1   To Carbohydrate Polymers
2   Effects of carboxylation of the side chains on the order-disorder transition in
3   aqueous solution of schizophyllan, a triple helical polysaccharide
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22
23 **Abstract**
24 Schizophyllan and scleroglucan are water-soluble polysaccharides having
25 repeating units consisting of three β-1,3-linked glucose residues in the main
26 chain and a single β-1,6-linked glucose residue as the side chain. This
27 polysaccharide dissolves as a triple helix in an aqueous solution and shows a
28 cooperative order-disorder transition between the side chain and solvent
29 molecules while retaining the triple helical conformation. Periodate and
30 subsequent chlorite oxidations selectively modify the side chain glucose to
31 provide the corresponding dicarboxylate units. Optical rotation measurements
32 and differential scanning calorimetry were performed on carboxylated
33 schizophyllan/scleroglucan (sclerox) samples to investigate the effects of the
34 degree of carboxylation on the order-disorder transition in deuterium oxide with
35 0.1 M NaCl. The transition curves for the sclerox samples are strongly dependent
36 on the degree of carboxylation. The modified side chains cannot take the ordered
37 structure, resulting in a reduction of the transition enthalpy. The transition
38 temperature for carboxylated schizophyllan becomes lowered and the transition
39 curve broadens with increasing the degree of carboxylation. The permanent
40 disordered units are included in a trimer by the carboxylation to inhibit a long
41 sequence of the ordered units.
42
43 Keywords: schizophyllan, periodate oxidation, order-disorder transition,
44 differential scanning calorimetry, optical rotation
45
46 **Introduction**
47   Schizophyllan is a water-soluble polysaccharide produced by *Schizophyllum*
48 *commune*, which consists of tetrasaccharide repeating units containing three β-
49 1,3-glucoses and one branched β-1,6-glucose (Kikumoto, Miyajima, Yoshizumi,

50 Fujimoto, Kimura, 1970; Kikumoto, Miyajima, Kimura, Okubo, Komatsu, 1971).
51 Scleroglucan is a structurally similar polysaccharide elaborated by a species of
52 the genus *Sclerotium* (Johnson et al, 1963). These polysaccharides dissolve in
53 water as triple helices, where three main chains form the triple helix and the side
54 chain glucoses are arranged outward the helical core to ensure high solubility to
55 water (Takahashi, Kobatake, Suzuki, 1984). The triple helix of schizophyllan and
56 scleroglucan shows strong molar mass dependencies of radius gyration
57 (Kashiwagi, Norisuye, Fujita, 1981; Yanaki & Norisuye, 1983) and intrinsic
58 viscosity (Norisuye, Yanaki, Fujita, 1980; Yanaki, Norisuye, Fujita, 1980) in
59 aqueous solutions. It was concluded on the analysis of the wormlike chain model
60 that the triple helix is a quite rigid rod represented with a large persistence
61 length above 150nm.
62   The schizophyllan triple helix has characteristic physical and biological
63 properties in the aqueous solutions, *e.g.*, high viscosity (Carriere, Amis, Schrag,
64 Ferry, 1985; Enomoto, Einaga, Teramoto, 1985; Fang & Nishinari, 2003), liquid
65 crystallinity (Van, Norisuye, Teramoto, 1980; Itou, Van, Teramoto, 1985),
66 anticancer activity (Norisuye, 1985; Okamura et al, 1986), and reassembly with
67 polynucleotides (Sakurai & Shinkai, 2000; Sakurai, Mizu, Shinkai, 2001;
68 Mochizuki & Sakurai, 2009). A cooperative order-disorder transition is one of the
69 characteristic features of the schizophyllan triple helix in aqueous solutions
70 (Asakawa, Van, Teramoto, 1984; Itou, Teramoto, Matsuo, Suga, 1986; Kitamura &
71 Kuge, 1989). The side chain forms an ordered structure along with hydrated
72 water molecules in the ordered triple helix state (Triple helix I). As a
73 temperature rises, the ordered structure is converted into the disordered triple
74 helix (Triple helix II). The transition has a strong molar mass dependency
75 because of its highly cooperative nature (Itou et al, 1986). In addition, the
76 transition temperature and the transition enthalpy depend largely on the solvent
77 conditions. The transition temperature shifts from $7^{o}C$ in H_2O to $18^{o}C$ in D_2O, and
78 the transition enthalpy of the D_2O solution is larger than in H_2O (Itou, Teramoto,
79 Matsuo, Suga, 1987). The same order-disorder transition was observed for other
80 branched β-1,3-glucans, and the number of branching per the main chain glucose
81 affects the order-disorder transitional behavior in DMSO-water mixtures
82 (Kitamura, Ozawa, Tokioka, Hara, Ukai, Kuge, 1990; Zhang, Xu, Zhang, 2008;
83 Wang, Zhang, Zhang, Ding, 2009), indicating that the hydration of the triple helix,
84 especially the side chains and solvent molecules, plays an important role in the
85 transition.
86   Chemical modifications generally alter the physicochemical and biological
87 properties of the parent polysaccharide. Periodate oxidation is well known as an
88 important reaction for the structural determination of polysaccharides (Perlin,
89 1980). This oxidation proceeds stoichiometrically with the glycol-cleavage
90 reaction at the C-C bond of the vicinal diols or successive hydroxyl groups.
91 Schizophyllan can be modified into ionic polysaccharide by periodate and
92 subsequently chlorite oxidations (Crescenzi, Gamini, Paradossi, Torri, 1983;
93 Gamini, Crescenzi, Abruzzese, 1984). The glucose ring of the side chain is
94 selectively modified into a linear dialdehyde chain, whereas the main chain of
95 schizophyllan is resistant to the periodate oxidation. Chlorite oxidation can
96 further alter the aldehyde groups into carboxyl groups. These oxidations allow
97 the preparation of different degrees of substitution (DS) of the carboxylated
98 derivative. Carboxylated schizophyllan (Sclerox, Figure 1) is soluble in water and

