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2014 REU Poster: Measuring Tryptophan Metabolism Using Analogs of Tryptophan

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Measuring Tryptophan Metabolism Using Analogs of Tryptophan

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Introduction
- Secondary metabolites are organic compounds produced through the modification of primary metabolites. Secondary metabolites typically do not play essential roles in growth as primary metabolites do.
- In plant, most secondary metabolites play roles in defense against insects and microbial pathogens.
- Many plant secondary chemicals have important uses for humans. For example, many pharmaceuticals are based on plant chemical structures, and secondary metabolites are widely used for recreation and stimulation.
- Arabidopsis thaliana is a small flowering plant that is widely used as a model organism in plant biology. Arabidopsis is a member of the mustard (Brassicaceae) family, which includes cultivated species such as cabbage and radish.

Why we use Arabidopsis
- Cheap/easy to maintain
- Self fertile
- Fast life cycle
- Transformable
- 5 chromosomes (diploid)
- Small genome: approx. 26,000

In Arabidopsis, tryptophan is the precursor of several secondary metabolites
- Glucosinolates are a class of defense compound used to protect the plant from insect attack. Those derived from tryptophan (Trp) are called indolic glucosinolates (IGs).
- Camalexin (CAMA) is another defense compound produced from Trp and it protects the plant from fungal attack.
- Indole-3-acetic acid (IAA) is a plant growth hormone called auxin.

Indolic glucosinolates made by Arabidopsis

Plants are grown in sterile liquid PNS medium and at 10 days after germination, they are fed with 25 micromolar 7AT or SMA and grown for 48 h.
- Plant tissue is harvested and “desulfo glucosinolates” are isolated.
- Desulfitoglucosinolates are then analyzed by reverse-phase HPLC using an increasing acetonitrile gradient.
- Different desulfitoglucosinolates were detected by their absorbance at 229 nm.
- For quantification, peak areas were normalized to plant tissue weight and a known weight standard.

Hypothesis 1
5-methyl-tryptophan (or 5-methylanthranilate) creates a new IG

Hypothesis 2
Mutants with altered endogenous Trp pools to be tested for incorporation of Trp analogs

Conclusions
- Analogs of Trp (or precursors) can be incorporated into indolic glucosinolates in vivo.
- The incorporation of the analogs into indole glucosinolate relative to the incorporation of endogenous Trp, reflects the size of the endogenous Trp pool.

References

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