Abstract:

Most herbivorous lepidopteran larvae ingest large amounts of chlorophyll. Free chlorophyll and its tetrapyrrole breakdown products could be phototoxic in the presence of oxygen, due to the light-driven generation of reactive oxygen species. We examined larvae of the cotton leafworm *Spodoptera littoralis* for adaptations to cope with this potential threat. Chlorophyllide-binding proteins, previously described from *Bombyx mori*, are also present in *S. littoralis*. When two genes encoding these proteins were knocked out by CRISPR/Cas9, *S. littoralis* larvae consuming potted lima bean plants survived in the dark, but died in the light. When artificial diet was supplemented with chlorophyll or other tetrapyrroles, double-knockout larvae survived in the dark but died in the light. Double-knockout larvae consuming lima bean leaves were found to have high concentrations of some tetrapyrroles in the hemolymph. Heterologously-expressed chlorophyllide-binding protein bound to some tetrapyrroles. We hypothesize that chlorophyllide-binding proteins protect the larvae from reactive oxygen species in the aerobic environment of the hemocoel, by sequestering tetrapyrroles in the anaerobic midgut for subsequent excretion via the feces. To further test this hypothesis, we are examining the hemolymph for light-dependent generation of reactive oxygen species in the presence of tetrapyrroles.
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