99   show different physical properties in aqueous solution from parent
100   schizophyllan, including polyelectrolyte properties (Coviello, Dentini, Crescenzi,
101   1995; Coviello, Dentini, Crescenzi, Vincenti, 1995), and related properties, such
102   as gelation with trivalent cations (Stokke, Elgsaeter, Smidrød, Christensen,
103   1995). The solubility to water is related to the hydration of this modified
104   polysaccharide, which may influence the order-disorder transition. However, the
105   effect of chemical modification of the side chains of schizophyllan has not been
106   investigated for the order-disorder transition.
107   In previous studies on the order-disorder transition of schizophyllan, the
108   molecular mechanism and the molecular motion were studied by optical
109   rotation, adiabatic calorimetry and dielectric relaxation (Teramoto, Gu, Miyazaki,
110   Sorai, Mashimo, 1995; Hayashi, Shinyashiki, Yagihara et al, 2001; Yoshiba, Ishino,
111   Teramoto Nakamura et al, 2002; Yoshiba, Teramoto, Nakamura, Kikuchi,
112   Miyazaki, Sorai, 2003; Yoshiba, Teramoto Nakamura et al, 2004). Chemical
113   modifications of polysaccharide side chains generally influence such transition
114   markers. For example, partial acid hydrolysis was used in a double helical
115   polysaccharide, xanthan, to investigate the influence of the removal of side
116   chains on the conformational transition of xanthan (Christensen, Knudsen,
117   Smidsrød, Kitamura, Takeo, 1993). Since the order-disorder transition of
118   schizophyllan is characteristic to the triple helical conformation (Kitamura &
119   Kuge, 1989), it is necessary to investigate both of the molecular conformation
120   and the transition behavior after the chemical modification. From
121   conformational aspects for sclerox, we showed that a sclerox at low DS remains a
122   trimer in 0.1 M NaCl (Yoshiba, Sato, Osumi, Ulset, Christensen, 2015). However,
123   size-exclusion chromatography combined with multi-angle light scattering
124   detection (SEC-MALS) indicated the global conformation of the trimer is different
125   from the triple helix of parent schizophyllan by becoming more flexible and
126   extended after the carboxylation. In the present study, we report effects of
127   carboxylation of the side chains on the cooperative order-disorder transition of
128   sclerox. The sclerox samples with different DS were investigated by differential
129   scanning calorimetry and optical rotation to characterize the transition behavior
130   of sclerox. Since schizophyllan in aqueous solutions has a low transition
131   temperature of 7 °C, D_2O was used as solvent to extend the temperature range of
132   the triple helix I. We added 0.1 M NaCl to essentially prevent the electrostatic
133   interaction between the trimers. Since the conformation of the sclerox trimer can
134   be determined from SEC-MALS measurements, the effects of chemical
135   modification on its specific hydration around the triple helix were analyzed.
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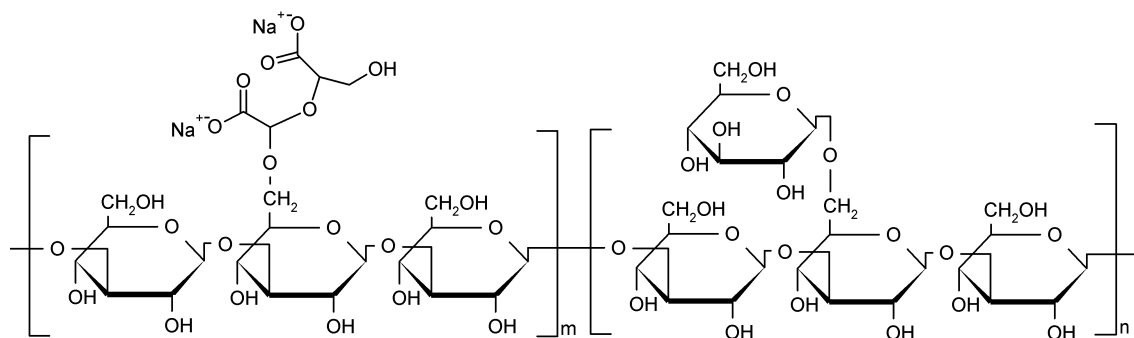
138   **Figure 1** Chemical structure of the carboxylated schizophyllan/scleroglucan
139   (Sclerox) by partial periodate and chlorite oxidation.
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141
