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ORIGINAL ARTICLE

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Structure of cellulose microfibrils and the hydration effect in *Cryptomeria japonica*: a small-angle X-ray scattering study

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Abstract The nanometer scale structure of cell walls in sugi wood (*Cryptomeria japonica* D. Don) and the hydration dependence were examined by the small angle X-ray scattering technique. Disk-shaped scattering patterns were observed for sugi wood. The radial average of two-dimensional data from the cross section could supply the scattering intensity with statistical accuracy much higher than that obtained from the sector average of the streak-shaped scattering pattern, and both the scattering intensities provided similar structural information. The scattering patterns from the cross section of the wood are characterized by rhombic or cross-shaped isointensity curves for the lower q region and by circularly symmetric isointensity curves for the higher q region. This shows that the disk-shaped scattering has two different kinds of scattering origins. The microfibril radii in the cell wall were determined by fitting the model scattering function of cylindrical fibrils to the scattering data. Values of 12.3 ± 0.3 Å and 12.2 ± 0.3 Å were obtained for the fibril radii of the neighboring earlywood and latewood, respectively, in dry specimens. A drastic structural change of the cell walls was detected with increasing water content from 40% to 100%. A low q rise in the scattering intensities below 0.1 $Å^{-1}$ became weak and changed into a flat pattern, and the rhombic isointensity curves changed to cross-shaped patterns in the two-dimensional scattering from the cross section. The calculated radii R increased from 12.2 ± 0.3 Å to 13.3 ± 0.1 Å.

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Introduction

In wood, the microstructure of the cell wall is known to dominate many properties that affect the human lifestyle, such as the mechanical strength achieved through the shape and arrangement of cellulose microfibrils in the cell wall. Many studies of the microstructures have been performed through measurements of the microfibril angle and the diameter and shape of the cross section of microfibrils in the cell walls of woods by means of small-angle X-ray scattering (SAXS). For example, Jakobs et al.[1] showed using SAXS measurements that the diameter of cellulose microfibrils stays remarkably constant at 2.5 nm throughout the neighboring earlywood and latewood of *Picea abies*.

The mechanical properties of wood change depending on the water content. Jakobs et al.[2] investigated hydration-dependent structural changes in the natural wood cell of *P. abies* using SAXS. They showed that considerable changes of the cell wall structure occurred for specimens dried below the fiber saturation point, x_F, while the structure of the cell wall was independent of the hydration in the native state for $x > x_F$.

Three different experimental techniques, transmission electron microscopy (TEM), wide-angle X-ray scattering (WAXS), and SAXS, have been used for structural characterization of wood. Using these three techniques, Jakobs et al.[3] obtained the same value of 2.5 ± 0.2 nm for the diameter of elementary cellulose fibrils in the cell walls of *P. abies*. Among these techniques, SAXS requires no serious sample shaping as performed for TEM experiments and can determine structural parameters such as the diameter and orientation of fibrils averaged over the area irradiated with X-rays, although it does not provide the direct images supplied by TEM. The average size of cellulose crystallites can also be determined using the Scherrer equation, but it must be taken into account that the Bragg reflections may be

broadened not only by the finite size of the crystallites but also by possible imperfections in the crystal lattice.

Sugi wood, *Cryptomeria japonica* D. Don, occupies about 60% of the accumulated artificial plantations in Japan and has been extensively employed for framed structures in houses and so forth. However, the microstructures of cell walls in sugi wood and the hydration dependence have rarely been examined through the determination of structural parameters, such as the microfibril angle and the diameter and shape of the cross section of microfibril by using SAXS.

The microstructures of the cell wall in wood largely consist of the S_2 and S_1 layers, and both layers contain systems of long but thin cellulose fibrils, parallel on average, and embedded in a matrix of hemicellulose and lignin. The direction of the fibrils in the S_2 layer is given by a spiral angle with respect to the stem axis. In the second largest layer S_1, the direction of the fibrils is roughly perpendicular to that of the S_2 layer.[1] Because the scattering from a very long cylinder is known to be in the shape of a thin disk perpendicular to the cylinder, the comparison of the observations of the scattering pattern in a thin disk shape from two different angles parallel (as a streak) and perpendicular to the disk pattern can be expected to provide much information about the microstructure of the cell walls in wood. Furthermore, the radial average of the two-dimensional data of the disk shape scattering over the wide range of the detector plane is expected to lead to one-dimensional data with statistical accuracy much higher than that obtained from the sector-averaged data from a streak-shaped scattering pattern. Therefore, measurement using a point-collimated instrument with an area detector is essential for the study of the structure of woods through the detection of an anisotropic scattering intensity.

