et al., 1963). These polysaccharides dissolve in water as triple helices, where three main chains form the triple helix and the side chain glucoses are arranged outward the helical core to ensure high solubility to water (Takahashi, Kobatake, Suzuki, 1984). The triple helix of schizophyllan and scleroglucan shows strong molar mass dependencies of radius gyration (Kashiwagi, Norisuye, Fujita, 1981; Yanaki & Norisuye, 1983) and intrinsic viscosity (Norisuye, Yanaki, Fujita, 1980; Yanaki, Norisuye, Fujita, 1980) in aqueous solutions. It was concluded on the analysis of the wormlike chain model that the triple helix is a quite rigid rod represented with a large persistence length above 150nm.

The schizophyllan triple helix has characteristic physical and biological properties in the aqueous solutions, e.g., high viscosity (Carriere, Amis, Schrag, Ferry, 1985; Enomoto, Einaga, Teramoto, 1985; Fang & Nishinari, 2003), liquid crystallinity (Van, Norisuye, Teramoto, 1980; Itou, Van, Teramoto, 1985), anticaner activity (Norisuye, 1985; Okamura et al, 1986), and reassembly with polynucleotides (Sakurai & Shinkai, 2000; Sakurai, Mizu, Shinkai, 2001; Mochizuki & Sakurai, 2009). A cooperative order-disorder transition is one of the characteristic features of the schizophyllan triple helix in aqueous solutions (Asakawa, Van, Teramoto, 1984; Itou, Teramoto, Matsuo, Suga, 1986; Kitamura & Kuge, 1989). The side chain forms an ordered structure along with hydrated water molecules in the ordered triple helix state (Triple helix I). As a temperature rises, the ordered structure is converted into the disordered triple helix (Triple helix II). The transition has a strong molar mass dependency because of its highly cooperative nature (Itou et al, 1986). In addition, the transition temperature and the transition enthalpy depend largely on the solvent conditions. The transition temperature shifts from 7°C in H₂O to 18°C in D₂O, and the transition enthalpy of the D₂O solution is larger than in H₂O (Itou, Teramoto, Matsuo, Suga, 1987). The same order-disorder transition was observed for other branched β-1,3-glucans, and the number of branching per the main chain glucose affects the order-disorder transitional behavior in DMSO-water mixtures (Kitamura, Ozawa, Tokioka, Hara, Ukai, Kuge, 1990; Zhang, Xu, Zhang, 2008; Wang, Zhang, Zhang, Ding, 2009), indicating that the hydration of the triple helix, especially the side chains and solvent molecules, plays an important role in the transition.

Chemical modifications generally alter the physicochemical and biological properties of the parent polysaccharide. Periodate oxidation is well known as an important reaction for the structural determination of polysaccharides (Perlin, 1980). This oxidation proceeds stoichiometrically with the glycol-cleavage reaction at the C-C bond of the vicinal diols or successive hydroxyl groups. Schizophyllan can be modified into ionic polysaccharide by periodate and subsequently chlorite oxidations (Crescenzi, Gamini, Paradossi, Torri, 1983; Gamini, Crescenzi, Abruzzese, 1984). The glucose ring of the side chain is selectively modified into a linear dialdehyde chain, whereas the main chain of schizophyllan is resistant to the periodate oxidation. Chlorite oxidation can further alter the aldehyde groups into carboxyl groups. These oxidations allow the preparation of different degrees of substitution (DS) of the carboxylated derivative. Carboxylated schizophyllan (Sclerox, Figure 1) is soluble in water and...
show different physical properties in aqueous solution from parent
schizophyllan, including polyelectrolyte properties (Coviello, Dentini, Crescenzi,
1995; Coviello, Dentini, Crescenzi, Vincenti, 1995), and related properties, such
as gelation with trivalent cations (Stokke, Elgsæter, Smidrød, Christensen,
1995). The solubility to water is related to the hydration of this modified
polysaccharide, which may influence the order-disorder transition. However, the
effect of chemical modification of the side chains of schizophyllan has not been
investigated for the order-disorder transition.

