Proton−Deuteron and H_2O−D_2O Exchange in Polysaccharides by X-ray and Small-Angle Neutron Scattering

Michael Gawronski, Harald Conrad,* and Tasso Springer

Institut für Festkörperforschung, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

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ABSTRACT: Rodlike fragments of the polysaccharide cinerean were examined in H_2O and D_2O with small-angle X-ray and neutron scattering (SAXS and SANS). From measurements of the macroscopic scattering cross section on an absolute scale the mass per length of the rods has been determined. The SANS measurements in D_2O yield a value very different from those obtained by other measurements (SAXS with H_2O and D_2O; SANS with H_2O). This effect has been used to determine the exchange of protons and bound H_2O from the polysaccharide against deuterons and D_2O. The number of H_2O molecules per repeating unit is determined to be about 17.

Introduction

The polysaccharide cinerean is a microbial β-(1,3)(1,6)-D-glucan[1] produced by the fungus *Botrytis cinerea*.[2,3] By electron microscopy we found that the native cinerean consists of wormlike molecules with a large persistence length.[4] By ultrasonic degradation, fragments are formed which can be considered as rigid rods.[4] The morphology of the rods is multihelical in water. The conformation of cinerean in different solvents is studied in details in another work.[7] We are interested in cinerean as a model substance for long and stiff molecules, which are technically used for spinning and the production of fibers.[5]

In this work we examine fragmented cinerean in H_2O and in D_2O with small-angle X-ray and neutron scattering (SAXS and SANS) in order to determine the mass per length M/L of the rods from the macroscopic scattering cross-section. The SANS measurements of cinerean in D_2O yield a value for M/L, which will be compared to other measurements (SAXS with D_2O and with H_2O; SANS with H_2O). The resulting difference can be interpreted by an exchange of protons of the polysaccharide and of water attached to the rod against deuterons of the solvent leading to a change in the contrast for neutrons. This can be used to determine the number of exchangeable H_2O molecules per repeating unit.

Experimental Section

The cultivation of *B. cinerea* and the sample preparation with ultrasound is descibed elsewhere.[7] The cinerean used for this experiments had a molecular weight $M_w = 200\,000$, and the samples were prepared from the same sample stock. The concentration of the solutions was between 21 and 28 mg/mL, and the solutions were clear at the measuring temperature of 25 °C. To make sure that the solutions were at equilibrium, the experiments were repeated after a waiting time and the results were reproduced.

Small-Angle X-ray Scattering (SAXS). The SAXS measurements were performed at the JUSIFA-small-angle scattering facility at DESY/Hasylab in Hamburg, Germany, with the DORIS storage ring as the X-ray source.[8] The scattered intensity is measured with a two-dimensional position-sensitive detector. To obtain the mass per unit length a standard scatterer of amorphous carbon was used for calibration on an absolute scale with an accuracy of about 5%. The X-ray wavelength was 0.1 nm.

Small-Angle Neutron Scattering (SANS). The SANS measurements were performed at the D 11 neutron small-angle camera[9] at the ILL in Grenoble, France, and at the KWS 1 facility[10] at the research reactor DIDO in Juelich, Germany. The wavelengths of the neutrons were 0.6 nm and 0.7 nm. For calibration on an the absolute scale, Lupolen and H_2O were used, the latter being a standard procedure at ILL.

Chemicals. D_2O (99.9% d) was purchased from Sigma.

Results and Discussion

Theoretical Background. The structure factor $S(Q)$ of a particle of volume V contains the information about the density distribution of the scattering centers in the particle, namely

$$S(Q) = \left|\frac{1}{V}\int_V \mathrm{d}V \exp(-iQr)\right|^2 \qquad (1)$$

Q is the scattering vector, with $Q = (4\pi/\lambda) \sin\theta \approx 4\pi\theta/\lambda$ with the scattering angle 2θ and the wavelength λ of the incident radiation. For an isotropic system an average over all particle orientations is to be taken. The intensity $\Delta I(Q)$ scattered into a resolution element $\Delta\Omega$ of the detector is connected to the macroscopic scattering cross-section by

$$\frac{\mathrm{d}\sum(Q)}{\mathrm{d}\Omega} = \frac{\Delta I(Q)/\Delta\Omega}{I_0 D T} = nK^2 S(Q) \qquad (2)$$

with the number density $n = cN_A/M$ of the particles. I_0 is the primary intensity, D the sample thickness, c the concentration in g/cm^3 of the particles in the solution, N_A Avogadro's number, M the molecular mass of the particles and K the "contrast" between particle and the solvent. For X-rays the contrast is

$$K^2 = \frac{\mathrm{d}\sigma_{Th}}{\mathrm{d}\Omega} V^2 (\Delta\rho_e)^2 = \frac{\mathrm{d}\sigma_{Th}}{\mathrm{d}\Omega} M^2 (\Delta z_e)^2 \qquad (3)$$

