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REVIEW ARTICLE

Efflux Transporters as a Novel Herbivore Countermechanism to Plant Chemical Defenses

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Abstract The recent discovery of efflux transporters in the gut has revolutionized our understanding of the absorption and bioavailability of pharmaceuticals and other xenobiotics in humans. Despite the celebrity of efflux transporters in the areas of pharmacology and medicine, their significance is only beginning to be realized in the area of plant–herbivore interactions. This review integrates reports on the importance of gut efflux transporters to diet selection by herbivores. The diets of herbivores are laden with toxic plant secondary metabolites (PSMs) that until recently were thought to be processed almost exclusively by detoxification enzymes in the liver. We describe how efflux transporters in the gut may play a critical role in regulating the absorption of PSMs in herbivores and dictating diet selection. Recent studies suggest that the role of efflux transporters in mediating diet selection in herbivores may be as critical as detoxification enzymes. In addition to diet selection, gut efflux transporters have implications for other aspects of plant–animal interactions. They may be significant components of the evolutionary arms race that influences chemical diversity in plants. Furthermore, in agricultural systems, gut efflux transporters may play an important role in the effectiveness of pesticides. This synthesis paper introduces a new direction in plant–herbivore interactions by providing a complementary mechanism, regulated absorption, to detoxification that may define tolerance to PSMs by herbivores.

Keywords Herbivores · Plant secondary metabolites · Regulated absorption · P-glycoprotein · Mammals · Efflux transporters · Diet selection · Herbivore tolerance · Transporter proteins

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Introduction

Two central foci in plant–herbivore interactions have been identified to explain the diversity of plant chemical defenses as well as the variation in herbivore tolerance to defenses. Much progress has been made in identifying and quantifying plant secondary metabolites (PSMs), as well as understanding their influence on herbivore feeding (Freeland and Janzen, 1974; Palo and Robbins, 1991; Rosenthal and Berenbaum, 1992; Cheeke, 1998; Foley et al., 1999). Diversity in PSMs has been attributed to the need of plants to defend themselves against a wide range of herbivores and pathogens (Berenbaum et al., 1991; Fritz and Simms, 1992; Berenbaum, 1995, 1999). In contrast, relatively little progress has been made with respect to the other central goal, i.e., explaining the variation in tolerance to PSMs by herbivores. Variation in detoxification capabilities has long been suggested as the underlying mechanism responsible for differential tolerances to PSMs among herbivores (Freeland and Janzen, 1974; Foley et al., 1999; Pass et al., 2001; Karban and Agrawal, 2002; Li et al., 2004). However, individuals and species with the highest PSM tolerance do not always have the highest activity levels of detoxification enzymes (Neal, 1987) or the highest rates of PSM elimination. For example, *Neotoma stephensi*, a juniper specialist, can tolerate more juniper and PSMs in juniper than its generalist counterpart, *N. albigula* (Sorensen et al., 2005), but it does not have a faster rate of juniper PSM elimination from the blood (Sorensen and Dearing, 2003).

We propose that, in addition to enzymatic detoxification, variation in mechanisms that regulate absorption of PSMs in the intestine may further explain the variation in herbivore tolerance to PSMs. Although the importance of PSM absorption in herbivores is not a new concept, previously proposed mechanisms have focused on passive barriers in the gut such as peritrophic membranes (PMs) (Lehane, 1997; Barbehenn, 2001). In this work, we argue that in addition to passive barriers, herbivores can actively transport PSMs out of enterocytes and back into the lumen of the gut, limiting PSM absorption. We refer to this process and its predicted influence on the foraging behavior of herbivores as the "regulated absorption model." We identify a plausible mechanism for regulated absorption and provide evidence that this mechanism can influence PSM tolerance in herbivores. In addition, we propose that regulated absorption of PSMs may further our understanding of chemical diversity in plants. Throughout, we provide several testable hypotheses regarding the role that regulated absorption plays in influencing the interactions between PSMs and herbivores. This synthesis paper introduces ecologists to a new direction in plant–herbivore interactions by providing a complementary mechanism, regulated absorption, to detoxification that may define tolerance to plant secondary metabolites by herbivores.

