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Potential detoxification of gossypol by UDP-glycosyltransferases in the two Heliothine moth species *Helicoverpa armigera* and *Heliothis virescens*

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ABSTRACT

The cotton bollworm *Helicoverpa armigera* and the tobacco budworm *Heliothis virescens* are closely related generalist insect herbivores and serious pest species on a number of economically important crop plants including cotton. Even though cotton is well defended by its major defensive compound gossypol, a toxic sesquiterpene dimer, larvae of both species are capable of developing on cotton plants. In spite of severe damage larvae cause on cotton plants, little is known about gossypol detoxification mechanisms in cotton-feeding insects. Here, we detected three monoglycosylated and up to five diglycosylated gossypol isomers in the feces of *H. armigera* and *H. virescens* larvae fed on gossypol-supplemented diet. Candidate UDP-glycosyltransferase (UGT) genes of *H. armigera* were selected by microarray studies and *in silico* analyses and were functionally expressed in insect cells. In enzymatic assays, we show that UGT41B3 and UGT40D1 are capable of glycosylating gossypol mainly to the diglycosylated gossypol isomer 5 that is characteristic for *H. armigera* and is absent in *H. virescens* feces. In conclusion, our results demonstrate that gossypol is partially metabolized by UGTs via glycosylation, which might be a crucial step in gossypol detoxification in generalist herbivores utilizing cotton as host plant.

1. Introduction

Generalist insect herbivores can cope with a large diversity of toxic secondary metabolites produced by their host plants by developing different strategies like behavioral avoidance, rapid excretion, target site mutation, or sequestration (Heckel, 2014; Heidel-Fischer and Vogel, 2015). They also utilize diverse mechanisms of metabolic detoxification to circumvent the toxicity of plant secondary metabolites. In many cases metabolic detoxification can be divided into three phases. In phase I, enzymes such as cytochrome P450 monooxygenases (P450s) or carboxylesterases act directly on the toxin molecule, introducing or releasing functional groups and thus increasing the reactivity and the hydrophilicity of the toxin. In phase II, enzymes like glutathione *S*-transferases, UDP-glycosyltransferases, and methyl-, acetyl-, phospho- and sulfotransferases conjugate endogenous molecules to the toxins (Robertson et al., 1999; Wilkinson, 1986). The resulting conjugates are less reactive and more water-soluble than the original toxins and thus lose the ability to diffuse through membranes. Phase III enzymes such as ATP-binding cassette transporters facilitate the active transport of toxins across membranes, thus enabling their excretion. However, the detoxification process is much more flexible than the classification into the three phases implies (Rowland et al., 2013).

Glycosylation of toxins is a particularly important detoxification mechanism, in which a lipophilic aglycone is converted into a more hydrophilic and readily excretable compound (Wilkinson, 1986). The underlying mechanism is a second order nucleophilic substitution (Radominska-Pandya et al., 2010) catalyzed by glycosyltransferases, which are found in animals, plants, fungi, bacteria, and viruses (Bock, 2015; Paquette et al., 2003). Insects use UDP-glucose

Abbreviations: DMSO, dimethyl sulfoxide; P450, cytochrome P450 monooxygenase; UGT, UDP-glycosyltransferase.

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as an activated sugar donor (Ahn et al., 2012) that is transferred by UDP-glycosyltransferases (UGTs), which are anchored in the endoplasmic reticulum (Fig. 1). While UGTs also fulfill important endogenous functions in insect olfaction (Bozzolan et al., 2014; Robertson et al., 1999), pigmentation (Hopkins and Kramer, 1992), UV-shielding (Daimon et al., 2010), and cuticular tanning (Kramer and Hopkins, 1987), several studies describe the involvement of UGTs in detoxification of plant secondary metabolites in insects (Ahmad and Hopkins, 1992; Ahn et al., 2011b; Kojima et al., 2010; Wouters et al., 2014). However, compared to other detoxification enzymes such as P450s (Feyereisen, 2012) knowledge about UGTs involved in detoxification of plant toxins in insects is rare (Heidel-Fischer and Vogel, 2015).

Helicoverpa armigera (Hübner), the cotton bollworm, and *Heliothis virescens* (Fabricius), the tobacco budworm, (Lepidoptera: Noctuidae) are important agricultural pests on many different crop plants. A favorite host plant of both noctuid moth species is cotton (*Gossypium* spp.), which contains a number of secondary metabolites to defend itself against herbivores and pathogens. A major defensive compound of cotton is gossypol, a yellow colored sesquiterpene dimer stored in subepidermal glands that is toxic to many insect species. The toxicity of gossypol can be mostly attributed to its highly reactive aldehyde groups that interact with amino acids of proteins and to the presence of six phenolic hydroxyl groups (Dodou, 2005). The rather hydrophobic nature of gossypol allows the molecule to diffuse across membranes (Laughton et al., 1989) and thus to harm the organism.

