Correlation Between Chain Architecture and Hydration Water Structure in Polysaccharides

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Supporting Information

ABSTRACT: The physical properties of confined water can differ dramatically from those of bulk water. Hydration water associated with polysaccharides provides a particularly interesting example of confined water, because differences in polysaccharide structure provide different spatially confined environments for water sorption. We have used attenuated total reflection infrared (ATR-IR) spectroscopy to investigate the structure of hydration water in films of three different polysaccharides under controlled relative humidity (RH) conditions. We compare the results obtained for films of highly branched, dendrimer-like phytoglycogen nanoparticles to those obtained for two unbranched polysaccharides, hyaluronic acid (HA), and chitosan. We find similarities between the water structuring in the two linear polysaccharides and significant differences for phytoglycogen. In particular, the results suggest that the high degree of branching in phytoglycogen leads to a much more well-ordered water structure (low density, high connectivity network water), indicating the strong influence of chain architecture on the structuring of water. These measurements provide unique insight into the relationship between the structure and hydration of polysaccharides, which is important for understanding and exploiting these sustainable nanomaterials in a wide range of applications.

INTRODUCTION

Polysaccharides are an important class of naturally occurring biopolymers that have attracted increasing recent interest because of their potential to replace synthetic polymers derived from depleting petroleum sources, while addressing consumer concerns about the environmental impact of current technologies and products. Polysaccharides are commonly used in a wide range of industrial and biomedical applications, for example, as moisturizers, rheology modifiers, and emulsifiers, with the utility of these hydrophilic biopolymers determined to a large extent by their interaction with water. It is clear that a detailed understanding of polysaccharide hydration is key to the development of new products and technologies based on these sustainable materials.

Water binds to the polar groups of polysaccharides, which typically results in a three-dimensional transient hydrogen bond network in which bonds are constantly breaking and reforming. Both the chemical composition and the structure of the polysaccharide affects the hydrogen bond network of the sorbed water molecules. In addition, confinement of the sorbed interstitial water leads to structure and dynamics that differs from that of bulk water.

Infrared (IR) absorption spectroscopy is well suited to the characterization of water structure in polysaccharides, providing insight into the strength and arrangement of chemical bonds. The O–H stretching mode of water is sensitive to the surrounding hydrogen bond environment. In general, hydrogen bonding between two water molecules leads to a weakening of the covalent water O–H bond, causing a decrease in the O–H stretching frequency. Furthermore, the strength of hydrogen bonding depends on the O–H···O bond angle. A hydrogen bond is said to be linear when the hydrogen atom sits along the axis defined by the two oxygen atoms (i.e., the HOO bond angle is 0°). Thus, the O–H stretching frequency also depends on the orientation of the hydrogen bonds and is sensitive to distortions of hydrogen bond linearity. An increase (decrease) in the number, linearity, or strength of hydrogen bonding results in a decrease (increase) in the frequency of the v(OH) IR absorption band. Thus, the IR spectrum of water provides semiquantitative molecular level information regarding the structural arrangement of the water hydrogen bond network.

In the present study, we used attenuated total reflection infrared (ATR-IR) spectroscopy to measure IR absorption spectra of films of three naturally occurring polysaccharides. We compare the results obtained for two linear molecules, hyaluronic acid (HA) and chitosan, with those obtained for a highly branched, dendrimeric molecule, phytoglycogen. The physical properties of HA and chitosan have been well characterized, but those of phytoglycogen are not as well-known. Although significant work has been done to understand the basic structure of glycogen-like molecules, as well as the action of enzymes, a detailed understanding of the interaction between water and the highly branched,
hydrophilic structure of the particles remains elusive. Recently, we used neutron scattering to study the structure and hydration of monodisperse phytoglycogen nanoparticles.32 We found that the phytoglycogen nanoparticles are very highly hydrated, containing between 250 and 285% of their weight in water, and that the dynamics of hydration water inside the nanoparticles were significantly slowed down. The neutron scattering study did not, however, provide information about the structure of the hydration water, and this is the focus of the present manuscript. By calculating the difference between ATR-IR spectra measured at different relative humidity (RH) values and that measured at a reference low RH value, we eliminate the chemical specificity of the OH band feature, allowing us to isolate and compare the structuring of water in the different polysaccharides. We find striking similarities between water structuring in the two linear polysaccharides, and significant differences between the linear molecules and highly branched phytoglycogen. In particular, the phytoglycogen nanoparticles exhibited a high degree of highly ordered network water connectivity, and a large increase in the fraction of disordered multimer water clusters with increasing RH, whereas the water structure for HA and chitosan was found to be relatively insensitive to changes in RH. These measurements suggest that the high degree of branching in phytoglycogen leads to a much more well-ordered water structure, indicating the strong influence of chain architecture on the structuring of water. The results of the present study provide new insights that are key to fully understanding and exploiting these materials in new technologies and therapies.

