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Crystal transition between hydrate and anhydrous (1→3)-β-D-xylan from *Penicillus dumetosus*

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ABSTRACT

The crystal structures of hydrated and anhydrous (1→3)-β-D-xylan and the crystal transition between them were investigated. Highly crystalline samples of (1→3)-β-D-xylan were prepared from a siphoneous green alga, *Penicillus dumetosus*. The crystal structure was analyzed using synchrotron X-ray diffraction and solid-state ^{13}C NMR spectroscopy. The hydrated form was converted to the anhydrous form with contraction in both the *a*-axis and *c*-axis directions, and the crystallinity decreased considerably at the same time. The crystal transition between hydrated and anhydrous (1→3)-β-D-xylan was monitored using synchrotron X-ray diffraction under controlled relative humidity. The transition took place at certain transition points, and was accompanied by a large hysteresis. From the difference in the weight and unit-cell volume at the transition points, the number of water molecules in the hydrated form was estimated as one per xylose residue.

1. Introduction

(1→3)-β-D-Xylan is a polysaccharide occurring in some green algae and red algae (Percival & McDowell, 1967). In particular, the cell walls of some siphoneous green algae such as *Bryopsis*, *Caulerpa*, *Udotea*, and *Penicillus* are known to consist mainly of fibrillar (1→3)-β-D-xylan rather than cellulose, which occurs in many plants as the fibrillar structural component in their cell walls (Frei & Preston, 1964).

The crystal structure of (1→3)-β-D-xylan was first investigated using X-ray fiber diffraction patterns obtained from the inner wall of *Penicillus dumetosus*, where the crystalline (1→3)-β-D-xylan was oriented in the longitudinal cell direction (Frei & Preston, 1964). Frei and Preston reported that hydrated and anhydrous forms existed and proposed the following hexagonal unit cells: $a = 15.4$ Å and $c = 6.12$ Å for the hydrate, and $a = 13.7$ Å and $c = 5.85$ Å for the anhydrous form. These results were supported by an electron-diffraction study (Chanzy, Guizard, & Vuong, 1977). Using the fiber X-ray diffraction pattern of the hydrated form, further analysis was subsequently carried out by combined with molecular modeling and infrared spectroscopy (Atkins & Parker, 1969; Atkins, Parker, & Preston, 1969). According to the proposed model for the hydrate, xylan chains form a 6/1 right-handed triple helix arranged in the hexagonal unit cell, where six xylose residues and six water molecules are included, the space group being $P6_3$. Calculation of the conformational energies also supported this triple-helical model (Sathyanarayana & Rao, 1970). Regarding the hydrogen bond, however, different schemes have been proposed as a result of reexamination of the X-ray diffraction pattern (Veluraja & Atkins, 1987) and a quantum mechanical calculation (Miyoshi, Uezu, Sakurai, & Shinkai, 2006).

One of the interests in (1→3)-β-D-xylan is its similarity to (1→3)-β-D-glucan, which occurs in many organisms (Stone & Clarke, 1992) and has attracted much attention because of its physiological effects (Ooi & Liu, 2000). The only difference between the chemical structures of (1→3)-β-D-glucan and (1→3)-β-D-xylan is the existence of a hydroxymethyl group, thus their conformations are also assumed to be similar. In fact, the crystallographic study of (1→3)-β-D-glucan showed that it forms a triple-helical structure (Chuah, Sarko, Deslandes, & Marchessault, 1983; Deslandes, Marchessault, & Sarko, 1980), which is quite similar to that of (1→3)-β-D-xylan, and it also has hydrated and anhydrous forms (Marchessault, Deslandes, Ogawa, & Sundararajan, 1977).

Recently, we reported the crystal transition between hydrated and anhydrous (1→3)-β-D-glucan using highly crystalline paramylon, and the study provided some information about its transition

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properties and the number of water molecules in the hydrated form (Kobayashi, Kimura, Togawa, Wada, & Kuga, 2010). In the case of (1→3)-β-D-xylan, the hydration and dehydration behaviors have not been precisely investigated yet. In this study, therefore, we describe the crystal transition between hydrated and anhydrous (1→3)-β-D-xylan using a siphoneous alga, *P. dumetosus*, and compare it with the case of (1→3)-β-D-glucan.

