

PHYTOGLYCOGEN NANOPARTICLES: EXCITING SCIENCE AND PROMISING TECHNOLOGIES FROM NATURE

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Nanoscale science and technology is a very active and multidisciplinary area of research. A significant amount of this research is inspired by materials and mechanisms found in nature [1]. Some of the most familiar examples from nature that rely on nanoscale structure include the iridescence of butterfly wings, the self-cleaning nature of lotus leaf surfaces, and the adhesive nature of gecko feet. At the level of single molecules, biology offers lipids, proteins and DNA, which demonstrate an exceedingly high level of precision of expression and structure that is required for their proper biological function. Assemblies of these molecules result in elegant nanoscale structures such as lipid vesicles, protein filaments, and molecular motors that form the basis of many essential biological processes, and are exquisite examples of self-assembled nanotechnology found in nature. It is certainly true that much can be learned from nature to produce new nanostructured materials for new technologies. As physicists, it is fascinating to look in detail at the amazing structures and mechanisms that have evolved at the molecular level.

In the present article, we focus on a special biopolymer, phytyglycogen. This is a highly branched, water-soluble polymer of glucose produced by plants such as sweet corn and rice, and is analogous to glycogen, an energy storage molecule in animals. The molecules are chemically simple, but have a special dendrimeric or tree-like structure (Fig. 1) that results in compact, monodisperse nanoparticles with many applications in personal care, food and nutrition, and biomedicine.

The particles were originally discovered in the Dutcher Lab in a fundamental, multidisciplinary research project focused on bacterial adhesion. They were the waste

product of a multistep chemical procedure and it was their special optical scattering (opalescence) that led to their serendipitous discovery. Our realization that similar particles, phytyglycogen, are produced by sweet corn led to the promising sustainable nanotechnology described in the present article.

The applications of phytyglycogen rely on exceptional properties that emerge from the structure of the phytyglycogen nanoparticles and their interaction with water, such as a remarkable capacity to retain water, and low viscosity and exceptional stability in aqueous dispersions [2,3]. To investigate the origin of these properties, we have chosen physical characterization techniques that are particularly well suited to the study of polysaccharides and water, such as neutron scattering, infrared spectroscopy, rheology, atomic force microscopy and ellipsometry. Below, we describe how these measurements have revealed important information about the structure and hydration of phytyglycogen nanoparticles. The results of these experiments provide new insights into the fundamental physical properties and promising applications of this remarkable sustainable nanomaterial.

STRUCTURE AND HYDRATION OF NANOPARTICLES

Neutron scattering is ideally suited to the study of biological materials at the molecular level [4]. Neutrons have a small wavelength (of the order of an Angstrom), and therefore scattering experiments provide information down to the molecular, and sometimes, the atomic level. Unlike x-rays, neutrons scatter well from low atomic number elements and so are well suited for the study of biological materials. In addition, deuteration (substituting deuterium D for hydrogen H) can be used to enhance or suppress scattering, since neutrons scatter with the opposite phase from H and D atoms. For colloidal dispersions of particles in water, deuterated water is used to vary the scattering contrast between the particles and the solvent in an experiment known as a contrast series, and to avoid the increase in the background signal due to the large incoherent scattering cross-section of hydrogen. In small angle neutron scattering (SANS), the scattering intensity I is measured as a function of



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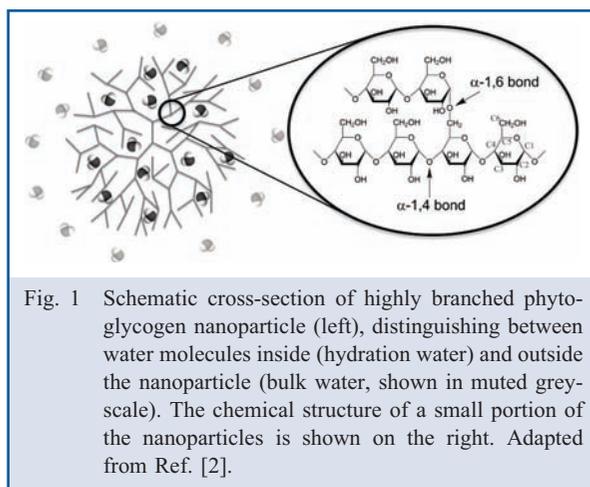
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SUMMARY

Nature offers amazing examples of nanostructured molecules and materials. We describe the unique structure, hydration and applications of naturally occurring phytyglycogen nanoparticles.