**RESULTS AND DISCUSSION**

Water molecules can form up to four hydrogen bonds, which allows for transient local structural arrangements.13–45 Numerous experiments and simulations show evidence of different local structures in bulk liquid water that arise from variations in the arrangement of hydrogen bonds.43–45 The ATR-IR spectrum of the O–H stretching mode of bulk liquid water is shown in Figure 1. The shape of the v(OH) band for bulk liquid water is complex, as it depends on the transient hydrogen bond network of the water molecules, and it consists of two prominent shoulders near 3225 and 3400 cm

**Figure 1.** ATR-IR spectrum of the O–H stretching region measured for bulk liquid water at ∼25 °C. The arrows indicate the positions of the network and multimer water subpopulations.

were acquired at a resolution of 4 cm

where I

and I

are the infrared single beam intensities of the film at controlled relative humidity and the bare ATR crystal background, respectively. The relative humidity (RH) was controlled by adjusting the relative amounts of constant flows of dry nitrogen gas and water-saturated nitrogen gas over the films with the RH value measured using a Sensirion SHT71 humidity sensor.16 Polysaccharide ATR-IR spectra were collected in RH steps of ~10% between 0 and 90% humidity at ~25 °C. The sample was allowed to equilibrate for at least 20 min at each RH setting prior to collecting the ATR-IR spectrum. Longer time-scale experiments were also performed to determine the equilibration time necessary to achieve steady-state spectra.

The broad O–H stretching absorbance band of sorbed water is the result of overlapping components. Fourier self-deconvolution was used to determine the frequencies of each component (band enhancement factors of 1.6–2.0 were used). The absorbance for a given component was calculated by integrating in a 40 cm

window around the component center, as determined by the Fourier self-deconvolution procedure. The center of gravity of the v(OH) band was determined for the 3800–2950 cm

frequency range.

### EXPERIMENTAL SECTION

**Polysaccharide Materials.** Three different polysaccharides were used in the present study: phytoglycogen, hyaluronic acid (HA), and chitosan. Phytoglycogen is a highly branched polysaccharide, produced in the form of monodisperse nanoparticles by some varieties of plants. It consists of linear chains of α-1,4 linked D-glucose units that undergo α-1,6 branching every 10–12 monomer units.33–35 Monodisperse phytoglycogen nanoparticles (hydrated molecular weight MW = 14.7 × 10^6 g/mol)32 were extracted and purified from sweet corn (provided by Mirexus Biotechnologies Inc.). Further details on the extraction, purification, and characterization of the phytoglycogen nanoparticles can be found in ref 32. Hyaluronic acid (HA) is a linear polysaccharide composed of alternating β-1,4 linked D-glucuronic acid and β-1,3 linked N-acetyl-D-glucosamine units. The hydration properties of HA have been extensively studied because of its use in medical and cosmetic applications such as moisturizers, lubricants, wound healing, and bodily fluid substitutes.11–17 HA (molecular weight MW = 1.3 × 10^5 g/mol) was obtained from Making Cosmetics Inc. and used as received. Chitosan is a linear polysaccharide composed of randomly distributed β-1,4 linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acylated unit), and it is characterized by its degree of acetylation (DA), with DA < 50% corresponding to chitosan and DA > 50% corresponding to the related polysaccharide, chitin. Its high cost of production typically limits chitosan to specialty, niche applications such as wound dressings, antimicrobial coatings, and drug delivery.23,30–41 Chitosan (molecular weight MW = 110 × 10^3 to 250 × 10^3 g/mol, DA = 24%) was obtained from Sigma-Aldrich and used as received.

