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Diurnal changes in *Sorghum* leaf starch molecular structure

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Abbreviations:
CLD, chain-length distribution
SEC, size-exclusion chromatography
DP, degree of polymerization
DMSO, dimethyl sulfoxide

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ABSTRACT

Control of the fine structure of transitory starch synthesized during the day in leaves is required for its normal degradation during the subsequent night. In this study, the molecular structure of transitory starch from *Sorghum* leaves over the diurnal cycle was characterized using size-exclusion chromatography. This is the first study of diurnal changes in the chain-length distribution (CLD) of amylopectin and amylose over the entire range of chain lengths, and in the size distribution of whole starch molecules. It was found that the outer layers of leaf starch granules, which were synthesized during the daytime and degraded during the night, contained more large molecules, including amylopectin with more short chains and more branching, than those in the inner layers. The outer layers also had lower amylose content. Starch molecular sizes in leaves are much smaller than in grain starch. The starch structures observed are likely to give optimal energy control during plant growth. Lack of this control may contribute to poor plant growth.

1. Introduction

Starch can be classified into two types: transitory starch, which is synthesized in photosynthetic organs and is often termed leaf starch, and reserve, or storage starch, which occurs in storage organs. Transitory starch is a primary product of photosynthesis in the leaves of most plants. In some plants, up to half the photo-assimilated carbon is stored as starch in leaves, to be remobilized later [1]. Transitory starch synthesized in chloroplasts during daytime photosynthesis is degraded that night, providing a continued supply of sugars to sustain metabolism in the leaf and for export to sink organs throughout the night.

There are up to five identifiable levels in the structure of transitory starch (Fig. 1) [2,3]. Glucose monomeric units are connected to form individual chains of starch (first level), which may be joined to form either amylose (containing a few long branches) or amylopectin (hyper-branched with many short branches) (second level). The first level is quantified as the chain-length distribution (CLD). The individual branches of amylopectin, forming clusters, intertwine as helices (third level) and form alternating layers of crystalline and amorphous lamellae (fourth level) with long linking amylopectin chains and amylose occupying the amorphous layers. These in turn form the semi-crystalline and amorphous shells, which make up a granule (fifth level).

The biosynthesis of reserve and transitory starches are facilitated by a number of core enzymes. Linear chains are formed by starch synthase (SS), which add glucose units to the non-reducing ends of starch branches by forming α-(1 → 4) glycosidic linkages. Linear chains are cleaved by starch-branching enzyme (SBE), with the chain that was removed then attached to another branch via an α-(1 → 6) branching linkage. SBE, the only enzyme responsible for the branching of starch in plants, has different isoforms (e.g. SBEI and SBEII), which show a wide variation in the chain-length transfer pattern during catalysis. These differences are related to their glucan substrate preferences, which in turn are associated with their different roles in starch synthesis. SBEI has a preference for amylose as a substrate and transfers relatively longer glucan chains, up to a degree of polymerization (DP, the number of anhydroglucose monomer units) of 30, with the majority being DP 10–13 [4]. SBEII transfers shorter chains (DP 6–14) and prefers amylopectin as a substrate [5]. In cereals, the SBEII class is subdivided into SBEIIa and SBEIIb, each with different kinetic characteristics

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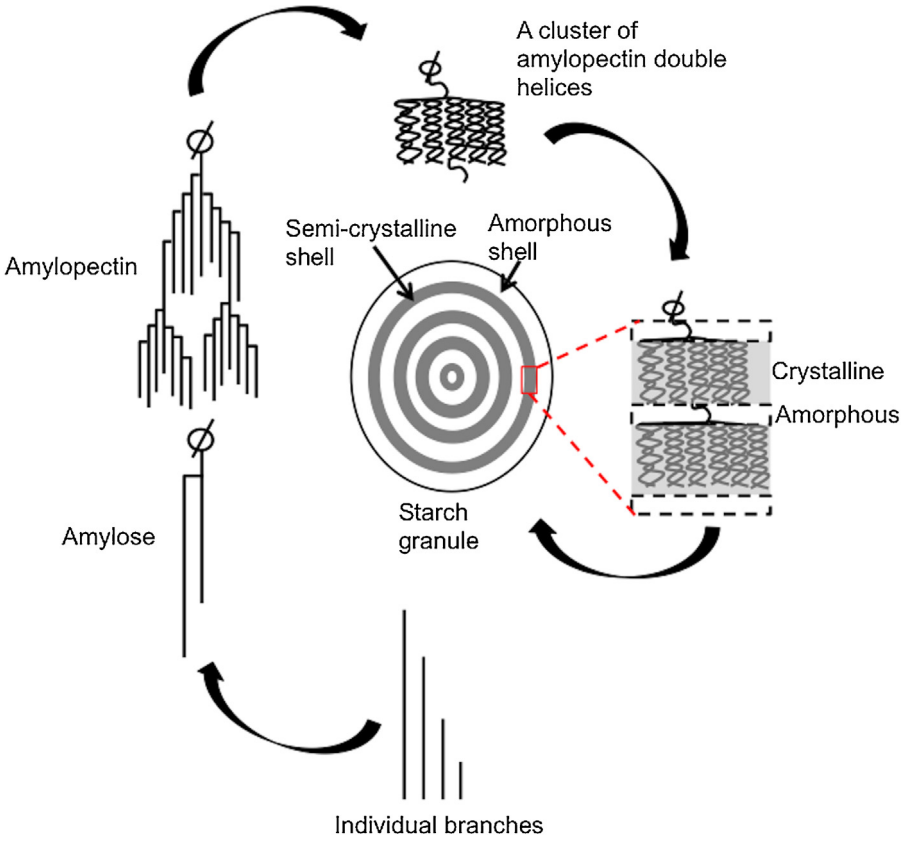