In the present study, the microstructure of the cell walls in *Cryptomeria japonica* D. Don was examined by SAXS measurements of the shape and diameter of the cross section of microfibril in the cell walls and their hydration dependence, using a pinhole instrument.

Materials and experimental method

Wood specimens of *Cryptomeria japonica*, about 8 × 10 mm in size and 0.4 mm thick, were prepared for SAXS measurements from straight grains cut out of the stem. To determine the optimum thickness of the SAXS specimen, we performed SAXS measurements on the dried specimens with varying specimen thickness from 0.3 to 1.2 mm, and found that the multiple scattering cannot be neglected for samples of 0.5-mm thickness or more. We used specimens that were 0.4 mm thick for all the SAXS measurements.

Two kinds of specimens were prepared from neighboring parts of *C. japonica*: one slice was cut along the longitudinal and radial direction of the stem (radial section), and another was sliced perpendicular to the longitudinal direction of the stem (cross section). Samples with hydration x of 0%, 15%, 30%, 50%–60%, 100%, and 200% were prepared by immersing the dried specimens in water up to the prescribed water content. The degree of hydration x was calculated using the formula

$$x = \left(m_x - m_0\right)/m_0 \tag{1}$$

where m_X is the weight of the sample at hydration x and m_0 is that of dry wood. The samples for the SAXS measurement were kept in a brass holder and sealed using an O-ring and two kapton sheets that were 25 or 50 μm thick, in order to avoid the change in the moisture condition of samples loaded in the evacuated sample chamber.

The overall features of the pinhole SAXS instrument utilized in this study were described previously.[4] The sealed sample was irradiated with a monochromatized beam collimated using two pinhole apertures separated by 800 mm, and the scattered radiation was recorded as two-dimensional data on the imaging plate. This instrument can give an effective q range of about $0.008 < q < 3.5\,\text{Å}^{-1}$ for the same instrumental geometry, where q is the modulus of scattering vector given by $q = 4\pi\sin\theta/\lambda$, where λ is the wave length of the incident radiation and 2θ is the scattering angle. The resolution of the apparatus for Cu-Kα radiation is $\Delta q_{\text{FWHM}} \sim 0.005\,\text{Å}^{-1}$. All the flight paths including the sample chamber were evacuated to a pressure of 1 Pa. SAXS data collected on the pinhole instrument at room temperature using Cu-Kα radiation were corrected only for background and the sample absorption. A beam spot at the sample position was 0.6 mm in diameter at 90% of the beam intensity. The position of samples can be changed by two micrometer screws in two mutually perpendicular directions in the plane normal to the incident X-ray beam.

Results

SAXS from the neighboring earlywood and latewood in dried specimens

Annual rings in *Cryptomeria japonica* were clearly recognized, and the widths of the latewood were more than 0.6 mm, the size of the incident beam at the sample position, and the widths of the earlywood were 1 mm or more. Therefore, the identification of the SAXS patterns from the neighboring latewood and earlywood could be made distinctly by the measurement of the SAXS intensities by shifting the sample in steps of 0.5 mm in the direction across the annual ring.

Two-dimensional scattering data plotted in Fig. 1a for the latewood in the radial section of the dried specimens shows clearly that the scattering pattern for the latewood consists of one strong streak in the equatorial direction normal to the stem axis and one weak streak in the meridional direction along the stem axis. The strong streak normal to the stem is expected to originate from cylindrical cellulose fibrils oriented along the vertical direction in the S_2 layer, and the weak streak in the direction of the stem axis is due to the superposition of many cellulose microfibrils of the equatorial direction in the S_1 layer from the description

