Morphology and structure of starch-like glucans in a branching enzyme mutant of Arabidopsis complemented with the glycogen branching enzyme from *E. coli*

Laura Boyer[1], Ofilia Mvundza Ndjindji[2], Christine Lancelon-Pin[2], Adeline Courseaux[1], Fabrice Wattebled[1], Xavier Roussel[1], Christophe D'Hulst[1], Bruno Pontoire[3], Jean-Luc Putaux[2]*

[1] *UGSF, UMR CNRS 8576, Université Lille 1, Bât. C9, F-59655 Villeneuve d'Ascq, France*
[2] *CERMAV, UPR CNRS 5301, Université Grenoble Alpes, BP 53, F-38041 Grenoble Cedex 9, France*
[3] *UR1268 Biopolymères Interactions Assemblages, INRA, F-44300 Nantes, France*

*jean-luc.putaux@cermav.cnrs.fr; Phone: +33 (0)476037604; Fax: +33 (0)476547203

1. INTRODUCTION

Starch and glycogen are the two main storage polysaccharides that accumulate in living cells. Although they are both made of α(1→4)-linked glucose residues branched in α(1→6) position, they differ in structure and properties [1]. The main difference lies in the distribution of the α(1→6) linkages. In glycogen, these branching points are homogeneously distributed whereas in amylopectin (the major and semicrystalline fraction of starch), they are concentrated in amorphous lamellae. The crystalline regions of amylopectin are mainly composed of short linear glucans that intertwine into parallel double helices. In starch metabolism, isoamylase-type debranching enzymes, that specifically cleave the α(1→6) linkages, control the distribution of the branching points by removing those in excess or misplaced, allowing the interlacing of the chains, an essential event to confer crystallinity to amylopectin [2,3]. The present study aimed at establishing the implication of branching enzymes, that introduce the α(1→6) linkages in the glucans, in the branch point distribution in amylopectin.

2. RESULTS

2.1 Experimental section

The Arabidopsis *be2be3* branching enzyme double mutant that accumulates maltose instead of starch [4] has been transformed, allowing the expression of the glycogen branching enzyme (GlgB) form *E. coli*. A transit peptide allowing chloroplast targeting of GlgB was added to the *E. coli* sequence and introduced in the *be2be3* mutant genome through Agrobacterium transformation. Transgenic plants were selected on MS medium containing hygromycin B [5]. Plants harboring low, intermediate and high levels of GlgB activity (referred to as β1, β12 and β20, respectively) were cultivated during 16 h day / 8 h night cycles. Strips of freshly cut leaves harvested at the end of the day were fixed and embedded in Epon. Ultrathin sections were post-stained with periodic acid thiosemicarbazide silver proteinate (PATAg) and observed with a Philips CM200 transmission electron microscope (TEM) at 80 kV. Purified polyglucan fractions were observed by TEM and scanning electron microscopy (SEM) with a FEI Quanta 250 microscope. The chain length distributions (CLDs) were determined by anion exchange chromatography (HPAEC-PAD) after complete debranching of the glucans by an isoamylase. The branching degree was determined from the CLDs using the method described by Szydlowski *et al* [6]. X-ray diffraction (XRD) diagrams of hydrated glucan fractions were recorded with a Bruker D8 Discover diffractometer.

2.2 Polyglucan morphology and distribution in the chloroplasts and after purification

We have compared the shape and distribution of the synthesized glucans, positively stained with PATAg, in chloroplasts of wild-type Arabidopsis, specific mutants and transformed plants with different levels of GlgB activity (Fig. 1). Wild-type chloroplasts contain a few typical flat starch granules (Figs. 1a and 2a). The *isa1isa3pu1* mutant does not contain any debranching activity and thus only accumulate phytoglycogen particles (Fig. 1b). The *be2be3* mutant does not contain any branching activity. It does not accumulate any polysaccharide and only maltose is synthesized (Fig. 1c). The GlgB-containing β mutants accumulate dense glucan aggregates with irregular shapes. Their size decreases with increasing GlgB activity (Figs. 1d-e and 2b,c). The particles purified from β12 and β20 retain the multilobular aspect of glycogen but are significantly larger and denser (Fig. 3). The CLDs of the glucans from transformed plants are intermediate between those of wild-type starch and phytoglycogen. The fraction of short chains and the calculated branching degree increase with increasing GlgB activity. The synthesized glucans are semicrystalline and exhibit a B-type XRD pattern similar to that of wild-type starch granules, with a crystallinity index decreasing with increasing GlgB activity.

