THE ROLE AND REGULATION OF SUCROSE-PHOSPHATE SYNTHASE FROM SUGARCANE (Saccharum species)

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Photosynthetic carbon assimilation and sucrose synthesis in plants

Exported to sink tissues
Sucrose-phosphate synthase (SPS) activities determine sucrose accumulation in leaves of Saccharum species

<table>
<thead>
<tr>
<th>Lines</th>
<th>PEPC (unit/mg prot)</th>
<th>NADP-ME (unit/mg prot)</th>
<th>SPS (µg/g FW)</th>
<th>Sucrose contents (µg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni9</td>
<td>1.68</td>
<td>1.08</td>
<td>37.74</td>
<td>77.50</td>
</tr>
<tr>
<td>NiF8</td>
<td>1.47</td>
<td>1.11</td>
<td>38.70</td>
<td>84.44</td>
</tr>
<tr>
<td>NCo310</td>
<td>1.14</td>
<td>0.98</td>
<td>25.77</td>
<td>55.90</td>
</tr>
<tr>
<td>Molokai</td>
<td>1.67</td>
<td>0.8</td>
<td>31.53</td>
<td>59.20</td>
</tr>
<tr>
<td>Babakan</td>
<td>1.75</td>
<td>0.72</td>
<td>28.45</td>
<td>52.70</td>
</tr>
<tr>
<td>Bois R</td>
<td>1.29</td>
<td>1.16</td>
<td>23.50</td>
<td>51.10</td>
</tr>
<tr>
<td>28NG</td>
<td>1.45</td>
<td>0.92</td>
<td>24.51</td>
<td>52.70</td>
</tr>
<tr>
<td>IN84</td>
<td>1.99</td>
<td>0.90</td>
<td>21.94</td>
<td>55.82</td>
</tr>
<tr>
<td>SES186</td>
<td>2.10</td>
<td>0.90</td>
<td>20.51</td>
<td>46.00</td>
</tr>
<tr>
<td>JW94</td>
<td>1.71</td>
<td>1.24</td>
<td>15.69</td>
<td>39.10</td>
</tr>
</tbody>
</table>

The enzyme activities are expressed as unit/mg prot and sucrose contents in µg / g FW

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PEPC : phosphoenolpyruvate carboxylase
NADP-ME: NADP malic enzyme
SPS : sucrose-phosphate synthase
Rubisco : ribulose-1,5-bisphosphate carboxylase/oxygenase

Sucrose contents in the leaf of Saccharum species were mainly fluctuated according to the SPS activities
Induction of SPS activity during drought stress in sugarcane

Change in sucrose contents (upper), SPS activities (middle) and SPS levels (lower) during 10 days water stress treatments.

Dry season is the best condition for harvesting sugarcane.

ABA induced SPS levels

Change in protein levels of SPS, GS1, GS2 and LSU-Rubisco proteins after treatment of ABA hormone at concentration 0, 10 and 100 µM. The proteins levels were detected by Western Blot analysis with a specific antibody.
Cloning and expression analysis of genes for sucrose-phosphate synthase from sugarcane

Detection of transcripts of SoSPS1 and SoSPS2 in different organ of sugarcane

Comparison of the amino acid sequences deduced from cDNAs for SPS from sugarcane. Box sequences I, II, III are functionally important regions. Dotted Ser residues are the regulatory phosphorylation sites.

Overexpression of \textit{SoSPS1}-cDNA increased SPS levels and sucrose accumulation in leaf of transgenic tomato

Western Blot analysis with antibody against SPS1
Overexpression of *SoSPS1* cDNA increased SPS activity and sucrose content in leaves of transgenic sugarcane

Semi-quantitative RT-PCR analysis of *SoSPS1* (upper) and *Actin* (lower) expression in leaf of transgenic sugarcane

SPS activity in leaf of *Wild type* and transgenic sugarcane

<table>
<thead>
<tr>
<th>Transgenic sugarcane (WT 1 2 3 4 5)</th>
<th>SoSPS1 levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPS activity in leaf (µg/µg prot/min)</td>
<td></td>
</tr>
<tr>
<td>Sucrose content in leaf (mg/g fresh weight)</td>
<td></td>
</tr>
</tbody>
</table>
Activity of plant SPS is regulated by diurnal cycles. Active under light and inactive during dark condition.

This allosteric regulation involving metabolites G6P

Increasing G6P levels up to around 5 mM increased the SPS activity.
Expression and purification of recombinant SPS1 in *Escherichia coli*

**Construct Preparation:**

**6xHis-tag**

- **Construct:** 6xHis-tag at N-terminus
- **Host:** *E. coli* BL21 (DE3)

**Expression:**

SPS recombinant *E. coli* was cultured at 20°C + 0.5mM IPTG
Expression of recombinant SPS protein in *E. Coli* under limited-proteolysis condition

Preparation under carefully regulated condition:

<table>
<thead>
<tr>
<th>Control vector</th>
<th>(kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Two major bands were detected in SPS gene transformed cells around 120kDa (A Form) and 100 kDa (B Form).