142   **Experimental**
143
144   *Schizophyllan and carboxylated schizophyllan samples*
145   The schizophyllan sample (Taito Co., now Mitsui sugar Co., Japan) was sonicated
146   in an aqueous solution with a Branson Sonifier 250 (Branson Ultrasonics Co.,
147   Danbury, USA) to prepare two degraded schizophyllan samples. The sonicated
148   sample was purified by the fractional reprecipitation in the aqueous solution
149   with ethanol to obtain middle fraction of each sample (SPG-1, SPG-2). The
150   carboxylation of the sample was performed in an aqueous solution according to
151   previous study on sclerox (Yoshiba et al, 2015). In brief, 30 mM aqueous sodium
152   periodate (Wako Pure Chemical Industries Ltd., Osaka, Japan) was dropped into
153   120 ml of 0.1wt% aqueous schizophyllan containing 13.3 ml of 1-propanol to
154   prepare different degree of oxidation of the samples. After dialysis against
155   deionized water, sodium chlorite (Kanto Chemical Co. Inc., Tokyo, Japan) and
156   acetic acid (Wako Pure Chemical Industries Ltd., Osaka, Japan) were added into
157   the solution to be 0.5 M for each reagent. These oxidations were performed in
158   dark at 10 °C for 24 hours. After oxidations, the solutions were dialyzed and
159   neutralized with 0.01 M NaOH to obtain the sodium salt of the freeze-dried
160   samples. The sclerox samples were corded as SC-1-*X* or SC-2-*X*, where *X* is
161   denoted by the mole fraction of sodium periodate for the repeating unit of
162   schizophyllan at the oxidation. The degree of substitution, DS, was determined
163   by the potentiometric titration for the acid form of the sample with 0.01 M NaOH
164   in aqueous 0.1 M NaCl after the immersion with Amberlite-120B (Organo Co.,
165   Ltd., Tokyo, Japan) for 24 hours. The degree of substitution of the samples was
166   listed in Table 1.
167
168   *Size Exclusion Chromatography equipped with a Multi-Angle Light Scattering*
169   *detector (SEC-MALS)*
170   The weight average molar mass and the molar mass dispersity of the samples
171   were determined by the size exclusion chromatography equipped with a multi-
172   angle light scattering detector (SEC-MALS). The SEC columns (SHODEX OHPak
173   SB-G, two SHODEX OHPak SB806MHQ, Showa Denko KK, Kanagawa, Japan) were
174   serially connected in the SEC system (GPC-101, Showa Denko KK, Kanagawa,
175   Japan). The SEC system was connected with a DAWN HELEOS II MALS detector
176   (Wyatt Technology Co., Santa Barbara, USA) and differential refractometer.
177   Aqueous 0.1 M NaCl was used as an eluent. The flow rate was fixed at 1.0
178     $cm^3 min^{-1}$ and the columns were kept at 40 °C in the measurement. The solution
179   dissolved in 0.1 M NaCl with 0.01 M NaOH was injected (injection volume, 0.1
180   cm^3) after the filtration with DISMIC-25AS (pore size 0.45 μm, Toyo Roshi Co.,
181   Ltd., Tokyo, Japan) and the measurement was operated by ASTRA® ver. 5.3
182   software (Wyatt Technology Co., Santa Barbara, USA). The refractive index
183   increment, $(\partial n/\partial c)_\mu = 0.141$ $cm^3 g^{-1}$, was used to calculate the weight average
184   molar mass, M_w, and molar mass dispersity M_w/M_n. Table 1 summarizes M_w, and
185   M_w/M_n of the samples used in following measurements determined from the
186   SEC-MALS measurements.