Introduction to Regulated Absorption

Ingestion of PSMs presents a physiological challenge for herbivores. PSMs that are ingested and absorbed exert a variety of deleterious effects on tissues and organs (Polya, 2003). Furthermore, the toxicity of PSMs is typically concentration-dependent and is therefore correlated with both the quantity of PSM ingested and the quantity of PSM that is absorbed and available throughout the body via the general circulation (Schumaher-Henrique et al., 2003; Orafidiya et al., 2004).

Because of the ubiquity of secondary metabolites in plants, herbivores cannot simply avoid them, but must employ mechanisms that minimize their levels in the circulation. Detoxification is currently regarded as the major physiological process that regulates the pharmacological distribution of PSMs in the body (Rosenthal and Berenbaum, 1992; Foley et al., 1999). In general, the process of detoxification biotransforms toxic PSMs into water-soluble metabolites that can be readily excreted. Although detoxification can lead to the formation of more toxic metabolites, the detoxification process typically results in a reduction in concentrations of toxic PSMs in the body. Moreover, several studies on insects have demonstrated that increased levels and activity of detoxification via enzymes are correlated with increased intake levels of certain PSMs in various herbivores. For example, the induction of cytochrome P-450 monooxygenases by PSMs, xanthotoxin and nicotine, is associated with high tolerance to these in the corn earworm (*Helicoverpa zea*) and tobacco hornworms (*Manduca sexta*), respectively (Snyder and Glendinning, 1996; Li et al., 2000; Sasabe et al., 2004). Although several studies have investigated detoxification enzymes in mammalian herbivores (Boyle et al., 1999, 2000a,b; Pass et al., 1999, 2001, 2002; Ngo et al., 2000; Lamb et al., 2001, 2004), few have directly linked higher PSM tolerance with higher detoxification enzyme activity in mammals (Miranda et al., 1991).

Although detoxification clearly plays a significant role in enhancing the excretion of PSMs and is predicted to facilitate PSM intake in herbivores, it is not the only mechanism that can regulate PSM concentrations in the body. For PSMs to gain access to tissues and organs, absorption across the gastrointestinal tract must first occur. Therefore, mechanisms in the gut that limit absorption provide the first line of defense against ingested PSMs. Detoxification in the intestine, whether via enzymes from microbes (Scheline, 1973; Jones and Megarrity, 1986; Hammond, 1995; Bhat et al., 1998) or the herbivore (Rosenthal and Berenbaum, 1992; Ilett et al., 1993; Watkins, 1997; Berenbaum, 1999; Von Richter et al., 2004), can regulate the absorption and subsequent distribution of ingested PSMs in herbivores. However, several studies demonstrate that many ingested PSMs that possess chemical properties conducive to absorption are neither detoxified nor absorbed in the gut (Ohmart and Larsson, 1989; Southwell et al., 1995; Schmidt et al., 2000; Cooper, 2001). For example, ingested terpenes, highly lipophilic PSMs, are excreted unchanged in the feces of two species of herbivorous mammals (*Neotoma* spp.; Sorensen and Dearing, 2003) and in frass of insects (Gómez et al., 1999; Müller and Hilker, 1999; Evans et al., 2000; Southwell et al., 2003). Physical barriers that limit the passive diffusion of PSMs across the gut, such as the peritrophic matrix and surfactants, have been the only mechanisms proposed to explain this phenomenon (Lehane, 1997; Barbehenn and Martin, 1998; Barbehenn, 2001). However, insights from biomedical and pharmaceutical research suggest that active transport of xenobiotics, including PSMs, out of enterocytes and into the gut lumen is also common and provides an efficient barrier against the absorption of lipophilic xenobiotics.