Here $\mathrm{d}\sigma_{Th}/\mathrm{d}\Omega$ is the differential scattering cross section of the electron, $\Delta\rho_e$ the average electron density difference between particle and solvent, V the volume of the particle and Δz_e the difference in mole electrons per gram between particle and solvent. The contrast for neutrons is given by

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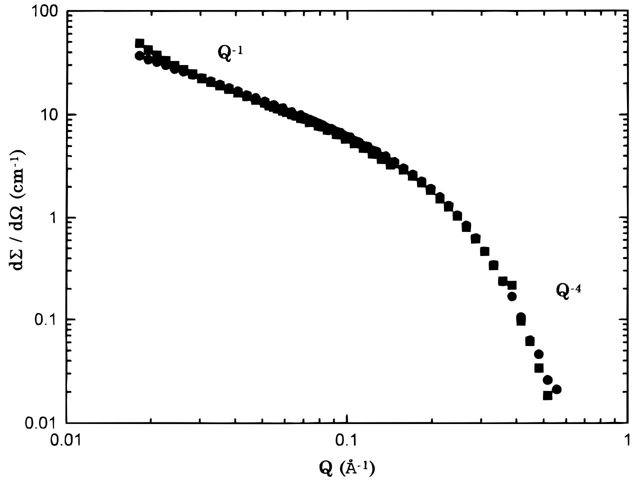

Figure 1. Macroscopic scattering cross section per solid angle of fragmented cinerean in H_2O (circles) and in D_2O (squares) with SAXS (normalized to the concentration *c*). The Q^{-1} and the Q^{-4} regimes can be clearly seen. The points are nearly indistinguishable.

$$K^2 = V^2(\Delta\rho_b)^2 = M^2(\Delta b)^2 \qquad (4)$$

where $\Delta\rho_b$ is the difference of the scattering length density between particle and solvent.

Assuming a cylindrical shaped particle of length L with an effective radius R, the macroscopic scattering cross-section for $1/L < Q < 1/R$ is[6, 7]

$$\frac{d\sum(Q)}{d\Omega} = (\Delta k)^2 c N_A \pi \frac{M}{L}\frac{1}{Q}\exp(-R^2Q^2/4) \qquad (5)$$

with $(\Delta k)^2 = (\Delta z_e)^2\, d\sigma_{Th}/d\Omega$ for X-rays and $(\Delta k)^2 = (\Delta b)^2$ for neutrons, respectively.

Cinerean in H_2O and D_2O with X-rays. Figure 1 shows the macroscopic scattering cross-section of fragmented cinerean in H_2O and D_2O measured with X-rays vs the scattering vector. A Q^{-1} and a Q^{-4} regime can be clearly seen. In the Q^{-1} regime the exponential function is close to unity, so that this region contains only information about the mass per length *M*/*L* of the rod. The result for *M*/*L* is the same for polydisperse systems with a length distribution, because the rods differ in length and not in mass per length. Both radius *R* and mass per length *M*/*L* have been determined from the slope and *Q* extrapolation, respectively, of a plot of $\ln(Q \times d\Sigma/d\Omega)$ vs Q^2 for the crossover regime between Q^{-1} and Q^{-4} according to eq 5.[6] For large scattering vectors ($Q \gg 2\pi/R$) an asymptotic Q^{-4} regime prevails. The mass per length of cinerean in D_2O ($M/L = 2250 \pm 450$ Da nm^{-1}) does not differ from samples in H_2O ($M/L = 2300 \pm 450$ Da nm^{-1}) within errors (see Table 1).