2. Experimental

2.1. Sample preparation

Dried samples of the siphoneous green alga, *P. dumetosus*, were kindly provided by Dr. H. Chanzy, CERMAV. After treatment with 0.1 N HCl to remove calcite, the algal cell walls were treated with 70% ethanol at 70 °C for 3 h and then boiled in water for 3 h. They were then treated with 0.3% $NaClO_2$ solutions buffered at pH 4.9 in 0.1 M acetate buffer at 70 °C for 3 h. For solid-state ^{13}C NMR and gravimetry measurements, an aliquot of the purified cell walls was homogenized using a double-cylinder-type homogenizer (Physcotron, Microtec Co. Ltd., Japan). Both the purified cell walls and homogenized samples were stored in deionized water for the wet samples, and over phosphorus pentoxide in a desiccator for the dry samples.

2.2. Synchrotron X-ray diffraction analysis

Synchrotron X-ray diffraction was carried out at the BL40B2 beam line located at the SPring-8 facility (Hyogo, Japan). A stack of several purified cell walls that were cut into 1 cm lengths was mounted on a goniometer head and synchrotron-radiated X-rays were irradiated orthogonal to the cell surface for a period of 120 s. Air with RH = 100% and 0%, generated from a humidity generator (Shinyei SRG-1R, Japan), was flowed over the samples to keep them in wet and dry conditions, respectively. The diffraction patterns were recorded using a camera system equipped with a flat imaging plate (IP) (R-Axis V^{++}, Rigaku, Japan) at room temperature and the sample-to-IP distance was calibrated using Si powder (d = 3.1355 Å).

The diffraction profiles were obtained from the diffraction patterns using the R-Axis display software package (Rigaku, Japan). The peak positions were determined by peak-fitting using a nonlinear least-square fitting program for pseudo-Voigt functions (Wada, Okano, & Sugiyama, 1997).

2.3. Solid-state CP/MAS ^{13}C NMR spectroscopy

Samples were inserted individually into tightly sealed 4-mm BL-type ZrO_2 rotors. ^{13}C CP/MAS NMR spectra were recorded with a Bruker Avance spectrometer equipped with a 4-mm BL-type probe and operated at 100 MHz. The spectra were acquired at room temperature with a 100 kHz proton dipolar decoupling field, matched cross-polarization (CP) fields of 80 kHz, a proton 90° pulse of 2.5 μs and magic angle spinning (MAS) at a spinning speed of 12 kHz. The CP transfer was achieved using a ramped amplitude sequence (RAMP-CP) for an optimized total time of 2 ms. The sweep width was 50 kHz to avoid baseline distortion with 2994 TD points, and the Fourier transformation was achieved without apodization over 8000 points. The repetition time was 4 s and an average number of 20 000 scans was acquired for each spectrum. The ^{13}C chemical shifts were determined relative to the carbon chemical shift of the carbonyl signal of glycine at 176.03 ppm.

2.4. Monitoring the crystal transition using synchrotron X-ray diffraction

Monitoring the drying and wetting process with change in humidity was carried out in the BL40B2 beam line at the SPring-8 facility, in the same as for the synchrotron X-ray diffraction analysis described above. Measurement of the drying process was performed from RH = 100% to 0% in decreasing steps of 10%, followed by a wetting process from RH = 0% to 100% in increasing steps of 10%. Each X-ray diffraction measurement was carried out after a stabilization time of 10–20 min.

2.5. Gravimetry with water adsorption and desorption

Water adsorption/desorption isotherms were obtained by gravimetry. The samples were stored over saturated salt solutions in a desiccator at 23 °C until their weight became constant at each RH.

