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Revealing the important role of allosteric property in sucrose phosphate synthase from sugarcane with N-terminal domain deletion

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Abstract. Sucrose occupies many essential roles to control regulation of carbon partitioning in plants, including prokaryotic cells. Sucrose phosphate synthase (SPS; EC 2.4.1.14) is a key enzyme to catalyze the form of sucrose in primary sucrose synthesis pathway. Plants SPS has a molecular size around 120 kDa, which consists of N-terminal domain, C-terminal domain, and central domain. We produced the recombinant sugarcane SPS (*So*SPS1) in *Escherichia coli*, however, the expression often appears to be a shorter form with retained enzyme activity. In our result, we reported that the shorter form is suggested to have a truncated N-terminal 20-kDa region. The truncated form of *So*SPS1 (ΔN-SPS) tends to enhance the specific activity 10-fold compared to full-length *So*SPS1. The full-lenght *So*SPS1 showed a remarkable allosteric activation by glucose-6-phosphate (G6P), while none of the N-terminal truncated form had such a characteristic. By kinetic analysis of full-length *So*SPS1, a higher substrate affinity was shown in the presence of G6P. Conversely, the ΔN-SPS showed a similar substrate affinity whether G6P was added or not. Based on these results, we revealed that N-terminal region of SoSPS1 has essential role for allosteric regulation by G6P and may function like a suppressor domain for the enzyme activity.

Keywords: sucrose phosphate synthase, N-terminus deletion, recombinant enzyme, sugarcane

1. Introduction

In most plants, sucrose is the major products of photosynthesis and common form of carbohydrate transported to various growing tissues and storage organs for allocation of carbon resources. Sucrose phosphate synthase (SPS) plays a crucial role to catalyze the formation of sucrose-6-phosphate (S6P) from fructose-6-phosphate (F6P) and uridine diphosphate-glucose (UDP-G) as substrates [1; 2; 3]. The activity of SPS is allosterically controlled by metabolites, such as glucose-6-phospate (G6P) and inorganic phosphate [4]. However, the precise allosteric mechanism of SPS is still unclear. Such molecular characteristics of SPS are believed to be crucial for playing the physiological role of regulating photosynthetic carbon flux into sucrose [18; 20].

Plants SPS has a molecular size around 120 kDa, which consists of N-terminal domain, C-terminal domain, and central domain. The central region contains glycosyltransferase domain responsible for the catalytic function of SPS [5]. The C-terminal region resembles sucrose phosphate phosphatase and the N-terminal region has no clear similarity with any other proteins [17].

It has previously cloned cDNAs from sugarcane leaves, *So*SPS1 and *So*SPS2, encoding two isoforms of SPS. *So*SPS1 is abundantly expressed in sugarcane leaves and is considered to be a representative of the isoenzyme responsible for photosynthetic carbon allocation with the regulatory function [6]. In this study, cDNA of *So*SPS1 was expressed in *E. coli* cells and recombinant enzymes with various lengths of the N-terminal deletion were produced. We report here that the N-terminal region of sugarcane SPS is not essential for the catalytic reaction itself, but plays a role in the allosteric regulation.

2. Experimental Method

A series of mutant SPSs with the N-terminal truncation was constructed and generated by PCR. The PCR product of the N-terminal truncated forms designated as HisΔN1, HisΔN2, HisΔN3, HisΔN4,

HisΔN5 and HisΔN6 were subcloned into pGEM-T vector (Promega) at the insertion site and then cut with SbfI and EcoRI. The resulting DNA fragments encoding the N-terminal truncated SPSs were inserted into *Pst*I and *Eco*RI sites of pTrcHisA. All the SPS genes thus constructed were verified by DNA sequencing.