Mechanisms of Regulated Absorption

Despite the lack of attention from scientists who study plant–herbivore interactions, the biochemical mechanisms that actively regulate the absorption of ingested compounds are well known to medical scientists (Hunter and Hirst, 1997; Sharom,

1997; Watkins, 1997; Benet and Cummins, 2001; Doherty and Charman, 2002; Schinkel and Jonker, 2003). The molecules best known for their capacity to regulate the absorption of xenobiotics are collectively known as ATP-binding cassette (ABC) transporters. Permeability glycoprotein, or P-glycoprotein (P-gp), is encoded by the Multidrug Resistance (MDR) gene, and is one of over 200 ABC transporters that has been identified as playing a significant role in regulating bioavailability, or tissue distribution, of xenobiotics (Dietrich et al., 2003; Chan et al., 2004). Like other ABC transporters, P-gp is primarily expressed in the apical membrane of cells in barrier tissues, including the intestine, liver, kidney, testes, uterus, and blood–brain barrier. However, unlike other ABC transporters, P-gp is not substrate-specific and is capable of transporting a variety of structurally diverse compounds, including natural products (Sharom, 1997; Seelig and Landwojtowicz, 2000; Schinkel and Jonker, 2003). Although P-gp is a promiscuous transporter, most P-gp substrates are organic, relatively lipophilic, and contain polar or nonpolar domains (i.e., amphipathic, Sharom, 1997; Schinkel and Jonker, 2003).

P-gp is a major factor influencing the relationship between dose, blood concentration, and response of many orally administered drugs in humans (Van Zuylen et al., 2000; Ayrton and Morgan, 2001; Funakoshi et al., 2003). In general, individuals with higher quantities of P-gp exhibit lower blood concentrations and greater fecal excretion of certain orally administered drugs (Debenham et al., 1982; Van Zuylen et al., 2000). P-gp is also known to govern the bioavailability of many drugs resulting in lower pharmacological effect (Van Asperen et al., 1996; Jonker et al., 1999; Schinkel, 1999). P-gp reduces the bioavailability of ingested xenobiotics in three ways (Fig. 1). First, it can directly limit absorption by actively effluxing ingested xenobiotics out of cells against a concentration gradient via an ATP-dependent pump (Sharom, 1997; Dietrich et al., 2003). Second, it can indirectly

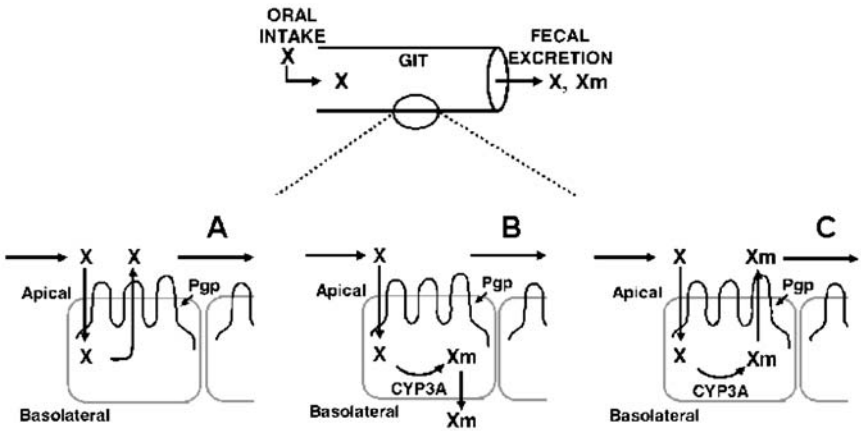

Fig. 1 Schematic representation of the functional role of P-glycoprotein (P-gp) and cytochrome P-450 3A (CYP3A) in regulating the absorption of ingested xenobiotics (X, i.e., drugs or plant secondary metabolites) in the gastrointestinal tract (GIT). P-gp and CYP3A can regulate the absorption of ingested xenobiotics (X) and detoxification metabolites (Xm) resulting in fecal excretion of these molecules via three scenarios. Ingested xenobiotics that are substrates of P-gp can be directly effluxed out of cells by P-gp (A). CYP3A metabolizes xenobiotics, which may result in absorption of the xenobiotic metabolite across the basolateral membrane (B). Finally, if the xenobiotic metabolite is a P-gp substrate, P-gp can efflux the metabolite out of cells (C)