3. Results and discussion

3.1. Synchrotron X-ray diffraction

Synchrotron X-ray diffraction diagrams of the wet and dry samples are shown in Fig. 1. The intensity of each reflection was not uniform in the azimuthal direction, indicating that the crystalline (1→3)-β-D-xylan was partly oriented along the fiber axis in the cell walls, as previously reported (Frei & Preston, 1964). Synchrotron X-ray diffraction profiles obtained by radial integration of the diffraction patterns (Fig. 1) are shown in Fig. 2.

The profile of the wet sample (Fig. 2a) shows many sharp peaks, indicating the high crystallinity of the sample. All of the observed peaks were indexed with a hexagonal unit cell and the unit-cell parameters were determined as: a = 15.34 Å and c = 6.07 Å. These values agree well with the values of the hydrated (1→3)-β-D-xylan reported in a previous study (Frei & Preston, 1964). In the profile of the dried sample, only a few broad peaks can be observed in the low Q region. The crystallinity of the samples considerably decreased on drying. Despite the quite low resolution of the profile, the peaks were also indexed with a hexagonal unit cell and gave the unit-cell parameters of: a = 14.2 Å and c = 5.8 Å. These values are also consistent with the values of anhydrous (1→3)-β-D-xylan previously reported (Frei & Preston, 1964), although the a-axis value was slightly larger.

Dehydration of the hydrated (1→3)-β-D-xylan produced a contraction in both the a- and c-axis directions. The difference in the unit-cell volumes between the hydrated and anhydrous forms was 224 Å^3. The number of water molecules in the unit cell of the hydrate, calculated using this value, was 6.9, assuming that the density of the water in the hydrated (1→3)-β-D-xylan is the same as the density of ice, 0.92 g/cm^3. Considering the structural model of (1→3)-β-D-xylan, the unit cell of which contains six xylose residues, the most appropriate number of water molecules contained in the unit cell of the hydrate is six.

3.2. Solid-state CP/MAS ^{13}C NMR spectroscopy

The wet and dry samples were also investigated using solid-state CP/MAS ^{13}C NMR spectroscopy. The spectrum of the hydrated (1→3)-β-D-xylan obtained from the wet sample (Fig. 3a) has a much better resolution than those reported previously (Saito, Yamada, Yoshioka, Shibata, & Erata, 1991). It clearly showed resonances from all of the carbon atoms split into two peaks, except for C4. The peak fitting of all of the resonances showed that they consist of two peaks with equal areas, indicating that the asymmetric unit contains two xylose residues. This is inconsistent with the model