The constructed plasmids were introduced into E. coli BL21 (DE3) competent cells (Novagen) to express the full length SPS and various N-terminal truncated forms. The transformed bacterial cells were expressed at 20ºC, then 0.05mM IPTG was added and continued overnight. The bacterial cells were suspended in extraction buffer [50mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM MgCl2, 1mM EDTA and 1mM PMSF], and disrupted by sonication on ice. Cell homogenate was centrifuged at 15,000 rpm for 15 min at 4ºC and the resulting supernatant was mixed gently with DE52 anion exchange cellulose (Whatman). The filtrate was directly loaded onto a column of cOmplete His-Tag Purification Resin (Roche) equilibrated with 50 mM Tris-HCl (pH 7.5)/150mM NaCl. The column was washed with the same buffer containing 30mM imidazole and 10% glycerol (w/v) and the resin binding proteins were eluted by increasing concentration of imidazole to 120mM. The eluted sample from the His-tag resin column was concentrated and applied to size exclusion chromatography on a Superdex 200 column (GE Healthcare Life Sciences) and eluted with 50mM Tris-HCl (pH 7.5)/150mM NaCl.

Proteins in fractions obtained during purification were separated by sodium dodecyl sulphate (SDS) polyacrylamide-gel (10%) electrophoresis (PAGE) as described by Apriasti [7]. The gels were directly stained with Coommassie Brilliant Blue. The quantification of protein bands stained with Coommassie Brilliant Blue and visualized by the colour development was performed by densitometry using ImageJ software (http://imagej.nih.gov/ij/).

SPS activity was assayed as described previously [8] with an appropriate modification. The assay mixture (50 ml) contained 50mM Hepes-NaOH (pH 7.5), 20mM MgCl2, 20mM F6P and 20mM UDP-G. The mixture was incubated at room temperature for 10 min and reaction was stopped by an addition of 35 ml of 1M NaOH, followed by incubation at 95ºC for 10 min to decompose unreacted F6P. To determine sucrose formed by the enzyme reaction, 125 ml of 0.1% (w/v) resolcinol in 95% ethanol and 30% (w/v) HCl was added and heated at 85ºC for 8 min. The developed color of sucrose derivative was measured at absorbance 520nm with using a microtiter plate reader.

3. Results and Discussion

Activity of the truncation forms of HisΔN2 to HisΔN6, using at least partially purified preparation (**Fig. 1A**), were measured in the presence of the saturating concentrations of F6P and UDP-G, together with that of the full length SPS. As shown in Fig. 1B, the truncated forms showed an increasing tendency of the specific activity and this was the most remarkable for HisΔN6 that showed an increase in the activity higher by 10-fold than that of the full length SPS. These results were in good agreement with previous report [9] that SPS activity in the *E. coli* cells became higher when accumulation level of Form B was increased. Form B was a proteolytic product of recombinant full-length SPS when it was produced from *E. coli* expression system.
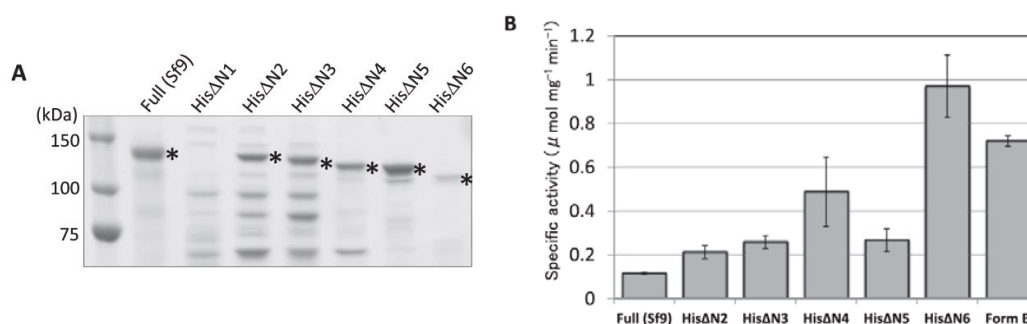
Figure 1 Comparison of activity of the N-terminal truncated and full length. (A) SDS-PAGE analysis of various forms of SPS used for activity measurement. (B) Specific activity of the N-terminal truncated and full-length forms of SPS
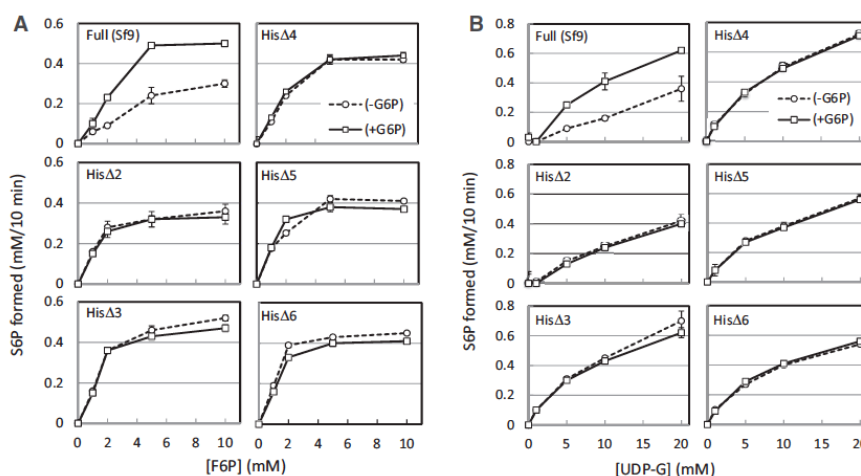
Figure 2 Kinetic properties of the N-terminal truncated and full-length. (A) Activity of various forms of SPS was measured in the assay mixture containing 20mM UDP-G and increasing concentrations of F6P from 0 to 10mM in the presence or absence of 6mM G6P. (B) Activity of various forms of SPS was measured in the assay mixture containing 10mM F6P and increasing concentrations of UDP-G from 0 to 20mM in the presence or absence of 6mM G6P.
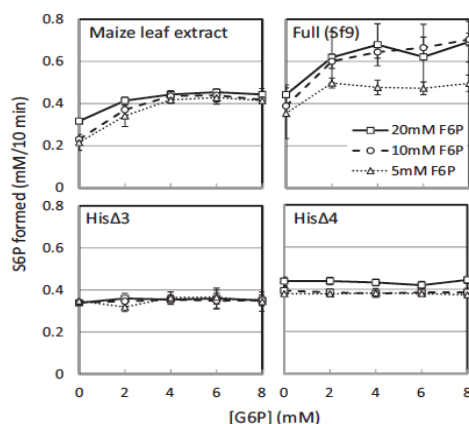
Figure 3 An allosteric effect of G6P on the activity of recombinant SPSs and authentic SPS from plant leaves (maize leaf)

