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ISSN: 0975-928X

Volume: 10

Number: 4

EJPB (2019) 10(4):1581-1585

DOI:10.5958/0975-928X.2019.00203.5

Electronic Journal of Plant Breeding, 10 (4): 1581-1585 (Dec 2019) DOI: 10.5958/0975-928X.2019.00203.5
ISSN 0975-928X

Research Note

Analysis of granule-associated Starch branching enzyme IIb, involved in *amylose extender* mutation of maize

Alla Singh*, DP Chaudhary, Ramesh Kumar, Sujay Rakshit.
ICAR-Indian Institute of Maize Research, P.A.U. campus, Ludhiana - 141004.
***E-Mail**: alla.singh@icar.gov.in, allasingh.panesar@gmail.com

(Received: 15 Nov 2019; Revised: 16 Dec 2019; Accepted: 20 Dec 2019)

Abstract
Maize starch is an important industrial commodity. Starch synthase, phosphorylase, branching and debranching enzymes act in concert and results in a particular starch architecture. Starch branching enzymes play an important role in defining the overall starch structure. In maize endosperm, Starch branching enzyme IIb is predominant. The computational structure of Starch branching enzyme IIb has been determined. The putative substrate binding site and active site pore channel have been elucidated. As the enzyme is known to prefer amylopectin as substrate, the disachharide melibiose containing α- 1,6 glycosidic bond was docked on the modelled protein. The substrate binding site and active site channel has been discussed. Understanding of starch structure is essential for its modification and processing in forms, that are desirable for specific end-use applications. Generation of feedstock specific for a particular starch modification would result in environmentally-friendly starch production system by minimizing the requirement of chemicals for starch modifications. This is also necessary for allele mining to direct further breeding goals in this area.

Starch is an important structural component of grains, that imparts strength and can also be used to obtain energy in times of need, especially during seed germination. It is built of polyglucan, resulting in a semicrystalline structure which is insoluble in water. The wrinkled phenotype of pea seeds, studied by Mendel for genetics, is result of an alteration in starch structure. The reduced starch content in seeds leads to osmotic pressure and development of wrinkles. Starch is polymer of glucose. Glucose molecules can be joined to each other in linear patterns, resulting in α-1,4 glycosidic bonds. The glucose molecules can also join in branched pattern, resulting from α-1,6 glycosidic bonds. The linear and branched structures are referred to as amylose and amylopectin, respectively. Four enzymes act in concert for the microstructure of starch [Kang *et al.* (2006)]. ADP-Glc pyrophosphorylase synthesizes sugar nucleotide precursors. Starch synthase extends the α -1,4-linked glucan chains. Starch branching enzymes (SBEs) introduce α -1,6 branch points in the linear α -1,4-linked polyglucan, while Starch debranching enzymes (DBEs) hydrolyze branches in glucans. Historically, the chain elongation and branching activities were referred to as P- and Q- enzyme activities. The structure of starch is significantly shaped by the frequency of branching via α- 1,6 glycosidic bonds and also by position of these branch points relative to each other. The glucan chain length plays an important role in an organism's physiology. Starch branching enzymes play an important role in determining the final microstructure of starch granules. It accepts polyglucan substrate and transfer glycosidic bonds in the α -1,6 orientation. This results in generation of non-reducing ends that can be further extended. Inactivation of Starch branching enzymes results in amylose extender mutation of cereals, which is desirable for its potential health benefits in the form of Resistant Starch (Wang *et al.* (2017)).

Understanding of the role of enzymes in shaping the starch structure is essential to determine useful alleles for utilization in plant breeding endeavours towards specific starch microstructures. SBEs are considered important targets for improvement of further features in starches, particularly for food industry. Structure-function analysis of key features, patterns or domains in an enzyme reveals the functional properties associated with particular features, which can be combined to result in an 'ideal' sequence that serves the desired purpose. Structural allele mining can be used for bio-prospecting of proteins and their variants for use in breeding programmes. In maize, SBEIIb is expressed at 50-fold higher level than SBEIIa (Tetlow and Emes (2014)). While SBEI prefers amylose as substrate, SBEII enzymes prefer amylopectin as substrate. Considering the importance of SBEIIb in maize, its structure was modeled and mimic of the potential substrate of the enzyme, melibiose, which has α -1,6-glycosidic bond between glucose and galactose, was docked on the modelled structure to reveal substrate binding site.