reduce the bioavailability of ingested xenobiotics by regulating concentrations of intracellular xenobiotics to levels within the capacity of associated detoxification enzymes in cells, particularly cytochrome P-450 3A enzymes (Watkins, 1997; Ayrton and Morgan, 2001; Benet and Cummins, 2001). P-gp and CYP3A are colocalized in the villus tip of enterocytes, share many of the same substrates, and demonstrate broad overlap in substrate specificities (Kim et al., 1999; Katoh et al., 2001; Zhang and Benet, 2001). These attributes suggest that P-gp and CYP3A act in concert as a barrier to drug absorption. Finally, P-gp can efflux detoxification metabolites out of cells, thereby reducing the absorption of potentially toxic metabolites in addition to ingested parent xenobiotics (Katoh et al., 2001). In terms of drug therapy, mechanisms that limit absorption, enhance excretion, and minimize delivery of drugs to target tissues are medically problematic. For example, P-gp present in the transmembrane of cancer cells prevents the absorption of chemotherapeutic drugs (Lin and Yamazaki, 2003). Furthermore, because P-gp is a promiscuous transporter, it effluxes a wide range of anticancer drugs out of cells, resulting in the multidrug resistance phenomenon in cancer patients (Bellamy, 1996; Sparreboom et al., 2003). However, the same mechanisms that limit drug distribution in humans may also minimize the distribution of deleterious PSMs and prove beneficial to herbivores. In the following sections, we provide evidence that P-gp, or similar active transporters, may be employed by herbivores to enhance excretion and limit the bioavailability of ingested PSMs.

Evidence for Efflux Transporters in Herbivores

The first line of evidence that active transporters such as P-gp may be present in herbivores is the ubiquity and conservation of P-gp-like molecules among organisms. P-gp has been identified within mammals (humans, mice, rats, primates, dogs) and among distantly related taxa, such as bacteria, nematodes, insects, fish, birds, and plants (Dudler and Hertig, 1992; Lanning et al., 1996a; Davies et al., 1997; Barnes, 2001; Doi et al., 2001; Saier and Paulsen, 2001; Buss et al., 2002; Smith and Prichard, 2002; Mikkaichi et al., 2004). Furthermore, the sequence of P-gp is highly conserved across taxa (UCS Genome Bioinformatics website, http://genome.ucsc.edu). For example, the P-gp homologue in the plant, *Arabidopsis thaliana*, shares seven of nine introns with mammalian P-gp to within a few nucleotides (Dudler and Hertig, 1992). The shared sequence, organizational structure, and function of P-gp among distantly related organisms indicate that insect and mammalian herbivores will likely have P-gp homologues. Although empirical studies on P-gp in herbivores are minimal, the few species that have been investigated do possess P-gp or P-gp-like mechanisms. For example, P-gp-like transporters have been identified in the tobacco budworm, and appear to actively transport xenobiotics out of cells in these insects (Lanning et al., 1996a; Gaertner et al., 1998). Studies on herbivorous woodrats also demonstrate that several species of mammalian herbivores possess P-gp (Green et al., 2004).

A second piece of corroborating evidence is that a large number of PSMs that are potentially consumed by herbivores are substrates for P-gp (Patel et al., 2004; Zhou et al., 2004). Alkaloids, such as vinblastine from *Vinca rosea* (periwinkle, Burns, 1972; Sharom, 1997) and more recently, piperine from black (*Piper nigrum* Linn) and long pepper (*P. longum* Linn), are P-gp substrates (Bhardwaj et al., 2002).

Others include diterpene from *Euphorbia* (spurges, Hohmann et al., 2002; Appendino et al., 2003), phenolic glycosides from foxglove (*Digtalis lanata*, Kim et al., 1999; Katoh et al., 2001; Funakoshi et al., 2003), curcumin from *Curcumin longa* (Limtrakul et al., 2004), ginsenosides from ginseng (Choi et al., 2003; Kim et al., 2003), catachins from green tea (*Camellia sinensis*, Wang et al., 2002), bergamotin from grapefruits (Ohnishi et al., 2000; Wang et al., 2001), and hypericin from Saint John's wort (*Hypericum perforatum*, Wang et al., 2004).

