Investigation of the roles of Vitamin B6 in carbohydrate metabolism in *Arabidopsis thaliana*

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Abstract

Vitamin B6 is a required coenzyme for many cellular processes, including amino acid metabolism, carbohydrate metabolism, ethylene and chlorophyll synthesis, and response to both biotic and abiotic stress. There are six different forms, or vitamers, of vitamin B6: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM) and their phosphorylated derivatives, pyridoxine 5'-phosphate (PNP), pyridoxal 5'phosphate (PLP), and pyridoxamine 5'-phosphate (PMP). PLP is the active form of the vitamers. In most organisms, PLP is synthesized by the "de novo pathway. " This pathway is found in most organisms. Animals, however, are unable to synthesize vitamin B6 and therefore must obtain this important nutrient from their diet. Another pathway of vitamin B6 metabolism is found in all organisms, including animals. This pathway, termed the "salvage pathway", is responsible for the interconversion of the six different vitamer forms. Deficiency of vitamin B6 in humans has been linked with gestational diabetes, depression and epilepsy.

My work is focused on two different mutants of vitamin B6 synthesis, *pdx1.3* and *sos4*. These are mutants of the de novo pathway and the salvage pathway, respectively. The *pdx1.3* mutant is deficient in vitamin B6 synthesis, while the *sos4* mutant has increased vitamin B6 content. Even though these two mutants have widely different levels of vitamin B6, they share a slate of common phenotypes, including chlorosis, stunted growth, root sensitivity to sucrose, altered sugar accumulation, altered starch structure and altered chloroplast ultrastructure. These phenotypes cannot be explained by known roles of vitamin B6. Currently, I am investigating the mechanism(s) which allow both mutants to display the same phenotypes even though they have very different levels of vitamin B6. Understanding these mechanisms may allow us to develop crops that are more resistant to biotic and abiotic stress, such as plant pathogens or drought, and to develop crops with higher nutritional value.

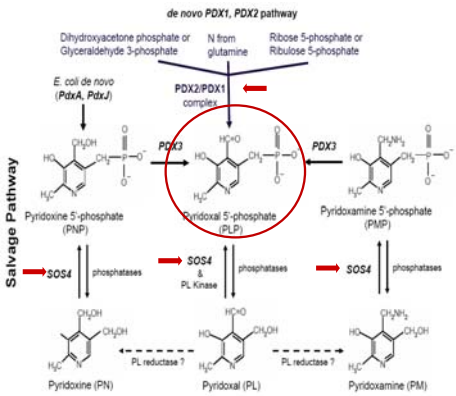

Figure 1. De novo and Salvage Vitamin B6 metabolic pathways. Two vitamin B6 metabolic pathways exist in nature. The de novo pathway is found in almost organisms but not in animals. *E. coli* and a few other eubacteria synthesize pyridoxine 5'-phosphate. However, the majority of organisms synthesize pyridoxal 5'-phosphate, which is the active form of the vitamin. There are six different forms of vitamin B6, termed vitamers. They are pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM), and the phosphorylated derivatives PNP, PLP and PMP respectively. These different forms of vitamin B6 are interconverted between each other by the salvage pathway, which is present in all organisms. Red arrows indicate the points in the pathways in which our mutants are deficient.

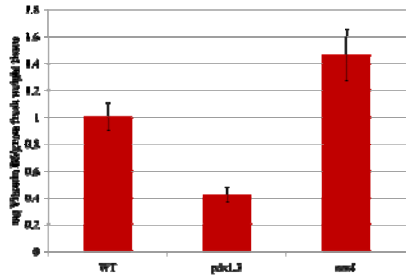

Figure 2. *pdx1.3* mutants are deficient in vitamin B6, while *sos4* mutants have more vitamin B6 than Wild Type (WT) plants. Vitamin B6 was extracted from six week old plants. Vitamin B6 was quantified using a bioassay with yeast auxotrophic mutants and comparison to a standard curve of known vitamin B6 concentrations.

