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From Glycogen to Amylopectin: A Model for the Biogenesis of the Plant Starch Granule

Minireview

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Starch constitutes the major source of calories in the human diet. Over 600 commercial products are generated from starch both for food and also non-food uses. Plant Starch can be distinguished from glycogen by the presence of a highly ordered and dense packing of glucan chains. This packing results in the growth of large insoluble granules in the plastids of the eukaryotic plant cell. The enzymes that have been described in the starch biosynthetic pathway are related to those that are involved in cyanobacterial glycogen synthesis. The genetic, molecular biological, and enzymological studies performed to date while allowing constant progress to be made have failed to uncover the biochemical reactions responsible for the synthesis of an ordered crystalline starch structure instead of glycogen. We believe that the order-generating steps have just been discovered in Chlamydomonas and maize. After briefly reviewing our current understanding of amylopectin structure and synthesis, we will present a model that explains the biogenesis of the plant starch granule. This model is consistent with the two-dimensional structures published for the amylopectin clusters, and it is sufficiently predictive to allow us to think in terms of the three dimensional pattern of granule growth.

Understanding Amylopectin Synthesis would be Sufficient to Explain the Major Features of Starch Biogenesis

Starch accumulates as a complex granular structure made of α glucans (α-1,4 linked and α-1,6 branched) both in the leaf cell chloroplast (transient starch) or in the amyloplast of the plant storage tissue cell (storage starch) (reviewed by Nelson and Pan, 1995). Both kinds of polysaccharides are synthesized in plastids by similar enzymes using ADP-glucose as a substrate. The storage polysaccharide is usually defined as a mix of two distinct fractions: amylopectin and amylose. Amylopectin is by far the major compound. It is of very high molecular weight (10^7-10^9 Da) and harbors 5% of α-1,6 branches (reviewed by Manners, 1989). Amylose is often referred to as a smaller linear molecule (molecular weight of 10^5-10^6 Da) with very few α-1,6 branches (less than 1%). Its association with amylopectin inside the granule remains to be determined. Amylopectin is sufficient to generate full size granules both in wild-type starch from photosynthesizing cells and in mutant starches devoid of amylose. No mutants lacking selectively amylopectin have ever been described in plants suggesting that an understanding of amylopectin biosynthesis will be sufficient to explain the major features of starch granule biogenesis.

Amylopectin is a Highly Ordered Crystalline Molecule Harboring Parallel Glucan Double Helices

The structure and position in the granule of individual amylopectin molecules are illustrated in Figure 1. The glucan chains are packed in clusters by an asymmetric distribution of α-1,6 branches within the molecule. The branches are tightly spaced at the root of each unit cluster thus generating a tight packing of parallel glucans that intertwine to form double helices. These double helices are responsible for the crystallinity of starch which can be monitored by X-Ray diffraction analysis. A unit amylopectin cluster is thus composed of an amorphous portion containing most of the tightly spaced branches (the amorphous lamella) and a crystalline (the crystalline lamella) segment containing the parallel glucans. The exact conservation of the amylopectin cluster size (9 nm) throughout the plant kingdom (Jenkins et al., 1993) suggests the existence of a highly ordered, precise, and well-conserved biosynthetic pathway. It is worth stressing that the cluster model of amylopectin allows for indefinite growth of macrogranular structures. By comparison, animal glycogen contains regularly spaced α-1,6 branches leading to small size (25 nm of average diameter) β particles with a molecular weight of 10^7 Da. Because of the regularity of the branching in glycogen, it is possible to predict theoretically the maximal size attainable by the unit glycogen particle before steric hindrance impairs further growth. Those calculations are in good agreement with the 10^7 Da value that is indeed measured for these particles (Geddes, 1985). Thus the major differences existing between water-soluble amorphous glycogen and insoluble crystalline starch are explained not only by the overall decrease of branching observed in the plant polysaccharide but also by the asymmetric distribution of the α-1,6 branches in the unit amylopectin cluster. How plant cells achieve this distribution has until very recently resisted all research efforts. Despite the finding and intensive study of elongation (starch synthases) and branching enzymes similar to those involved in bacterial glycogen synthesis, attempts at synthesizing amylopectin's typical asymmetric pattern of branching in vitro or in vivo