The third line of evidence that active transporters such as P-gp are present in intestines of herbivores is the excretion of unmetabolized PSMs in the feces. A single study demonstrated that mammals (*Neotoma* spp.) excrete large quantities of ingested PSMs unmetabolized in the feces (Sorensen et al., 2004). Other examples of fecal excretion of unchanged compounds are found in herbivorous insects. For example, a large proportion of ingested nicotine is excreted unmetabolized in the feces of several insects specializing on tobacco (Self et al. 1964a,b; Snyder et al., 1994). Several other insect herbivores excrete terpenes in the feces that are identical in chemical structure and proportion to those found in the plants they consume (Gómez et al., 1999; Evans et al., 2000).

We propose that active transport of PSMs out of enterocytes is a plausible explanation for the fecal excretion of many unmetabolized PSMs in herbivores. Although passive barriers, such as peritrophic membranes (PMs), may be important in limiting the absorption of large complex PSMs, such as tannins (Barbehenn, 2001), these barriers would not be effective against small lipophilic compounds. Many of the PSMs excreted unmetabolized in the feces of herbivores, such as terpenes, are small molecules, highly lipophilic and, therefore, not likely to be passively filtered by PMs. Moreover, PMs cannot explain the excretion of terpenes unchanged in the feces of mammalian herbivores (Sorensen et al., 2004) because mammals do not possess PMs (Lehane, 1997). Instead, the excretion of many unmetabolized PSMs in the feces of herbivores is likely attributable to an active transporter such as P-gp. In support, a nicotine pump similar to P-gp has been identified in both malpighian tubules and in the blood–brain barrier of the tobacco hornworm (Murray et al., 1994; Gaertner et al., 1998). Finally, the strong correlation between the lipid solubility of a compound and its ability to interact with P-gp suggests that lipophilic PSMs are substrates for transporter proteins (Zamora et al., 1988; Schinkel and Jonker, 2003).

Although the excretion of unmetabolized lipophilic PSMs in the feces suggests that P-gp-like mechanisms are present in herbivores, the majority of studies fail to detect large quantities of unmetabolized PSMs in the feces of herbivores (Foley et al., 1987; Boyle et al., 1999, 2000a,b). We argue that these results do not necessarily indicate that most herbivores do not possess or utilize transporter proteins to minimize PSM bioavailability. Instead, the lack of unmetabolized PSMs in the feces may be attributable to the complex interactions between transporter proteins, detoxification enzymes, and microbes in the intestine prior to absorption. PSMs that are not absorbed may be detoxified or degraded by enzymes or microbes in the hindgut prior to excretion. For example, detoxification enzymes, particularly cytochrome P-450 3A, are locally associated and share substrate affinity with P-gp (Watkins, 1997; Kim et al., 1999; Zhang and Benet, 2001; Doherty and Charman, 2002). In addition, intestinal microbes from a variety of herbivores are known to metabolize and degrade PSMs (Carlson and Breeze, 1984; Jones and Megarrity, 1986; Hammond, 1995). Specifically, many microbes metabolize and degrade

lipophilic terpenes (Harder and Probian, 1995; Misra et al., 1996), which are likely P-gp substrates. Therefore, it is possible for P-gp to regulate the absorption of ingested PSMs in herbivores, but unmetabolized PSMs in the feces would not be present because of detoxification and modification of PSMs by enzymes and microbes prior to excretion. Alternatively, the rate of absorption of highly lipophilic compounds may exceed the rate of efflux by P-gp-like mechanisms resulting in absorption of PSMs that are actually P-gp substrates.

New Directions

It is essential to identify ways to isolate the contribution of active transport proteins in regulating PSM absorption in herbivores to better understand foraging constraints and disparate foraging strategies among herbivorous species. In this work, we suggest several areas of future research for chemical ecologists to better understand the interactions between PSMs and herbivores. Because of the preponderance of research on the function of P-gp, the following hypotheses will focus on assays and predictions for P-gp, but could be potentially applied to any efflux transporter protein.