187

188 **Table 1** The degree of substitution and SEC-MALS results for carboxylated
189 schizophyllan and schizophyllan samples in aqueous 0.1 M NaCl.

Sample	DS	M_w / g mol^{-1}	M_w/M_n
SPG-1	0	34.1×10^4	1.4
SC-1-0.2	0.16	32.4×10^4	1.4
SC-1-0.4	0.20	32.4×10^4	1.4
SPG-2	0	16.0×10^4	1.4
SC-2-0.4	0.21	13.2×10^4	1.4

190

191 *Optical rotation (OR) measurement*
192   Optical rotation measurements were performed with a P-1000 digital
193 polarimeter (JASCO Co., Tokyo, Japan) at the wavelength of 589 nm. 1 wt%
194 solution was prepared with D_2O (Sigma-Aldrich Co., USA) with 0.1 M NaCl. The
195 solution was filled in a cylindrical cell of the optical pass length of 5.0 cm with a
196 water jacket. The cell was thermo-stated by circulating water and the
197 temperature was recorded by thermocouples attached on the cell wall. The mass
198 concentration was calculated with the solution density to determine the specific
199 rotation, $[\alpha]_{589}$.

200

201 *Differential scanning calorimetry (DSC)*
202   Differential scanning calorimetry for schizophyllan and carboxylated
203 schizophyllan solutions was made on a Pyris-1 differential scanning calorimeter
204 (Perkin Elmer Co., Massachusetts, USA). D_2O containing 0.1 M NaCl were injected
205 into dried sample in a large volume stainless steel pan (Perkin Elmer Co.,
206 Massachusetts, USA). The measurements were performed at the scanning rate of
207 2.0 K min^{-1} with the solvent as a reference at nitrogen gas flow of 10 cm^3 min^{-1}.
208 The temperature scale of the calorimeter was calibrated with the melting
209 temperature of cyclohexane and indium and the heat flow was corrected with
210 the enthalpy of fusion of indium. The measurement was operated by a Pyris®
211 software (Perkin Elmer Co., Massachusetts, USA). The measurements were
212 performed repeatedly to confirm the heat flow curves to reproduce each
213 measurement within the experimental error.

214

215

216 **RESULTS and DISSCUSSION**

217

218 *DSC curves and optical rotation*
219   Figure 2 shows DSC curves for 10 wt% solutions of SPG-1, SC-1-0.2, SC-1-0.4
220 and SPG-2, SC-2-0.4, respectively, in D_2O containing 0.1 M NaCl. The heating rate
221 was 2.0 K min^{-1}. The DSC curves were reproduced by repeated heating/cooling
222 cycles. Hence, the transition is thermally reversible in this temperature range. All
223 DSC curves show a thermal peak corresponding to the order-disorder transition.
224 The transition temperature, T_r, was taken from the peak of the DSC curve (Table
225 2). By comparing SPG-1 and SPG-2 it is observed that both T_r and the sharpness
226 of the peak increase with increasing M, which reflect the molar mass dependence
227 of the order-disorder transition (Itou et al, 1987; Yoshiba et al, 2002). In
228 contrast, the DSC curves for the carboxylated SC-1-0.2, SC-1-0.4 and SC-2-0.4
229 have broader peaks and are shifted to lower temperatures. The transition

230 enthalpies, ΔH_r, were calculated from the peak areas of the DSC curves in Figure
231 2, where the thin lines indicated were chosen as baselines for the samples (Table
232 2). Compared with previous study of schizophyllan, the present ΔH_r is slightly
233 underestimated by a tailing at low temperature side. Nonetheless, the values of
234 ΔH_r for the sclerox solutions were appreciably smaller than those for the parent
235 schizophyllan, indicating that the carboxylated side chains do not contribute to
236 the heat of the order-disorder transition.
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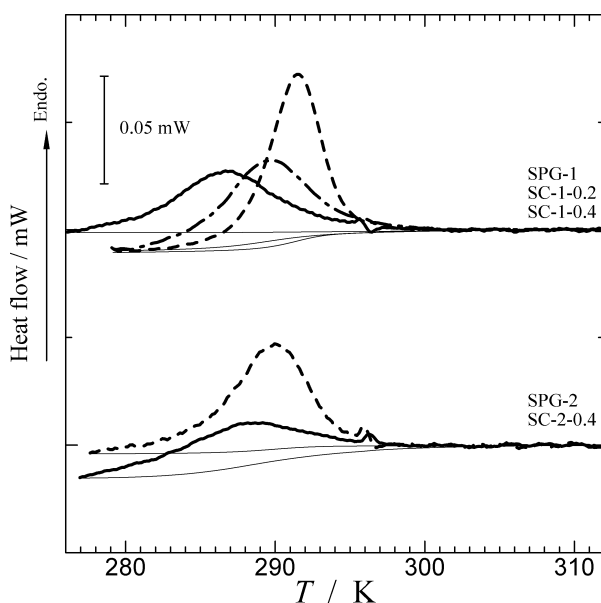
239
240 **Figure 2** DSC curves for 10 wt% solutions of schizophyllan and carboxylated
241 schizophyllan in D_2O containing 0.1 M NaCl at the heating rate of 2.0 K min^{-1}.
242 Dashed curves: SPG-1 and SPG-2. Dash-dotted curve: SC-1-0.2. Solid curves: SC-
243 1-0.4 and SC-2-0.4. The data were vertically shifted for clarity.
244
245
246 **Table 2** The transition enthalpy and transition temperature obtained from DSC
247 and OR (T_r only)

| Sample	ΔH_r / kJmol^{-1}	T_r / K (DSC)	T_r / K (OR)[a]
SPG-1	3.9	291.3	291.0
SC-1-0.2	3.0	289.3	288.8
SC-1-0.4	2.7	286.5	287.1
SPG-2	3.4	289.8	288.3
SC-2-0.4	2.3	288.0	286.0