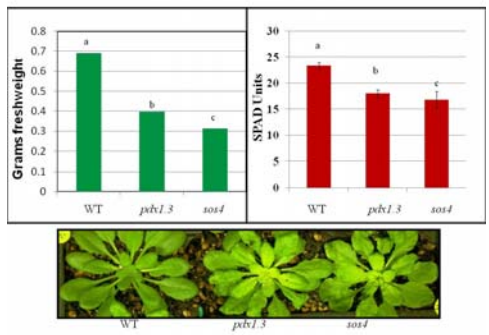

Figure 3. Both *pdx1.3* and *sos4* mutants are smaller and chlorotic when compared to WT plants. Plants were harvested at six weeks of age. SPAD values (measurement of chlorophyll fluorescence) were measured using a Minolta SPAD-502 chlorophyll meter. SPAD values have been closely correlated with chlorophyll content.

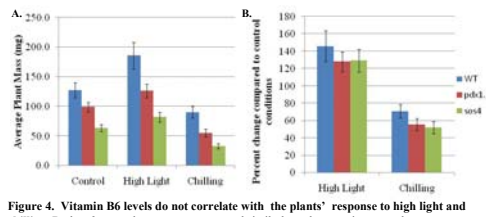

Figure 4. Vitamin B6 levels do not correlate with the plants' response to high light and chilling. Both *pdx1.3* and *sos4* mutants respond similarly to these environmental stresses. Plants were grown for three weeks under control (20˚C, 8 hour photoperiod, 200µmol s^{-1} m^{-2} light), high light (20˚C, 8 hour photoperiod, 1000µmol s^{-1} m^{-2} light) or chilling (5˚C, 8 hour photoperiod, 200µmol s^{-1} m^{-2} light) conditions. **A.** Total dry weight of plants grown under control, high light and chilling conditions. **B.** Relative dry weight of plants grown under control, high light, and chilling conditions. Relative dry weight is compared to the same line of plants grown under control conditions.

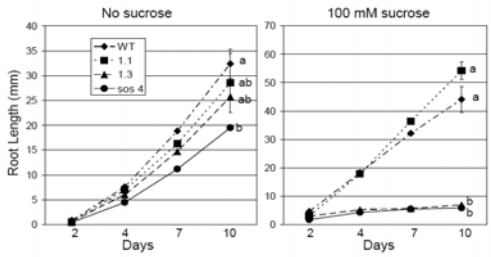

Figure 5. Roots of *pdx1.3* and *sos4* mutants are inhibited by sucrose. Plants were germinated and grown in vitro on MS plant cell culture medium supplemented with sucrose and B6 and other vitamins for one week and then transplanted to MS medium (no vitamins) with and without 100mM sucrose supplementation. Cell culture plates were oriented vertically, and plants were grown for two weeks and root growth was measured every two to three days.

B6 supplementation (2.5 µM)	Root Length (mm) at 10 days ± SE MS media with 100 mM Sucrose Wild type	*pdx1.3*	*sos4*
None	31.0 ± 1.7	7.2 ± 0.6	4.3 ± 0.3
Pyridoxine	25.2 ± 1.2	29.7 ± 3.0	3.4 ± 0.6
Pyridoxal	33.2 ± 2.0	28.2 ± 1.9	5.3 ± 1.2
Pyridoxal 5'-phosphate	30.5 ± 2.9	29.8 ± 1.7	4.2 ± 0.5

Table 1. Root growth of the *pdx1.3* mutants can be rescued by addition of vitamer supplementation. Root growth of sos4 mutant cannot be rescued by supplementation, which is consistent with overabundance of vitamin B6 in the sos4 mutant.

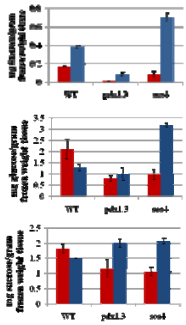

Figure 6. Sugar metabolism and carbon partitioning is altered in both *pdx1.3* and *sos4* mutants. *pdx1.3* plants had less fructose than wild type, and showed increased sucrose accumulation during the light period. *sos4* mutants showed higher accumulation of all sugars during the light period. Plants were harvested at six weeks of age at the beginning of the day (red) or the end of the day (8 hours of light, blue). Sugar was extracted by grinding with liquid nitrogen followed by extraction buffer. Soluble sugars were quantified by enzymatic assay and compared to a standard curve of known sugar concentrations.

