# Conformational Characteristics of Oxidized Scleroglucan

**Tommasina Coviello,**[†] **Hitoshi Maeda, Yoshiaki Yuguchi, Hiroshi Urakawa, and Kanji Kajiwara***

*Faculty of Engineering & Design, Kyoto Institute of Technology, Kyoto, Sakyo-ku, Matsugasaki 606, Japan*

**Mariella Dentini and Vittorio Crescenzi**

*Department of Chemistry, La Sapienza University, I-100185 Rome, Italy*

*Received November 4, 1997; Revised Manuscript Received January 7, 1998*

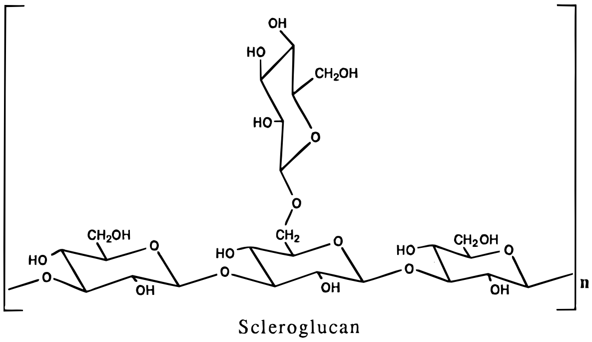
ABSTRACT: The conformation of oxidized scleroglucan (sclerox) was characterized in water and in NaCl aqueous solution by means of small-angle X-ray scattering (SAXS). The observed SAXS profiles were analyzed in terms of a modified broken rod model, where each rod component was replaced with the molecular models of a triple-stranded helix and a single chain simulated by the Monte Carlo method. The electrostatic interaction due to carboxylate groups was taken into account by assuming a Gaussian-type interaction potential. The results confirmed that the triple-stranded helix of scleroglucan was disentangled to a single chain by the oxidation of $\beta$-D-glucosyl side residues. The triple-stranded structure is partly recovered by shielding charge on the side chains.

## Introduction

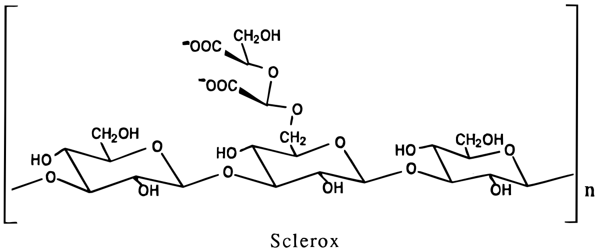
Scleroglucan (Figure 1) is a branched (1 → 3)-$\beta$-D-glucan, secreted by fungi of the genus sclerotium, and it is known to assume a triple-stranded helical conformation in aqueous solution.[1] Scleroglucan is nonionic, but carboxylate groups can be introduced onto the $\beta$-D-glucosyl side residues by periodate−chlorite oxidation[2] to afford the polyelectrolyte characteristics.[3,4] The polyelectrolyte behavior was quantitatively analyzed as a function of ionic strength by adding NaOH[5] or NaCl.[6] Here a sclerox chain was found to be flexible at high pH from the viscosity data and thus is speculated to assume a single-stranded conformation. The triple-stranded conformation is considered to be destabilized by an oxidation reaction.

In the NaCl aqueous solution, a normal polyelectrolyte effect was observed at low salt concentration, where the intrinsic viscosity decreases monotonically with ionic strength. However, the intrinsic viscosity was found to take the minimum value in the 0.4 M NaCl aqueous solution and then increase abruptly with a further increase of salt concentration.[6] An annealing effect is noticed in almost all ranges of salt concentration investigated except at the highest concentration (5 M NaCl). The set of experimental data indicates a recovery of the triple-strand helical conformation of sclerox by salt and/or thermal treatment.

This paper examines the conformational characteristics of sclerox in aqueous solution by means of small-angle X-ray scattering (SAXS). Owing to its short wavelength, the SAXS is capable of analyzing the (amorphous) structure on the order of a few angstroms to several hundreds angstroms.[7] Since the conformational transition is thought to induce the structural change on the order of 10−100 Å, the SAXS is expected to provide the quantitative view for the structural change of sclerox in solution. Here the observed SAXS profile is fitted to the calculated scattering factor from an appropriate molecular model. The molecular model may visualize the structural change of sclerox induced by added salt and/or heat.