248 a, at f=0.5
249
250
251 Figure 3A and 3B show the temperature dependence of the specific rotation
252 $[\alpha]_{589}$ for the five samples. The wavelength was 589 nm, and the solvent was the
253 same as in the DSC experiments (D_2O with 0.1 M NaCl). Generally, all samples
254 displayed a typical transition (decrease in specific optical rotation) in the same
255 temperature range as in the DSC experiments. The linear part of the curves on
256 the high temperature side (dashed lines) was taken as the baseline $[\alpha]_{dis}$ for the
257 disordered state of each sample. Baselines $[\alpha]_{ord,SPG}$ for the ordered states of SPG-

258 1 and SPG-2, were drawn parallel to those of the disordered states (dash-dotted
259 lines).
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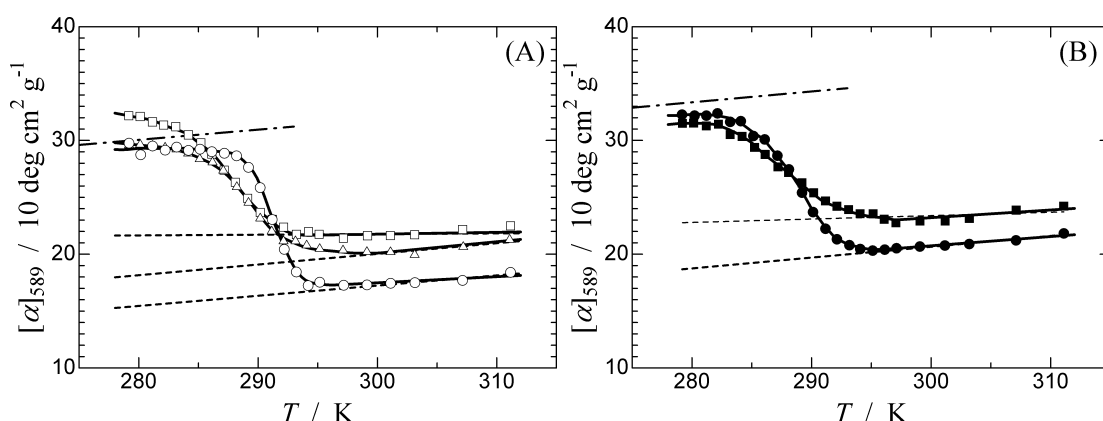
262
263 **Figure 3** Specific rotations $[\alpha]_{589}$ at the wavelength of 589 nm for schizophyllan
264 and carboxylated schizophyllan in 0.1 M NaCl D_2O solutions: (A), unfilled circles,
265 SPG-1, unfilled triangles, SC-1-0.2, unfilled squares, SC-1-0.4; (B), filled circles,
266 SPG-2, filled squares, SC-2-0.4. Dashed lines are the baselines for the disordered
267 state, $[\alpha]_{dis}$, the dash-dotted lines are the baselines for the ordered state, $[\alpha]_{ord}$,
268 for SPG-1 and SPG-2, and solid curves are eye guides.
269
270
271 It is first noted that the optical rotation in the disordered state shifts to higher
272 values with increasing DS. This suggests that the intact side chain (glucose
273 residue) and the modified side chain (Figure 1) contribute differently to the total
274 optical rotation, reflecting differences in the number of asymmetric carbons in
275 the two cases (Jackson & Hudson, 1937). This finding parallels the observations
276 for xanthan with partially hydrolysed (removed) side chains (Christensen,
277 Knudsen, Smidsrød, Kitamura, Takeo, 1993), where the total optical rotation
278 (and transition enthalpy) changed linearly with changes in the number of side
279 chains. Secondly, it is noted that the transition temperatures obtained from OR
280 agree well with those obtained by DSC, i.e. a decrease with increasing DS (Table
281 2). Thirdly, the transitions detected by OR become broader with increasing DS,
282 again in qualitative agreement with DSC. Finally, the unmodified SPG-2 appears
283 to have a broader transition curve than that of SPG-1. This is attributed to the *M*
284 dependence of the transition, as mentioned above.
285 The fraction *f* of repeating units in the ordered state was calculated by