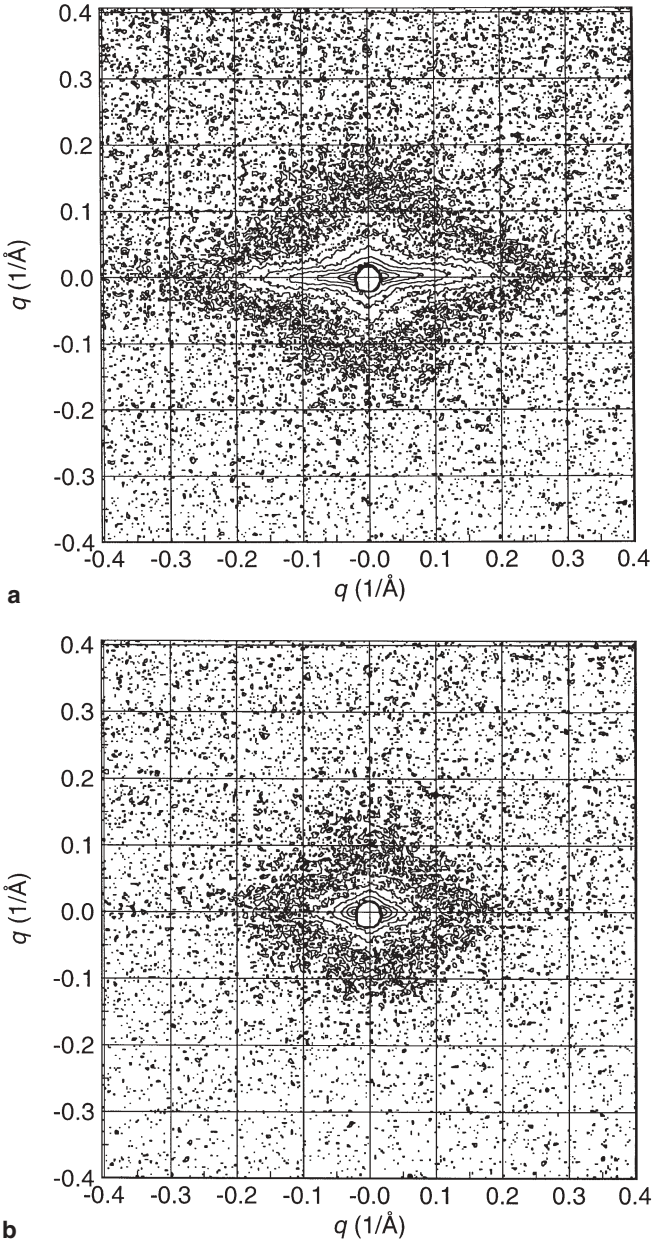

Fig. 1. Isointensity contour maps with logarithmic spacing of scattering intensities from a radial section of neighboring latewood (**a**) and earlywood (**b**) of *Cryptomeria japonica* D. Don. The longitudinal direction of the stem is vertical

given by Jakobs et al.[1] Scattering from the earlywood shown in Fig. 1b shows a weak pattern of diamond-shaped isointensity elongated along the equator in the relatively lower q region. The same scattering patterns were found for ramie fibers, and the main source of the scattering was attributed to voids elongated in elliptical shapes in the direction of fibers.[5]

Scattering patterns from the cross sections of *C. japonica* corresponding to those plotted in Fig. 1 are plotted in Fig. 2a for the latewood and in Fig. 2b for the earlywood. Both scattering patterns are characterized by rhombic isointensity curves for the q region less than about $0.15\,\text{Å}^{-1}$. With regard to the q region above about $0.15\,\text{Å}^{-1}$, circularly symmetric isointensity curves were observed for the scattering pattern from the latewood, and for the weak scattering intensity from the earlywood.

Consideration of the SAXS patterns from the cross sections of *C. japonica* may lead to the view that these scattering intensities are composed of two different types of scattering contributions; one consists of the scattering pattern characterized by rhombic isointensity curves becoming pronounced less than $0.15\,\text{Å}^{-1}$, and the other consists of the scattering pattern which extends over the q region above $0.15\,\text{Å}^{-1}$ in the form of circularly symmetric isointensity curves. Comparison of the scattering patterns from the latewood with incident X-rays parallel and perpendicular to the direction of the stems indicates clearly that the circularly symmetric scattering spreading over the q region above $0.15\,\text{Å}^{-1}$ is disk-shaped, and confirms the description by Jakobs et al.[1] for the strong streak vertical to the stem.

The reduction of two-dimensional data to one-dimensional data depending on a scalar q is required in order to perform quantitative investigations on the observed scattering data which exhibit disk-shaped patterns. The scattering intensities $I(q)$ were calculated by radial averages over a circle for the circularly symmetric scattering patterns from the cross section of the wood, and over the sectors symmetric about the X-axis with an open angle of 20° for the radial sections of wood that exhibited streaks in the scattering pattern. The typical scattering intensities versus a scalar q are plotted in Fig. 3 for the radial and cross sections of the dried specimens. It is noteworthy that the scattering intensity from the radial section almost overlaps that from the cross section of the latewood in *C. japonica*, in the wide q range. Error bars added in Fig. 3 were due only to statistical errors in the count rates introduced by the radial average. Much higher precision was obtained for the scattering intensity from the cross section than from the radial section for the same specimen, as seen in Fig. 3.