Scleroglucan



Sclerox

**Figure 1.** Scleroglucan and sclerox.

## Experimental Section

Oxidized scleroglucan (sclerox) samples were prepared as described elsewhere.[2,3] The solutions for SAXS experiments were prepared by dissolving the sodium salt or TBA salt of sclerox in distilled water or NaCl (0.1 or 1 M) aqueous solution and were filtered through a membrane of 0.8-$\mu$m pore size.

---

* Corresponding author.

[†] On leave from Department of Chemistry and Technology of Biologically Active Compounds, La Sapeinza University, I-00185 Rome, Italy.

S0024-9297(97)01628-8 CCC: $15.00

**Table 1. Optical Activity ([α] at 302 nm), the Constant Term in Eq 2 or Eq 5, and the Estimated Triple-Stranded/Single Fraction of Sclerox and Scleroglucan**

| sample name | added NaCl conc | optical activity [α] at 302 nm | constant term | triple-stranded/single chain |
|---|---|---|---|---|
| scleroglucan (20 °C) | none | +81 | 2.5 | 100/0 |
| (sclerox)Na (20 °C) | none | +88 | 2 | 0/100 |
| | 0.1M NaCl | +90 | 1.5 | 0/100 |
| | 1 M NaCl | +166 | 3.5 | 85/15 |
| (sclerox)Na (35.6 °C) | 1 M NaCl | +122 | 4 | 0/100 |
| (sclerox)Na (55 °C) | 1 M NaCl | +101 | 2.5 | 80/20 |
| (sclerox)Na (back to 20 °C) | 1 M NaCl | +121 | 2 | 80/20 |
| (sclerox)TBA (20 °C) | none | +68 | 2 | 0/100 |
| | 0.1 M NaCl | | 1 | 0/100 |
| | 1 M NaCl | | 3 | 80/20 |

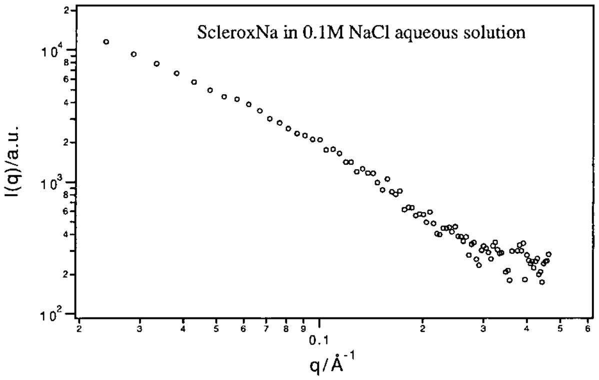


**Figure 2.** Observed small-angle X-ray scattering (the double logarithmic plots) from (sclerox)Na in 0.1 M NaCl aqueous solution.

The SAXS measurements were performed at BL-10C of the Photon Factory, Tsukuba, Japan. An incident X-ray was monochromatized to $\lambda = 1.49$ Å and focused to a focal point with a bent focusing mirror. The scattered X-ray was detected by a one-dimensional position-sensitive proportional counter positioned at a distance of about 1 m from the sample holder. A flat sample cell of 0.2-cm path length made of stainless steel provided with windows of 20-$\mu$m-thick quartz plates was used. The temperature of the cell was controlled by circulating water of a constant temperature through the cell holder. The solutions were injected into the cell, which was placed in the cell holder at least 10 min prior to the SAXS measurements. The SAXS intensities were accumulated for a total of 600 s by repeating the measurements for a period of 60 s each time from a fresh solution in order to ensure enough statistical precision without degrading the polysaccharide samples by X-ray irradiation. The scattering intensities were corrected with respect to the variation of the incident X-ray flux by monitoring with an ion chamber in front of the cell holder. The X-ray absorption of the solution was compensated by measuring the incident and transmitted X-ray intensities. The excess scattering intensities were calculated by subtracting the scattering intensities of the solvent from those of the polysaccharide solutions.