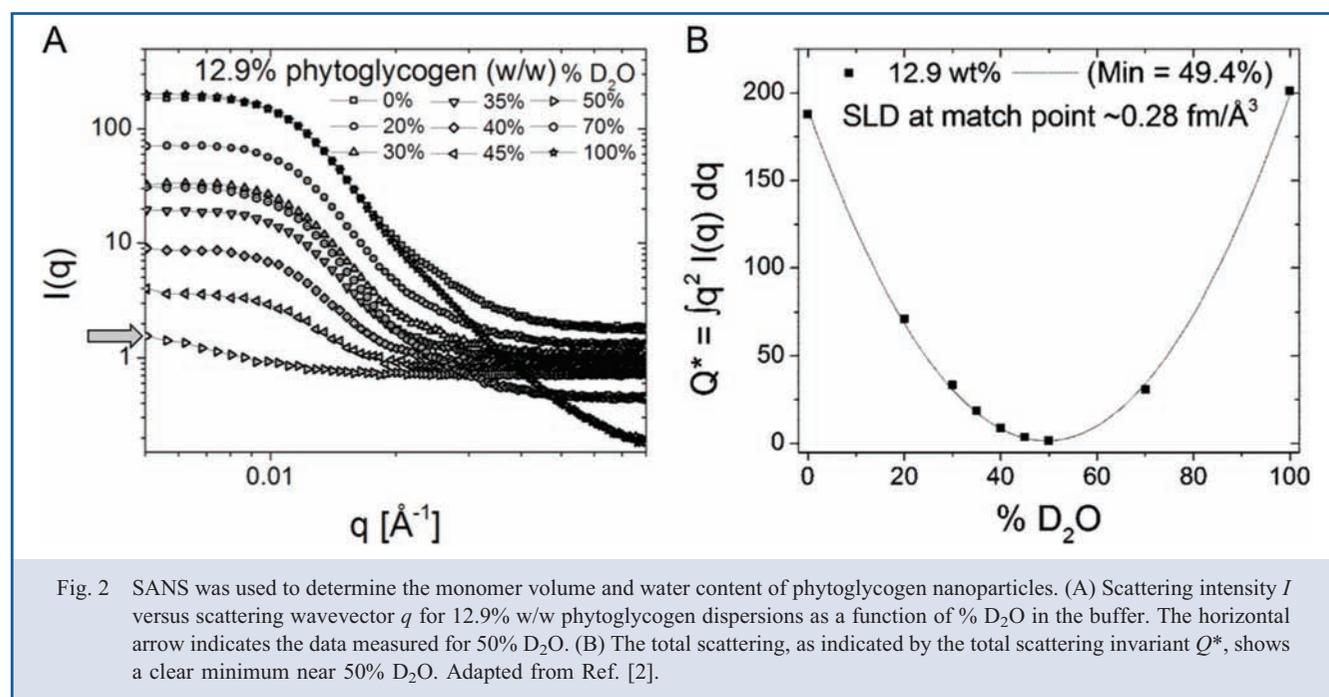


scattering wavevector q (such as the curves shown in Fig. 2A). SANS can be used to determine the particle diameter for dilute dispersions, and the average spacing between particles for concentrated dispersions [2]. These measurements revealed a particle diameter of 35 nm for the phyto-glycogen nanoparticles, with a small polydispersity of $\sim 10\%$. This value of the particle diameter is consistent with direct imaging of the nanoparticles using atomic force microscopy (AFM) [5]. In measurements on high concentration dispersions, we observed that the particle spacing was equal to the particle diameter at a concentration of $\sim 20\%$ w/w, indicating the onset of jamming of the nanoparticles that was consistent with the results of rheology measurements on aqueous dispersions [6].

Contrast series were performed in which the ratio of $D_2O:H_2O$ in the solvent was varied between 0 and 100% (Fig. 2). We found that, at a particular value of $\% D_2O$, the neutron scattering length density (NSLD) of the solvent was almost exactly matched to the NSLD of the particles, suppressing the scattering intensity. Specifically, we observed a quadratic dependence of the total scattering (Porod) invariant $Q^* = \int q^2 I(q) dq$ on $\% D_2O$ with a single minimum in the scattered intensity. The results for 12.9% w/w phyto-glycogen are shown in Fig. 2, and a similar dependence was observed for a higher phyto-glycogen concentration of 22.4% w/w [2]. These results indicated that the radial particle density of the nanoparticles is uniform, confirming that the flexible glucose chains uniformly fill in the space available within each nanoparticle [2].