Hydration effects on the SAXS intensities of earlywood and latewood in sugi

Scattering intensities were measured for wood specimens with hydration x of 0%, 15%, 30%, 50%–60%, 100%, and 200%, and the hydration dependence was examined. Almost all of the two-dimensional scattering patterns from the radial sections and the cross sections of the hydrated specimens exhibited strong streaks vertical to the stem and circularly symmetric isointensity patterns above $0.15\,\text{Å}^{-1}$, respectively, similar to those of the dried wood. Elliptical isointensity patterns exhibited by the cross section of the only specimen with hydration of 50%–60% may have resulted from the possibility that the specimen was sliced in a direction deviating from perpendicular to the stem. With regard to the scattering patterns less than about $0.1\,\text{Å}^{-1}$, it is interesting that cross-shaped patterns as shown in Fig. 4 appear for the cross section of the specimens with hydration above 100% in the q region where rhombic isointensity

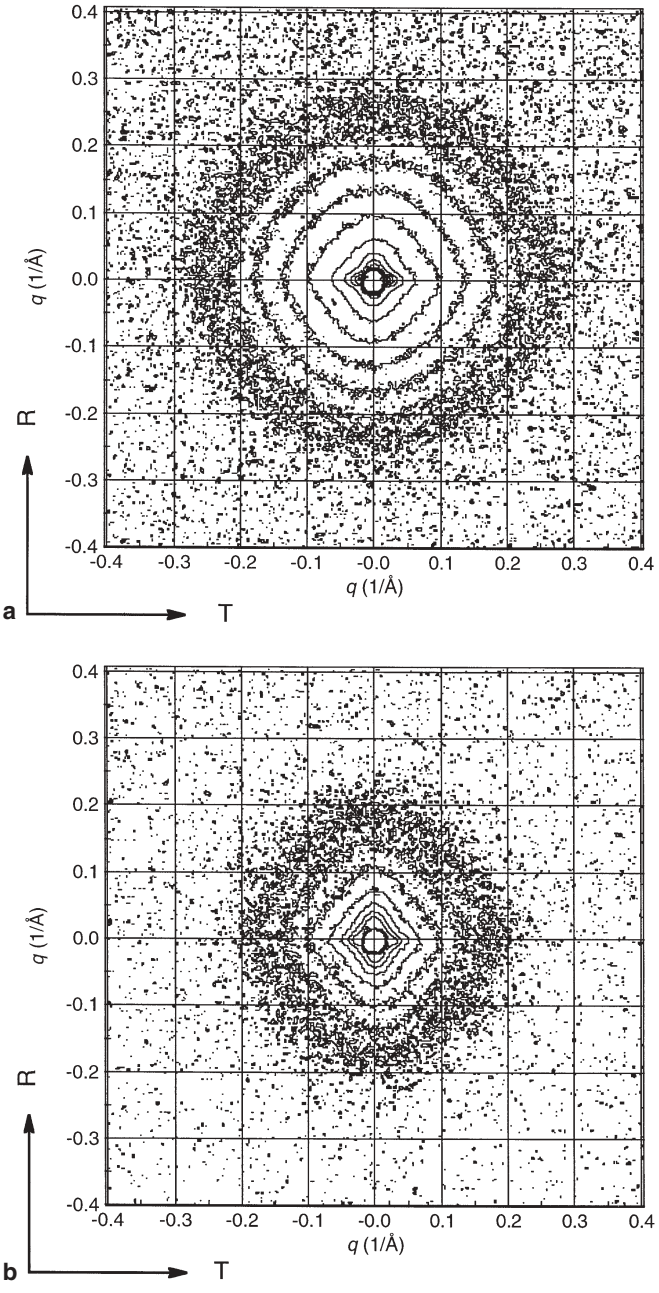

Fig. 2. Isointensity contour maps with logarithmic spacing of scattering intensities from a cross section of neighboring latewood (**a**) and earlywood (**b**) of *Cryptomeria japonica* D. Don