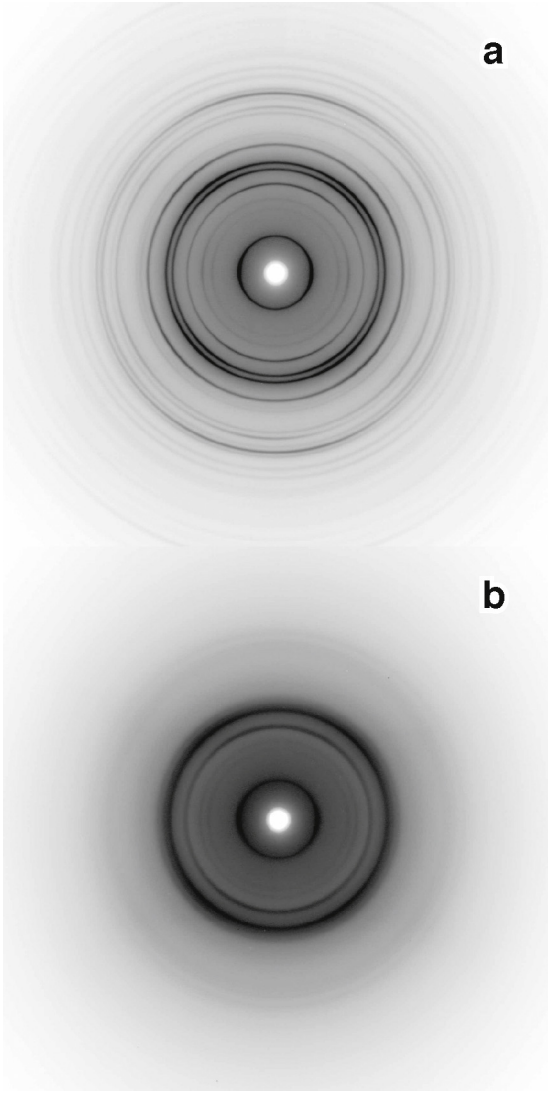

Fig. 1. Synchrotron X-ray fiber diffraction patterns of (a) hydrated and (b) anhydrous (1→3)-β-D-xylan from the wet and dry samples of *Penicillus dumetosus*, respectively. The pattern of the hydrate was recorded under a RH = 100%.

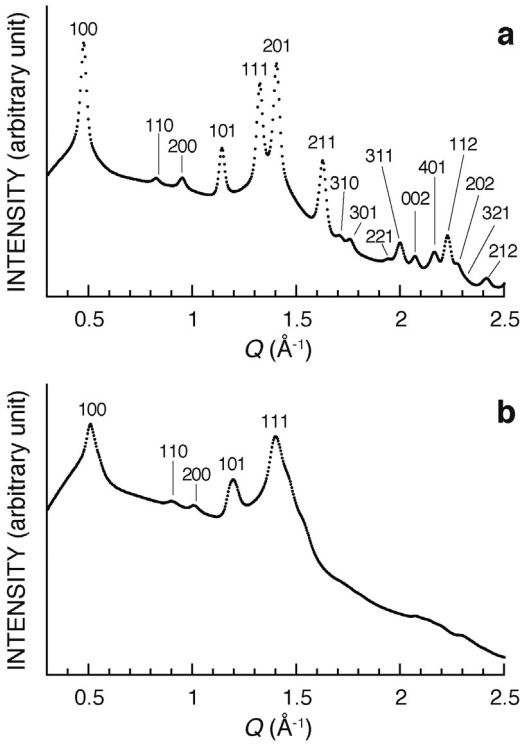

Fig. 2. Synchrotron X-ray fiber diffraction profiles of (a) hydrated and (b) anhydrous (1→3)-β-D-xylan obtained from intensity integration of the patterns in Fig. 1.

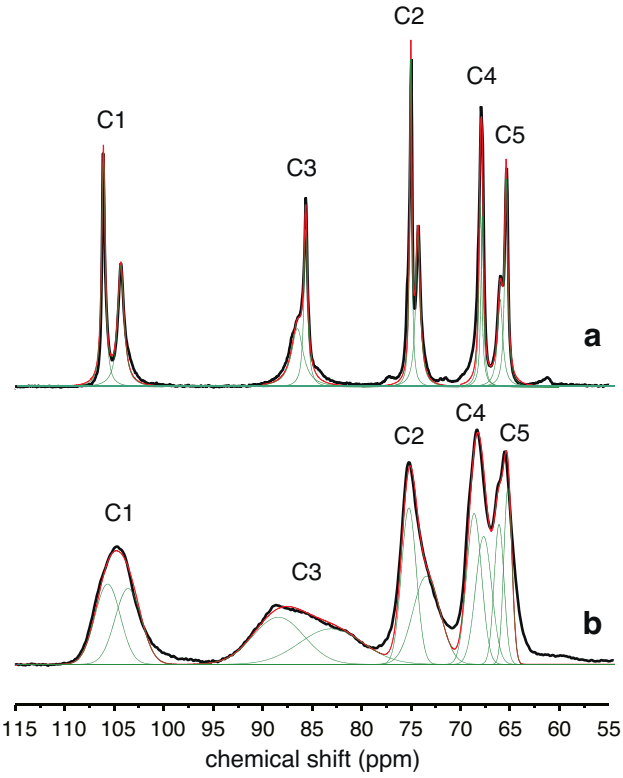

Fig. 3. Solid-state ^{13}C NMR spectra of (a) hydrated and (b) anhydrous (1→3)-β-D-xylan with the peaks obtained by peak fitting (green lines) and their integration (red lines). The peaks are assigned according to Saito et al. (1991). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

proposed previously, where the asymmetric unit contains only one xylose residue.