## Results and Discussion

Table 1 summarizes the scleroglucan and sclerox samples used in this work. The small-angle X-ray scattering (SAXS) was observed from the aqueous solutions of respective polysaccharide samples. A typical example of the SAXS measurements is shown in Figure 2 in terms of the double logarithmic plots for (sclerox)Na in 0.1 M NaCl aqueous solution. The observed scattering profiles are analyzed by decomposing in two or three components, representing a single chain or a triple-strand helix of (1 → 3)-$\beta$-D-glucan, and a constant term, representing the spatial correlation between single chain and/or triple-strand helix fragments. Here the scattering profile is assumed to be described in terms of a broken rod model[8] as

$$q^2 I(q)/c \approx \sum_i \pi q w_i M_{\mathrm{li}} \cdot \frac{4 J_1^{\,2}(q r_i)}{(q r_i)^2} + \text{constant} \quad (1)$$

$$q = (4\pi/\lambda)\,\sin(\theta/2)$$

where $J_1$ denotes the first-order Bessel function of the first kind and $w_i$ is the weight fraction of the $i$th component specified by the cross-sectional radius $r_i$ and the linear mass $M_{\mathrm{li}}$. The scattered intensity is normalized by the concentration $c$. The constant term in eq 1 takes into account the intercylinder correlation due to the flexible (broken) part distributed randomly along a chain. In more detail, the constant term is composed of three factors: the number of rod ends per unit length, the number of kinks per unit length, and the number of crossings per unit length with other rods.[9] Assuming a sclerox chain is composed of triple-stranded helices and disentangled single chains, eq 1 is rewritten as

$$q^2 I(q)/c \approx \sum_i w_i M_{\mathrm{li}} \Theta_i(q) + \text{constant} \quad (2)$$

Here $i$ denotes either a triple-stranded helix component or a single-chain component and $\Theta_i(q)$ is a corresponding particle scattering factor calculated from the atomic coordinates of the respective molecular models. The constant term is considered to account for the structural characteristics of the boundary of triple-stranded helixes and disentangled chains and thus to give an estimate for the deviation of the chain conformation at the boundary from the stable (1 → 3)-$\beta$-D-glucan chain conformation. Expected scattering profiles are shown in Figures 3 and 4 in terms of the Kratky plots and the cross-sectional Guinier plots for a triple-stranded helix and a single chain of scleroglucan, respectively. The scattering profiles were calculated from the atomic coordinates of a triple-stranded helix and a single chain of scleroglucan according to the Debye formula:

$$I(q) = \sum_{i=1}^{n} f_i^{\,2} g_i^{\,2}(q) + 2 \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} f_i f_j g_i(q)\, g_j(q) \cdot \frac{\sin(d_{ij} q)}{d_{ij} q} \quad (3)$$

where $f_i$ and $d_{ij}$ denote the atomic scattering factor of the $i$th atom and the distance between the $i$th and $j$th atoms, respectively. The form factor $g_i(q)$ of a single atom is assumed to be represented by the form factor