In previous studies on the order-disorder transition of schizophyllan, the
molecular mechanism and the molecular motion were studied by optical
rotation, adiabatic calorimetry and dielectric relaxation (Teramoto, Gu, Miyazaki,
Sorai, Mashimo, 1995; Hayashi, Shinyashiki, Yagihara et al, 2001; Yoshia, Ishino,
Teramoto Nakamura et al, 2002; Yoshia, Teramoto, Nakamura, Kikuchi,
modifications of polysaccharide side chains generally influence such transition
markers. For example, partial acid hydrolysis was used in a double helical
polysaccharide, xanthan, to investigate the influence of the removal of side
chains on the conformational transition of xanthan (Christensen, Knudsen,
Smidsrød, Kitamura, Takeo, 1993). Since the order-disorder transition of
schizophyllan is characteristic to the triple helical conformation (Kitamura &
Kuge, 1989), it is necessary to investigate both of the molecular conformation
and the transition behavior after the chemical modification. From
conformational aspects for sclerox, we showed that a sclerox at low DS remains a
trimer in 0.1 M NaCl (Yoshia, Sato, Osumi, Ulset, Christensen, 2015). However,
size-exclusion chromatography combined with multi-angle light scattering
detection (SEC-MALS) indicated the global conformation of the trimer is different
from the triple helix of parent schizophyllan by becoming more flexible and
extended after the carboxylation. In the present study, we report effects of
carboxylation of the side chains on the cooperative order-disorder transition of
sclerox. The sclerox samples with different DS were investigated by differential
scanning calorimetry and optical rotation to characterize the transition behavior
of sclerox. Since schizophyllan in aqueous solutions has a low transition
temperature of 7 °C, D2O was used as solvent to extend the temperature range of
the triple helix I. We added 0.1 M NaCl to essentially prevent the electrostatic
interaction between the trimers. Since the conformation of the sclerox trimer can
be determined from SEC-MALS measurements, the effects of chemical
modification on its specific hydration around the triple helix were analyzed.
**Figure 1** Chemical structure of the carboxylated schizophyllan/scleroglucan (Sclerox) by partial periodate and chlorite oxidation.

**Experimental**

**Schizophyllan and carboxylated schizophyllan samples**

The schizophyllan sample (Taito Co., now Mitsui sugar Co., Japan) was sonicated in an aqueous solution with a Branson Sonifier 250 (Branson Ultrasonics Co., Danbury, USA) to prepare two degraded schizophyllan samples. The sonicated sample was purified by the fractional reprecipitation in the aqueous solution with ethanol to obtain middle fraction of each sample (SPG-1, SPG-2). The carboxylation of the sample was performed in an aqueous solution according to previous study on sclerox (Yoshida et al., 2015). In brief, 30 mM aqueous sodium periodate (Wako Pure Chemical Industries Ltd., Osaka, Japan) was dropped into 120 ml of 0.1wt% aqueous schizophyllan containing 13.3 ml of 1-propanol to prepare different degree of oxidation of the samples. After dialysis against deionized water, sodium chlorite (Kanto Chemical Co. Inc., Tokyo, Japan) and acetic acid (Wako Pure Chemical Industries Ltd., Osaka, Japan) were added into the solution to be 0.5 M for each reagent. These oxidations were performed in dark at 10 °C for 24 hours. After oxidations, the solutions were dialyzed and neutralized with 0.01 M NaOH to obtain the sodium salt of the freeze-dried samples. The sclerox samples were coded as SC-1-X or SC-2-X, where X is denoted by the mole fraction of sodium periodate for the repeating unit of schizophyllan at the oxidation. The degree of substitution, DS, was determined by the potentiometric titration for the acid form of the sample with 0.01 M NaOH in aqueous 0.1 M NaCl after the immersion with Amberlite-120B (Organo Co., Ltd., Tokyo, Japan) for 24 hours. The degree of substitution of the samples was listed in Table 1.

