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

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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 all 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 all organisms, except in animals. E. coli and a few other organisms synthesize pyridoxine 5'-phosphate. However, the majority of organisms synthesize pyridoxine 5'-phosphate, which is the active form of the vitamers. There are six different forms of vitamin B6: pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM), and their 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 amastigote mutants and compared 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 those environmental stresses. Plants were grown for three weeks under control (20°C, 8 hour photoperiod, 200µmol s⁻¹ m⁻² light), high light (25°C, 8 hour photoperiod, 1000µmol s⁻¹ m⁻² light) or chilling (15°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 orientated vertically, and plants were grown for two weeks and root growth was measured every two to three days.

Figure 9. Both pdx1.3 and sos4 mutants are drought tolerant. Plants were grown for four weeks at 22°C and short day length conditions (8 hours light). Plants were watered with a water-soluble fertilizer for three weeks. Plants shown are approximately 7 weeks old. Drought tolerance is consistent with the more highly branched starch of the mutant plants.

Figure 6. Sugar metabolism and carbon partitioning are 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 (0h) and at the end of the day (8 hours of light, blue). Sugar was extracted by protocols with liquid-liquid extraction followed by extraction buffer. Soluble sugars were quantified by enzymatic assay and compared to a standard curve of known sugar concentrations.

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

Table 2. Protein localization prediction software predicts that the SOS4 protein localizes in chloroplasts and other cellular organelles, and not in the cytosol. 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.

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