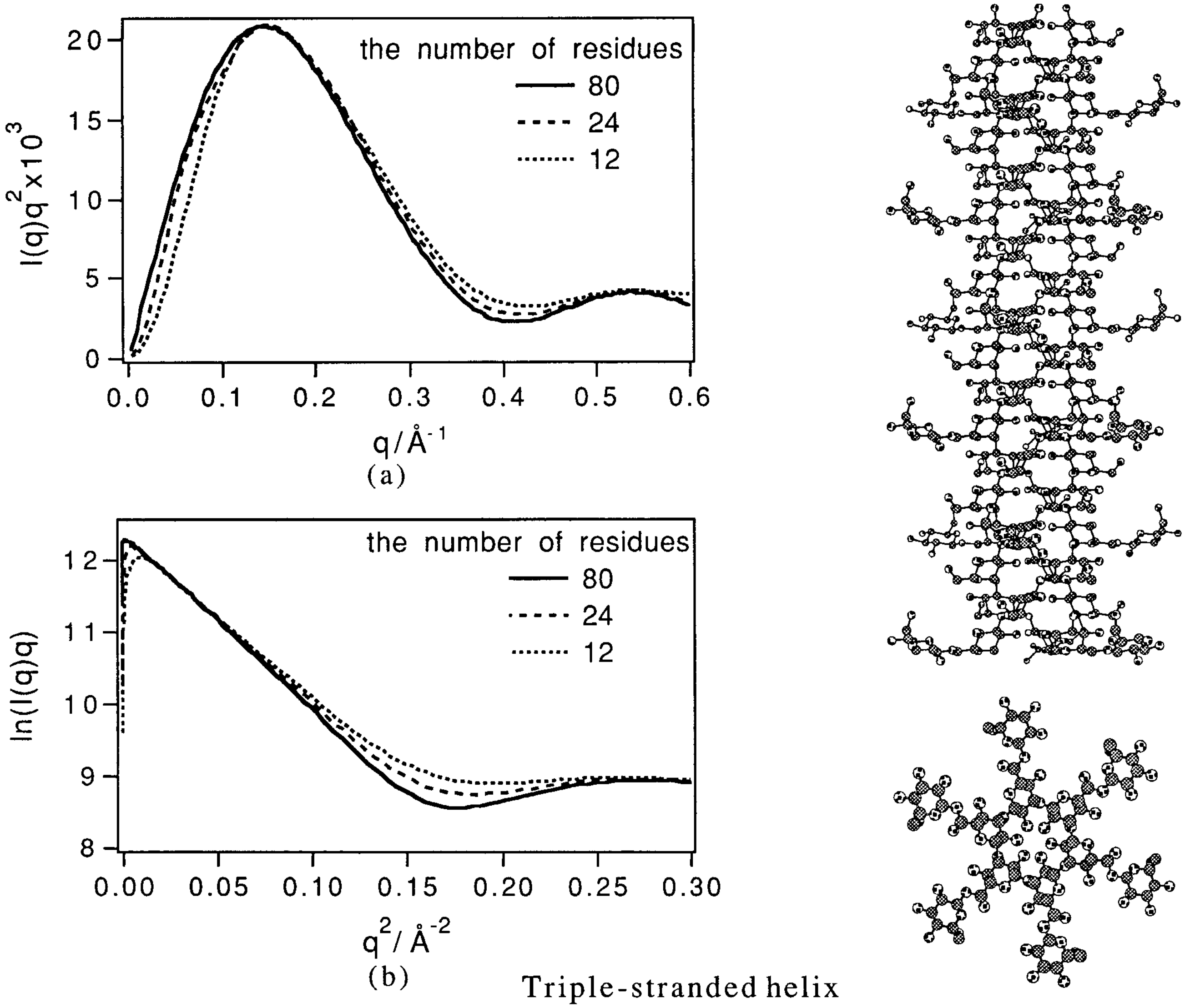


**Figure 3.** Scattering profiles calculated from the atomic coordinates of a triple-stranded helix of scleroglucan as a function of the number of residues (indicated in the figure): (a) the Kratky plots; (b) the cross-sectional Guinier plots. The molecular models are shown to the right of the corresponding figures.

of a sphere possessing the radius equivalent to the van der Waals radius of the *i*th atom as

$$g_i(q) = \frac{3[\sin(R_iq) - (R_iq)\cos(R_iq)]}{(R_iq)^3} \qquad (4)$$

Here $R_i$ denotes the van der Waals radius of the *i*th atom and is set to 1.67 or 1.50 Å for a carbon or an oxygen atom, respectively. Single chains of scleroglucan were generated by the Monte Carlo method according to the energy map of laminari-biose,[10] and the scattering profile of a single chain was calculated as an ensemble average of the particle scattering factors over all generated chains. The scattering profiles calculated from two molecular models (a triple-stranded helix and a single chain) confirm the stiff nature of (1 → 3)-β-D-glucan chains. A model (1 → 3)-β-D-glucan chain is composed of 80 residues. The calculated scattering profile depends on the number of residues in a (1 → 3)-β-D-glucan chain, as seen in Figures 3 and 4, but no number-of-residues dependency is observed in the scattering profile at $q > 0.1$ Å$^{-1}$ when the number of residues exceeds 80. Since no criterion is available on the number of residues constituting a triple-stranded helix or a single chain in the molecular model, the number of residues is fixed to 80 in the present analysis from the viewpoint of the consistency of fitting in a lower $q$ region.

The observed scattering profiles were decomposed into three terms (a triple-stranded helix term, a single-chain and a constant term) according to eq 2. The fitting allows estimating the weight fractions of the residues involved in a triple-stranded helical conformation and a single-chain conformation with an accuracy of ±4%, respectively. The results are summarized in Table 1, and a fitting example is shown in Figure 5 for (sclerox)-Na in water. The scattering profile observed from (sclerox)Na exhibits an interference peak due to a strong electrostatic repulsion between charged carboxylate groups in water. The interference due to the electrostatic interaction in (sclerox)Na in water was taken into account by the interference term $S(q)$ incorporated in eq 2 as

$$q^2I(q)/c \approx (\sum_i w_iM_{li}\Theta_i(q) + \text{constant})\cdot S(q) \qquad (5)$$