Generation of homology model of *Zea mays* SBEIIb. The homology model of *Zea mays* SBEIIb was generated using SWISS-MODEL program (Waterhouse *et al.* (2018)). The secondary structure of the model was computed using PDBsum program (Laskowski *et al.* (2018)). The estimates of model quality, Global Model Quality Estimation (GMQE) and QPEAM were obtained from the server (Benkert *et al.* (2011)).

Docking of melibiose on *ZmSBEIIb* model. The structure of melibiose was obtained from ZINC ligand repository [Sterling *et al.* (2015)]. Melibiose was docked onto *ZmSBEIIb* model using rigid docking algorithm based on shape complimentarity via Patch Dock program (Duhovny *et al.* (2002), Schneidman-Duhovny *et al.* (2005)). The active site pore channel of the protein was determined using Pore Walker program (Pellegrini-Calace *et al.* (2009)).

The homology model of *Zea mays* SBEIIb was determined. It showed a good sequence identity of 59.61% with rice SBEI. The crystal structure of rice SBEI has been solved (Noguchi *et al.* (2011)) and is available on PDB (ID: 3AML). The structure has a GMQE and QMEAN score of 0.73 and -2.58, respectively. Both the scores, which represent the quality of model, indicate that the modelled structure is of high-quality. The modelled structure contains 28 α-helices and 30 β-sheets. The structure roughly consists of three domains. Figure 1(A) shows the 3-dimensional representation of the *ZmSBEIIb* model. Figure 1(B) displays the secondary structure of the tertiary molecule. The three domains have been colored in magenta, yellow and orange. As SBEII enzymes are known to prefer amylopectin as substrate, a disaccharide with α -1,6-glycosidic linkage was used to determine the substrate binding site on *ZmSBEIIb*, since melibiose mimics amylopectin in its glycosidic linkage [Figure 2(A)]. Melibiose was docked onto *ZmSBEIIb* model using rigid docking algorithm based on shape complimentarity. Melibiose bound to target site containing 9 β-helices in the N-terminal region of the protein [Figure 2(B)]. This region corresponds to the domain coloured in magenta in Figure 1(A). SBEs utilize glucans as their substrate and transfer the glycosidic bonds in different orientation. They are known to have an extended active site where the processes of hydrolysis and transglycosylation occur. In order to elucidate the active site channel of *ZmSBEIIb* enzyme, PoreWalker program was used. Although PoreWalker program is used for transmembrane proteins, which possess long channels, the program was used with *ZmSBEIIb* due to the similarity of an extended active site with channel in a typical transmembrane protein. Figure 2(C) shows that channel extends throughout the length of the protein, spanning all the domains. This is consistent with the molecular function of the SBE proteins.

The structure of maize SBEIIb and the melibiose binding site have been determined computationally. The putative pore channel of the protein has been indicated. Breeding of crops for improvement requires elucidation of useful alleles for a particular trait. Usually, beneficial alleles are clustered along with linkage drag-causing sequences. Genetic recombination is employed to remove the component of linkage drag (Leung *et al.* (2015)). An understanding of the structure-function of a protein, thereby determining the key residues, can be helpful in discovery of novel, useful variants. A direct extension of the above work is elucidation of the logotype of amino acid residues that line the channel of the *ZmSBEIIb* protein. The sequence logo, thus obtained, would be useful in determining the desirable amino acid residues at important sites in the protein structure. This can be combined with EcoTILLING to identify useful alleles (Barkley and Wang (2008)). EcoTILLING allows screening of germplasm for natural mutations. The maize populations segregating for differential starch qualities can be screened for mutations along the pore channel and active site, to determine their role in regulating the starch structure. Mechanistic understanding of starch granule biosynthesis and the role of branching enzyme would result in informed screening and categorization of useful alleles towards desired starch modifications. There is demand for continuous supply of specific starch functionalities. In order to minimize chemical modifications, the starch crops need to be specifically altered to result in functionalized or near-functionalized starch, as required. This requires robust understanding of starch structure and determination of useful allelic diversity in natural mutants, to realize environmental friendly starch production with relevance for industry. This study elucidates the structural features in the protein, including the pore where polyglucan chain can enter and the melibiose binding site, which can constitute one of the factors leading to recognition of the amylopectin structure. The allelic diversity inherent in the *ZmSBEIIb* enzyme's sequence and its role in influencing starch structure may be understood in terms of the structure-function relationship of the key sites of maize *SBEIIb* enzyme, as determined in the above study.

