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Studies on Functional Analysis of Plant Starch Biosynthetic Enzymes

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About three centuries have already passed since Antoni van Leeuwenhoek observed the gelatinization of starch granule under a microscope in 1719. Now, many people know not only the word “starch,” but also the reaction in which starch is digested by α-amylases contained in our saliva. However nobody can correctly address the question how plant starch is made. The final aims of our research were to elucidate the mechanism of plant starch biosynthesis and then to take advantage of the knowledge in our lives. For that purpose, we tried to understand the characteristics of various enzymes related to starch biosynthesis. We have isolated cDNA clones for ADPglucose pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (DBE) from kidney bean (*Phaseolus vulgaris* L.) plants. Northern blot analyses showed that transcripts for all AGPases and SBEs isolated in this study accumulate in both leaves and seeds, whereas the profiles of organ-specific expression for SSs and DBEs differ with each isozyme. To investigate enzymatic properties, several recombinant enzymes were purified from *Escherichia coli* cells. Three SS isozymes (designated rPvSSI, rPvSSIIb and rPvGBSSIa) showed distinct chain-length specificities for the extension of glucan chains. rPvGBSSIa acts on chains of approximately DP=15 of amylopectin and elongates them processively to synthesize ultimate long chains. The elongation properties of chains by PvGBSSIa isozyme must have a pivotal role in amylose biosynthesis. Two SBE isozymes (designated rPvSBE1 and rPvSBE2) were also purified from *E. coli* cells and their enzymatic properties were determined. Western blot analysis with antisera raised against rPvSBE1 and rPvSBE2 showed that these two SBEs were located in different amyloplast fractions of developing seeds of kidney bean: PvSBE2 was present in the soluble fraction, whereas PvSBE1 was associated with starch granule fraction. These differences in location suggest that these two SBE isozymes have different roles in amylopectin synthesis in kidney

bean seeds. Moreover, we showed that a larger form of PvSBE2, LF-PvSBE2, exists which contains an extended N-terminal region. Unlike the soluble location of PvSBE2, LF-PvSBE2 is observed in both the soluble and starch-granule fractions. We also demonstrated that the extended N-terminal region in LF-PvSBE2 alters not only its subcellular location but also its kinetic properties as well. The two isoforms are encoded by the same gene, which produces two distinct transcripts generated by alternative splicing of the first two exons. Our results shed new insights on our understanding of the regulatory mechanism of amylopectin biosynthesis.

Key words: starch biosynthesis, starch branching enzyme, starch synthase, subcellular localization