286
$$f = \frac{1}{1-DS}\left(\frac{[\alpha]_{589}-[\alpha]_{dis}}{[\alpha]_{ord,SPG}-[\alpha]_{dis,SPG}}\right) \qquad (1)$$

287 where $[\alpha]_{dis,SPG}$ is the baseline for the disordered state of SPG. The midpoint of
288 the transition was taken as the temperature at *f* = 0.5. The values are included in
289 Table 2. Figure 4A and 4B show the comparison of *f* between the DSC and the OR
290 measurements for SPG-1, SC-1-0.2, SC-1-0.4, and SPG-2, SC-2-0.4 to confirm the
291 consistency between DSC and OR data. The data from the optical rotation were
292 indicated with same symbols of Figure 3A and 3B. The fraction *f* of repeating
293 units in the ordered state can be calculated from DSC curves by

294 $$f = \int_{T_i}^{T} \Delta h \, \mathrm{d}T \left/ (1-DS) \int_{T_i}^{T_f} (\Delta h)_{\mathrm{SPG}} \, \mathrm{d}T \right.$$ (2)

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

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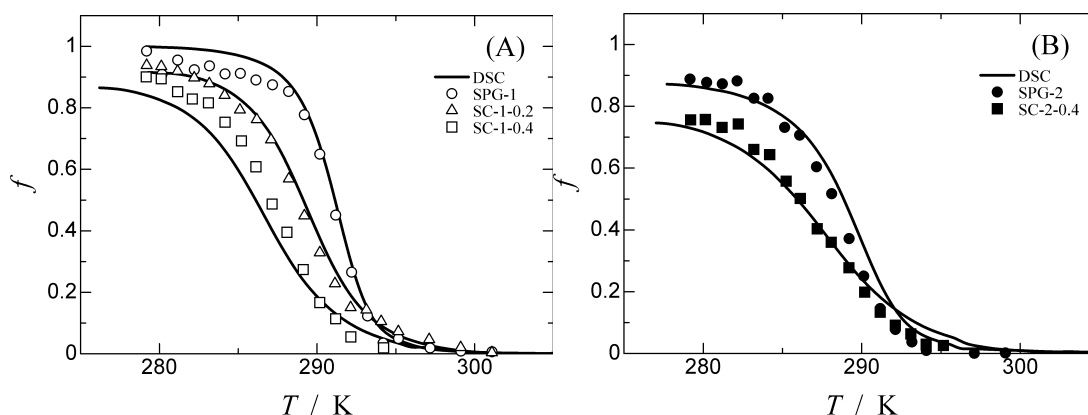
302 T / K T / K
303 **Figure 4** Comparison of the fraction of the repeating unit in the ordered state
304 between DSC and OR measurements; the data points, the values from the specific
305 rotation $[\alpha]_{589}$; (A), SPG-1, SC-1-0.2 and SC-1-0.4; (B) SPG-2 and SC-2-0.4. Solid
306 curves are the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

307

308

309 *Analysis of the transition curve*
310   We can summarize present results of SEC-MALS, DSC, and optical rotation (OR)
311 measurements performed on the order-disorder transition of sclerox, as follows:
312 Firstly, the sclerox samples have almost the same M before and after the
313 oxidations. In contrast, a dissociation or aggregation of the trimer would largely
314 affect M_w and the molar mass distribution (Yoshiba et al, 2015). However, these
315 are almost unchanged for present samples by the chemical modification, which is
316 attributed to the low DS. This indicates that the sclerox samples maintain the
317 trimeric state. The second is the dependencies of ΔH_r and OR data on DS. The
318 modified side chains are most probably randomly distributed along the sclerox
319 trimer. Since only the unmodified side chains contribute to form the ordered
320 structure, the reduction of ΔH_r with increasing DS suggests that the modified side
321 chain cannot take the ordered structure. Thirdly, T_r becomes lower, and the
322 transition curve broadens with increasing DS. These effects are concerned with
323 the molecular mechanism of the order-disorder transition. As demonstrated
324 previously, the order-disorder transition occurring in the schizophyllan triple
325 helix is described by a statistical theory of one-dimensional cooperative systems
326 (Itou et al, 1986; Itou et al, 1987; Yoshiba et al, 2002). Therefore, we discuss the
327 above-mentioned effects of the carboxylation on the transition using the same
328 statistical theory in what follows.
329   As pointed out by Itou et al (1986), this transition depends strongly on the
330 sample molar mass, indicating that the transition can be regarded as a linear
331 cooperative phenomenon. It has been shown that such transition can be