Fig. 1. The hierarchical structural levels of organization in leaf starch. It shows the progression from individual branches (level 1) through to the starch granule (level 5).

and tissue expression patterns. SBEIIb in monocots is exclusively expressed in non-photosynthetic storage tissues (e.g. endosperm) and has a narrower range of glucan transfer preference (DP 6–7) than SBEIIa, which is ubiquitously expressed [5]. Another important starch biosynthetic enzyme is debranching enzyme (DBE), which is required for the trimming of improperly positioned branches. DBE and SS, as with SBE, have different isoforms [6]. Recent reviews provide more detail regarding starch biosynthesis [7–10].

Transitory starch in leaves is degraded primarily by hydrolysis of the constituent glucans into maltose and glucose, both of which can be exported from the chloroplast and metabolized in the cytosol [10]. There is good evidence that starch degradation is dependent on the reversible phosphorylation of glucans at the surface of the starch granule, which serves to solubilize the granule's surface, thus allowing hydrolases access to the glucan chains [11–16]. Hydrolysis of the linear chains is catalyzed primarily by β-amylases. The complete degradation of amylopectin also requires hydrolysis of branch points by DBE, because β-amylases can neither hydrolyze α-(1 → 6) branching linkages nor act immediately adjacent to them. Disproportionating enzyme (D-enzyme) is also involved in starch degradation downstream of starch granule hydrolysis, transferring a maltosyl group from maltotriose to another glucan, generating glucose and a longer glucan that can be further degraded by β-amylase.

The fine structure of transitory starch synthesized during the day influences degradation during the following night, to ensure a regular carbon flux and energy supply for plant growth; inappropriate fine structure impairs the proper diurnal cycling of starch in plant leaves [17]. For example, in the absence of properly branched transitory amylopectin, irregular starch granules are formed that cannot be efficiently degraded and mobilized at night, with the accumulation of these granules within the chloroplast, triggering senescence [17]. Alterations in starch turnover in maternal plants may also have a major effect on fruit growth and seed composition [18].

Despite the importance of transitory starch in plant growth and development, very few studies have been conducted to investigate the molecular structure of transitory starches. Among the few studies undertaken, most used *Arabidopsis*, with the characterization of starch structure being focused on the granular level and the first level of starch structure, with the CLD being characterized only up to low DPs (~DP 50) [19–24]. There is potential new knowledge to be obtained by extending such studies to higher DPs and to other plants, given the significant differences between species in the factors controlling starch metabolism and the structure of the starch itself [17]. The C_4 photosynthetic plants such as *Sorghum*, maize and sugarcane have very high rates of biomass accumulation, yet little is known about the fine structure and turnover of transitory starch in these important food and biomass species.

To better understand the dynamics of transitory starch formation and degradation, we characterized transitory starch levels 1 and 2 structures by size-exclusion chromatography (SEC), from *Sorghum* leaves harvested at various times across a diurnal cycle, exploiting the natural diurnal rhythms of starch structure turnover. Natural light and mature leaves were used in order to closely emulate natural conditions. Knowledge of the structural changes of transitory starch across a diurnal cycle is a prerequisite to understanding its regulation.

2. Materials and methods

2.1. Plant material and growth conditions

Five individual *Sorghum* plants (inbred line Tx430) [25,26] were used for sampling. Seeds were planted in a temperature-controlled glasshouse (18–28 °C) without supplementary light or humidity