The interference term is given by assuming the Gauss-

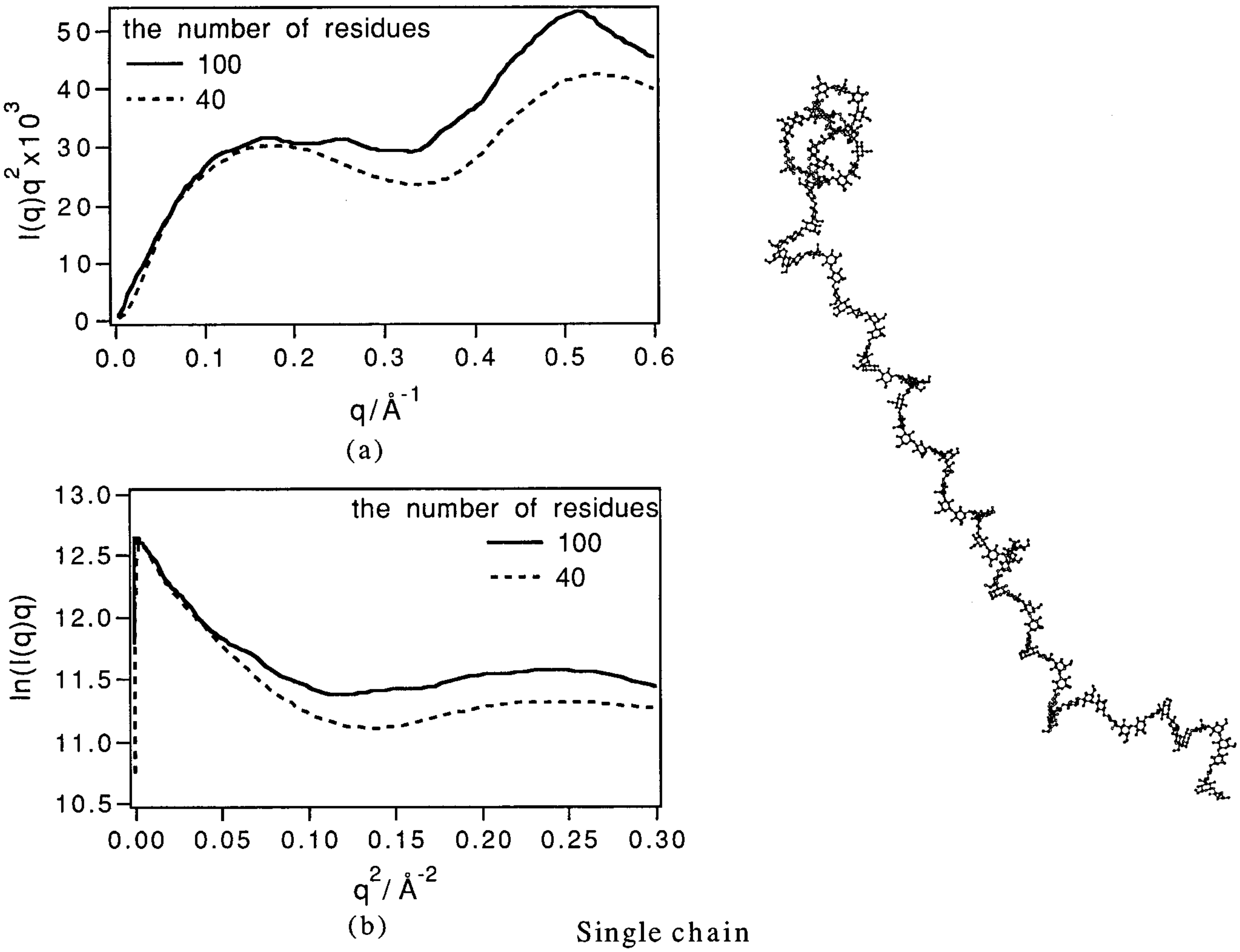


**Figure 4.** Scattering profiles calculated from the atomic coordinates of a single chain of scleroglucan as a function of the number of residues (indicated in the figure): (a) the Kratky plots; (b) the cross-sectional Guinier plots. The molecular models are shown to the right of the corresponding figures.

ian type interaction potential[11] between charged carboxylate groups as

$$S(q) \approx \frac{1}{1 + cK \exp(-\xi^2 q^2)} \tag{6}$$

where $K$ is a constant including the molecular weight and the second virial coefficient, $c$ is the concentration, and $\xi$ denotes the correlation length of the interaction, representing a range of interaction. The correlation lengths of interaction were estimated as $\xi = 59$ Å and $\xi = 72$ Å for (sclerox)Na and (sclerox)TBA, respectively, from the curve fitting. An example is shown for (sclerox)Na in Figure 5.