**ATR-IR Experimental Methods.** Solutions of 1% w/w phytoglycogen nanoparticles and hyaluronic acid (sodium salt) were prepared using Milli-Q water (resistivity of 18.2 MΩ-cm) and 1% w/w chitosan in 5% acetic acid. To prepare the polysaccharide films (thickness > 3 μm), solutions were spread onto a ZnSe-diamond ATR crystal and the water was evaporated under a stream of dry nitrogen gas. All spectra were collected on a Bruker Vertex 70 FTIR spectrometer equipped with an MCT-A detector using an incident angle of 45°. The spectra were acquired at a resolution of 4 cm

where I

and I

are the infrared single beam intensities of the film at controlled relative humidity and the bare ATR crystal background, respectively. The relative humidity (RH) was controlled by adjusting the relative amounts of constant flows of dry nitrogen gas and water-saturated nitrogen gas over the films with the RH value measured using a Sensirion SHT71 humidity sensor.16 Polysaccharide ATR-IR spectra were collected in RH steps of ~10% between 0 and 90% humidity at ~25 °C. The sample was allowed to equilibrate for at least 20 min at each RH setting prior to collecting the ATR-IR spectrum. Longer time-scale experiments were also performed to determine the equilibration time necessary to achieve steady-state spectra.
molecules that are more weakly coupled in a distorted tetrahedral structure that includes some nonlinear (or absent) hydrogen bonds, that is, distorted pentamers and tetramers.9,10,46 Both of these components are associated with network water, that is, water in a hydrogen-bonded network. The weak high-frequency shoulder near 3600 cm\(^{-1}\) is attributed to water molecules in a highly disturbed hydrogen bond network. This type of water is sometimes termed multimer water,9,51,52 which consists of water clusters containing two or less hydrogen bonds per water molecule.9,10,46

The structure of the water hydrogen bonding network and the relative populations of the different water types can be modified from that of bulk liquid water upon sorption by polysaccharides. In the present study, we use this well-established model of water structuring to interpret the shape of the \(\nu(OH)\) band arising from interstitial water molecules sorbed by phytoglycogen, HA, and chitosan films.

ATR-IR Spectroscopy of Polysaccharide Films. In Figure 2a–c we show the relative humidity (RH) dependence of ATR-IR spectra of phytoglycogen, HA, and chitosan films within the 3800–2600 cm\(^{-1}\) range, corresponding to the \(\nu(OH)\) band. The IR absorbance in this region is dominated by the O–H stretching vibrations of sorbed water but also contains contributions due to O–H stretching vibrations arising from the pyranose ring hydroxyl groups (\(\sim\)3600–3000 cm\(^{-1}\)), various C–H stretching vibrations (\(\sim\)3000–2800 cm\(^{-1}\)) and, in the case of HA and chitosan, N–H stretching vibrations (\(\sim\)3300–3000 cm\(^{-1}\)). Because of their different chemical compositions, the spectra shown in Figure 2a–c for the different polysaccharides have significantly different shapes. For each polysaccharide, the IR absorbance in the 3600–3000 cm\(^{-1}\) region increases with increasing RH (Figure 2), corresponding to the uptake of water.

As described above, each of the ATR-IR spectra presented in Figure 2a–c is a convolution of IR bands arising from the vibrational modes of the sorbed water and the polysaccharide. To remove the contributions of the polysaccharide vibrational modes from the IR spectra, difference spectra were calculated by subtracting the absorbance spectrum measured at the lowest RH (<4% RH, which we refer to as 0% RH) from spectra collected at higher RH values. In this way, information specific to the nature of the sorbed water can be obtained. This approach has been used to study water in HA films and other systems.9,13–17,49–51

In Figure 2d–f, we show difference spectra for the phytoglycogen, hyaluronic acid, and chitosan films as a function of RH. These sequences of spectra are remarkably similar for the two linear polysaccharides and significantly different for the highly branched phytoglycogen, suggesting differences in the water hydrogen bonding network. The center of gravity (COG) of these \(\nu(OH)\) bands reflects the average frequency distribution of the O–H stretching vibrations and serves as a measure of the average strength of hydrogen bonding for the sorbed interstitial water molecules. In Figure 3, we show the COG for the \(\nu(OH)\) band of the water sorbed by each of the polysaccharide films. An increase (decrease) in the frequency of the \(\nu(OH)\) band is indicative of a decrease (increase) in the degree of hydrogen bonding. For phytoglycogen, the \(\nu(OH)\) COG remains nearly unchanged at 3263 cm\(^{-1}\) before increasing slightly (by 3 cm\(^{-1}\)) above 80% RH. The results for HA are similar with the \(\nu(OH)\) COG remaining nearly constant at 3317 cm\(^{-1}\) throughout the studied RH range, which is
chains and decreasing the glass transition temperature (T_g) have a plasticizing effect, increasing the mobility of the polymer chains and decreasing the glass transition temperature (T_g) of the polymer.61,62 The resulting changes to the mechanical and barrier properties of hydrophilic polymers have been exploited in a variety of applications including food processing and packaging, and controlled delivery of bioactives.19 At constant temperature, water sorption can cause a glass transition to occur. In the case of chitosan, previous studies using dynamic mechanical thermal analysis and differential scanning calorimetry have shown that a glass transition occurs at ~25 °C for water contents of ~15−20% by mass.19,20 Our gravimetric water sorption isotherm (25 °C) data for chitosan (Figure S1, Supporting Information) show that the film is ~15−20% water by mass at ~50% RH, suggesting that a glass transition occurs near this RH value. Therefore, our observed increase in the ν(OH) band COG with increasing RH between 40% and 60% RH for the chitosan film (Figure 3), corresponding to a decrease in the degree of hydrogen bonding of the interstitial water molecules, is consistent with previously published T_g data for chitosan.19,20