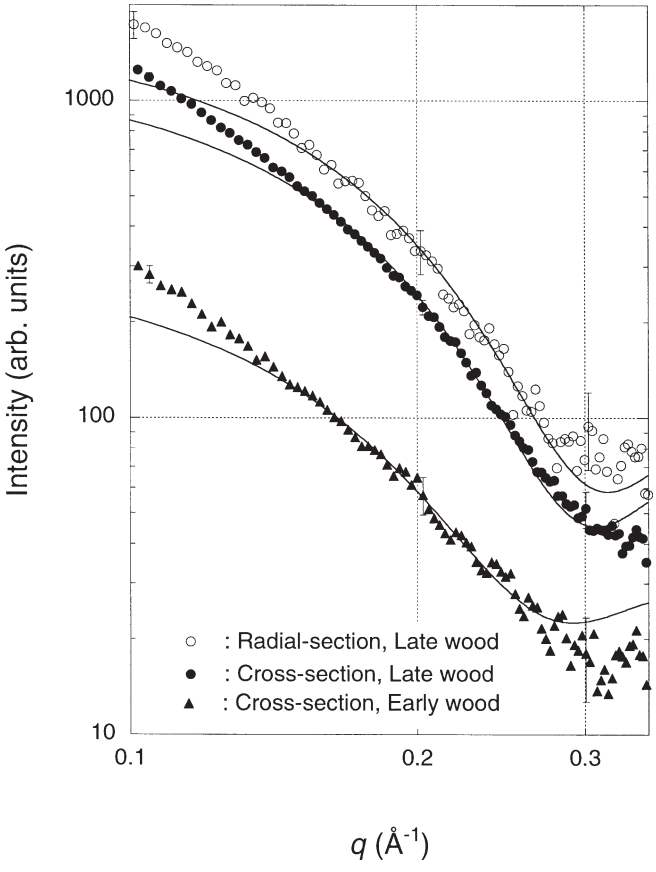

Fig. 3. Typical scattering intensities from the radial and cross sections of dried specimens, and the fitted curves of Eq. 2. Scattering intensity from the radial section almost overlaps that from the cross section, hence it was shifted by a factor 1.5 for clarity

curves are observed for the specimens with hydration less than 50%–60% (Fig. 2).

Typical hydration dependence is shown in Fig. 5 for the scattering intensities obtained by radially integrating the circularly symmetric isointensity data from the latewood in the cross sections of *C. japonica*. Features of the scattering intensities change distinctly with increasing degree of hydration from 55% to 100%. A remarkable low q rise in the scattering intensities is observed below 0.1 $Å^{-1}$ for the specimens with degree of hydration less than 55%, while a flat pattern is observed for the specimens with hydration

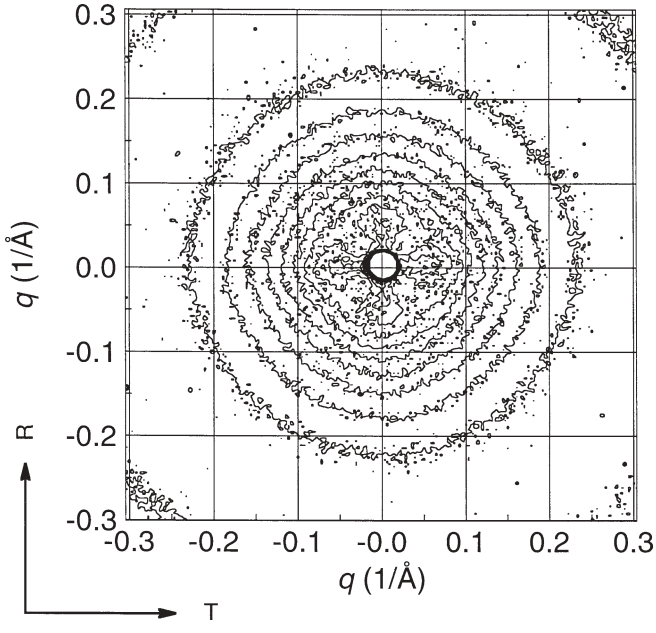

Fig. 4. Isointensity contour map with spacing of scattering intensities from a cross section of the latewood of *Cryptomeria japonica* D. Don with hydration of 200%