**Size Exclusion Chromatography equipped with a Multi-Angle Light Scattering detector (SEC-MALS)**

The weight average molar mass and the molar mass dispersity of the samples were determined by the size exclusion chromatography equipped with a multi-angle light scattering detector (SEC-MALS). The SEC columns (SHODEX OHPak SB-G, two SHODEX OHPak SB806MHQ, Showa Denko KK, Kanagawa, Japan) were serially connected in the SEC system (GPC-101, Showa Denko KK, Kanagawa, Japan). The SEC system was connected with a DAWN HELEOS II MALS detector (Wyatt Technology Co., Santa Barbara, USA) and differential refractometer. Aqueous 0.1 M NaCl was used as an eluent. The flow rate was fixed at 1.0 cm³min⁻¹ and the columns were kept at 40 °C in the measurement. The solution dissolved in 0.1 M NaCl with 0.01 M NaOH was injected (injection volume, 0.1 cm³) after the filtration with DISMIC-25AS (pore size 0.45 μm, Toyo Roshi Co., Ltd., Tokyo, Japan) and the measurement was operated by ASTRA® ver. 5.3 software (Wyatt Technology Co., Santa Barbara, USA). The refractive index increment, \((\partial n/\partial c)\)=0.141 cm³g⁻¹, was used to calculate the weight average molar mass, \(M_w\), and molar mass dispersity \(M_w/M_n\). Table 1 summarizes \(M_w\) and \(M_w/M_n\) of the samples used in following measurements determined from the SEC-MALS measurements.
Table 1 The degree of substitution and SEC-MALS results for carboxylated schizophyllan and schizophyllan samples in aqueous 0.1 M NaCl.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DS</th>
<th>$M_w$ / g mol$^{-1}$</th>
<th>$M_w/M_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPG-1</td>
<td>0</td>
<td>$3.1 \times 10^4$</td>
<td>1.4</td>
</tr>
<tr>
<td>SC-1-0.2</td>
<td>0.16</td>
<td>$3.2 \times 10^4$</td>
<td>1.4</td>
</tr>
<tr>
<td>SC-1-0.4</td>
<td>0.20</td>
<td>$3.2 \times 10^4$</td>
<td>1.4</td>
</tr>
<tr>
<td>SPG-2</td>
<td>0</td>
<td>$1.6 \times 10^4$</td>
<td>1.4</td>
</tr>
<tr>
<td>SC-2-0.4</td>
<td>0.21</td>
<td>$1.3 \times 10^4$</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Optical rotation (OR) measurement

Optical rotation measurements were performed with a P-1000 digital polarimeter (JASCO Co., Tokyo, Japan) at the wavelength of 589 nm. 1 wt% solution was prepared with D$_2$O (Sigma-Aldrich Co., USA) with 0.1 M NaCl. The solution was filled in a cylindrical cell of the optical pass length of 5.0 cm with a water jacket. The cell was thermally attached by circulating water and the temperature was recorded by thermocouples attached on the cell wall. The mass concentration was calculated with the solution density to determine the specific rotation, $[\alpha]_{589}$.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry for schizophyllan and carboxylated schizophyllan solutions was made on a Pyris-1 differential scanning calorimeter (Perkin Elmer Co., Massachusetts, USA). D$_2$O containing 0.1 M NaCl were injected into dried sample in a large volume stainless steel pan (Perkin Elmer Co., Massachusetts, USA). The measurements were performed at the scanning rate of 2.0 K min$^{-1}$ with the solvent as a reference at nitrogen gas flow of 10 cm$^3$ 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). The measurements were performed repeatedly to confirm the heat flow curves to reproduce each measurement within the experimental error.

