Archives of Biochemistry and Biophysics 596 (2016) 63–72

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

# Comparative *in vitro* analyses of recombinant maize starch synthases SSI, SSIIa, and SSIII reveal direct regulatory interactions and thermosensitivity

Binquan Huang [a, 1], Peter L. Keeling [b], Tracie A. Hennen-Bierwagen [a], Alan M. Myers [a, *]

[a] Roy J. Carver Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50011, USA
[b] Center for Biorenewable Chemicals, 1140K Biorenewables Research Laboratory, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

*Article history:*
Received 8 December 2015
Received in revised form
6 February 2016
Accepted 28 February 2016
Available online 3 March 2016

*Keywords:*
Starch synthase
Starch structure
Maize
Endosperm
Thermosensitivity

ABSTRACT

Starch synthases SSI, SSII, and SSIII function in assembling the amylopectin component of starch, but their specific roles and means of coordination are not fully understood. Genetic analyses indicate regulatory interactions among SS classes, and physical interactions among them are known. The N terminal extension of cereal SSIII, comprising up to 1200 residues beyond the catalytic domain, is responsible at least in part for these interactions. Recombinant maize SSI, SSIIa, and full-length or truncated SSIII, were tested for functional interactions regarding enzymatic activity. Amino-terminal truncated SSIII exhibited reduced activity compared to full-length enzyme, and addition of the N terminus to the truncated protein stimulated catalytic activity. SSIII and SSI displayed a negative interaction that reduced total activity in a reconstituted system. These data demonstrate that SSIII is both a catalytic and regulatory factor. SSIII activity was reduced by approximately 50% after brief incubation at 45 °C, suggesting a role in reduced starch accumulation during growth in high temperatures. Buffer effects were tested to address a current debate regarding the SS mechanism. Glucan stimulated the SSIIa and SSIII reaction rate regardless of the buffer system, supporting the accepted mechanism in which glucosyl units are added to exogenous primer substrates.



## 1. Introduction

Starch, the main carbon reserve in plants, supports metabolic function owing in large part to the molecular architecture of its most abundant component amylopectin. A key feature of amylopectin structure is the length distribution of "linear", i.e., α(1 → 4)-linked, chains that are joined to each other by α(1 → 6) branches. Linear chains are synthesized by starch synthases (SS) (EC 2.4.1.21) that catalyze addition of glucosyl units to the non-reducing end of an existing polymer using ADPglucose (ADPGlc) as the hexose donor [1–3]. All known genomes of chloroplast-containing organisms contain separate genes encoding five different SS classes designated granule-bound starch synthase (GBSS), SSI, SSII, SSIII, and SSIV [4]. In some species one or more of the genes have duplicated and taken on apparent tissue-specific functions, but in all green algae and land plants it appears that at least one gene for each class is active. Such evolutionary conservation suggests that each SS class provides a unique function. Some specific roles have been described, as follows [2,5].

GBSS functions primarily in synthesis of the amylose component of starch, and the role of SSIV appears to be in granule initiation. The current study addresses SSI, SSII, and SSIII, which all participate in determining amylopectin structure. SSI appears to be primarily responsible for generating linear chains with degree of polymerization (DP) 8–12. SSII is thought to elongate such chains to achieve the full length present in the crystalline regions of mature starch grains, approximately DP 16–22. The substrate and product

*List of abbreviations:* ADPGlc, ADP glucose; SS, starch synthase; DP, degree of polymerization; SSIII-HD, residues 770–1225 of full-length SSIII constituting the "SSIII homology domain"; SSIII-N, residues 90–769 of full-length SSIII constituting the 670 N terminal residues of the predicted mature protein as it exists *in vivo*; SSIII-CAT, residues 1226–1674 of full-length SSIII constituting the catalytic domain including the entire GT-B structural fold; SSIII-HDCAT, residues 770–1674 of full-length SSIII containing both the SSIII homology domain and the catalytic domain; SSIII-NHD, residues 90–1225 of full = length SSIII containing both the amino terminal domain and the SSIII-homology domain.

* Corresponding author. 2110 Molecular Biology Building, Ames, IA 50011, USA.
*E-mail address:* ammyers@iastate.edu (A.M. Myers).
[1] Present address. Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK.

http://dx.doi.org/10.1016/j.abb.2016.02.032
0003-9861/

specificities of SSI and SSII overlap, and there appears to be competition between them *in vivo* for access to the acceptor substrate. The roles of SSIII appear to be more complex and to involve regulatory interactions separable from enzymatic activity, as discussed in a following section.