The A form was close in size with SPS from sugarcane leaves.
Characterization of two forms of SPS through enzymatic activity

The total extracts prepared from the comparable amounts of bacterial cells:

- The SPS activity profile from time course of *E. coli* cultivation was increased according to the accumulation profile of B Form
- B Form was successfully purified by several steps of chromatography systems
- Addition of allosteric effector of G6P did not increase SPS B activity and the allosteric property was not observed in B Form of SPS.
- So, the truncated SPS or B form SPS is not regulated by allosteric effector of G6P
Production of the N-terminal truncated forms of SPS

Expression of the N-terminal truncated forms of SPS in *E. coli*

<table>
<thead>
<tr>
<th>4 hr growth</th>
<th>Overnight growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>HisFull</td>
<td>HisFull</td>
</tr>
<tr>
<td>HisΔN1</td>
<td>HisΔN1</td>
</tr>
<tr>
<td>HisΔN2</td>
<td>HisΔN2</td>
</tr>
<tr>
<td>HisΔN3</td>
<td>HisΔN3</td>
</tr>
<tr>
<td>HisΔN4</td>
<td>HisΔN4</td>
</tr>
<tr>
<td>HisΔN5</td>
<td>HisΔN5</td>
</tr>
<tr>
<td>HisΔN6</td>
<td>HisΔN6</td>
</tr>
</tbody>
</table>

Anti-SPS
The full-length SPS was produced in insect cells and purified using GFP nanotrap system. The full length SPS was resulted after treatment with TEV protease.

GFP Nanotrap purification of Full length SPS

[imidazole]

0 mM  20 mM  250 mM
Effect of the N-terminal truncation on SPS specific activity

Preparation of SPS forms by partially purification

Specific activity of full-length and truncated form of SPS

- Full-length SPS has the lowest specific activity
- ΔN6 (without N-terminal region) increased in the activity of SPS by 10-fold than full-length
- Longer truncated of N-terminal region enhances the specific activity
Kinetic properties of SPS forms, full length and series N-terminal truncation

- The N-terminal region give significant influence on the SPS activity. Addition of G6P increased activity full length SPS, but the activity of N-terminal truncated SPS was not significantly influenced by addition G6P.
- The full-length SPS activity is regulated by allosteric effector G6P, but the N-terminal truncated SPS is not effected by the effector G6P.
- So, the N-terminal domain play a significant role on the regulation of SPS.
Proposed model for the regulation of plant SPS

**Full-length**

UDP-G

UDP-G

UDP-G

N

N

N

N

Less Activate

N-terminal region as a suppressor for enzyme activity

Activated

+ G6P

Activated

N-terminal truncated form

UDP-G

UDP-G

UDP-G

N

N

N

N

+ G6P

Activated

Actived

UDP-G

UDP-G

UDP-G

N

N

N

N

Activated

G6P

G6P

G6P

G6P

G6P
IN PLANTA STUDY
Overexpression of engineered SPS1-cDNA in tomato plants

Fig. 3. Growth of two weeks tomato seedling (A), meristematic tomato shoot tips during 2 days co-cultivation (B), and tomato shoots in selection MS media containing antibiotic kanamycin (C). Putative transformant tomato shoot survived in the selection media, but the non-transformant shoot turned to chlorosis, browning and died.

On going experiment
Purification and trial crystallization to determine biological structure SPS protein

**Sample:** HisΔN6

**Vector:** pTrcHisA  
**Host:** *E. coli* BL21 (DE3)  
**Medium:** LB medium  
**Expression:** 16L (+0.06mM IPTG); 20°C

- Resuspend in extraction buffer  
- Sonication  
- Centrifuge 15,000rpm at 4°C, 15 min

**Purification steps:**  
1. DE52  
2. DEAE  
3. SourceQ  
4. cOmplete His-tag  
5. Superdex200 (+5mM F6P)

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**The result of size exclusion chromatography:**

**Difficult to obtain the crystal**

**Problems:**  
- Easy to precipitate  
- Not enough sample for crystallization  
- High percent polydispersity (over than 40%)

**Further purification is necessary**
Conclusion

1. Sucrose-phosphate synthase (SPS) is a key enzyme for sucrose synthesis and accumulation in sugarcane leaves. Enhancement of SPS activity increases sucrose accumulation.

2. The mechanism of allosteric regulation in plant SPS may involve the upstream N terminal region. Truncation of N terminal resulted in an increased of SPS specific activity.

3. We propose to redesign SPS protein through genome editing for improving in sucrose accumulation and plant growth.
THANK VERY MUCH
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