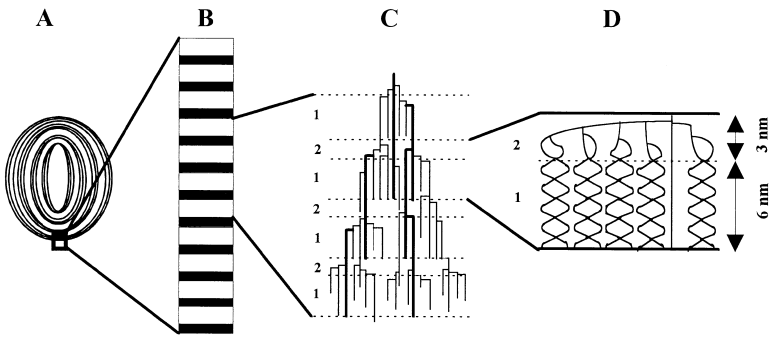

Figure 1. An Overview of Starch Granule Organization

(A) A schematic view of a 1.5 μm thick starch granule with its succession of amorphous and crystalline growth rings.

(B) A section of a crystalline growth ring of the granule is related to the molecular organization of amylopectin. Each shaded and plain section represents an amorphous and a crystalline lamella respectively. Thus the crystalline growth ring enlarged in this panel contains a regular succession of 11 amorphous and crystalline lamellae. This would amount to a 0.1 μm thick growth ring.

(C) This panel enlarges a succession of 7 lamellae and relates them to the primary structure of a portion of an amylopectin molecule. Each line represents an α-1,4 linked glucan chain. The chains are hooked together by α-1,6 branches. The dotted line delimits the sections appearing in the crystalline (1) and amorphous (2) lamellae. Note that most α-1,6 branches are included in the amorphous lamellae at the root of the chain clusters and that the glucans are pointing towards the granule's surface.

(D) This panel relates a part of primary structure depicted in (C) to the secondary structure of a single cluster displaying the double helical structures. The 6 nm size of the crystalline portion corresponds to a length of 18 glucose residues.

in E. coli have not been successful. Of particular significance is the finding that both maize starch-branching enzymes expressed alone or in combination in an E.coli strain lacking its own glycogen branching enzyme have generated glycogen-like products (Guan et al., 1995) and not granular starch. These observations have raised the possibility that amylopectin synthesis might require an additional biochemical step distinct from classical elongation and branching.

Glucan Trimming: a Requirement for our Understanding of Starch Granule Biosynthesis

The failure to generate granular starch from selective action of the branching enzymes in vitro or in E. coli has prompted us to search for novel essential functions involved in starch biosynthesis both in maize and Chlamydomonas. The *su1* (*sugary 1*) mutants of maize were known for decades as mutants defective in some aspect of starch granule biosynthesis (Correns, 1901). The mutants have reduced amounts of starch and a substantial amount of a novel species of water-soluble polysaccharide whose structure is reminiscent of those reported for glycogen (Sumner and Somers, 1944) and for this reason was named "phytoglycogen." The presence of phytoglycogen in these mutants led Erlander in 1958 to speculate that amylopectin would be generated by debranching of glycogen and that amylose would be produced by further debranching of amylopectin (Erlander, 1958). This hypothesis was revived and modified by Pan and Nelson (1984) who did find a selective defect in the *su1* mutants with respect to one particular debranching enzyme isoform. Pan and Nelson thus proposed that branching and debranching have to be precisely balanced during starch biosynthesis. Similar mutants were reported in rice that also displayed a decrease in debranching enzyme activity (Nakamura et al., 1996). These observations were recently confirmed by cloning a *Su1* gene and sequencing its cDNA (James et al., 1995). The protein bears the strongest homologies to bacterial isoamylases (debranching enzymes). Moreover the *Su1* gene product expressed in E.coli does harbor debranching activity. The case for debranching enzymes participating in some aspect of starch biosynthesis in maize is thus compelling. Maize plants carrying disruptions in the *Su1* genes still accumulate reduced but significant amounts of granular starch questioning the absolute requirement of debranching activity for granular starch synthesis. However, in maize, different genes encoding enzymes of similar function are expressed at different times during endosperm development. Thus additional debranching enzyme isoforms of similar function might be present and active in *su1* mutants. This could explain the presence of an incompletely defective phenotype. In Chlamydomonas reinhardtii, seven independent alleles were generated at the *STA7* locus. All mutants completely lacked granular starch and replaced it by 5% (with respect to what would have been the normal starch amount) of a water-soluble polysaccharide that turned out to be phytoglycogen (Mouille et al., 1996). The defect could be correlated to the selective disappearance of a specific debranching activity. Since no other enzyme activities appear deficient in these strains, these results imply that debranching is mandatory to obtain starch biosynthesis in plants. We believe that glucan trimming is required to generate order in the amorphous lamella for subsequent synthesis of the crystalline lattice. We are now in a position to propose a unifying model explaining the major features of starch granule biogenesis in plants.