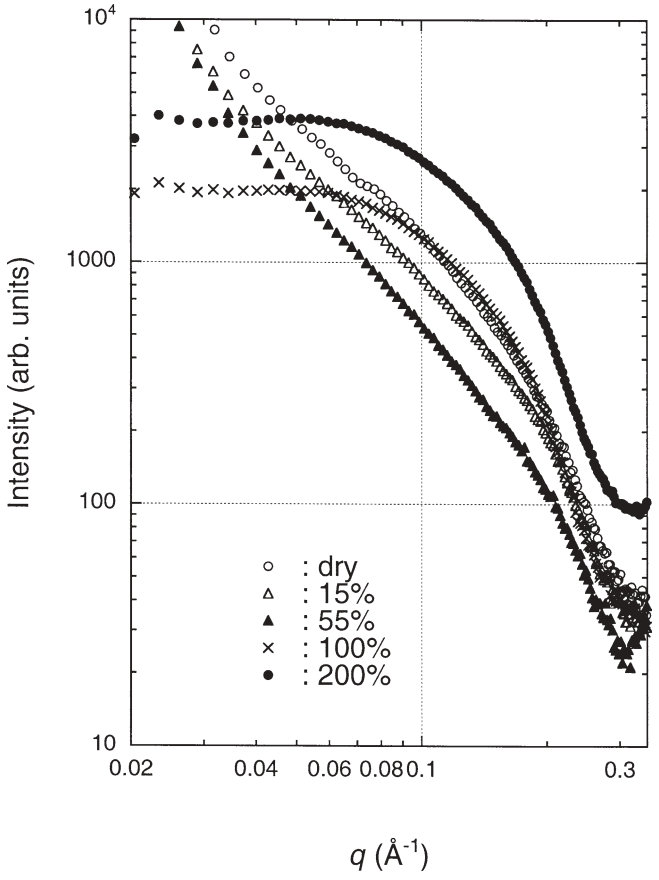

Fig. 5. Scattering intensities from the cross sections of the latewood of *Cryptomeria japonica* D. Don with hydration of 0%, 15%, 50%–60%, 100%, and 200%

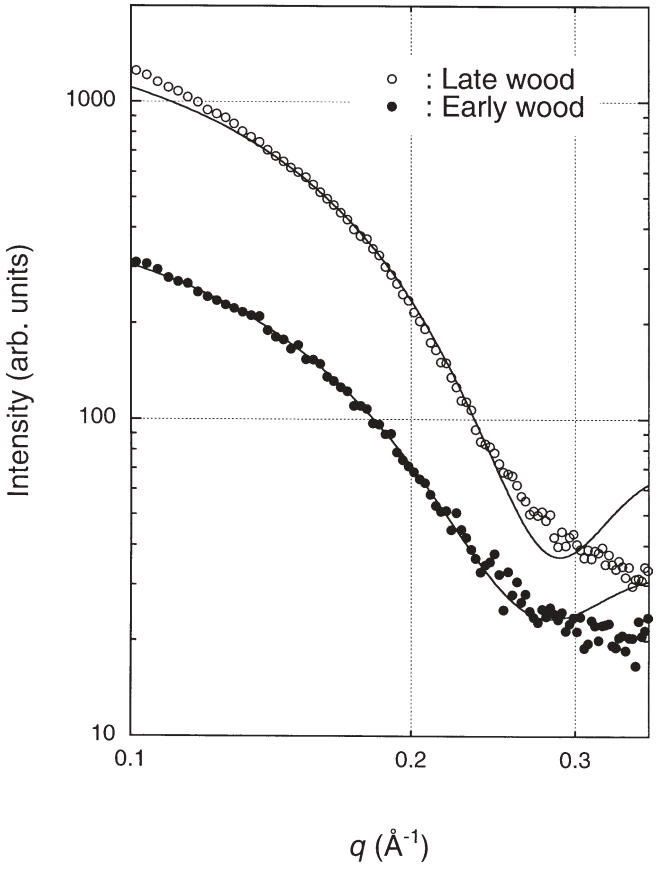

Fig. 6. Fits of Eq. 2 to the scattering intensities of the neighboring earlywood and latewood for the cross section of *Cryptomeria japonica* D. Don with hydration of 100%, together with the experimental scattering intensities

higher than 100%. Similar behavior of the hydration dependence of the scattering intensities is observed for the latewood in the radial section and for the earlywood in the cross section.