While generally very little is known about the detoxification of gossypol in lepidopteran larvae that feed successfully on cotton plants, a particular P450 enzyme, CYP6AE14, has been associated with gossypol detoxification, due to its induction in *H. armigera* larvae after gossypol ingestion (Celorio-Mancera et al., 2011; Mao et al., 2007). However, evidence for a direct involvement of this particular P450 enzyme in gossypol metabolism is still missing. Rojas et al. (1992) reported gossypol glycosides in feces of *H. virescens* after ingestion of a gossypol diet, but the identity of the conjugates and the enzymes involved was not clearly described.

In this study we detected and characterized several isomers of gossypol glycosides in the feces from *H. armigera* and *H. virescens* that fed on gossypol-supplemented diet. We found species-specific differences in the pattern of glycosylated gossypol isomers. Heterologous expression of *H. armigera* UGTs revealed two UGTs that showed specific gossypol glycosylation activity. With these results we shed some light upon the mechanism that enables two Heliothine pest species to utilize cotton as a host plant without being poisoned by gossypol.

2. Materials and methods

2.1. Insects and bioassay

H. armigera larvae were collected from Toowoomba, Queensland, Australia, in 2003 (TWB strain). *H. virescens* larvae were provided by North Carolina State University and had been originally collected in Clayton, North Carolina, USA, in 1988 (JEN strain). *H. armigera* larvae were reared on Bio-Serv diet (General Purpose Lepidoptera) and *H. virescens* larvae on pinto bean diet (Joyner and Gould, 1985) under laboratory conditions (26 °C, 55% relative humidity, 16:8 h light:dark photoperiod) in Jena, Germany. Pinto bean diet was used for feeding assays with both species.

Two gossypol-supplemented diets were prepared with a racemic mixture of gossypol isolated from cotton seeds (TimTec) as described previously by Stipanovic et al. (2006) with slight modifications. Gossypol (800 mg or 1600 mg) was dissolved in ethyl acetate (35 mL), added to alphacel (15 g; MP Biomedicals), a non-nutritive cellulose bulk, and dried at room temperature for 24 h. Hexane (50 mL) was added and evaporated for 36 h. Freshly prepared pinto bean diet (490 g) was cooled down to about 47 °C and

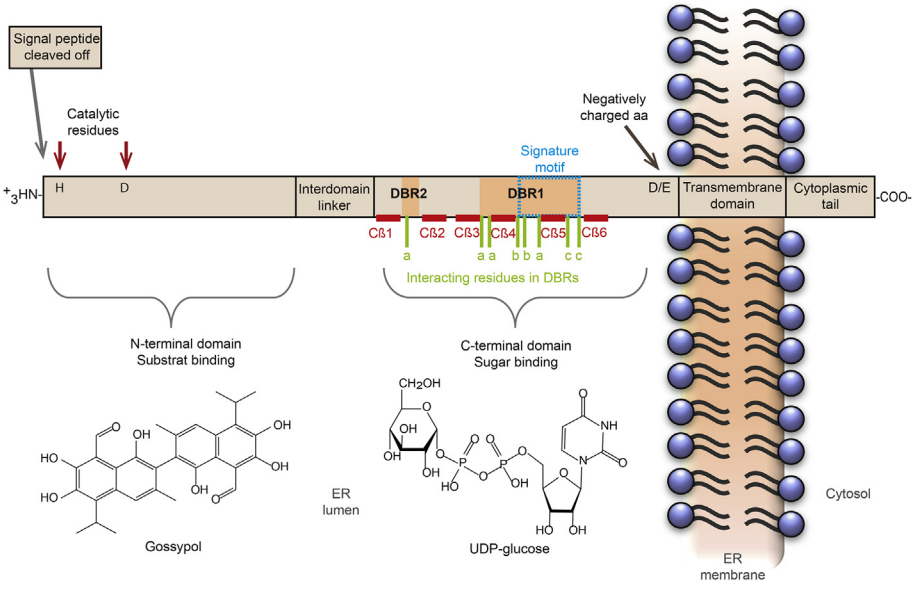

Fig. 1. Schematic drawing of the general structure of a membrane bound animal UDP-glycosyltransferase (UGT). After cleavage of the N-terminal signal peptide that directs the protein to the ER, only a short cytoplasmic tail remains in the cytosol. A short transmembrane domain anchors the protein in the ER membrane flanked by a highly conserved, negatively charged residue [glutamic acid (E) or aspartic acid (D)] at the intersection to the globular part of the enzyme located in the ER lumen. This part consists of two major domains, the C-terminal sugar-donor binding domain and the N-terminal substrate-binding domain (Magdalou et al., 2010). Both domains are connected via an interdomain linker. The signature motif (blue box) that is located in the C-terminal domain is conserved across all organisms (Ahn et al., 2012). It overlaps with the sugar-donor binding region 1 (DBR1, orange box), which acts in concert with the sugar-donor binding region 2 (DBR2, orange box) and several (a) nucleotide-, (b) phosphate- and (c) glucoside-interacting residues. All putative β-sheets in the C-terminal domain are indicated in red font color. In the N-terminal domain, two catalytic residues [histidine (H) and aspartic acid (D); red arrows] are located which interact with the substrate.