Identifying the Source of β-Alanine Production in *Arabidopsis thaliana* by GC/MS

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Introduction

Plants are essential to the survival of many organisms on Earth. Plants sustain life, serving as a source of oxygen and consumable energy. The world is increasingly reliant on plant biochemical research to assist the development of sustainable, environmentally friendly infrastructure and large scale food production to meet the challenges of growing populations. Investing resources into understanding how energy is converted, used, and stored in plants through various metabolic pathways is worthy research for our expanding world.

The branched-chain amino acids (BCAAs) serve as an alternative energy form in most organisms. Specifically in plants, prior to the start of photosynthesis, the BCAAs - valine, leucine, and isoleucine - can be metabolized and used for energy.[1] BCAAs contain aliphatic side chains and can be converted into propionyl-CoA and acetyl-CoA, common energy sources that fuel cellular respiration.[2] In order to fully understand the mechanism of degradation of the BCAAs, understanding the enzymes and intermediates involved in these metabolic pathways is crucial. Specifically, in valine degradation, understanding the role and importance of methylmalonate semialdehyde dehydrogenase (MMSD), an enzyme that catalyzes the conversion methylmalonate semialdehyde to propionyl-CoA, will assist our understanding as to how valine is used effectively as an energy source.

Our previous research of *A. thaliana* seeds examined the role of MMSD in valine degradation. When seeking to identify the metabolic intermediates by Gas Chromatography/Mass Spectrometry (GC/MS), there was an enormous, exciting presence of β-alanine present in both *mmsd-1* (an *mmsd* knockout) and valine-treated wild-type seed lines. β-alanine is a nonessential, naturally occurring amino acid in all living organisms. Initially, we attributed the poor germination rate of the *mmsd-1* seed line to the lack of MMSD. However, we cannot be certain of that conclusion with the abnormal quantity of β-alanine discovered.

We proposed that β-alanine is formed from isoleucine and valine via propionyl-coA production. The formation of β-alanine in relation to BCAA degradation has not been studied in plants. Our overall objective was to explore this mechanism in *A. thaliana* seedlings to further identify the source producing the excess β-alanine. We explored this pathway using ^{13}C-labeled precursors and GC/MS.

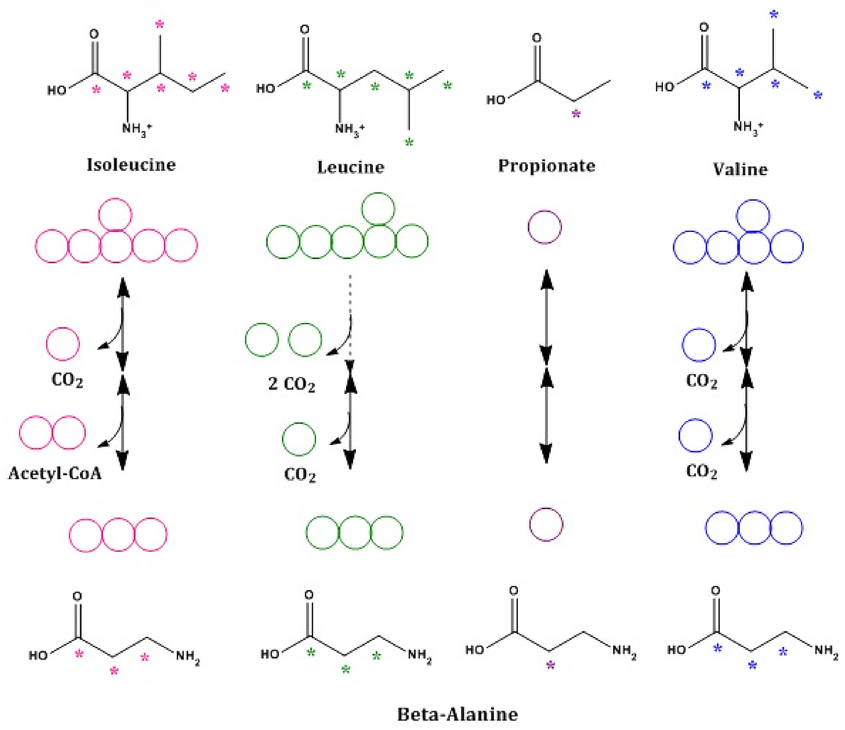

Figure 1: The BCAA degradation pathway with respect to β-alanine production. The metabolic pathway above highlights the important intermediates in BCAA degradation, leading to the conversion of the various ^{13}C precursors to make β-alanine.