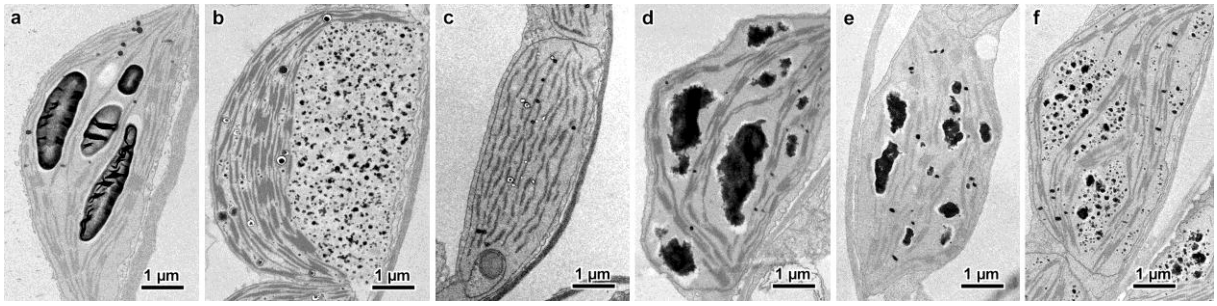

Figure 1. TEM images of ultrathin sections of Arabidopsis leaf plastids from wild type (a), *isa1isa3pu1* (b) and *be2be3* (c) mutants, and transformed plants (d-f), positively stained with PATAg. The level of GlgB activity in the three transformants varies from low (d) to intermediate (e) and high (f).

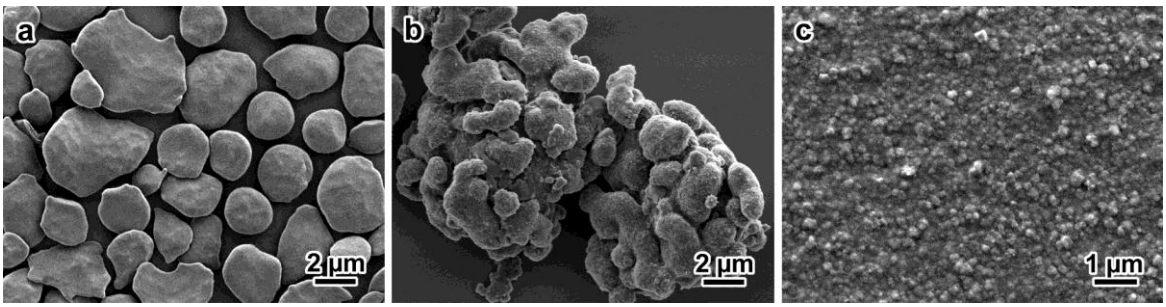

Figure 2. Secondary electron FEG-SEM images of purified wild-type *Arabidopsis* starch granules (a) and β1 (b) and β20 (c) polyglucan fractions. The specimens were coated with Au/Pd.

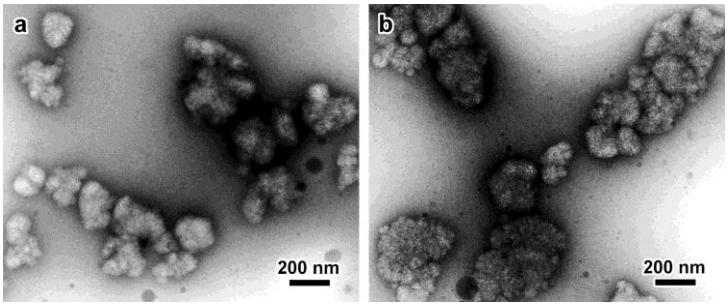

Figure 3. TEM images of purified polyglucans negatively stained with Uranyless^{TM} : a) β12; b) β20.

3. CONCLUSION

We have shown that replacing the endogenous Arabidopsis branching enzymes by a protein of bacterial origin with the same function could restore the synthesis of a polymer with characteristics resembling those of the wild-type amylopectin. A relation exists between the level of branching enzyme activity and the morphology and structure of the synthesized polysaccharides. Work is in progress to explain the peculiar shapes of the large polyglucan aggregates and determine if they are composite objects and if they have a molecular ultrastructure similar to that of wild-type starch granules.

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