332   characterized by the statistical theory for the helix-coil transition established by
333   Zimm, Bragg (Zimm & Bragg, 1959) and Nagai (Nagai, 1960, 1961). The
334   schizophyllan triple helix is divided into N transition units. In present analysis,
335   the unit is taken to be three times of the repeating units of the chemical
336   structure, i.e. $3M_0$ for schizophyllan (M_0 =648.6 g mol^{-1}). At the low temperature
337   side of the transition, the units except for the terminals are in the ordered state
338   (the fraction of the ordered units, $f \sim 1$). The sequence of the ordered units is
339   shortened with a progress of the transition, and finally all of the units become
340   the disordered state ($f = 0$). The statistical weight of the ordered unit, s, is related
341   to the Gibbs energy difference, ΔG_r^∞ by

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

343   where R is the gas constant and T is the absolute temperature. ΔH_r^∞ and T_r^∞ are
344   the transition enthalpy and the transition temperature at infinite N, respectively.
345   Assuming that the transition is concerned only between the adjacent units, the
346   partition function for the triple helix is given by

347     $$Z_N = \begin{pmatrix} 0 & 1 \end{pmatrix} \mathbf{M}^{N-2} \begin{pmatrix} 1 \\ 1 \end{pmatrix} \qquad (4)$$

348   where $\mathbf{M}$ is the matrix of 2×2 defined by

349     $$\mathbf{M} = \begin{pmatrix} s & 1 \\ \sigma^{1/2}s & 1 \end{pmatrix} \qquad (5)$$

350   with the cooperative parameter $\sigma^{1/2}$, characterizing the sequence number of the
351   ordered units at the half of the transition at infinite N (Teramoto & Fujita, 1975).
352   The fraction of the ordered units in the trimer is expressed formally by the
353   relation,

354     $$f = \frac{1}{N}\frac{d\ln Z_N}{d\ln s} \qquad (6)$$

355 Teramoto (2001) summarized the formulas to calculate the numerical value of
356 the transition curve and the physical meanings of the theoretical parameters
357 concerned.
358   For the sclerox trimer, some of the side chains should be replaced by the
359 modified side chains. The transition unit in the trimer consists of three side
360 chains, which can take either the ordered or disordered structure. The periodate
361 oxidation most likely modify the side chains randomly, so that three types of
362 modified units are produced after the chemical modification, in addition to the
363 unmodified unit. If all of the side chains in the unit are modified, the unit takes
364 only the disordered state. Here, this unit is termed the permanent disordered
365 unit. Since this unit in the trimer interrupts the formation of long sequence of the
366 ordered units as a terminal, the sequence of the ordered units becomes shorten
367 even at satisfactorily low temperature. Consequently, the transition curve may
368 become broader like a shorter chain. This situation is illustrated in Figure 5. In
369 this figure, the filled units and unfilled units represent the inactive units and the
370 active units to the order-disorder transition, respectively. The inactive unit has
371 three modified side chains, that is, the permanent disordered unit, whereas the
372 others belong to the active units. In this consideration, N can be regarded as the
373 successive number of the active units for the transition. Using theoretical
374 parameters (ΔH_r^∞, T_r^∞, $\sigma^{1/2}$) for D_2O solution of schizophyllan, the transition