RESULTS and DISSCUSSION

DSC curves and optical rotation

Figure 2 shows DSC curves for 10 wt% solutions of SPG-1, SC-1-0.2, SC-1-0.4 and SPG-2, SC-2-0.4, respectively, in D$_2$O containing 0.1 M NaCl. The heating rate was 2.0 K min$^{-1}$. The DSC curves were reproduced by repeated heating/cooling cycles. Hence, the transition is thermally reversible in this temperature range. All DSC curves show a thermal peak corresponding to the order-disorder transition. The transition temperature, $T_r$, was taken from the peak of the DSC curve (Table 2). By comparing SPG-1 and SPG-2 it is observed that both $T_r$ and the sharpness of the peak increase with increasing $M$, which reflect the molar mass dependence of the order-disorder transition (Itou et al, 1987; Yoshida et al, 2002). In contrast, the DSC curves for the carboxylated SC-1-0.2, SC-1-0.4 and SC-2-0.4 have broader peaks and are shifted to lower temperatures. The transition
enthalpies, $\Delta H_r$, were calculated from the peak areas of the DSC curves in Figure 2, where the thin lines indicated were chosen as baselines for the samples (Table 2). Compared with previous study of schizophyllan, the present $\Delta H_r$ is slightly underestimated by a tailing at low temperature side. Nonetheless, the values of $\Delta H_r$ for the sclerox solutions were appreciably smaller than those for the parent schizophyllan, indicating that the carboxylated side chains do not contribute to the heat of the order-disorder transition.

![DSC curves for 10 wt% solutions of schizophyllan and carboxylated schizophyllan in D$_2$O containing 0.1 M NaCl at the heating rate of 2.0 K min$^{-1}$. Dashed curves: SPG-1 and SPG-2. Dash-dotted curve: SC-1-0.2. Solid curves: SC-1-0.4 and SC-2-0.4. The data were vertically shifted for clarity.]

**Figure 2**

**Table 2** The transition enthalpy and transition temperature obtained from DSC and OR ($T_r$ only)

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Delta H_r$ / kJ mol$^{-1}$</th>
<th>$T_r$ / K (DSC)</th>
<th>$T_r$ / K (OR)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPG-1</td>
<td>3.9</td>
<td>291.3</td>
<td>291.0</td>
</tr>
<tr>
<td>SC-1-0.2</td>
<td>3.0</td>
<td>289.3</td>
<td>288.8</td>
</tr>
<tr>
<td>SC-1-0.4</td>
<td>2.7</td>
<td>286.5</td>
<td>287.1</td>
</tr>
<tr>
<td>SPG-2</td>
<td>3.4</td>
<td>289.8</td>
<td>288.3</td>
</tr>
<tr>
<td>SC-2-0.4</td>
<td>2.3</td>
<td>288.0</td>
<td>286.0</td>
</tr>
</tbody>
</table>

$a$, at $f=0.5$

**Figure 3A and 3B** show the temperature dependence of the specific rotation $[\alpha]_{589}$ for the five samples. The wavelength was 589 nm, and the solvent was the same as in the DSC experiments (D$_2$O with 0.1 M NaCl). Generally, all samples displayed a typical transition (decrease in specific optical rotation) in the same temperature range as in the DSC experiments. The linear part of the curves on the high temperature side (dashed lines) was taken as the baseline $[\alpha]_{\text{dis}}$ for the disordered state of each sample. Baselines $[\alpha]_{\text{ord,SPG}}$ for the ordered states of SPG-
1 and SPG-2, were drawn parallel to those of the disordered states (dash-dotted lines).

![Figure 3](image)

**Figure 3** Specific rotations $[\alpha]_{589}$ at the wavelength of 589 nm for schizophyllan and carboxylated schizophyllan in 0.1 M NaCl D$_2$O solutions: (A), unfilled circles, SPG-1, unfilled triangles, SC-1-0.2, unfilled squares, SC-1-0.4; (B), filled circles, SPG-2, filled squares, SC-2-0.4. Dashed lines are the baselines for the disordered state, $[\alpha]_{\text{dis}}$, the dash-dotted lines are the baselines for the ordered state, $[\alpha]_{\text{ord}}$, for SPG-1 and SPG-2, and solid curves are eye guides.

