Glucan, Water Dikinase Activity Stimulates Breakdown of Starch Granules by Plastidial β-Amylases[1][W][OA]

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Glucan phosphorylating enzymes are required for normal mobilization of starch in leaves of Arabidopsis (*Arabidopsis thaliana*) and potato (*Solanum tuberosum*), but mechanisms underlying this dependency are unknown. Using two different activity assays, we aimed to identify starch degrading enzymes from Arabidopsis, whose activity is affected by glucan phosphorylation. Breakdown of granular starch by a protein fraction purified from leaf extracts increased approximately 2-fold if the granules were simultaneously phosphorylated by recombinant potato glucan, water dikinase (GWD). Using matrix-assisted laser-desorption ionization mass spectrometry several putative starch-related enzymes were identified in this fraction, among them β-AMYLASE1 (BAM1; At3g23920) and ISOAMYLASE3 (ISA3; At4g09020). Experiments using purified recombinant enzymes showed that BAM1 activity with granules similarly increased under conditions of simultaneous starch phosphorylation. Purified recombinant potato ISA3 (StISA3) did not attack the granular starch significantly with or without glucan phosphorylation. However, starch breakdown by a mixture of BAM1 and StISA3 was 2 times higher than that by BAM1 alone and was further enhanced in the presence of GWD and ATP. Similar to BAM1, maltose release from granular starch by purified recombinant BAM3 (At4g17090), another plastid-localized β-amylase isoform, increased 2- to 3-fold if the granules were simultaneously phosphorylated by GWD. BAM activity in turn strongly stimulated the GWD-catalyzed phosphorylation. The interdependence between the activities of GWD and BAMs offers an explanation for the severe starch excess phenotype of GWD-deficient mutants.

Starch consists of the two Glc polymers, amylose and amylopectin, and is deposited as semicrystalline granules inside plastids. The structure of amylopectin, which normally accounts for 70% or more of the dry weight of starch, is responsible for the semicrystalline nature of starch. In amylopectin α-1,4-linked glucan chains are connected by α-1,6 branches. The chain length distribution and the arrangement of the branch points in amylopectin lead to the formation of ordered arrays of densely packed double helices in the semicrystalline zones of the starch granule. The essentially linear amylose is probably present in the so-called amorphous zones of the granule that also contain amylopectin in a less ordered structure (Smith, 2001).

Enzymes required for starch synthesis are ADP-Glc pyrophosphorylase, starch synthases, branching enzymes, and also distinct isoforms of debranching enzymes (Tetlow et al., 2004; Zeeman et al., 2007). The breakdown of the starch particle is less well understood. α-Amylase, which cleaves α-1,4 bonds within the polyglucan, plays an important role in the degradation of cereal endosperm starch (Smith et al., 2005). However, this enzyme is not essential for starch breakdown in Arabidopsis (*Arabidopsis thaliana*) leaves (Yu et al., 2005). In contrast, Arabidopsis plants in which the plastidial β-amylase isoform β-AMYLASE3 (BAM3; BMY8, At4g17090) is repressed by means of RNAi show a starch excess phenotype in their leaves (Kaplan and Guy, 2005). The same phenotype was observed in potato (*Solanum tuberosum*) antisense plants with reduced expression of the plastidial β-amylase PCT-BMY1, the putative ortholog of BAM3 (Scheidig et al., 2002). β-Amylases are exoamylases that release maltose from the nonreducing ends of glucans or dextrins by cleavage of α-1,4 linkages. α-1,6 linkages are hydrolyzed by debranching enzymes. Most higher plants contain four different debranching enzymes: three isoforms of isoamylase and one limit dextrinase (Lloyd et al., 2005). It has been shown that the debranching

[1] This work was supported by the Deutsche Forschungsgemeinschaft (grant nos. SFB 429 TP–B2 to M.S. and TP–B7 to G.R.). S.M.S. acknowledges receipt of an Australian Research Council Federation Fellowship and Discovery Grant (grant no. DP0666434) to support this research.

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The author responsible for the distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Gerhard Ritte (ritte@uni-potsdam.de).

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www.plantphysiol.org/cgi/doi/10.1104/pp.107.104224