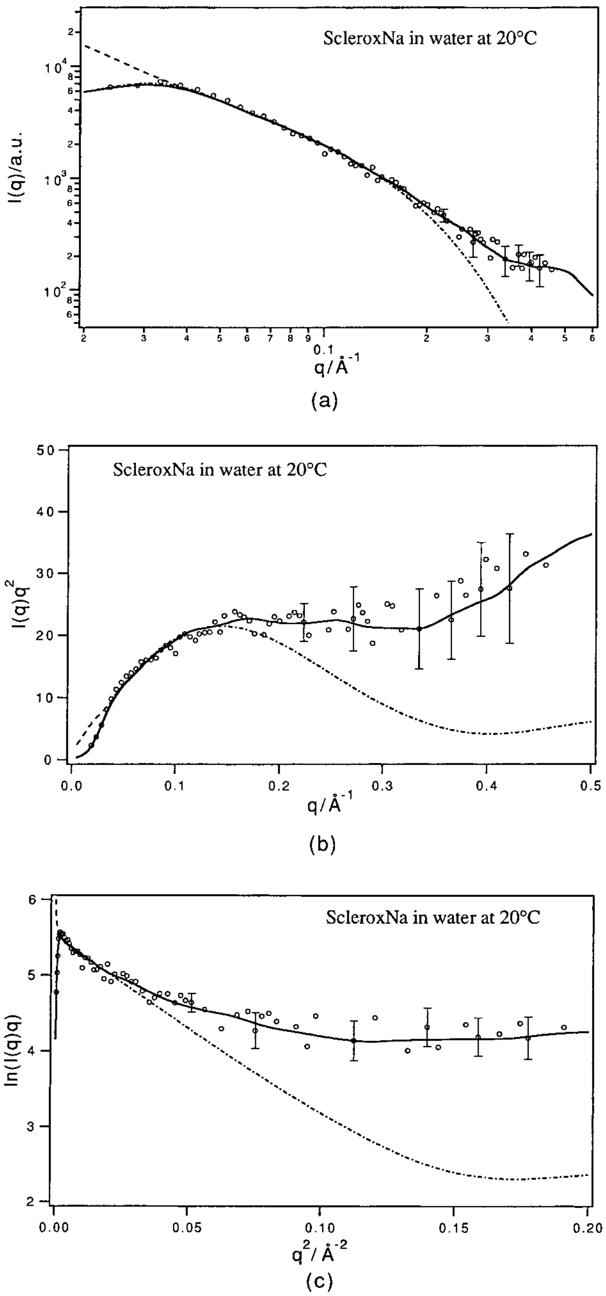
Added salt shields the charges on carboxylate groups, and no interference peak was observed in the small-angle X-ray scattering profiles from sclerox in NaCl aqueous solutions. Thus the fitting was made according to eq 2 without taking into account the interference term $S(q)$. A considerable portion (over 80%) of both (sclerox)Na and (sclerox)TBA chains assumes a triple-stranded helical conformation in 1 M NaCl aqueous solutions (see Table 1), whereas sclerox is represented by a sequence of simulated single chains in pure water. This result suggests that the introduction of charges in the side groups promotes the disentanglement of triple-stranded chains but that sclerox chains become triple-stranded again by shielding electric charges on carboxylate groups.

In the aqueous solution, most of the sclerox chains retain a single-chain conformation, but the electric charge will be shielded by adding NaCl (less than 0.1 M), so that the viscosity decreases as observed before.[5,6] Here the sclerox behaves as an ordinary polyelectrolyte. With more NaCl (over 0.4 M) in solution, the sclerox chain starts resuming a triple-stranded helical conformation, and the chain becomes more rigid to increase the viscosity.

We now examine the consistency of the present conformational analysis with the earlier data[5,6] of the optical activity and viscosity of sclerox under various conditions. The earlier results can be summarized as below:

The viscosity (in terms of $[\eta]$ or $\eta_{sp}/c$) decreases and the optical activity (in terms of $[\alpha]$ at 302 nm) increases at first by adding NaCl in the aqueous solutions. When more salt is added, the viscosity increases by taking the minimum value at 0.4 M NaCl concentration, and the optical activity decreases by taking the maximum value at 1.5 M NaCl concentration. The behavior of the viscosity and the optical activity as a function of the added salt concentration can be attributed to a recovery of a triple-stranded helical conformation by added salt screening the charges on the sclerox chain.