Data analysis

A rod-like particle having infinite length causes a scattering pattern with the directions of vector ***q*** distributed within the flat disk perpendicular to the direction of the rod. Considering the rods with a circular cross section of radius R, the scattering intensity in the disk plane from N single rods is given by

$$I_{\text{model}}(q) = N\left(2\frac{J_1(qR)}{qR}\right)^2, \tag{2}$$

where J_1 is the Bessel function of the first order.[6,7] The radius R should be understood as an averaged radius for the case of the cross sections of the rod with noncircular shapes, such as square or hexagonal shapes.[1] Because the disk-shaped scattering patterns observed in the q region above 0.15 Å^{-1} were interpreted as being caused by a single long fibril aligning along the stem axis, data analysis using Eq. 2 is expected to describe the characteristic features of the scattering intensities well and lead to a reliable estimate for the radius R of the cross section of cylindrical cellulose microfibrils.

The scattering intensities were fitted to Eq. 2 and values for radius R were calculated by solving the nonlinear least squares problem over the q range between 0.15 and 0.3 Å^{-1} using a modified Marquardt method in the SALS system.[8] Well-fitted $I_{\text{model}}(q)$ curves were obtained in the same q range between 0.15 and 0.3 Å^{-1}, as illustrated in Fig. 3 for the dried specimens, and in Fig. 6 for the cross section of the specimen with hydration of 100%. Values for the radius of the fibril cross section determined by the fits are plotted versus the hydration x in Fig. 7. Radius R of the fibril can be seen to increase above 13 Å for the specimens with the hydration above 100%, within the uncertainty in our analysis. Uncertainties shown in Fig. 7 depend on the goodness of fit between the observed and model scattering intensities. Values were obtained with high precision for the scattering data especially from the cross section of the latewood for which the data was well described by Eq. 2 over the wide q range as exemplified by Fig. 6.

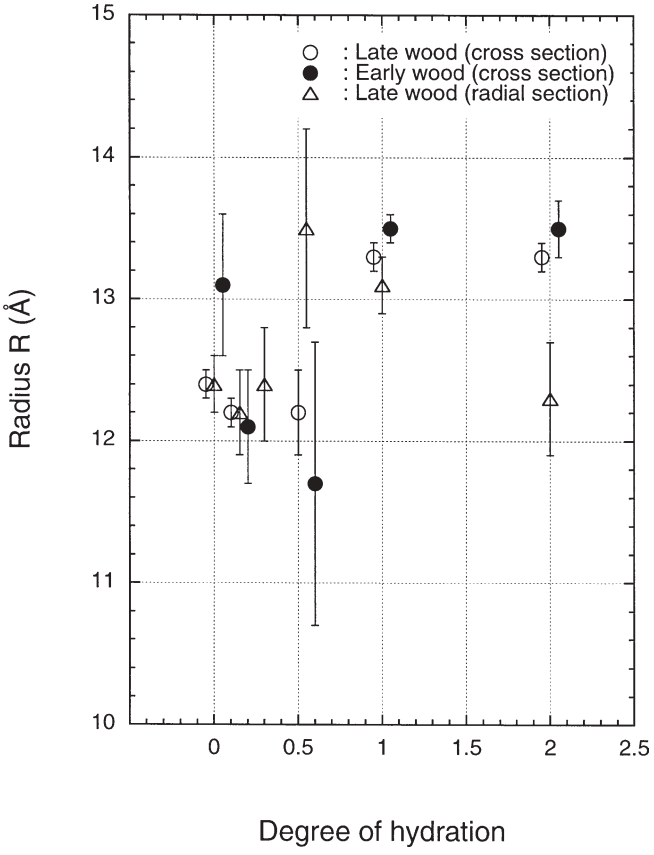

Fig. 7. Hydration dependence of the calculated radius *R* for the microfibril in the cell walls. Plots of the radius obtained from the latewood and earlywood in the cross section are shifted a little left and right, respectively, for clarity

Discussion

Two kinds of SAXS measurements with the X-rays incident on the sample along the stem axis (cross section) and vertical to the stem axis (radial section) led to disk-shaped scattering patterns from *Cryptomeria japonica*. The thin disk-shape scattering can be seen to arise from two kinds of scattering origins from Figs. 2a and 4 in which the scattering patterns from the cross section are drawn. The scattering patterns are characterized by rhombic isointensity curves for the q region less than about $0.15\,\text{Å}^{-1}$ and by circularly symmetric isointensity curves above about $0.15\,\text{Å}^{-1}$. The thin disk-shaped scattering pattern in the direction perpendicular to the stem axis shows that the scattering rods align along the stem axis on average. Weak streaks that are parallel to the stem axis in the scattering from radial sections (Fig. 1a) due to cellulose fibrils in the S_1 layer can be neglected compared with the strong streak in the equator of the q region less than about $0.15\,\text{Å}^{-1}$.