Results

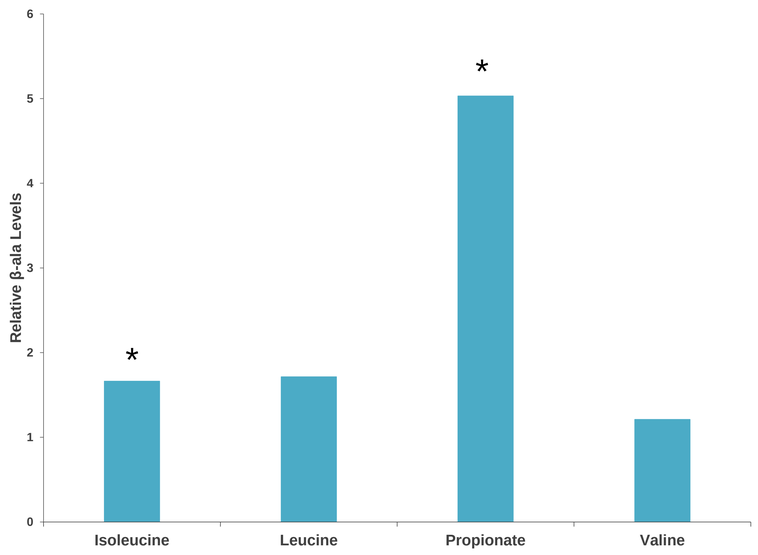

Figure 2: The effect of isoleucine, leucine, propionate, and valine treatment on β-alanine production in wild-type seedlings relative to the β-alanine levels of untreated wild-type seedlings. * denotes the ^{13}C-labeled precursor was detected in the β-alanine mass spectra.

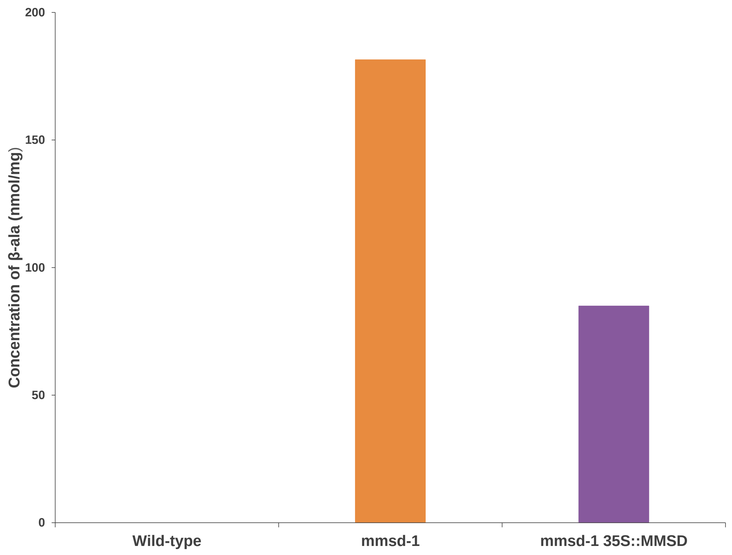

Figure 5: β-alanine levels in wild-type, *mmsd-1*, and *mmsd-1 35S::MMSD* seeds.

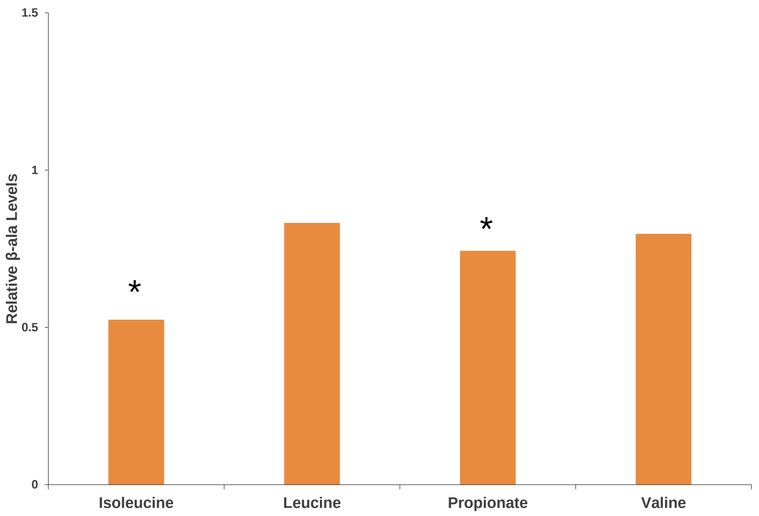

Figure 3: The effect of isoleucine, leucine, propionate, and valine treatment on β-alanine production in *mmsd-1* (*MMSD* knockout) seedlings relative to the β-alanine levels of untreated *mmsd-1* seedlings. * denotes the ^{13}C-labeled precursor was detected in the β-alanine mass spectra.