It is interesting to note that at large RH values, after the glass transition has occurred in chitosan, the band shape and COG of the ν(OH) band for both linear polysaccharides are similar to each other and to those for bulk liquid water (COG ~ 3320 cm⁻¹). This suggests that the average hydrogen bond strength of the water sorbed by the linear polysaccharides is similar and more closely resembles bulk liquid water than that sorbed by highly branched phytoglycogen, which exhibits stronger average hydrogen bonding.

To further quantify the spectral changes, it is useful to calculate absorbance ratios that compare the intensity of the IR components that arise from the different water subpopulations. The absorbance of each water band component was calculated by integrating a 40 cm⁻¹ window about each of the three component centers, as identified by Fourier deconvolution. The integrated area is proportional to the number of absorbing water molecules in the spectral range.9 Using these absorbance values, the spectral parameters R_network (R_network = A_3225/A_3400) and R_multimer (R_multimer = A_3600/A_3225) were calculated for each spectrum. These parameters provide information about the relative populations of the different water types and are known to be sensitive to the structural arrangement of hydrogen bonds.9,10,47−49,52−58 The parameter R_network describes the hydrogen bond structure of the network water and serves as a measure of the distortion of the hydrogen bond network and the degree of intermolecular coupling between water molecules. For example, the cooling of bulk water causes a progressive increase in R_network as the water molecules adopt a more rigid tetrahedral structure with less hydrogen bond defects.48,53 The parameter R_multimer provides a measure of the relative subpopulations of water molecules in a disrupted hydrogen bond network (multimer water) to those in a well-ordered hydrogen bond network (network water). Because of the complex shape of the ν(OH) band, the parameters R_network and R_multimer serve as a convenient means of comparing hydration between the different polysaccharides.

In Figure 4, we plot R_network and R_multimer as a function of RH for the phytoglycogen, HA, and chitosan films. We see that the R_network values calculated for the linear polysaccharides are similar and significantly smaller than those measured for highly branched phytoglycogen. We also see that the R_multimer values for the linear polysaccharides are significantly larger than for phytoglycogen, indicating a more disordered hydrogen bonding
Together, the values of motion leads to an increased fraction of multimer water, suggesting that increased polysaccharide chain connectivity of the network water is decreased following the glass transition that occurs as the RH is increased from 40% RH, as discussed earlier. This suggests that neither the network water nor the multimer water fraction changes with increasing water sorption, which is consistent with the observed insensitivity to RH for water sorbed by the HA film and indicates that the incremental water is incorporated into a similar hydrogen bonding environment throughout the sorption process.

We now discuss the $R_{\text{network}}$ and $R_{\text{multimer}}$ values for the three polysaccharides in more detail. For phytoglycogen, there is a small increase in $R_{\text{network}}$ from $\sim2.15$ at 0% RH to a plateau value of $\sim2.3$ at 40% RH, suggesting that the sorbed water fills the well-ordered primary hydration shell hydrogen bond network. The corresponding value of $R_{\text{multimer}}$ increases continuously from $\sim0.21$ to $\sim0.27$ with increasing humidity, showing that the multimer water fraction is increasing with increased water sorption. The greatest increase occurs at low RH between 0 and 30% RH, after which it remains essentially constant before increasing again above 80% RH. It is interesting to note that both $R_{\text{network}}$ and $R_{\text{multimer}}$ increase most steeply at values of RH between 0 and 40% RH. This implies that in the early stages of water sorption, the water molecules are incorporated into both the well-ordered (network) and disordered (multimer) water subpopulations.