Cinerean in H_2O and D_2O with Neutrons. Figure 2 shows the macroscopic scattering cross section in H_2O and D_2O measured with neutrons. In both cases the Q^{-1}-regime is well-developed. The crossover regime suffers from the subtraction of the background scattering of the solvent (especially in H_2O, because of the strong incoherent scattering of the protons). For D_2O the rise for very small *Q* is caused by agglomeration, because cinerean tends to precipitate in D_2O at relatively low concentrations and temperatures in contrast to H_2O (the solution properties of cinerean in H_2O are examined elsewhere[4]). In principle the shape of the curve for cinerean in D_2O with neutrons is the same as found in H_2O and in the SAXS measurements. But the value deduced for the mass per length is only $M/L = 120 \pm 25$ Da nm^{-1} differing strongly from the value in H_2O and with SAXS. We would like to stress at this point already that an apparent value as low as 120 Da/nm does not necessarily mean a change in either the chemical composition or the structural conformation of the polymer but can instead be explained by a dramatic change in contrast for neutrons (cf. eq 4). We will elaborate on this in the following paragraph. For the SANS value of cinerean in H_2O, on the other hand, we obtain $M/L = 1350 \pm 650$ Da nm^{-1} (see Table 1). In view of corrections due to the strong incoherent scattering of the protons, the agreement with the X-ray data can be considered as fair. A trivial concentration effect by precipitation of cinerean in D_2O or calibration errors cannot account for the large difference of the neutron results for H_2O and for D_2O, respectively. These errors do not exceed 10%.

Table 1. Mass Per Length (*M*/*L*) of Cinerean Measured in H_2O and D_2O with SAXS and SANS and Radius of the Rod Cross Section (from SAXS Only)[a]

		in H_2O	in D_2O
SAXS	*M*/*L* (Da nm^{-1})	2300 ± 450	2250 ± 450
	R (nm)	0.82 ± 0.02	0.82 ± 0.02
SANS	*M*/*L* (Da nm^{-1})	1350 ± 650	120 ± 25

[a] The relatively large error bars are due to the uncertainty in the density.

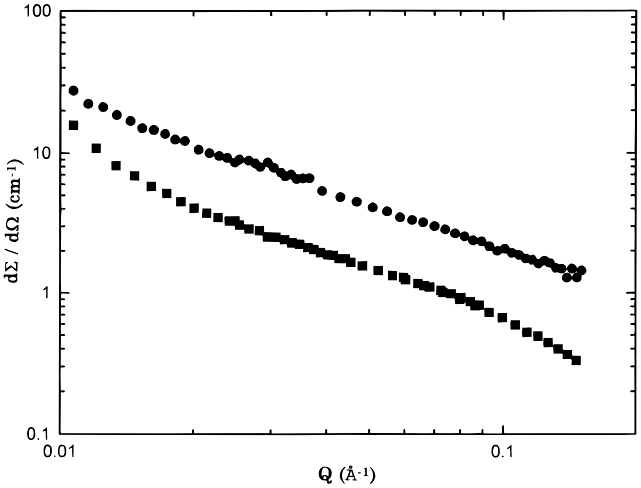

Figure 2. Macroscopic scattering cross section per solid angle of fragmented cinerean in H_2O (circles) and in D_2O (squares) with SANS (normalized to the concentration *c*). The mass per length calculated from the measurement in D_2O is far to small.

Proton/Deuteron and H_2O/D_2O Exchange

As indicated above, an obvious explanation for the observed difference were a contrast change for neutron scattering due to a proton/deuteron exchange. In cinerean 30% of the protons are bound to oxygen (cf. Figure 3). These are protons readily exchangeable. However, even a complete exchange of the OH-protons cannot account for the dramatic difference in the *M*/*L* values (cf. Table 1). Thus we were led to assume that there is an a priori unknown number of H_2O molecules bound to the polysaccharide strands. Dissolving such a hydrogenous cinerean/water compound in D_2O will then result in an exchange of bound water molecules as well. The fact that water can be associated to polymers is described in the literature.[11]

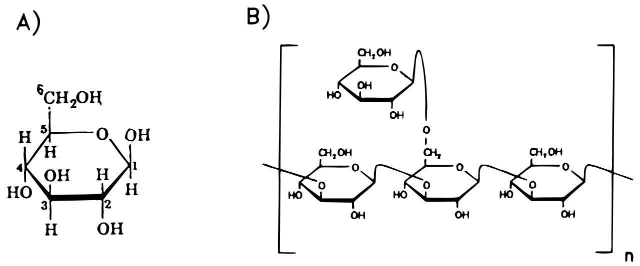

Figure 3. (A) β-D-Glycopyranose ring with the numbers for the carbon atoms in the ring and (B) repeating unit of cinerean (β-(1,3)−(1,6) connected).

The stoichiometric formula for a repeating unit is $C_{24}H_{40}O_{20}$ (polycondensation of glucose) (cf. Figure 3). After the proton−deuteron exchange at the O−H bonds only, it is $C_{24}H_{28}D_{12}O_{20}$. Taking into account the number x of exchangeable water molecules the protonated cinerean is $C_{24}H_{40}O_{20}(H_2O)_x$ or $C_{24}H_{28}D_{12}O_{20}(D_2O)_x$ for the deuterated cinerean, respectively. Knowing the stoichiometry the contrast of cinerean in H_2O and D_2O for SAXS and SANS can be calculated according to eqs 3 and 4. Thus the mass per length can be determined from the measured macroscopic scattering cross section with x as a parameter.