With increasing degree of hydration x, a low q rise in the scattering intensities below $0.1\,\text{Å}^{-1}$ becomes weak and changes into flat pattern for hydration higher than 100%, for all the measured scattering intensities as shown in Fig. 5. Correspondingly, the rhombic isointensity curves (Fig. 2a) change to cross-shaped patterns (Fig. 4) for the scattering from the cross section of the specimens with increasing degree of hydration above 100%. Usually, water is absorbed by wood as bound water in the cell wall in cases of hydration lower than the fiber saturation point, about 30% for *Picea abies*. At higher water content, water is mainly absorbed as free water or vapor in the voids and lumina.[2] The change in the scattering profiles from the low q rise to the flat pattern can be attributed to a decrease in the scattering contrast in electron density between pores and the surrounding cell media, by the absorption of water in the voids, and lumina having a length scale corresponding to the q region less than $0.1\,\text{Å}^{-1}$. The low q rise in the scattering intensities below $0.1\,\text{Å}^{-1}$ can be mainly attributed to pores, as previously described for the scattering from ramie fibers.[5] The rhombic pattern of the isointensity curve in the two-dimensional data from the cross section is considered to reflect the shape of the voids. The cross-shaped patterns detected for the specimens with the degree of hydration above 100% show only very weak patterns superimposed on the strong isotropic scattering extending over a wider q region above $0.1\,\text{Å}^{-1}$. Detection may be prevented by the strong low q rise for the scattering from the specimens with degree of hydration less than 55%. Therefore, it is difficult to conclude that the structural changes occur with increasing hydration above 100%. The cross-shaped patterns are considered to reflect rectangular structures somewhat shorter in the direction parallel to the annual rings having a length scale of the order of 100 Å, such as the correlation between cellular walls.

The observations of the scattering pattern in the shape of a thin disk from two different angles parallel (as a streak) and perpendicular to the disk pattern could provide the similar hydration dependence of the radius of the microfibril in the cell walls in sugi wood, as plotted in Fig. 7. The two-dimensional data from the cross section could supply one-dimensional data with much higher precision by the radial average over the wide range of the detector plane than that obtained from the sector-averaged data from the streak-shaped scattering pattern. Values calculated for the radius from the scattering of the latewood in the cross section increase from 12.2 ± 0.3 Å to 13.3 ± 0.1 Å with increasing water content from 55% to 100%. Values calculated from the other scattering patterns have a similar hydration dependency, except for the value from the scattering for the specimen of radial section at $x = 200\%$. However, the interpretation of an increase in the fibril radius seems difficult based only on these scattering data. The uncertainties of the radius are only due to statistical error in the counting rates.

Conclusions

Disk-shaped scattering patterns were detected for sugi wood (*Cryptomeria japonica*) using a pinhole SAXS instrument. The scattering patterns from the cross section of the wood are characterized by rhombic or cross-shaped isointensity curves for the lower q region and by circularly

symmetric isointensity curves for the high q region. This shows that the disk-shaped scattering has two different kinds of scattering origins. Furthermore, the radial average of two-dimensional data from the cross section could supply the scattering intensity with statistical accuracy much higher than that obtained from the sector average of the streak-shaped scattering pattern. Both the scattering intensities provided similar structural information.

SAXS measurements detected a drastic structural change of the cell walls in sugi wood with increasing the water content from 50% to 100%. A low q rise in the scattering intensities below $0.1\,\text{Å}^{-1}$ becomes weak and changes into flat pattern, and the rhombic isointensity curves change to cross-shaped patterns in the two-dimensional scattering from the cross section of the wood.

The cellulose microfibril radii R in the cell walls were calculated from the least-squares fit of Eq. 2. The calculated microfibril radii of the neighboring earlywood and latewood in the cross section give equal values 12.3 ± 0.3 Å and 12.2 ± 0.3 Å, respectively, for the dry wood. The calculated radii R tend to decrease slightly up to a water content of 50% and increase from 12.2 ± 0.3 Å to 13.3 ± 0.1 Å with increasing the water content from 50% to 100%. The fibril radii are constant for water content higher than 100%. The interpretation of an increase in the microfibril radius requires further investigation.

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