The $R_{\text{network}}$ and $R_{\text{multimer}}$ values for the linear polysaccharides HA and chitosan agree closely with one another, reflecting the resemblance between their respective difference spectra (Figure 2e,f). For HA, $R_{\text{network}}$ and $R_{\text{multimer}}$ remain essentially constant at values of $\sim1.27$ and $\sim0.37$, respectively, throughout the entire RH range. This suggests that neither the network water connectivity nor the multimer water fraction changes for HA with increased water sorption, which is consistent with the essentially constant value of the COG of the $\nu$(OH) band (Figure 3). For chitosan, $R_{\text{network}}$ remains essentially constant at a value of $\sim1.4$ as the RH is increased to $\sim40$% RH, and then decreases to a value of $\sim1.2$ at 60% RH, after which it remains essentially constant. This suggests that the order and connectivity of the network water is decreased following the glass transition that occurs as the RH is increased from 40% RH to 60% RH, as discussed earlier. $R_{\text{multimer}}$ for chitosan increases significantly ($\sim0.34$ to $\sim0.4$) in passing through the glass transition, suggesting that increased polysaccharide chain motion leads to an increased fraction of multimer water. Together, the values of $R_{\text{network}}$ and $R_{\text{multimer}}$ show that the chitosan glass transition induces disorder in the water structure by both perturbing the network water connectivity and increasing the multimer water fraction. This is consistent with a decrease in the average hydrogen bonding strength, as indicated by the decrease in the $\nu$(OH) band COG for the chitosan film (Figure 3). Such behavior has been observed for sorbed water at the gel-to-liquid phase transition of phospholipid multibilayers for which the disordering of the phospholipid polar headgroups perturbs the water structure.9

We also note that the values of $R_{\text{network}}$ measured for all three polysaccharides are larger than the value of $R_{\text{network}}$ $\sim0.95$ measured for bulk liquid water at 25 °C with the values for phytoglycogen being significantly larger. The enhanced values of $R_{\text{network}}$ for the polysaccharides, and in particular phytoglycogen, indicate stronger hydrogen bonding that could correspond to a greater fraction of linear hydrogen bonds relative to that of bulk liquid water.2,8

The difference spectra in Figure 2d–f and the spectral parameters in Figure 4 contain information about the average interstitial water structure in the polysaccharide films at a given RH. Further details of the water structure can be obtained by calculating difference spectra between successive RH steps (Figure S2, Supporting Information). In this way, incremental changes in sorbed water structure can be resolved. This approach has been used to gain insight into the hydration characteristics of biological polymers and lipid films.5,17 The RH dependence of the incremental $\Delta R_{\text{network}}$ and $\Delta R_{\text{multimer}}$ values for the three polysaccharides are shown in Figure 5. These plots magnify the changes in water structure reported in Figure 4, emphasizing the changes induced by each RH step, and we focus on the main features in these incremental data.

For phytoglycogen, $\Delta R_{\text{network}}$ has a maximum value at $\sim30$% RH, indicating that at low RH values the sorbed water molecules are contributing to an increasingly ordered and connected hydrogen bond network. This occurs together with an increase in $\Delta R_{\text{multimer}}$ at low RH values, indicating that both highly ordered and disordered water structure forms. For RH > 80%, there is an increase in $\Delta R_{\text{multimer}}$ and a corresponding decrease in $\Delta R_{\text{network}}$. This indicates that the water sorbed at large RH values is disordered, which is consistent with the observed decrease in the average strength of hydrogen bonding by sorbed water molecules in the phytoglycogen film at high RH (as shown by the increase in the $\nu$(OH) band COG frequency in Figure 3).

For HA, the incremental $\Delta R_{\text{network}}$ and $\Delta R_{\text{multimer}}$ parameters exhibit the same behavior (and numerical values) as the average water structure parameters presented in Figure 4. This is consistent with the observed insensitivity to RH for water sorbed by the HA film and indicates that the incremental water is incorporated into a similar hydrogen bonding environment throughout the sorption process.
The RH dependence of the incremental $\Delta R_{\text{network}}$ and $\Delta R_{\text{multimer}}$ parameters for chitosan are similar to those for the other linear polysaccharide HA with the exception that near the chitosan glass transition ($\sim$50% RH) both $R_{\text{network}}$ and $R_{\text{multimer}}$ deviate from the values observed for the HA film. The decrease in $\Delta R_{\text{network}}$ and the corresponding increase in $\Delta R_{\text{multimer}}$ near the glass transition show that the water molecules that are sorbed during the glass transition are forming a more disordered hydrogen bond network. These observations are consistent with the notion of increased chain motion and conformational rearrangement inducing disorder in the water hydrogen bond network.