The molecular weight and water content of the nanoparticles could also be determined from the SANS experiments. By dividing the sum of the atomic scattering lengths corresponding to the chemical formula for a glucose monomer by the neutron scattering length density measured at the contrast matching condition, we were able to calculate the glucose monomer volume. This value, together with the measured particle diameter, allowed us to determine the number of glucose units in each nanoparticle. By comparing the scattering at the contrast match condition with that in pure D_2O , we were able to determine that each nanoparticle sorbed $\sim 250\%$ of its own weight in water [2].

Because of the high water content of the nanoparticles, the dynamics of hydration water inside the particles can be compared to that of bulk water outside the particles using



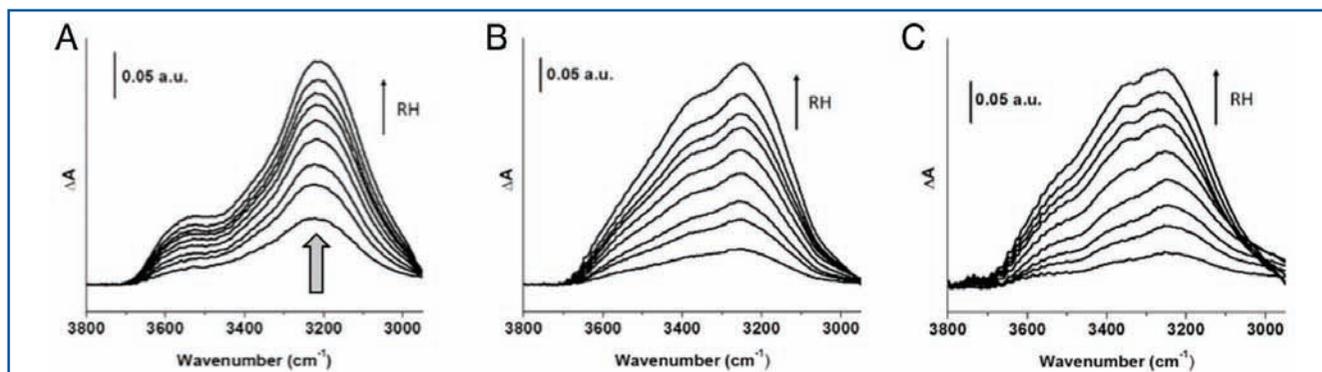


Fig. 3 Surface-sensitive infrared absorption difference spectra [$\Delta A = A(RH\%) - A(0\%)$] for (A) phytoglycogen, (B) hyaluronic acid, and (C) chitosan. The narrow arrows indicate the progression of spectra with increasing RH, and the wide arrow in (A) indicates the peak corresponding to highly ordered water. Adapted from Ref. [3].

quasi-elastic neutron scattering (QENS). QENS allows the observation of atomic/molecular motions on the time scale of tens of picoseconds to ~ 1 ns, over length scales of 0.3 to 3 nm, which is ideal for studying the diffusion of water. These measurements revealed a significant slowing down or retardation of the hydration water relative to bulk water by an average factor of $\xi \sim 5.8$. By performing the QENS measurements at different scattering wavevectors q , we observed that the retardation in the water dynamics was length-scale dependent [2]. This result showed for the first time that the measured value of the retardation factor ξ depends on the length scale probed in the experiment, and may help to reconcile the often conflicting range of hydration water retardation factors reported in the literature for different experimental techniques [7]. QENS also provided an additional independent and consistent estimate of the amount of water in each phytoglycogen nanoparticle, showing that the particles sorbed 285% of their own mass in water. Taken together, the SANS and QENS measurements provided a wealth of essential information about the structure and hydration of the phytoglycogen nanoparticles.