It is first noted that the optical rotation in the disordered state shifts to higher values with increasing DS. This suggests that the intact side chain (glucose residue) and the modified side chain (Figure 1) contribute differently to the total optical rotation, reflecting differences in the number of asymmetric carbons in the two cases (Jackson & Hudson, 1937). This finding parallels the observations for xanthan with partially hydrolysed (removed) side chains (Christensen, Knudsen, Smidsrød, Kitamura, Takeo, 1993), where the total optical rotation (and transition enthalpy) changed linearly with changes in the number of side chains. Secondly, it is noted that the transition temperatures obtained from OR agree well with those obtained by DSC, i.e. a decrease with increasing DS (Table 2). Thirdly, the transitions detected by OR become broader with increasing DS, again in qualitative agreement with DSC. Finally, the unmodified SPG-2 appears to have a broader transition curve than that of SPG-1. This is attributed to the $M$ dependence of the transition, as mentioned above.

The fraction $f$ of repeating units in the ordered state was calculated by

$$f = \frac{1}{1-DS} \left( \frac{[\alpha]_{589} - [\alpha]_{\text{dis}}}{[\alpha]_{\text{ord,SPG}} - [\alpha]_{\text{dis,SPG}}} \right)$$

where $[\alpha]_{\text{dis,SPG}}$ is the baseline for the disordered state of SPG. The midpoint of the transition was taken as the temperature at $f=0.5$. The values are included in Table 2. Figure 4A and 4B show the comparison of $f$ between the DSC and the OR measurements for SPG-1, SC-1-0.2, SC-1-0.4, and SPG-2, SC-2-0.4 to confirm the consistency between DSC and OR data. The data from the optical rotation were indicated with same symbols of Figure 3A and 3B. The fraction $f$ of repeating units in the ordered state can be calculated from DSC curves by
\[ f = \int_{T_i}^{T_f} \frac{\Delta h dT}{(1 - DS) \int_{T_i}^{T_f} (\Delta h)_{SPG} dT} \]  

(2)

where \( \Delta h \) is the difference between the DSC curve and the baseline, \( T_i \) and \( T_f \) are the temperatures where the DSC curve reaches the baseline at low and high temperature sides, respectively, and \( DS \) is the degree of substitution. In Figure 4A and 4B, solid curves indicate the results of \( f \) for SPG-1, SC-1-0.2, SC-1-0.4 and SPG-2, SC-2-0.4 obtained by DSC. Both data agreed satisfactorily in this experimental temperature range.

**Figure 4** Comparison of the fraction of the repeating unit in the ordered state between DSC and OR measurements; the data points, the values from the specific rotation \([\alpha]_{589}\); (A), SPG-1, SC-1-0.2 and SC-1-0.4; (B) SPG-2 and SC-2-0.4. Solid curves are the calculated values from DSC curves.

**Analysis of the transition curve**

We can summarize present results of SEC-MALS, DSC, and optical rotation (OR) measurements performed on the order-disorder transition of sclerox, as follows:

Firstly, the sclerox samples have almost the same \( M \) before and after the oxidations. In contrast, a dissociation or aggregation of the trimer would largely affect \( M_w \) and the molar mass distribution (Yoshiba et al., 2015). However, these are almost unchanged for present samples by the chemical modification, which is attributed to the low DS. This indicates that the sclerox samples maintain the trimeric state. The second is the dependencies of \( \Delta H_r \) and OR data on DS. The modified side chains are most probably randomly distributed along the sclerox trimer. Since only the unmodified side chains contribute to form the ordered structure, the reduction of \( \Delta H_r \) with increasing DS suggests that the modified side chain cannot take the ordered structure. Thirdly, \( T_r \) becomes lower, and the transition curve broadens with increasing DS. These effects are concerned with the molecular mechanism of the order-disorder transition. As demonstrated previously, the order-disorder transition occurring in the schizophyllan triple helix is described by a statistical theory of one-dimensional cooperative systems (Itou et al., 1986; Itou et al., 1987; Yoshiba et al., 2002). Therefore, we discuss the above-mentioned effects of the carboxylation on the transition using the same statistical theory in what follows.

