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A mode of action of glucosinolate-derived isothiocyanates: Detoxification depletes glutathione and cysteine levels with ramifications on protein metabolism in *Spodoptera littoralis*

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ABSTRACT

Glucosinolates are activated plant defenses common in the order Brassicales that release isothiocyanates (ITCs) and other hydrolysis products upon tissue damage. The reactive ITCs are toxic to insects resulting in reduced growth, delayed development and occasionally mortality. Generalist lepidopteran larvae often detoxify ingested ITCs via conjugation to glutathione (GSH) and survive on low glucosinolate diets, but it is not known how this process influences other aspects of metabolism. We investigated the impact of the aliphatic 4-methylsulfinylbutyl-ITC (4msob-ITC, sulforaphane) on the metabolism of *Spodoptera littoralis* larvae, which suffer a significant growth decline on 4msob-ITC-containing diets while excreting ITC-glutathione conjugates and their derivatives in the frass. The most striking effects were a decrease of GSH in midgut tissue and hemolymph due to losses by conjugation to ITC during detoxification, and a decline of the GSH biosynthetic precursor cysteine. Protein content was likewise reduced by ITC treatment suggesting that protein is actively catabolized in an attempt to supply cysteine for GSH biosynthesis. The negative growth and protein effects were relieved by dietary supplementation with cystine. Other consequences of protein breakdown included deamination of amino acids with increased excretion of uric acid and elevated lipid content. Thus metabolic detoxification of ITCs provokes a cascade of negative effects on insects that result in reduced fitness.

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1. Introduction

Insect herbivores are often deterred by specialized (or "secondary") plant metabolites (Wittstock and Gershenzon, 2002). Well-studied among these specialized metabolites is the glucosinolate-myrosinase system, a two-component activated defense found in plants of the order Brassicales, including important crops such as cabbage, canola, and mustard. The glucosinolate core structure is composed of an *S*-glucosylated thiohydroximate sulfate ester with a variable, amino acid-derived side-chain (Fig. 1A). Depending on the amino acid used as precursor in biosynthesis, glucosinolates can be classified as aliphatic (derived from Met, Leu, Ile, Val, or Ala), indolic (from Trp) or benzenic (from Phe or Tyr) (Agerbirk and Olsen, 2012; Fahey et al., 2001). Intact glucosinolates are not toxic, but are converted to toxic derivatives upon enzymatic activation by plant myrosinases (β-thioglucoside glucohydrolases, EC 3.2.1.147) (Fig. 1A). Myrosinases, spatially separated from glucosinolates in plant tissue (Andréasson and Jørgensen, 2003), cleave the glucose moiety of glucosinolates, detonating the so-called "mustard oil bomb" to form an unstable aglucone, which spontaneously rearranges (Matile, 1980) (Fig. 1A). The glucosinolate-myrosinase system is therefore a two-compartment system that is activated upon tissue disruption and loss of cell integrity (e.g. by chewing herbivores). Depending on the side-chain chemistry, as well as different protein factors and reaction conditions (pH, metal cations) present, the rearranged products include different ratios of isothiocyanates, nitriles, epithionitriles, and others (Wentzell and Kliebenstein, 2008; Wittstock and Burow, 2010).

All known natural isothiocyanates (ITCs) are formed by Lossen rearrangement of the glucosinolate aglucone. These compounds are regarded as the most toxic of the glucosinolate hydrolysis products and cause delays in insect growth and development, and

Abbreviations: GSH, L-glutathione (reduced); ITC, Isothiocyanate; 4msob, 4-Methylsulfinylbutyl; CysGly, Cysteinylglycine; Cys, Cysteine; FMOC, Fluorenylmethyloxycarbonyl.