If the asymmetric unit contains two xylose residues in the hexagonal unit cell, the hydrated (1→3)-β-D-xylan should have a structure with a lower symmetry than the proposed model, possibly with a space group of *P*3. Although the X-ray diffraction analysis was consistent with the proposed hexagonal unit cell, a larger unit cell containing two triple helices, which might be arranged in an anti-parallel manner, is also a possible explanation for the larger asymmetric unit. Another possibility is that there are two phases of the hydrated (1→3)-β-D-xylan. However, this is unlikely because the two peaks of each carbon atom in the NMR spectrum have equal areas. In any case, the cause of the inconsistency between the proposed model and the present NMR data cannot be identified from the current data. Further analysis is required to understand the precise structure of (1→3)-β-D-xylan.

On dehydration, all of the resonances became much broader (Fig. 3b). The anhydrous (1→3)-β-D-xylan has a more disordered structure than the hydrate, which is consistent with the result of the X-ray diffraction analysis. Although the peaks appeared to overlap each other because they were quite broad, all of the resonances were separated into two peaks by peak fitting, the same as for the

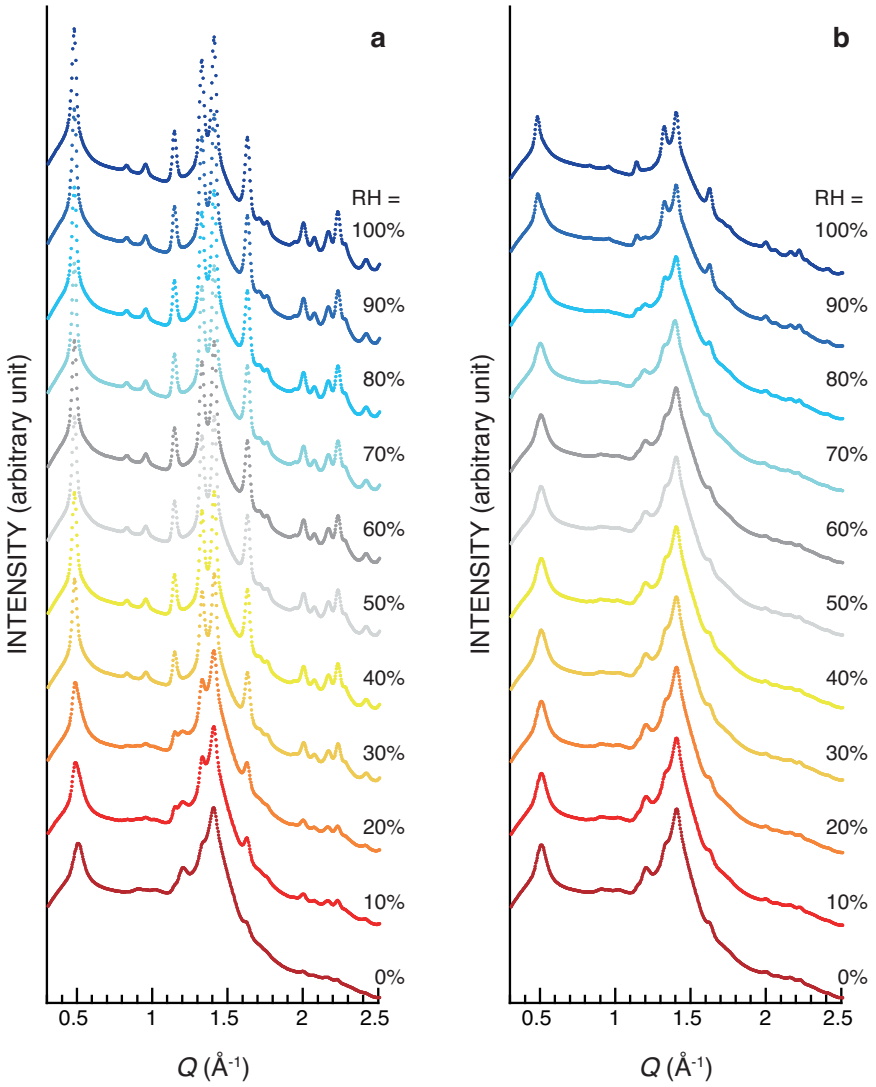

Fig. 4. Changes in synchrotron X-ray diffraction profiles during (a) the drying of the hydrated (1→3)-β-D-xylan with a decrease in RH and (b) the subsequent wetting with an increase in RH.