375 curve for sclerox solutions can be fitted by eq. (6) with *N* as an adjustable
376 parameter.
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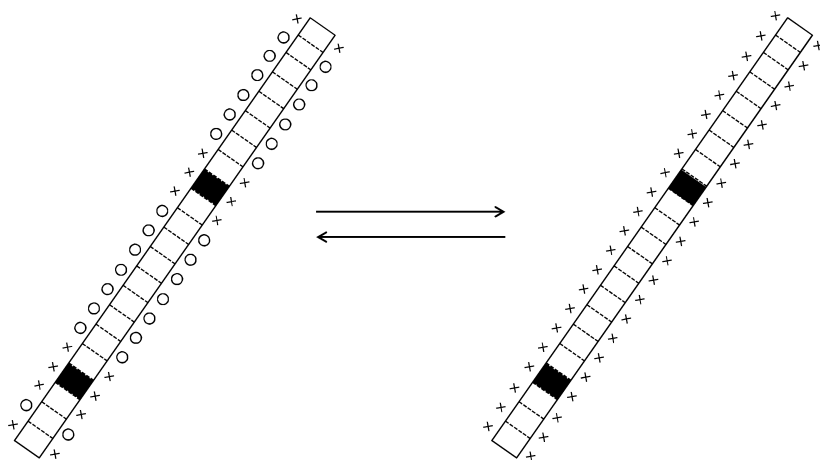
378
379 **Figure 5** Schematic illustrations for the order-disorder transition for
380 carboxylated schizophyllan. The polysaccharide units in the ordered and
381 disordered states designate circles and crosses at the side of the units,
382 respectively. Filled units represent the permanent disordered units.
383
384
385   Figure 6A shows the fitting results for SC-1-0.2 and SC-1-0.4 by using eq. (6).
386 For the calculation of the theoretical curve, known parameters were used; ΔH_r^∞ =
387 4600 Jmol^{-1}, T_r^∞ = 292.3 K, $\sigma^{1/2}$= 0.0135 (Itou et al, 1987). For SPG-1, the number
388 average molar mass was used for the theoretical calculation; *N*=125. The
389 theoretical curve well represents the *f* data for SPG-1. The results for SC-1-0.2
390 and SC-1-0.4 can be fitted with *N*= 69 and *N*=51, respectively. It is noted that *N*
391 for SC-1-0.2 bocomes nearly half the parent sample. This means that the
392 sequence of the ordered units of SC-1-0.2 is divided into two parts by including
393 the permanent disordered unit in the trimer. *N* for SC-1-0.4 becomes smaller
394 than SC-1-0.2. Since the number of the permanent disordered unit in the trimer
395 is further produced with increasing DS, the sequence of the ordered units
396 becomes shorter than SC-1-0.2.
397
398
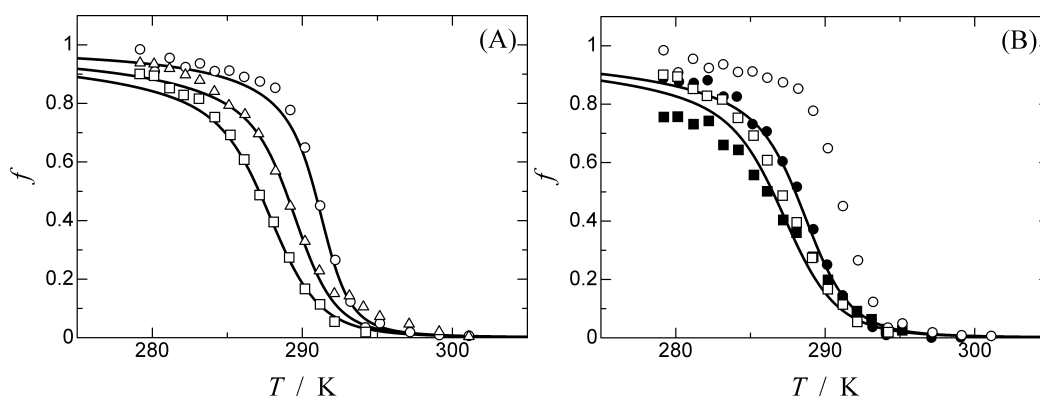
399
400 **Figure 6** Comparison between theoretical curves and experimental data from
401 the specific rotation for the order-disorder transition of sclerox in D$_2$O
402 containing 0.1 M NaCl: (A) unfilled circles, SPG-1 (*N*=125); unfilled triangles, SC-
403 1-0.2 (*N*=69); unfilled squares, SC-1-0.4 (*N*=51); (B) filled circles, SPG-2 (*N*=59);

404   filled squares, SC-2-0.4 (N=47); unfilled circles and unfilled squares, same as
405   those in Panel A. The solid curves were calculated with ΔH_r° =4600 Jmol^{-1}, T_r°
406   =292.3 K, $\sigma^{1/2}$ = 0.0135.
407
408
409   Figure 6B shows the f data for SPG-2 and SC-2-0.4. The f data for SPG-1 and SC-
410   1-0.4 are also shown in Figure 6(B) to compare the DS and M dependencies. It
411   can be seen that the strong M dependence between SPG-1 and SPG2 is well
412   expressed by the theoretical curve with N=59 for SPG-2 without fitting
413   parameter. The data for SC-2-0.4 can be fitted by N=47, which is comparable to
414   SC-1-0.4. Although the carboxylated side chains of SC-1-0.4 and SC-2-0.4 are
415   distributed with almost equimolar ratio against total number of the side chains,
416   the number of carboxylated side chain per a trimer becomes smaller for SC-2-0.4
417   because of low M. Therefore, the permanent disordered unit in a trimer of SC-2-
418   0.4 is less produced compared with SC-1-0.4. Consequently, the transition curve
419   becomes insensitive to M after the carboxylation.
420
421
422   **Conclusion**
423   Carboxylated schizophyllan ('sclerox') synthesized by partial periodate oxidation
424   followed by chlorite oxidation dissolves as a trimer, and shows a cooperative
425   order-disorder transition in D$_2$O solution containing 0.1 M NaCl, analogous to the
426   parent schizophyllan. The transition enthalpy decreases and the transition
427   temperature is lowered with increasing degree of carboxylation. The
428   carboxylated side chains of the trimer introduced by oxidation cannot take the
429   ordered structure, leading the decreasing the enthalpy of the transition.
430   Adopting the linear cooperative theory for the order disorder transition of
431   schizophyllan solution, the length of the ordered unit for sclerox becomes
432   shorter than those of parent schizophyllan, where the disordered unit produced
433   at carboxylation acts as a terminal of the successive ordered sequence.
434   Consequently, the transition temperature and transition enthalpy for sclerox
435   solution depend largely on the degree of carboxylation.
436
437
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