A Two Dimensional Model: Discontinuous Synthesis of the Amorphous and Crystalline Lamellae

A simplified two dimensional view of amylopectin biosynthesis is illustrated in Figure 2. Once the crystalline lamella has reached the critical size, allowing the branching enzymes to use its constitutive glucans as substrate, preamylopectin a branched intermediate will be produced in a fashion similar to what has been observed either in vitro or in vivo in E.coli with the maize starch branching enzymes. In order to generate the next planar crystalline lamella, the primers for elongation by the starch synthases must be either coplanar or arranged in a regular fashion as predicted by the crystalline lattices reported for starch (Imberty et al., 1988). We propose that preamylopectin trimming will proceed through the selective action of debranching enzymes such as those encoded by the maize *Su1* or Chlamydomonas *STA7* genes. The debranched oligosaccharides generated by these splicing events will then be used

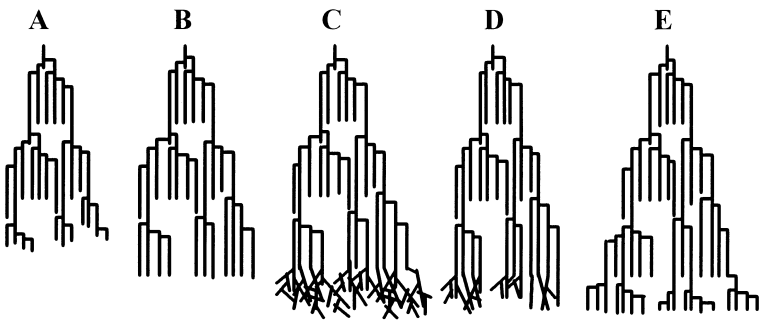

Figure 2. A Discontinuous Synthesis Model for Amylopectin Biosynthesis

This figure shows a two dimensional model for starch biogenesis occurring at the surface of the granule. Elongation starts from a trimmed amorphous lamella depicted in A, proceeds through B until the critical size needed to accomodate the branching enzymes is reached. Then because of the presence of high branching enzyme–specific activities, random branching occurs (C). Debranching activities will simultaneously trim down the loosely-branched glucans (D). This will prevent phytoglycogen synthesis and leave out the tightly spaced branches that will generate the next amorphous lamella (E).

with varying degrees of efficiency by the three distinct plant starch synthases (Maddelein et al., 1994). In all cases, in the presence of a physiological excess of branching enzyme the next amorphous lamella will be produced by preamylopectin processing as soon as the glucans elongated by the starch synthases have reached the critical minimal size to accomodate the catalytic site of the branching enzymes. Thus, it is the precision of the different chain-length-minimums of each different enzyme that provides the ground-rules for discontinuous synthesis.