hydrate. The chemical shifts of all of the peaks also changed with the transition from hydrated to anhydrous form (Table 1), indicating that dehydration produced changes in the molecular conformation of the xylan chains. In particular, these changes in the widths and shifts of the peaks were remarkable in the resonance from C3, as were also observed in the case of (1→3)-β-D-glucan.

3.3. *Crystal transition between hydrated and anhydrous (1→3)-β-D-xylan*

The drying process of the wet sample and the subsequent wetting process with varying RH were monitored using synchrotron X-ray diffraction (Fig. 4). At the beginning of the drying process (Fig. 4a, RH = 100%), the wet samples showed the pattern of the hydrated form, the same as the pattern in Fig. 2a. Until the RH reached 30%, the pattern of the profile remained basically unchanged with slightly decreasing intensity of each reflection. At RH = 20%, the pattern of the profile clearly changed and peaks from the anhydrous form appeared. The peaks from the hydrated form still remained subsequently, but they were almost absent at RH = 0%. In the subsequent wetting process, on the other hand, the profile remained basically unchanged up to RH = 70%. The peaks from the hydrated form gradually increased above RH = 80%, and the profile at RH = 100% showed completely the pattern of the hydrated form, although the sharpness of the peaks was lost compared with that of the initial wet sample. The dehydration and hydration occurred around RH = 20% and 80%, respectively, indicating that the crystal transition between hydrated and anhydrous forms took place with hysteresis. Hysteresis in the transition between hydrated and anhydrous forms was also observed in the case of other hydrated polysaccharides such as β-chitin and (1→3)-β-D-glucan (Kobayashi, Kimura, Togawa, & Wada, 2010; Kobayashi, Kimura, Togawa, Wada, & Kuga, 2010).

Fig. 5 shows changes in the unit-cell parameters and volume at each step determined from the profiles in Fig. 4. At the transition

Table 1
Chemical shifts of hydrated and anhydrous (1→3)-β-D-xylan.

	Chemical shifts (ppm)				
	C1	C2	C3	C4	C5
Hydrate	104.3 106.1	74.2 75.0	85.6 86.4	67.7 68.0	65.3 65.9
Anhydrous	103.7 105.8	73.8 75.6	83.4 88.7	68.1 69.1	65.7 66.6