The viscosity and the optical activity exhibit a thermal hysteresis.[6] For example, the optical activity drops from approximately 170 to approximately 100 in the temper-



(a)

(b)

(c)

**Figure 5.** Observed and calculated small-angle X-ray scattering profiles for (sclerox)Na in water at 20 °C: (a) the double logarithmic plots; (b) the Kratky plots; (c) the cross-sectional Guinier plots. Solid lines show the calculated scattering profiles from a single-chain model according to eq 5, and broken lines show those according to eq 2 before taking into account the interference effect *S*(*q*). One-dot broken lines represent the fitting results with a triple-stranded helix model with an interference effect.

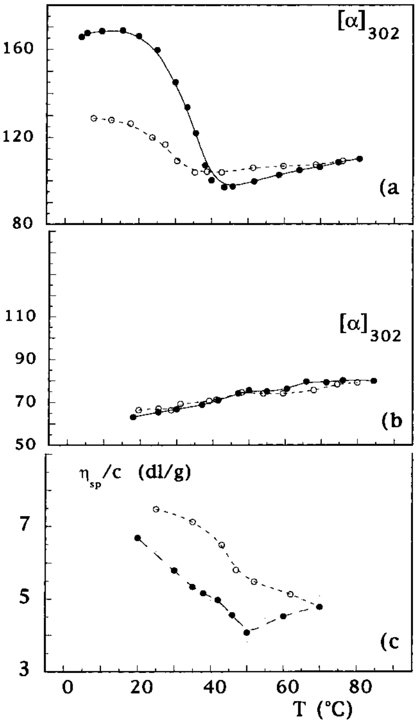


**Figure 6.** Temperature dependence of the optical activity (a and b) and viscosity (c) of sclerox in NaCl aqueous solutions with different salt concentrations: (a) 1 M NaCl; (b) 5 M NaCl; (c) 1.5 M NaCl. The closed circles denote the heating process, and the open circles denote the cooling process.

ature range between 20 and 40 °C by heating (in the (sclerox)Na/1 M NaCl aqueous solution), as shown in Figure 6. Figure 6 also demonstrates that the viscosity decreases at first and then increases by taking a minimum value at 50 °C (in the (sclerox)Na/1.5 M NaCl aqueous solution). The thermal hysteresis disappears after several cycles of heating and cooling, where the viscosity decreases gradually with temperature. The optical activity behaves in a similar way and settles to the intermediate value around 110, where the optical activity is observed to increase slightly with temperature. At a higher added-salt concentration (5 M NaCl), no thermal hysteresis was observed in both viscosity and optical activity.

The sclerox chain undergoes a conformational change from a single chain to a triple-stranded helix according to the ambient ionic strength and temperature. A broken rod seems to represent a whole chain of (sclerox)Na and (sclerox)TBA with two components specified by a single chain and a triple-stranded helix of (1 → 3)-β-D-glucan in general. Native scleroglucan assumes a triple-stranded helix, and (1 → 6)-β-D-glucan side chains will be charged by oxidation that causes disentangling of triple-stranded helixes. By adding salt, the charges are shielded and a (1 → 3)-β-D-glucan chain resumes a triple-stranded helical conformation in part. Here the (1 → 3)-β-D-glucan chain conformation is not fundamentally different in terms of a triple-stranded helix and a single chain, but the partial formation of a triple-stranded helix may involve a large conformational change at the boundary of a triple-stranded part and a disentangled part. Assuming a large optical activity at the boundary, the increase of the optical activity is accounted for by the formation of the boundary due to the resumption of the triple-stranded helical conformation when salt is added. The chain conformation at the boundary is considered to be in the metastable state of relatively high potential energy frozen by the formation of a triple-stranded helix. Large constant terms, for example in (scelrox)Na in the 1 M NaCl aqueous solution at 35.6 °C, which corresponds to the melting temperature of (sclerox)Na in the 1 M NaCl aqueous solution (see Table 1), reflect to some extent the degree of metastability. The thermal motion of the chains loosens the frozen metastable state and induces the conformational transition at around 50 °C at the boundary of a triple-stranded helix to a disentangled part. At around 50 °C, both the optical activity and the viscosity