A Three Dimensional Model: Induced Fit and Glucan Trimming

A major feature of the model we propose is that it gives us access to the third dimension of granule growth. The crystal lamella is a planar arrangement allowing for the three dimensional piling of glucan double helices (Figure 1). The amorphous lamella on the other hand will not be planar but space-filling as can be predicted by the synthesis of phytoglycogen. At this stage the processing of phytoglycogen can lead to a variety of three dimensional structures that will allow for three dimensional extension of the amylopectin molecule. It is easy to understand how this is needed to accomodate regular concentric growth of the starch granule. Oostergetel and van Bruggen (1993) have very recently examined sections of potato starch granules by electron optical tomography and by cryo–electron diffraction. Their data imply a superhelical arrangement of both amorphous and crystalline lamellae. Moreover distinct superhelices are interlocked through their respective amorphous and crystalline lamellae to yield a tetragonal symmetry (Figure 3). In this three dimensional arrangement, the double helical glucans are pointing in the axis of the superhelix towards the surface of the granule. This will of course allow for synthesis and growth of the crystals at the surface. This structure raises several questions with respect to biosynthesis, namely what determines the superhelical growth and how can this unidirectional growth account for concentric growth of the starch granule. We believe these questions can be presently addressed by our model. If we assume that the branching enzymes are setting the invariant amylopectin cluster size through their minimal catalytic requirements (see above), then once the first turn of the superhelix is synthesized the following turns will be dictated through this requirement. Concentric growth of the granule will call for synthesis

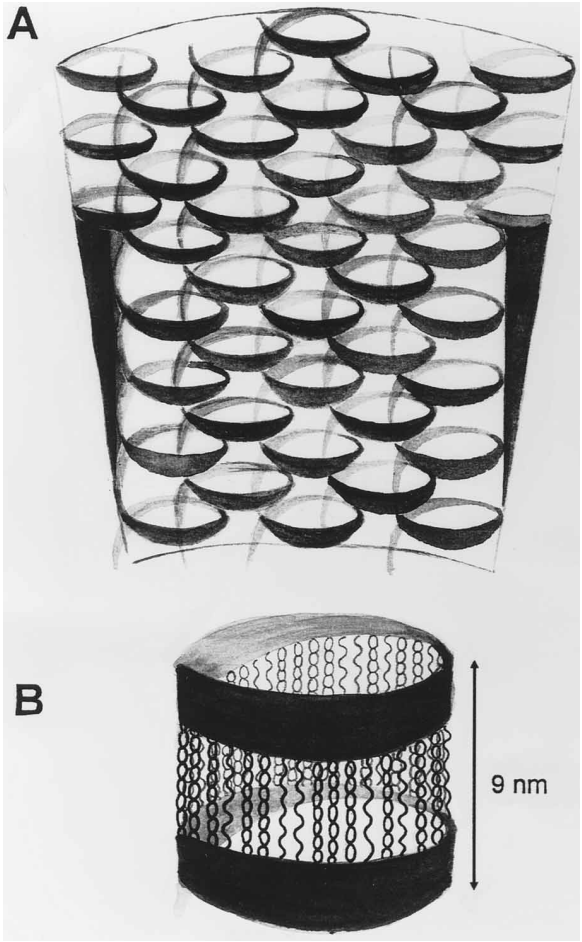

Figure 3. A Superhelical Model for the Three Dimensional Organization of Starch

(A) The superhelical three dimensional organization of a section of the starch granule (based on Oostergetel and van Bruggen, 1993). The top of the figure corresponds to the granule's surface. The shaded areas correspond to the amorphous lamellae of the amylopectin molecules.

(B) An enlargement of a single turn of the superhelix to display the double helices of the crystal lamellae. The shaded section would have overall structures similar to those shown for the amorphous lamellae in Figure 1. Each superhelix is interlocked to neighboring superhelices to generate a tetragonal organization. We propose that vacant spaces are filled with amorphous material until sufficient room is available to yield a novel superhelix.

of novel superhelices. These can be readily synthesized by allowing the amorphous lamella to fill vacant spaces between the growing superhelices. When sufficient space is available a novel superhelix will be made to grow by induced fit with the neighboring tetragonal organization. Debranching enzymes remain required at the surface to prevent glycogen synthesis and allow the trimming of the amorphous lamellae. The induced fit hypothesis for starch growth only requires the understanding of amylopectin cluster synthesis as proposed in our two dimensional model. Understanding how the first turn of the superhelices are generated will require further insight as to the priming events occurring at the granule core.

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