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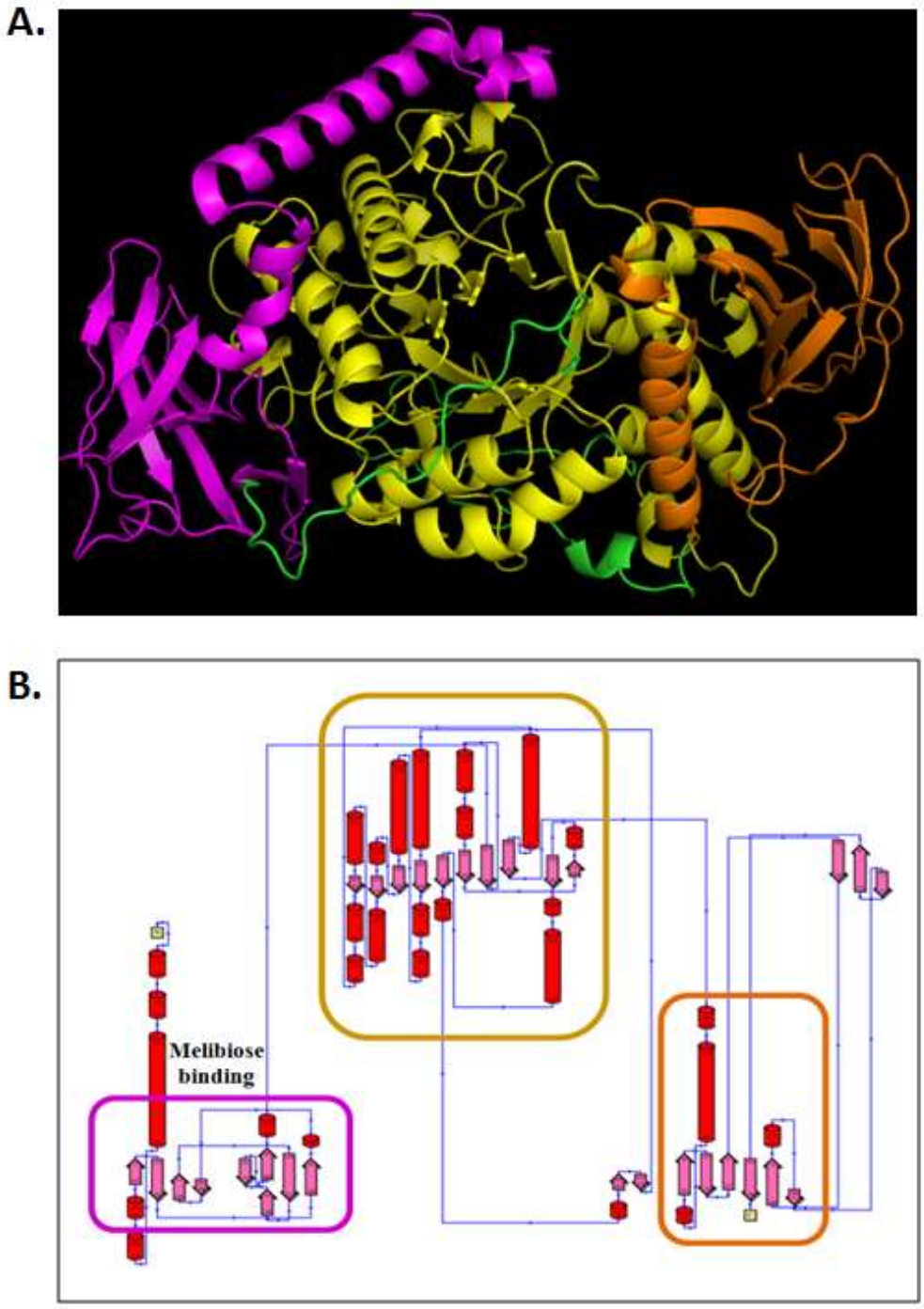

Fig. 1. Structure of *ZmSBEIIb* protein. A. 3-dimensional orientation of the molecule. The three major domains are coloured in magenta, yellow and organe. **B.** Secondary structure representation of the protein. The coloured domain in 1(A) are encircled in same colour in this diagram. The melibiose binding site is encircled.