By kinetic analysis of the full length and truncated forms of sugarcane SPS thus prepared, three important results were obtained: (i) the full length enzyme showed a remarkable allosteric activation by G6P, while none of the truncated enzymes tested here had such characteristics (**Fig. 3**), (ii) specific activity of the full length and truncated SPSs showed a tendency that longer truncation has higher activity (**Fig. 1**) and (iii) whether G6P was added or not, the truncated forms showed similar substrate affinity with F6P and UDP-G, while the full length enzyme showed a higher substrate affinity when G6P was present (**Fig. 2A** and **2B**).

Plant SPSs consist of three domains, glucosyltransferase domain (GTD) (55 kDa), the C-terminal phosphohydrolase domain (30 kDa) and the N-terminal domain (20 kDa). The N-terminal domain has no apparent homology with known motifs [10]. Notably, cyanobacteria SPSs [11] and H. orenii SPS (HoSPS) [12], which lack the N-terminal domain of plant SPSs, are not allosterically regulated, implying an involvement of the N-terminal domain in the allosteric regulation. Our present data give a clear piece of evidence that the N-terminal region composed of at least 171 residues of sugarcane SPS is crucial for its allosteric sensitivity to G6P and that the N-terminal truncation as short as 37 residues leads the mutant enzyme to be constitutively active with the allosteric sensitivity lost. The N-terminal region of plant SPS was postulated to be intrinsic disordered by bioinformatics analysis. It has been proposed that polypeptide regions with intrinsic disorders play a role for modulation of allostery [13; 15; 16], such as optimizing

high or low affinity binding and controlling the ability to interact the protein surface [14]. Therefore, plant SPS might be one of examples for such allosteric modulation.

4. Conclusions

Eventually, present biochemical studies has clarified the functional roles of the N-terminal region of sugarcane SPS. It would be advantageous to analysis both the full length and the N-terminal truncated forms for elucidation of the precise model of the allostery. In addition, dynamic simulations analysis may useful for mapping the energy landscape of allosteric process. These combined studies would provide an insight into molecular mechanism of SPS for controlling carbon partitioning in plant sucrose biosynthesis [19].

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