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Coordinated redox regulation of transferases involved in starch biosynthesis in *Arabidopsis thaliana*

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Introduction

Important structural and catalytic functions of many enzymes are dependent on regulation determined by **the redox state of the cell**. This regulation occurs through the breaking and reformation of disulfide bonds of the target proteins and has been described especially for many chloroplastic enzymes.

In the chloroplast, reducing equivalents produced during the day by photosynthesis are transported from photosystem I via the **ferredoxin-thioredoxin system** to the target proteins. Hence, this system links enzyme activity to light, ensuring **coordination between photosynthesis and metabolism** by reductive activation of enzymes during the day.

The approach

Starch biosynthetic enzymes: Investigation of activity → **Manipulation of redox potentials** → Native gels / Enzyme assays

Extracts of *Arabidopsis* wildtype plants were treated with a total concentration of **40 mM DTT** in varying ratios of reduced to oxidized DTT. The redox potential was calculated using the Nernst equation and the midpoint potential of -380 mV for DTT (at pH 7.9).

Redox range: **from -380 mV to -300 mV**

The activity of most chloroplastic redox regulated metabolic enzymes characterized so far, fall well within this range.

Main starch biosynthetic enzymes in *Arabidopsis thaliana*

- ADP-Glucose pyrophosphorylase
- **Starch Synthase (SS)** — catalyzes the transfer of a glucosyl unit from ADP-Glucose to a growing polymer chain through an α-(1 → 4) glycoside bond
  - **5 isoforms :** 1) GBSS (granule-bound SS), 2) SSI, 3) SSII, 4) SSIII, 5) SSIV
- **Starch Branching Enzyme (SBE)** — cleaves an internal α-(1 → 4) linkage and transfers the released linear chain to a C-6 hydroxyl, thus forming a new α-(1 → 6) branch point
  - **3 isoforms :** 1) SBEI, 2) SBEII , 3) SBEIII
- starch debranching enzyme

Some specific **redox regulated enzymes** active in starch metabolism have been identified in *Arabidopsis*:

- ✓the **ADPglucose pyrophosphorylase**,
- ✓the **beta-amylase BAM1,**
- ✓the **starch phosphorylator GWD1.**

All of them are **reductively activated** *in vitro*

Our goal:

- ➢**To identify, which isoforms of SS and SBE are redox regulated**
- ➢**To understand, how does the regulation work**

Results: Starch Synthase

The activity of starch synthases was characterized on glycogen containing gels (fig. 1). The identity of the various activities identified on the gel was confirmed by analysis of *Arabidopsis* mutants (data not shown).

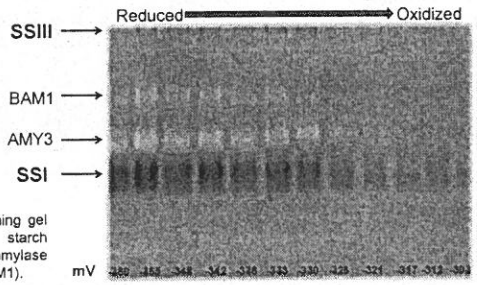

Figure 1. Glycogen containing gel revealing the activity of starch synthase (SSI, SSIII), α-amylase (AMY3) and β-amylase (BAM1).

The influence of the redox potential on the total activity of soluble starch synthases was investigated using enzyme assay (fig. 2). It clearly demonstrates the redox influence on soluble starch synthases.

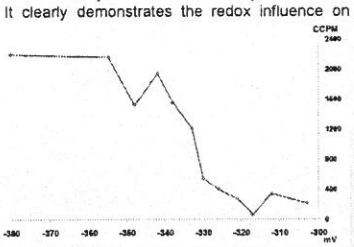

Figure 2. Total activity of soluble starch synthases of the measured extracts.

To determine the contribution of SSI and SSIII to the total activity of soluble starch synthases, enzyme assays were performed (fig. 3).

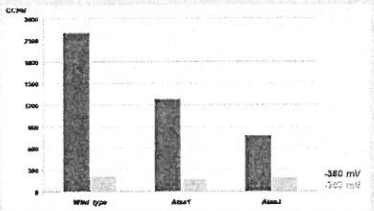

Figure 3. Total activity of soluble starch synthases in wild-type plants, *Atss1* and *Atss3* mutants. The redox point at -303 mV corresponds to the most oxidized state and -380 mV to the most reduced state.

Results: Starch Branching Enzyme

The activity of starch branching enzymes was characterized on a gel without substrate (fig. 4).

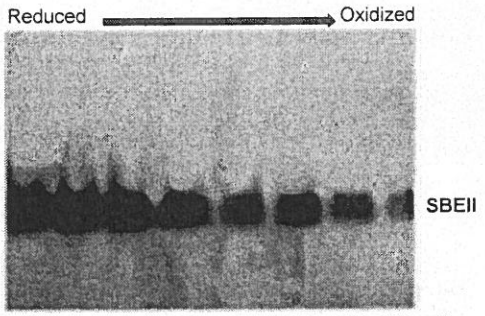

Figure 4. Gel revealing the activity of starch branching enzyme SBEII

One of three isoforms of SBE – SBEII- seems to be under redox control as well.

Conclusion

❖ Our data specifically demonstrates that at least 2 isoforms of the starch synthase, **SSI and SSIII**, and 1 isoform of the starch branching enzymes, **SBEII**, are **reductively activated**

❖ The data also suggests the presence of a coordinated regulative mechanism of starch biosynthesis providing simultaneous coordination of the multitude of enzymes responsible for correctly structuring the starch granule and its close link with the flow of photosynthetically fixed carbon into starch