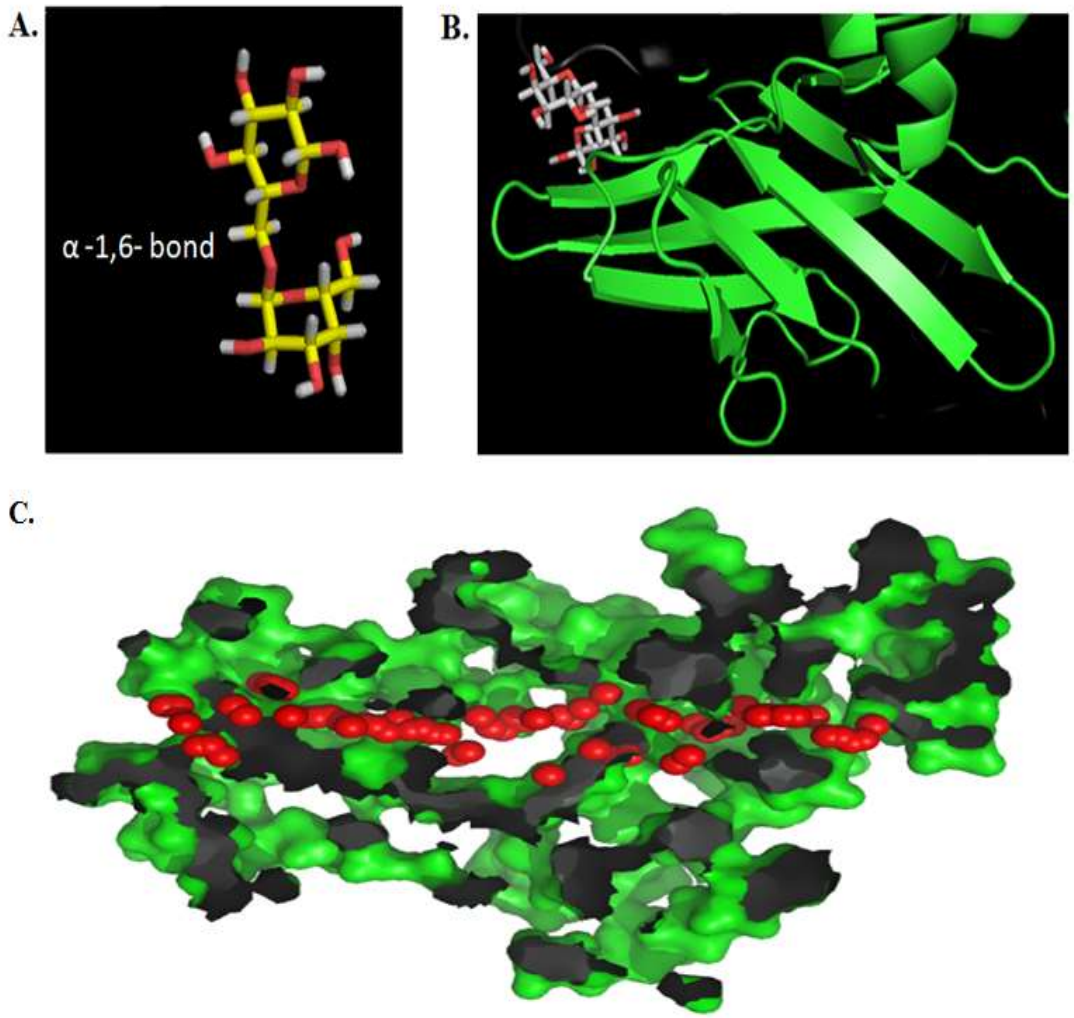

Fig. 2. Features of maize Starch Branching Enzyme IIb isoform. A. The disaccharide melibiose containing α -1,6-glycosidic bond is shown. B. Melibiose (coloured in white, with oxygen atoms in red) binds to domain with 9 β-sheets [coloured magenta in Figure 1(A)]. C. Putative channel (depicted in red) in *ZmSBEIIb* in which the polyglucan substrate binds and the processes of hydrolysis, transglycosylation and exit of the product take place.

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