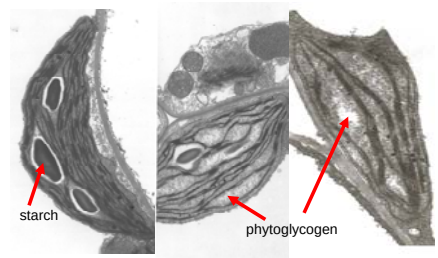

Figure 7. Chloroplast ultrastructure is altered in both *pdx1.3* and *sos4* mutants. Chloroplasts of mutant plants are swollen, have fewer thylakoid membranes and grana stacks. In addition, starch crystals are mostly absent and replaced with what resembles phytoglycogen, a complex carbohydrate which is more highly branched than that of crystalline starch. Starch was extracted from WT and mutant plants and digested with α-amylase and amyloglucosidase. These results also indicate that starch in the mutant plant are more highly branched (not shown).

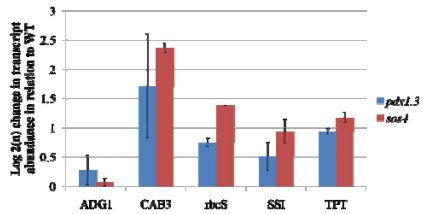

Figure 8. Both *pdx1.3* and *sos4* mutants share similar expression of key metabolic genes, despite differing levels of vitamin B6. Transcript abundance of six week old rosettes harvested at the end of the day (eight hours light). Transcript abundance is measured by qPCR and reported as log2(n) in relation to WT. Transcript abundance was normalized to actin. Error bars represent standard error and results are the product of at least two independent experiments. (ADG1 – ADP Glucose Pyrophosphorylase Small Subunit I; CAB3 – Chlorophyll a/b Binding Protein 3; rbcS - RuBisCO small subunit, SSI – Starch Synthase I; TPT – Triose Phosphate Transporter)

Figure 9. Both *pdx1.3* and *sos4* mutants are drought tolerant. Plants were grown for four weeks at 22˚C under short day length conditions (8 hours light). Plants were watered once a week. Water was withheld from plants for three weeks. Plants shown are approximately 7 weeks old. Drought tolerance is consistent with the more highly branched starch of the mutant plants.

Protein	Splice Variant	Prediction Software	Predicted sub cellular localization
SOS4	AT5G37850.1	WoLF PSORT	chloroplast
		TargetP	chloroplast
		SUBAII	mitochondria/plastid
SOS4	AT5G37850.2	WoLF PSORT	chloroplast
		TargetP	undetermined
		SUBA2	undetermined

Table 2. Protein localization prediction software predicts suggests that the SOS4 protein localizes in chloroplasts and other cellular organelles, and not in the cytoplasm. By contrast, PDX1.3 has been experimentally shown to localize in the cytoplasm and in cellular organelles. Subcellular localization of the SOS4 protein has not been experimentally determined.

Summary and Conclusions

- ***pdx1.3*** **and *sos4* mutants have altered levels of vitamin B6. *pdx1.3* contains less vitamin B6 than WT while *sos4* contains more vitamin B6 than WT.**
- **Despite differing levels of vitamin B6, both *pdx1.3* and *sos4* mutants share a slate of common phenotypes indicative of alterations in carbohydrate metabolism.**
- **Unlike the PDX1.3 protein which localizes in the cytoplasm as well as in organelles, localization of the SOS4 protein to the chloroplast is supported by bioinformatics data.**
- **We hypothesize that the phenotypes of both mutants may be due to a deficiency of vitamin B6 in the chloroplast, indicating that SOS4 plays a role in transport of vitamin B6 into the organelle.**
- **Investigation is currently underway to quantify vitamin B6 in chloroplasts of *sos4* mutants.**

Acknowledgements:

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