As pointed out by Itou et al. (1986), this transition depends strongly on the sample molar mass, indicating that the transition can be regarded as a linear cooperative phenomenon. It has been shown that such transition can be
characterized by the statistical theory for the helix-coil transition established by Zimm, Bragg (Zimm & Bragg, 1959) and Nagai (Nagai, 1960, 1961). The schizophyllan triple helix is divided into \( N \) transition units. In present analysis, the unit is taken to be three times of the repeating units of the chemical structure, i.e. \( 3M_0 \) for schizophyllan (\( M_0 = 648.6 \) g mol\(^{-1}\)). At the low temperature side of the transition, the units except for the terminals are in the ordered state (the fraction of the ordered units, \( f \sim 1 \)). The sequence of the ordered units is shortened with a progress of the transition, and finally all of the units become the disordered state (\( f = 0 \)). The statistical weight of the ordered unit, \( s \), is related to the Gibbs energy difference, \( \Delta G_r^{\infty} \) by

\[
\ln s = \frac{3\Delta G_r^{\infty}}{RT} = \frac{3\Delta H_r^{\infty}}{RT} \left( 1 - \frac{T}{T_r^{\infty}} \right)
\]

(3)

where \( R \) is the gas constant and \( T \) is the absolute temperature. \( \Delta H_r^{\infty} \) and \( T_r^{\infty} \) are the transition enthalpy and the transition temperature at infinite \( N \), respectively. Assuming that the transition is concerned only between the adjacent units, the partition function for the triple helix is given by

\[
Z_N = \left( \begin{array}{c} 1 \\ 0 & 1 \\ 1 & 1 \end{array} \right) M^{N-2} \left( \begin{array}{c} 1 \\ 1 \\ 1 \end{array} \right)
\]

(4)

where \( M \) is the matrix of \( 2 \times 2 \) defined by

\[
M = \left( \begin{array}{cc} s & 1 \\ \sigma^{1/2} s & 1 \end{array} \right)
\]

(5)

with the cooperative parameter \( \sigma^{1/2} \), characterizing the sequence number of the ordered units at the half of the transition at infinite \( N \) (Teramoto & Fujita, 1975).

The fraction of the ordered units in the trimer is expressed formally by the relation,

\[
f = \frac{1}{N} \frac{d \ln Z_N}{d \ln s}
\]

(6)

Teramoto (2001) summarized the formulas to calculate the numerical value of the transition curve and the physical meanings of the theoretical parameters concerned.

For the sclerox trimer, some of the side chains should be replaced by the modified side chains. The transition unit in the trimer consists of three side chains, which can take either the ordered or disordered structure. The periodate oxidation most likely modify the side chains randomly, so that three types of modified units are produced after the chemical modification, in addition to the unmodified unit. If all of the side chains in the unit are modified, the unit takes only the disordered state. Here, this unit is termed the permanent disordered unit. Since this unit in the trimer interrupts the formation of long sequence of the ordered units as a terminal, the sequence of the ordered units becomes shorten even at satisfactorily low temperature. Consequently, the transition curve may become broader like a shorter chain. This situation is illustrated in Figure 5. In this figure, the filled units and unfilled units represent the inactive units and the active units to the order-disorder transition, respectively. The inactive unit has three modified side chains, that is, the permanent disordered unit, whereas the others belong to the active units. In this consideration, \( N \) can be regarded as the successive number of the active units for the transition. Using theoretical parameters (\( \Delta H_r^{\infty}, T_r^{\infty}, \sigma^{1/2} \)) for D\(_2\)O solution of schizophyllan, the transition
curve for sclerox solutions can be fitted by eq. (6) with N as an adjustable parameter.