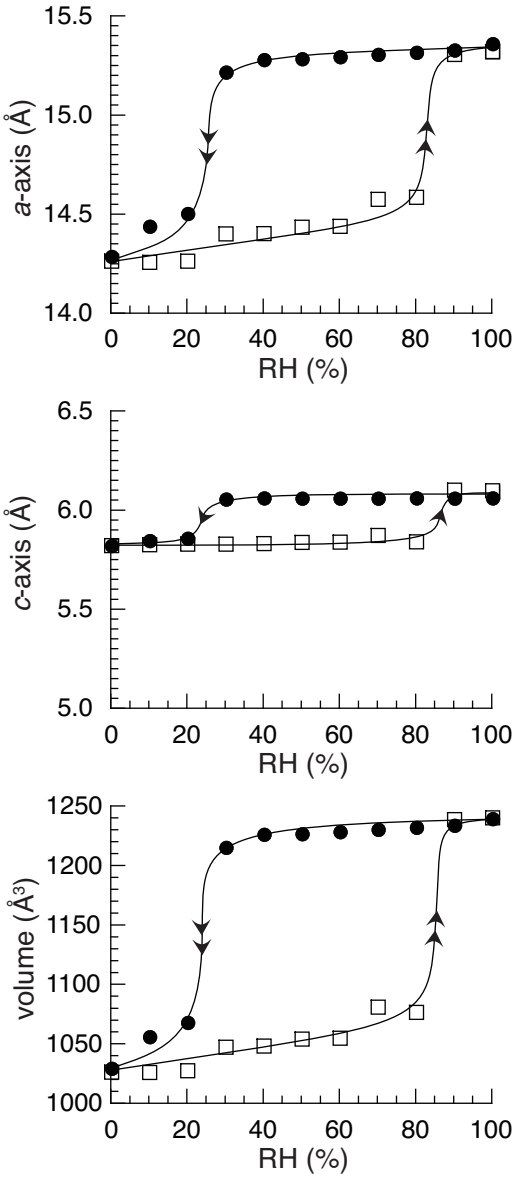

Fig. 5. Changes in unit-cell parameters calculated from each profile in Fig. 4. *Key*: filled circles = drying process; empty squares = wetting process.

points, both the *a*-axis and the *c*-axis decreased and increased steeply with dehydration and hydration, respectively, although the change in the *c*-axis was quite small. These values were basically constant outside the transition points, but only the *a*-axis in the anhydrous form varied somewhat with humidity change. Such a change in the *a*-axis value, i.e., the distance between the triple helices, outside the transition points was also observed in the case of (1→3)-β-D-glucan (Kobayashi, Kimura, Togawa, Wada, & Kuga, 2010). This behavior would result from adsorbed water on the crystalline surface, which relaxed and/or plasticized the crystals.

The isotherm of (1→3)-β-D-xylan obtained by gravimetry is shown in Fig. 6. In the drying process of the wet sample, the weight decreased greatly from the beginning to RH = 60% followed by a slight decrease around RH = 50%, but it decreased greatly again from RH = 40% to 20%. In the wetting process, a large increase in the weight was observed from RH = 60% to 80%. Combined with the results of monitoring the crystal transition using X-ray diffraction (Fig. 4), the decrease from RH = 40% to 20% in the drying process and the increase from RH = 60% to 80% in the wetting process resulted

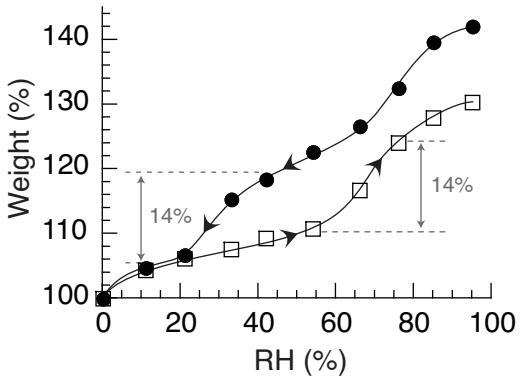

Fig. 6. Water-desorption/adsorption isotherm of (1→3)-β-D-xylan. *Key*: filled circles = drying process; empty squares = wetting process.

from the crystal transition between hydrated and anhydrous forms. The decrease and increase are approximately 14%, which corresponds to one water molecule per one xylose residue. This agrees with another stoichiometric value obtained from the difference in unit-cell volume as described above, and also agrees with the structural model (Atkins & Parker, 1969).

4. Conclusions

The crystal transition between hydrated and anhydrous (1→3)-β-D-xylan occurred as follows:

1. The crystallinity and the homogeneity of the molecular conformation decreased when the hydrated form converted to the anhydrous form.
2. The transition was reversible and there was a large hysteresis.
3. Both the distance between the triple helices and the pitch decreased and increased with dehydration and hydration, respectively.

Such transition properties are the same as those of (1→3)-β-D-glucan, another abundant polysaccharide that has a similar chemical and higher-order structure to (1→3)-β-D-xylan.

The unit cell and stoichiometric values of water obtained in this study agreed with the earlier proposed model. However, the solid-state ^{13}C NMR spectroscopy analysis provided an inconsistent result, suggesting that further analysis on the crystal structure is required.

Acknowledgments

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