The first needed area of research is to test the overall presence and level of transporter proteins in herbivores by using molecular techniques. The presence and quantity of particular proteins such as P-gp can be determined by using a combination of Western blots and quantitative polymerase chain reactions (qPCR). Western blots with monoclonal antibodies have been used to quantify P-gp in a variety of organisms, including herbivorous woodrats (Scheffer et al., 2000; Barnes, 2001; Doi et al., 2001; Green et al., 2004). Application of this method is accessible to a number of species because of the high conservation of monoclonal antibodies across species. Although this approach has been successfully applied to several species of animals, a potential limitation is the possible cross-reaction with proteins other than P-gp (Van Den Elsen et al., 1999) and the semiquantitative nature of the assay. Quantification of P-gp mRNA provides a more robust approach to identify and quantify P-gp in specific species of herbivores. Because sequences have not been determined for many wild species, species-specific sequences must be generated. In general, this process involves designing primary PCR primers from homologous sequences of nearest relatives, sequencing and verifying PCR products against known homologues to confirm identity, and then designing species-specific probes for qPCR. This approach was successfully used for plants (Dudler and Hertig, 1992; Yazaki et al., 2001; Sasaki et al., 2002), and will likely provide similar success in other species. An example of sequence alignment for a P-gp-like gene in plants and mammals is found in Dudler and Hertig (1992) and can provide an initial starting place for designing primers. In addition, homologous sequences can be designed based on P-gp (often referred to as MDR) gene alignments generated from the UCS Genome Bioinformatics website (http://genome.ucsc.edu). Quantitative PCR is beneficial in that it provides P-gp specificity and an opportunity to identify particular changes in P-gp sequence with respect to species, foraging strategies, and the type of PSM consumed. Quantification of P-gp-like transporters should not be restricted to segments of the gastrointestinal tract and should include studies in "barrier" tissues, such as the Malpighian tubules, liver, kidney, brain, testes, and uterus.

Given the likely applicability of these assays for P-gp quantification in herbivores, several hypotheses can be tested. First, these assays can be used to indirectly test the hypothesis that specific PSMs ingested by herbivores are substrates for P-gp. In general, ingestion of P-gp substrates yields an increase in P-gp concentrations (Benet and Cummins, 2001; Fromm, 2003, 2004; Lin and Yamazaki, 2003; Zhou et al., 2004). Determining whether a PSM is a P-gp substrate can be further verified with *in vitro* assays that use P-gp expressing cell lines (Varma et al., 2003; Moaddel et al., 2004; Wang et al., 2004) and/or *in vivo* studies that use P-gp knockout mice (Schinkel et al., 1997).

Second, assays can be employed to test the hypothesis that specialist herbivores rely on regulated absorption to a greater extent than generalist herbivores. Several studies that investigate the excretion of unchanged metabolites indirectly support this hypothesis. For example, the juniper specialist (*N. stephensi*) excreted a larger proportion (28%) of orally ingested alpha-pinene, the predominant PSM in juniper, in the feces than a closely related juniper generalists (*N. albigula*; 15%, (Sorensen et al., 2004). Similarly, specialist koalas excreted 15% of ingested eucalyptus oils, primarily composed of terpenes, in the feces (Eberhard et al., 1975) compared to 3% in a generalist counterpart, the brushtail possums (Foley et al., 1987). These results emphasize the need to quantify and compare P-gp activity, PSM intake, and fecal excretion of PSMs in additional specialist and generalist herbivores. Analytical techniques for quantification of unchanged PSMs include gas chromatography and high-pressure liquid chromatography; however, specific analysis is dependent on the chemistry of the particular PSM.