Finally, it is interesting to note that the incremental $\Delta R_{\text{multimer}}$ values for the three polysaccharides essentially converge at $\sim$90% RH. This suggests that as the polysaccharides approach saturation at very high humidity, the fraction of water molecules sorbing as multimer water becomes very similar.

Together, the data presented in Figures 4 and 5 provide a detailed description of water structure in the polysaccharide films as a function of RH. They demonstrate that the interstitial water in phytoglycogen experiences stronger hydrogen bonding, as indicated by both greater network water connectivity and less disordered multimer water, compared to the interstitial water in the linear polysaccharides.

Previous studies on confined interstitial water in polymer, micelle, liposome, and nanoporous glass systems have found differences in water structure compared to bulk liquid water. These differences have been found to depend on the size of the interstitial spatial environment occupied by the water molecules and are insensitive to interstitial chemical properties such as charge and polar group identity. Generally, the confinement of water in small spaces introduces hydrogen bond defects. In reverse micelles and liposomes, network water connectivity has been observed to decrease as the water molecules were confined in progressively smaller spaces (a few nm). In cross-linked poly(acrylamide) gels, network water connectivity decreases as cross-linking density is increased. As with the micellar and liposomal systems, this behavior was attributed to the decreased interstitial volume in the highly cross-linked gels that induces hydrogen bond defects. However, reducing the interstitial volume does not always lead to reduced water connectivity. The opposite trend was observed for water confined in cylindrical glass nanopores; network water connectivity was found to increase as the cylinder radius was decreased (from 320 to 8 nm). In this system, the reduction in interstitial space is most pronounced in one dimension (the cylindrical pore radius). For the cylindrical nanopores, increased confinement stabilized the network water hydrogen bond structure across long distances along the cylinder axis.

These results suggest that the significant differences in water structure observed between the highly branched phytoglycogen and the linear polysaccharides HA and chitosan in the present study are predominately due to differences in the interstitial spatial environment, as dictated by the chain architecture, rather than differences in the pyranose ring substituents. The denser highly branched phytoglycogen chain architecture may better promote, via narrower interstitial spacing, a more ordered water hydrogen bond network along the axis of the polysaccharide chain than the larger spacing associated with the linear chain architecture.

The large differences in water structure that we have observed between the linear and highly branched polysaccharides have direct implications for their applications. The larger values of $R_{\text{network}}$ for phytoglycogen (Figure 4), indicating stronger bonding of water with the highly branched polysaccharide than for the linear polysaccharides, means that phytoglycogen will less readily sorb and desorb water in response to changes in RH. This provides an advantage for the use of phytoglycogen as a long-term moisturizing agent in cosmetics, with more consistent delivery of moisture in environments in which considerable changes in RH occur. The high degree of water structuring for phytoglycogen will also contribute to its other remarkable physical properties such as the low viscosity and exceptional stability of aqueous dispersions.

**CONCLUSIONS**

Polysaccharide hydration, a key property that determines their physical characteristics and applications, depends on different sorption processes, such as the strong binding of water to various polysaccharide polar groups, and the extent of water layering that is determined by the spatial arrangement of the monomers. The relative contributions of different sorption processes can vary with RH, and this is also reflected in the structure of the sorbed interstitial water. In this work, ATR-IR spectroscopy was used to investigate the structure of water in films of phytoglycogen, hyaluronic acid, and chitosan under controlled relative humidity conditions. This detailed comparison has revealed strong similarities between the results for the linear polysaccharides, and significant differences between the results for the linear polysaccharides and highly branched phytoglycogen. The data show that the interstitial water sorbed by phytoglycogen is on average more strongly hydrogen bonded with a greater fraction of the water molecules incorporated into a well-ordered hydrogen network compared to the interstitial water in the linear polysaccharides ($R_{\text{network}}$ is on average a factor of 2 larger for phytoglycogen than for the two linear polysaccharides). This has allowed us to attribute significant differences in the ordering of the sorbed water to differences in chain architecture, which provides important insight into the physical properties of sustainable polysaccharide materials and their use in a wide range of applications.

**ASSOCIATED CONTENT**

* Supporting Information

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Additional technical details and two supporting data figures. (PDF)

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**Notes**

The authors declare the following competing financial interest(s): We declare that one of the authors (J.R.D.) is a founder of Mirexus Biotechnologies Inc., who has supplied the monodisperse phytoglycogen nanoparticles for the present study.
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