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Large scale structure of wheat, rice and potato starch revealed by ultra small angle X-ray diffraction

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ABSTRACT

Rice, wheat, and potato starches were investigated using ultra-small angle X-Ray diffraction (USXRD) in the range of 100–58,000 Å. The results showed trends consistent with the known sizes of starches. However, the observed Rg values for the scattering substances lie in the 100–300 nm range, very much in the low end of the known starch granule size distributions (and below the resolution of the light microscope) suggesting different, perhaps interesting, structures than those observed by light microscopy. Thus what were detected may possibly be the sizes of the crystalline regions postulated to occur in individual starch granules.

1. Introduction

Starch, the main storage polysaccharide in plants, is made of glucose molecules which attach to one another with α-D-(1 → 4) and α-D-(1 → 6) glycoside bonds. This macromolecule contains two different subunits called amylose and amylopectin [1,2]. Starches are distributed widely throughout plants but particularly they are stored in the seeds and in roots or tubers. In the leaves and stems of plants, the starch granules are closely associated with the green chloroplasts, where photosynthesis occurs [3]. During refining processes, the extraneous plant structures are separated from the small, rodlike (banana), spherical (corn), disk-shaped (wheat), or egg-shaped (potato) particles [4–6]. Commercial corn, potato, or other starch powders consist of these particles [1,7,8].

By ordinary microscopic observation it can be seen that granules possess a faintly visible layered structure, similar on a small scale to that of an onion, the annual rings of a tree, or the layers of a shell [1]. When the granule is excessively dried, or when attempts are made to crush the granule, it has a tendency to split radially, i.e., along lines from the center to the periphery. Study of the granules with polarized light helps to understand why this radial splitting occurs. With polarized light, the granule can be shown to have the ability to refract light doubly or to tilt light and the way in which this double refraction occurs suggests the idea that the long chainlike starch macromolecules are oriented radially in the granule [4].

X-ray diffraction is another technique that is widely used to study the starch structure. The parallel molecular chains in the granule appear to be arranged with a regularity of structure surpassing that of a simple molecular orientation, since the starch granules diffract X-rays nicely [2,8,9,10]. In other words, the parallel chains occasionally have crystalline arrangements in local regions of submicroscopic size [1,2], that makes X-ray diffraction a suitable approach to study starch.

Colloidal dimensions (between tens and several thousand Å) are enormously large compared to the X-ray wave length (e.g. the frequently used CuKα-line of 1.54 Å) which makes the angular range of observable scattering correspondingly small. Inhomogeneities in electron density of colloidal size in the sample causes small angle X-ray scattering [11]. Detailed analysis based on modeling and scattering curve for different starch granules have been reported [12]. By using lamellar microemulsion system as a point of reference, model analysis of the SAXS data were used to quantitatively characterize the lamellar structure of starch [13]. For randomly dispersed particles in solution or in a matrix, the data are radially symmetric around the incident beam, so it is only necessary to collect data along a line (in the case of conventional geometry (Fig. 12), or over an arc (in the case of an angle sensitive measurement (Fig. 13). Sampling of the pattern, however, is not continuous but occurs at discrete points by the process of digitizing the data.

Starch granules typically have dimensions exceeding 10,000 Å, much larger than the dimensions accessible (∼1000 Å) with the conventional X-ray setup described above. To access the ultra small angles required for the study of micrometer-scale structures, one

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may use a Bonse–Hart camera [14,15]. In this geometry (Fig. 13), a channel-cut germanium crystal monochromator is used to direct a very well collimated X-ray beam of well-defined wavelength onto the sample. The scattered X-rays, instead of being imaged on a detector, are directed onto a second channel-cut monochromator, which is scanned over the range of accessible scattering angles. The intensity at a particular Bragg angle (2θ) is measured directly using a scintillation counter. Because the pattern is taken as a function of angle, the beam width has no smearing effect. The energy spread of the monochromatic beam is also small since essentially only the CuKα emission line from the target is accepted. Thus, at least for initial analysis, the only correction necessary is the one for the length of the beam, thereby simplifying the calculation.

A Bonse–Hart camera of this type was utilized in this study to investigate a size regime for starch particles that was formerly inaccessible using X-ray diffraction. In order to accomplish this goal we have acquired and adapted existing analysis tools to a novel application (see Section 2). Then we surveyed potato and some starch-bearing seed grains (wheat and rice) to see if they could potentially yield interpretable data using this technique.