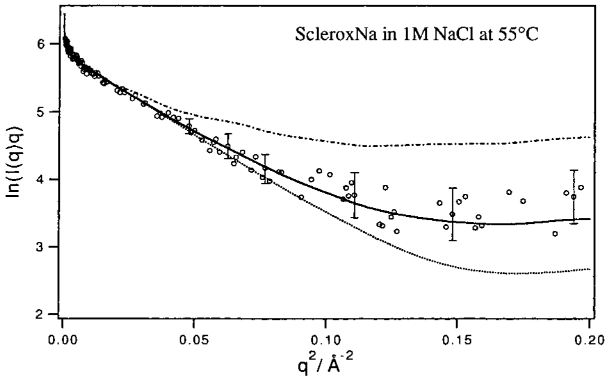


**Figure 7.** Observed and calculated small-angle X-ray scattering profiles for (sclerox)Na in 1 M NaCl aqueous solutions at 55 °C. A triple-stranded helix is formed by heating, as shown by the solid line calculated according to eq 2. The dotted line and the one-dot broken line represent the fitting results with a triple-stranded helix and a single chain, respectively.

of (sclerox)Na are observed to take a minimum value in the 1 M NaCl aqueous solution.[6] The conformational transition allows the rearrangement of the (1 → 3)-β-D-glucan chain conformation to a more stable one. This process is observed as a disappearance of the triple-stranded helixes at the intermediate temperature (35.6 °C) and a reappearance of the triple-stranded helixes over 50 °C in the (sclerox)Na/1 M NaCl aqueous solution when the temperature is increased (see Figure 7 and Table 1). When the heating/cooling cycles are repeated, sclerox forms a stable structure represented by a broken rod composed of triple-stranded and single-chain portions, as confirmed by the curve fitting shown in Figure 7. Here the intermediate value of the optical activity indicates that the chain structure at the boundary differs from the expected (1 → 3)-β-D-glucan chain conformation of a triple-stranded helix or a single chain. The small constant value 2 was estimated for (sclerox)-Na in the 1 M NaCl aqueous solution at 20 °C according to eq 2 (see Table 1) in comparison with the constant value 3.5 estimated before the first heating/cooling cycle. A small constant value suggests that a stable structure is formed by repeated heating/cooling. The stable structure thus formed undergoes no further conformational change with increasing temperature. This result suggests that a structural equilibrium exists between a triple-stranded chain and a disentangled single chain.

In conclusion, the charge on the side chains functions to untie the triple-stranded structure. The triple-stranded structure is formed partially by shielding charge, and the whole chain is frozen in a metastable state by the topological restriction at the partial triple-stranded helical conformation. The rearrangement of the chain conformation takes place by repeated heating/cooling so as to assume a more stable conformation.

**Acknowledgment.** This work was performed under the approval of the Photon Factory Advisory Committee (Proposal No. 94G-291).

## References and Notes

(1) Norisuye, Y.; Yanaki, T.; Fujita, H. *J. Polym. Sci., Polym. Phys.* **1980**, *18*, 547.
(2) Crescenzi, V.; Gamini, A.; Paradossi, G.; Torri, G. *Carbohydr. Polym.* **1983**, *3*, 273.
(3) Gamini, A.; Crescenzi, V.; Albrizzese, R. *Carbohydr. Polym.* **1984**, *4*, 461.
(4) Crescenzi, V.; Gamini, A.; Rizzo, R.; Meille, S. V. *Carbohydr. Polym.* **1988**, *9*, 169.
(5) Coviello, T.; Dentini, M.; Crescenzi, V. *Polym. Bull.* **1995**, *34*, 337.
(6) Coviello, T.; Dentini, M.; Crescenzi, V.; Vincenti, A. *Carbohydr. Polym.* **1995**, *36*, 5.
(7) See, for example: Glatter, O., Kratky, O., Eds.; *Small-Angle X-ray Scattering*; Academic Press: London, 1982.
(8) Guenet, J.-M. *Thermoreversible Gelation of Polymers and Biopolymers*; Academic Press: London, 1992.
(9) Luzzati, V.; Benoit, H. *J. Appl. Crystallogr.* **1961**, *14*, 297.
(10) Kitamura, S.; Minami, T.; Nakamura, Y.; Takeo, K.; Kobayashi, H.; Mimura, M.; Kajiwara, K. *J. Mol. Struct.* **1997**, *395–396*, 425.
(11) Shimode, M.; Mimura, M.; Yamanaka, S.; Urakawa, H.; Kajiwara, K. *Sen'i Gakkaishi* **1996**, *52*, 63.

MA9716286