**Figure 5** Schematic illustrations for the order-disorder transition for carboxylated schizophyllan. The polysaccharide units in the ordered and disordered states designate circles and crosses at the side of the units, respectively. Filled units represent the permanent disordered units.

Figure 6A shows the fitting results for SC-1-0.2 and SC-1-0.4 by using eq. (6). For the calculation of the theoretical curve, known parameters were used; $\Delta H_r^\infty = 4600 \text{ J mol}^{-1}$, $T_r^\infty = 292.3 \text{ K}$, $\sigma^{1/2} = 0.0135$ (Itou et al., 1987). For SPG-1, the number average molar mass was used for the theoretical calculation; $N=125$. The theoretical curve well represents the $f$ data for SPG-1. The results for SC-1-0.2 and SC-1-0.4 can be fitted with $N=69$ and $N=51$, respectively. It is noted that $N$ for SC-1-0.2 becomes nearly half the parent sample. This means that the sequence of the ordered units of SC-1-0.2 is divided into two parts by including the permanent disordered unit in the trimer. $N$ for SC-1-0.4 becomes smaller than SC-1-0.2. Since the number of the permanent disordered unit in the trimer is further produced with increasing DS, the sequence of the ordered units becomes shorter than SC-1-0.2.

**Figure 6** Comparison between theoretical curves and experimental data from the specific rotation for the order-disorder transition of sclerox in D$_2$O containing 0.1 M NaCl: (A) unfilled circles, SPG-1 ($N=125$); unfilled triangles, SC-1-0.2 ($N=69$); unfilled squares, SC-1-0.4 ($N=51$); (B) filled circles, SPG-2 ($N=59$);
filled squares, SC-2-0.4 (N=47); unfilled circles and unfilled squares, same as those in Panel A. The solid curves were calculated with \( \Delta H_{r}^{\infty} = 4600 \text{ J mol}^{-1} \), \( T_{r}^{\infty} = 292.3 \text{ K} \), \( \sigma_{1/2} = 0.0135 \).

Figure 6B shows the \( f \) data for SPG-2 and SC-2-0.4. The \( f \) data for SPG-1 and SC-1-0.4 are also shown in Figure 6B to compare the DS and \( M \) dependencies. It can be seen that the strong \( M \) dependence between SPG-1 and SPG2 is well expressed by the theoretical curve with \( N=59 \) for SPG-2 without fitting parameter. The data for SC-2-0.4 can be fitted by \( N=47 \), which is comparable to SC-1-0.4. Although the carboxylated side chains of SC-1-0.4 and SC-2-0.4 are distributed with almost equimolar ratio against total number of the side chains, the number of carboxylated side chain per a trimer becomes smaller for SC-2-0.4 because of low \( M \). Therefore, the permanent disordered unit in a trimer of SC-2-0.4 is less produced compared with SC-1-0.4. Consequently, the transition curve becomes insensitive to \( M \) after the carboxylation.

**Conclusion**

Carboxylated schizophyllan (‘sclerox’) synthesized by partial periodate oxidation followed by chlorite oxidation dissolves as a trimer, and shows a cooperative order-disorder transition in \( D_2O \) solution containing 0.1 M NaCl, analogous to the parent schizophyllan. The transition enthalpy decreases and the transition temperature is lowered with increasing **degree of carboxylation**. The carboxylated side chains of the trimer introduced by oxidation cannot take the ordered structure, leading the decreasing the enthalpy of the transition. **Adopting the linear cooperative theory for the order disorder transition of schizophyllan solution, the length of the ordered unit for sclerox becomes shorter than those of parent schizophyllan, where the disordered unit produced at carboxylation acts as a terminal of the successive ordered sequence. Consequently, the transition temperature and transition enthalpy for sclerox solution depend largely on the degree of carboxylation.**

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