2. Materials and methods

2.1. Ultra small angle X-ray diffraction experiments

Samples of various cereal seeds and polystyrene microspheres (Table 1) were mounted in a sample holder and scanned using a Bonse–Hart type camera [15]. The camera was mounted on a rotating-anode generator (RU200; Rigaku USA Inc., Danvers, MA) employing a 0.2 mm × 2 mm focus. Starting at an angle well away from the direct beam, the sample diffraction was scanned in 2θ, stepwise across the main beam to a comparable angle on the other side of the direct beam. The counting time per step was 1 second, but this time could be lengthened in order to improve the counting statistics. The step width varied; the smallest value was 4 arcsec while the largest value was 20 arcsec, in units of 2θ. The K-α1 and K-α2 lines from a copper target were selected by the defining channel-cut Ge crystal. Coordinated data collection and scanning of the analyzer crystal was done by using a Databox (Radix Inc. Chicago, USA).

Cereal samples were obtained from a local natural foods market. All of the scans (Table 1) were conducted twice; once with a copper foil in front of the sample to attenuate the direct beam, and a second time without the copper foil in order to improve the precision of the weaker scattering. By comparing the scans with and without a sample in place, one can observe the contribution of the main beam to the diffraction from the sample. Here the scans where Cu foil was not placed in front of the beam were used since these scans will yield more precise diffraction at larger angles.

In order to make best use of the available X-ray flux, several seeds were stacked up along the length of the direct beam. Scans were recorded with the seeds in two different orientations. In both orientations, the long dimension of the direct beam was parallel to the hilum. In the one orientation, the direct beam was incident directly onto the hilum. To obtain the other orientation, the seeds were rotated by 90° about their long axis. No difference was seen between these orientations and the patterns with the best counting statistics are shown in the results.

Images of the starch grains from potato, wheat and rice were obtained by an Olympus BX50 Phase Contrast Binocular Microscope with a camera lucida after staining with Lugol's iodine. Samples were obtained from local markets and their hydration levels are known as around 13–14% for rice, about 8% for wheat, and about 80% for potato.

Table 1
Description of experimental scans.

Sample	Description of the diffraction experiments
Sphere1	Polystyrene microspheres of 0.358 μm, no Cu foil used
Sphere2	Polystyrene microspheres of 0.481 μm, no Cu foil used
Sphere3	Polystyrene microspheres of 0.765 μm, no Cu foil used
Potato	∼3 mm thick slice, no Cu foil used
Wheat	6 Wheat berries stacked one above the other, no Cu foil used
Rice	4 Rice grains stacked one above the other, no Cu foil used

2.2. Desmearing the data

To obtain enough diffraction intensity using conventional laboratory-based X-ray sources, it is often necessary to use a line X-ray source. The difficulty with this technique is that patterns that would be circularly symmetric from a point source are convoluted with the shape of the line, with a consequent loss of detail in the X-ray pattern as well as shifts in the position of significant diffraction features. Desmearing small angle data, i.e. deconvoluting the data, is a difficult computational problem [16–18]. Glatter have produced a well established computer software package, ITR, suitable for this problem [19]. With this software it was possible to attempt to desmear the data. There is a very significant learning curve attached to using these programs since the experimental geometry assumed by the authors is not identical to the one used in the present experiments.

In the present work, the program was tested by taking data from polystyrene spheres with measured average diameters ranging from 0.358 to 0.765 μm in diameter. The beam dimensions were measured directly from a film image at a known distance from the source, which allowed the width and height to be expressed in terms of diffraction angle, and hence in hours. After confirming that the program was operating properly for the polystyrene spheres, with the Bonse–Hart setup, the program was used to desmear the data from the unknown samples.

2.3. Analysis of guinier plots

Microsoft Excel, Version 5.0 (Microsoft, Inc.) was utilized to analyze the Guinier Plots. For this, the same region used to calculate the slope, was used to find the standard error of the slope and hence the radius of gyration and its error. After running the "regression" tool of Microsoft Excel (Microsoft, Inc.), for the range mentioned, the error percentage was obtained by dividing the "standard error" value of the "x variable" by the "coefficient" value of the same variable. Confidence intervals of 95% were assumed in this calculation. Calculated Rg values were, then, multiplied by the percentage errors and their error ranges were obtained. Those values mentioned are shown as "±" signs after their respective values (see Section 3).

3. Results

3.1. Analysis of the scattering data

Initial experiments examined the diffraction patterns of polystyrene microspheres in order to test the program. Evaluating the effect of changing critical parameters was one of the major objectives in this process. After establishing that the program operated properly [20], diffraction patterns from rice, wheat, and potato starches were collected using USXRD and processed to yield desmeared $I(h)$ functions for each sample. Scattering data from microspheres of three different average diameters were first analyzed. Figs. 1–3 show the smeared and desmeared curves of 0.358 μm, 0.481 μm, and 0.765 μm polystyrene microspheres, respectively. After correctly desmearing diffraction patterns from microspheres of known sizes, the data from potato and the cereal