The modified differences $\Delta z_{\mathrm{H,D}}$ and $\Delta b_{\mathrm{H,D}}$ (cf. eq 5) are given by

$$\Delta z_{\mathrm{H,D}} = \frac{Z}{M'_{\mathrm{H,D}}} - \frac{\rho_{\mathrm{H_2O/D_2O}}}{\rho^{\mathrm{cin}}_{\mathrm{H,D}}}\frac{Z_{\mathrm{H_2O}}}{M_{\mathrm{H_2O/D_2O}}} \qquad (6)$$

$$\Delta b_{\mathrm{D}} = \frac{B}{M'_{\mathrm{D}}} - \frac{\rho_{\mathrm{D_2O}}}{\rho^{\mathrm{cin}}_{\mathrm{D}}}\frac{B_{\mathrm{D_2O}}}{M_{\mathrm{D_2O}}} \qquad (7)$$

respectively, where $Z = Z_{\mathrm{cin}} + xZ_{\mathrm{H_2O}}$ is the number of electrons, $M'_{\mathrm{H,D}} = M_{\mathrm{H,D}} + xM_{\mathrm{H_2O,D_2O}}$ is the mass and $\rho^{\mathrm{cin}}_{\mathrm{D}} = (M'_{\mathrm{D}}/M'_{\mathrm{H}})\rho^{\mathrm{cin}}_{\mathrm{H}}$ is the density of the cinerean/water compound. $B = B^{\mathrm{cin}}_{\mathrm{D}} + xB_{\mathrm{D_2O}}$ is the sum of the scattering lengths b_i of all atoms i in the cinerean/water compound.

The results for M/L from these calculations are shown in Figure 4 assuming a complete exchange of the OH protons. The M/L values from SAXS in H_2O and in D_2O are almost the same, because the electron density difference is hardly affected by the deuteration. These two curves and the curve of M/L from SANS in D_2O are intersecting for $x \approx 17$. We conclude that there are about 17 exchangeable water molecules per repeating unit of the polymer strand.

Conclusions

In aqueous solutions fragmented cinerean has a rodlike conformation. In dissolving hydrogenous cinerean in D_2O, protons from OH groups of the polysaccharide as well as bound water molecules are exchanged. This gives rise to a dramatic contrast change in a neutron scattering experiment. Exploiting this effect, assuming complete H−D exchange in the OH groups, we have shown by combined small-angle X-ray and neutron scattering that cinerean is reversibly binding about 17 water molecules per repeating polymer unit. Probably, the water molecules are held by hydrogen bonds at the oxygen atoms of the glycopyranose rings and at the β-(1,3) and β-(1,6) bonds with two water molecules per oxygen atom. Besides obtaining information on binding a fair amount of water, our method enables us to determine the “true” contrast of cinerean (and other polysaccharides) in D_2O for further neutron scattering experiments.

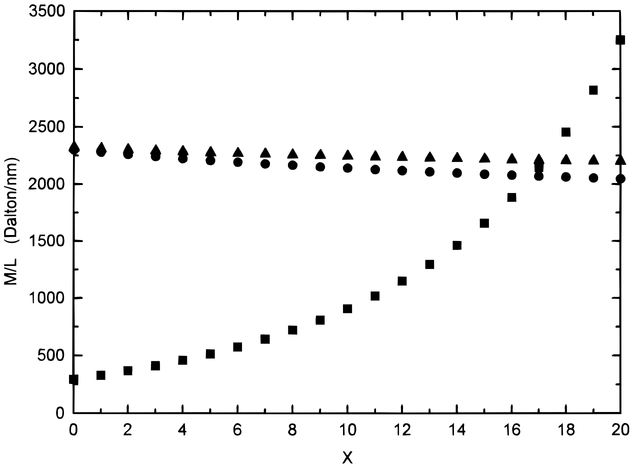

Figure 4. Mass per length calculated from the scattering cross-section with eqs 3−5 using eqs 6 and 7 depending on x, the number of attached H_2O molecules as parameter. The curves (SAXS in H_2O, circles; SAXS in D_2O, triangles; SANS in D_2O, squares) intersect for $x \approx 17$. This shows that there are about 17 H_2O molecules attached to one repeating unit. Due to the problems of the large incoherent scattering of the protons, the SANS experiments can only be seen as an qualitative hint and are not included in this figure.

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