The SS catalytic domain exhibits the conserved GT-B fold typical of glycogen synthase and other glycosyl transferases, comprising two separate Rossman folds designated as GT_1 and GT_5 (Pfam database IDs PF00534 and PF08323, respectively) [6,7]. N terminal extensions beyond the catalytic domain, of unknown structure, are present in each SS class and are conserved to various extents (Fig. 1A). For example, the N terminal extensions of SSI from potato and maize are 22% identical over 78 aligned residues. SSIII contains the longest N terminal extension among these enzymes. This includes a conserved sequence of approximately 450 residues, designated as the SSIII homology domain (SIII-HD), that is adjacent to the catalytic domain (Fig. 1A). As a typical example, SIII-HD of maize and potato exhibit 57% identity over 456 residues with no gaps in the alignment [8]. Both glucan binding and protein-protein interaction functions have been attributed to the SSIII-HD domain [9–12]. Additional sequences of various lengths ranging from approximately 130 to 700 or more amino acids are present in the SSIII N terminus adjacent to SSIII-HD (Fig. 1A). This 700 amino acid region in maize SSIII, designated as SSIII-N, is conserved at a relatively low level in other monocot species, but not between monocots and dicots. Structural motifs are not predicted from N domain primary sequences. In at least some instances the N domain is involved in protein-protein interactions, as shown by a positive signal between part of SSIII-N and maize SSI in *in vivo* protein-protein interaction tests [11].

The current study utilized recombinant maize SSI, SSIIa, and SSIII, and fragments of SSIII, to address three aspects of SS function in amylopectin biosynthesis. The first aspect is regulatory interactions between different SS classes. SSIII has been implicated as a regulatory factor in addition to its biosynthetic role. SSIII mutation in maize or rice endosperm resulted in elevated rather than decreased total SS activity in cell extracts [13–15], and such mutations in *Arabidopsis* leaf caused elevated starch content rather than decreased amounts as predicted simply from loss of a catalytic activity [16,17]. From these results SSIII appears to be a negative regulator of other SSs. Additional evidence for a regulatory role is that in cereals the effects of SSIII mutations on amylopectin structure are discontinuous with regard to the specific chain lengths that change in abundance [15,18,19]. In contrast, mutations affecting SSI or SSII result in alterations in continuous ranges of chain lengths [5]. SSI and SSII, therefore, appear to be involved in generation of

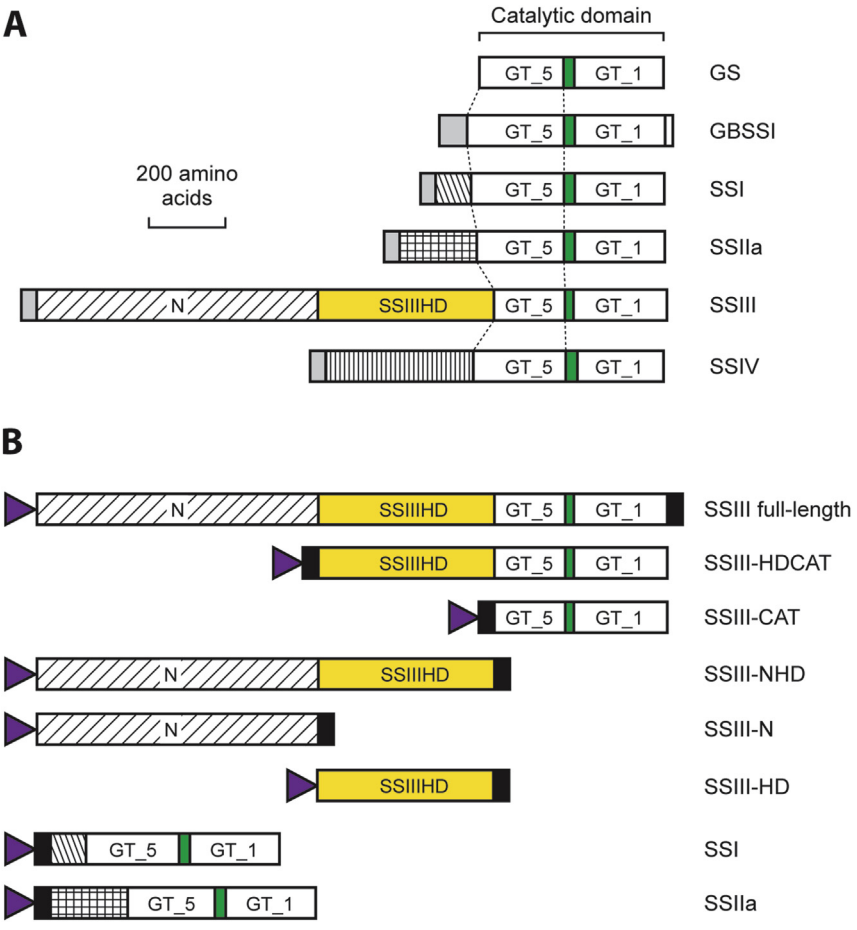


**Fig. 1.** SS domains and expressed proteins. Figures are drawn to scale except for promoters and affinity tags. A, Maize enzymes predicted from cDNA sequences, with *E. coli* glycogen synthase (GS) included for comparison. GT_1 and GT_5 are conserved domains that together constitute the conserved GT-B fold of the catalytic domain of glucosyl transferases such as SS. Green boxes indicate linker sequences that connect GT_1 and GT_5. Gray boxes indicate known or predicted plastid transit peptides. Crosshatched boxes and the solid yellow box indicate N terminal extension regions of distinct sequence. B, Proteins expressed in *E. coli*. Domain designations are as in panel A with the additions that purple triangles indicate the phage T7 promoter and black boxes indicate affinity tags.