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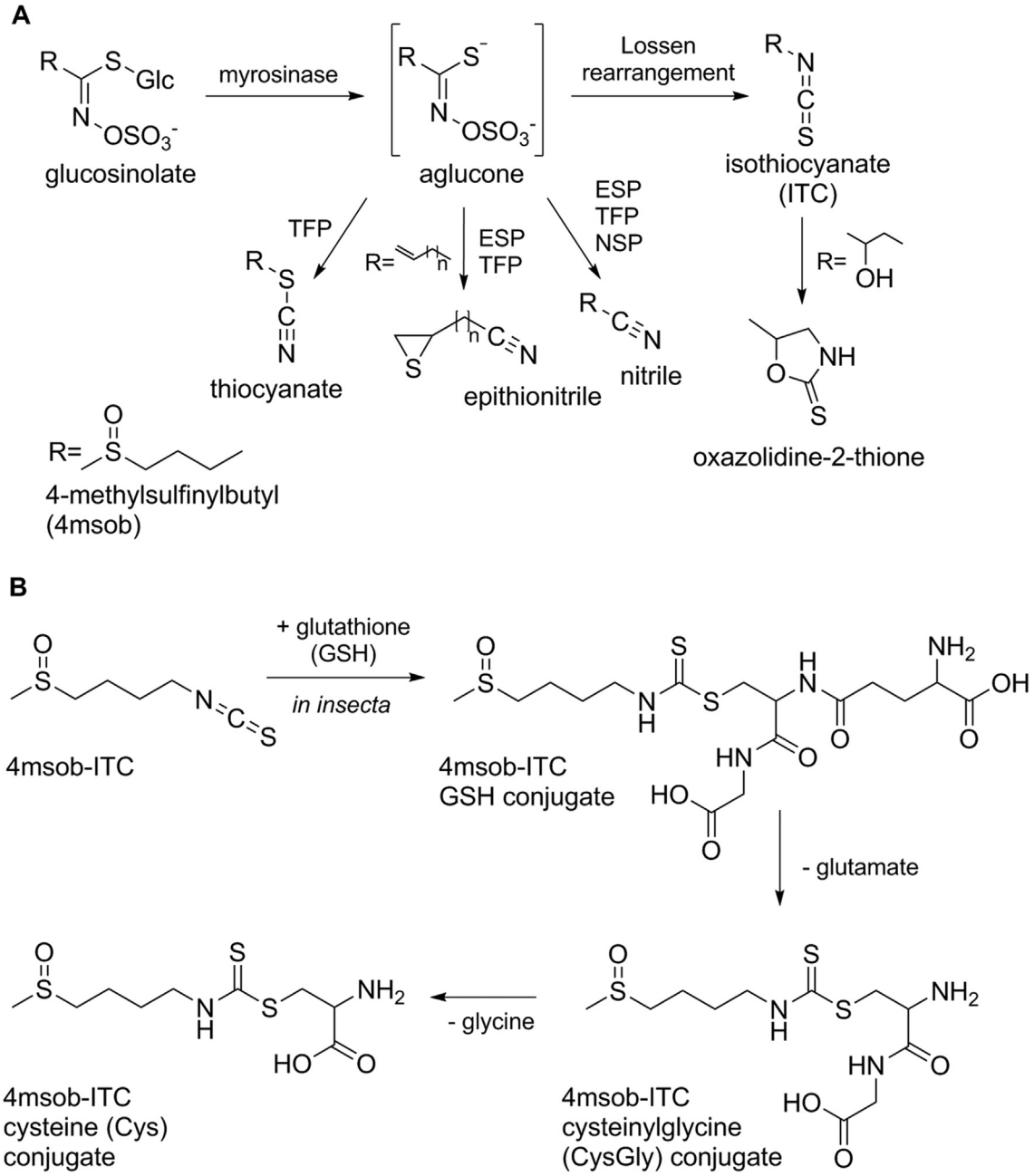

Fig. 1. A. Glucosinolates are activated by plant myrosinases, which remove the glucose moiety. The resulting aglucone is unstable and rearranges to different hydrolysis products. Among them, the isothiocyanates (ITCs) are considered the most toxic. 4-Methylsulfinylbutyl glucosinolate (with the R group depicted) is the most abundant glucosinolate in the rosette leaves of *Arabidopsis thaliana* (Col-0 accession). **B.** Generalist herbivores detoxify ITCs via conjugation to glutathione (GSH), depicted here with 4msob-ITC. The electrophilic ITC group forms a dithiocarbamate with the nucleophilic thiol of the GSH. Further hydrolysis via the mercapturic acid pathway leads to the corresponding cysteinylglycine (CysGly) and cysteine (Cys) conjugates.

sometimes death (Agrawal and Kurashige, 2003; Beekwilder et al., 2008; Li et al., 2000; Lichtenstein et al., 1962; Müller et al., 2010; Wadleigh and Yu, 1988; Wittstock et al., 2003). Toxicity is mediated by the typically lipophilic character of the side chain as well as the electrophilic nature of the ITC group. ITCs can passively diffuse across cellular membranes from the food bolus within the gut lumen, reaching the intracellular environment of the gut epithelium and from there can pass into the insect hemolymph. The reactive −N═C═S group of ITCs causes biological damage due to its high reactivity toward nucleophiles, functioning as a Michael acceptor for thiol or amine side chains of glutathione (GSH) and proteins at physiological conditions (Kawakishi and Kaneko, 1987; Kawakishi and Namiki, 1982). However, ITCs are apparently not directly reactive towards DNA or RNA (Xiao et al., 2012). The covalent reaction of ITCs with protein thiols and amines leads to alterations in secondary and tertiary protein structure, and ultimately to changes or loss of function of those proteins (Kawakishi and Kaneko, 1987; Kawakishi and Namiki, 1982; Mi et al., 2011) and to initiation of signaling cascades (Cross et al., 2007, 2009; Hu et al., 2011). A common detoxification of ITCs and other electrophilic xenobiotics is conjugation to the nucleophilic thiol (−SH) group of GSH, a so-called phase-II detoxification

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