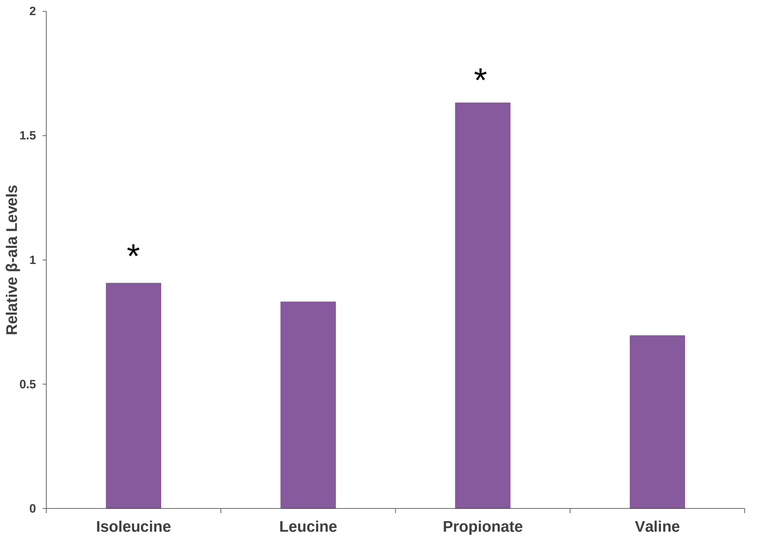

Figure 4: The effect of isoleucine, leucine, propionate, and valine treatment on β-alanine production in *mmsd-1 35S::MMSD* (*MMSD* knockout mutants complemented with the MMSD gene) seedlings relative to the β-alanine levels of untreated *mmsd-1 35S::MMSD* seedlings. * denotes the ^{13}C-labeled precursor was detected in the β-alanine mass spectra.

Methods

Seed Amino Acid Extraction Protocol: Seeds were homogenized in 1 ml 100 °C sterile dH_2O, rinsed with 500 µl of 100 °C sterile dH_2O, heated for 10 minutes at 100 °C, chilled on ice, and centrifuged for 14000 g at 4 °C. The supernatant was extracted, frozen in N_2 (l), and lyophilized for 24 hours. Samples were resuspended in 300 µl of sterile dH_2O and centrifuged for 14000 g at 4 °C. Samples were then derivatized using the EZ:FAAST Physiological Amino Acid Derivitization and analyzed by GC/MS.

Seedling Amino Acid Extraction Protocol: Powered seedling samples were placed in 5% perchloric acid, neutralized with 10M KOH, and centrifuged for 30 minutes at 1200 g at 4 °C. The supernatant was extracted, frozen in N_2 (l), and lyophilized for 24 hours. Samples were resuspended in 300 µl of sterile dH_2O and centrifuged for 14000 g at 4 °C. Samples were then derivatized using the EZ:FAAST Physiological Amino Acid Derivitization and analyzed by GC/MS.

Conclusions

- Contrary to wild-type seeds, β-alanine was found in *mmsd-1* and *mmsd-1 35S::MMSD* seeds.
- Our proposed hypothesis is confirmed: β-alanine can be produced through the products of isoleucine degradation in all Arabidopsis seed lines.
- Valine and Leucine do not directly produce β-alanine as evidence by no ^{13}C label present in the β-alanine mass spectra. However, there may be an indirect metabolic link to alter the level of β-alanine due to metabolic flux and amino acid homeostasis, which is supported by altered levels of free amino acids (data not shown).
- Wild-type and *mmsd-1 35S::MMSD* seedlings are able to accumulate β-alanine while *mmsd-1* did not accumulate additional β-alanine.

References and Acknowledgements

1. Goodwin GW., Rougraff PM., Davis EJ., and Harris RA. "Purification and characterization of methylmalonate-semialdehyde dehydrogenase from rat liver and identity to malonate-semialdehyde dehydrogenase." *The Journal of Biological Chemistry,* (**1989**) **264** (25): 14965-14971.
2. Zolman BK., Monroe-Augustus M., Thompson B., Hawes JW., Krukenberg KA., Matsuda SP., and Bartel B. "CHY1, an Arabidopsis mutant with impaired β-oxidation, is defective in a peroxisomal β-hydroxyisobutyryl-CoA hydrolase." *The Journal of Biological Chemistry,* (**2001**) **273** (33): 31037–31046.

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