To learn about the structure of the hydration water inside the phytoglycogen nanoparticles we used infrared (IR) spectroscopy, which reveals details of molecular vibrations. By focusing on the band corresponding to the OH stretching vibration, IR spectroscopy can be used to determine the relative amounts of different sub-populations of structured water. The OH stretching region in IR absorption spectra of hydrated polysaccharides is complicated because it contains contributions from different sub-populations of water as well as contributions from bonds within polysaccharides, e.g., C-H, O-H and N-H stretching. We eliminated the contribution from the polysaccharide bonds by subtracting the IR absorption spectrum collected for thin films of polysaccharides at a low value of relative humidity (RH) from spectra collected at higher RH values [3]. This technique allowed us to compare

the water structuring in chemically different polysaccharides. We found striking similarities between water structuring in two linear polysaccharides, hyaluronic acid and chitosan, and significant differences between the linear molecules and highly branched phytoglycogen (Fig. 3). In particular, the hydration water in the phytoglycogen nanoparticles is significantly more highly ordered and tightly bound than in the linear polysaccharides, as indicated by the large peak at ~ 3200 cm^{-1} in the phytoglycogen spectra (Fig. 3). 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.

We also studied the interaction of the nanoparticles with water by measuring the equilibrium swelling of ultrathin films of the nanoparticles [8]. Ellipsometry is a sensitive optical technique that is ideally suited to this measurement, allowing precise determinations of the thickness and index of refraction of thin films [9]. The swelling of polysaccharide films, produced by changing the RH of the air surrounding the films, provides insight into short-range repulsive forces acting between the chains at low RH, and hydration forces at high RH [10,11]. The hydration forces can be interpreted in terms of an exponential decay of the disjoining pressure acting across the film: $P = P_0 \exp(-D/\lambda_0)$. We find that the highly branched phytoglycogen nanoparticles have hydration properties that are intermediate to those of cellulose, a semi-crystalline glucose-based polysaccharide, and those of dextran, a less branched glucose-based polysaccharide. These results reiterate the strong influence of chain architecture on the hydration properties. We also compared the swelling results with IR measurements of the water structure, and we find a correlation between the structural rearrangement of the hydrogen-bonding network of the tightly bound hydration water and the inter-chain separation in the highly branched phytoglycogen nanoparticles [8].

TECHNOLOGY TRANSFER AND COMMERCIALIZATION

The unique interaction of water with phyto glycogen nanoparticles suggests a wide range of promising technological applications. For example, the strong bonding of water to the nanoparticles provides a unique advantage for use as a moisturizing agent in cosmetics. In response to changes in *RH*, phyto glycogen nanoparticles will sorb and desorb water less readily than other polysaccharides. This provides an advantage for the use of phyto glycogen as a long-term moisturizing agent in cosmetics, with more consistent delivery of moisture in environments in which considerable changes in *RH* occur. Hydrophobic modifications of the nanoparticles extend the range of applications to solubilizing and stabilizing hydrophobic bioactive compounds in foods and medicines.

Initially, the technology was developed in the Dutcher Lab at the University of Guelph, supported by over \$1M in government grants. The technology is now being commercialized by Mirexus Biotechnologies Inc. (MBI), which is a spinoff company from the Dutcher Lab [12]. Through its subsidiary companies Mirexus, Fortis Ingredients and Glysantis, MBI is pursuing opportunities in personal care, food and nutrition, and biomedical applications, respectively. MBI has been successful in raising \$19M in investment, and operates an R&D facility in Guelph with 18 fulltime employees (8 PhDs). MBI works closely with a network of university researchers to gain new knowledge and to discover and evaluate new applications of the phyto glycogen nanoparticles.

CONCLUSIONS

By applying sophisticated physical characterization techniques to the study of naturally occurring polysaccharide nanoparticles, we have learned in detail about the interplay between the unique particle architecture and hydration water. The results of these studies have also provided valuable input for the design of new technologies that are being commercialized by Mirexus Biotechnologies Inc. This work demonstrates the value of physics-based strategies for the discovery and characterization of sustainable nanomaterials.

ACKNOWLEDGEMENTS

We thank Phil Whiting and Anton Korenevski for discussions that have greatly benefitted this work. Mirexus Biotechnologies Inc. generously supplied the phyto glycogen nanoparticles. This work was funded by grants from the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA), the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Ontario Centres of Excellence, the Advanced Foods and Materials Network, and Mirexus Biotechnologies Inc. (J.R.D.). J.R.D. is the recipient of a Senior Canada Research Chair in Soft Matter and Biological Physics. Support for J.K. was provided through the Scientific User Facilities Division of the DOE Office of Basic Energy Sciences under US DOE Contract No. DE-AC05-00OR22725. Small-angle neutron scattering was performed at ORNL using the EQ-SANS instrument at the Spallation Neutron Source, a facility managed by UT-Battelle, LLC under US DOE Contract No. DE-AC05-00OR22725.

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