Quantitative assays can also be used to investigate the hypothesis that mechanisms regulating PSM absorption are positively correlated with expression of P-450 enzymes, specifically CYP3A, in the intestine. This prediction is based on the observation of large substrate overlap in CYP3A and P-gp within some species (Kim et al., 1999; Benet and Cummins, 2001; Katoh et al., 2001; Zhang and Benet, 2001). Furthermore, these molecules are typically located near one another and are thought to function in tandem. Chemical and physiological ecologists could, therefore, simultaneously compare P-gp and CYP3A levels along with the excretion of unmetabolized and metabolized PSMs in the feces when determining the role of P-gp in herbivory.

Finally, the ability to sequence, quantify, and test substrate affinity for P-gp makes it possible to investigate how generalists cope pharmacologically with a chemically diverse diet. Generalists are typically exposed to a wide and unpredictable array of PSMs because they consume taxonomically, and therefore, chemically diverse plant species, whereas specialists typically consume higher concentrations of a less diverse spectrum of PSMs because they consume plants within a narrow taxon range. It is theorized that generalists possess greater structural and functional flexibility in counterdefense mechanisms against PSMs (Gatehouse, 2002; Li et al., 2004). Greater polymorphisms in P-450 detoxification enzymes have been identified in a population of generalist insect herbivores (*H. zea*), and these are associated with wider substrate breadth compared to a population of specialists (*Papilio polyxenes*; Li et al., 2004). We predict that investigation on P-gp sequence and substrate affinity will reveal that generalist populations have greater P-gp polymorphisms that broaden substrate affinity compared to specialists. These tests will further describe the molecular and genetic disparity in counterdefenses between specialist and generalist herbivores.

Transporter proteins may not only be involved in the excretion of PSMs in feces, but may also explain the mechanisms for their sequestration in insects. Many herbivores "shuttle" ingested PSMs against a concentration gradient and sequester them unmetabolized in diverticular pouches, defensive glands, or hemolymph (Eisner et al., 1974; Müller and Hilker, 1999; Schmidt et al., 2000; Nishida, 2002; Pasteels and Hartmann, 2004). To date, no specific transporter has been implicated for the movement of these PSMs. However, there is recent evidence that selective transport systems are responsible for sequestration of certain metabolites in tissues of insects (Pasteels and Hartmann, 2004). In addition, transporter proteins have been proposed as a mechanism employed by plants to sequester PSMs in vacuoles, thereby protecting plant cells from the consequences of PSM exposure (Debeaujon et al., 2001; Sakai et al., 2002).

P-gp and Secondary Compound Diversity

P-gp transporters and the secondary compounds that interfere with them may be key factors that contribute in part to the bewildering diversity of chemical defenses in plants. The astounding diversity of secondary compounds in many species of plants is thought to be the result of a biochemical "arms race," or reciprocal evolution between plants and herbivores (Berenbaum et al., 1991; Fritz and Simms, 1992; Berenbaum, 1995, 1999; Castellanos and Espinosa-Garcia, 1997). The presence and abundance of P-gp transporters in their guts could represent an evolutionary counterresponse on the part of herbivores that reduces the physiological effect of secondary compounds by decreasing the quantity of "potent" PSM absorbed into the tissues. As a consequence of P-gp transporters, plants may have evolved secondary compounds that directly interfere with P-gp transporters that thereby enhance toxicity through increased absorption of potent secondary compounds. We refer to the P-gp inhibitors as "potency enhancers" because they act to increase the absorption of compounds that are biologically potent, or bioactive. Examples of PSMs that reduce the effectiveness of P-gp and enhance the absorption of potent compounds include furanocoumarins from grapefruits (Ohnishi et al., 2000; Wang et al., 2001; Honda et al., 2004), quercetin in oaks and birches (Shapiro and Ling, 1997; Conseil et al., 1998), and diterpenoids in spurges (Hohmann et al., 2002; Appendino et al., 2003). Although no work exists on the synergism between "potency enhancers" that inhibit P-gp and secondary compound diversity, some plant species with P-gp inhibitors also produce potent lipophilic compounds (e.g., terpenes in grapefruit) that could be P-gp substrates. Obviously, more research is necessary to critically evaluate these speculations.

In contrast, secondary compounds have been described that activate P-gp transporters, thereby reducing absorption of other compounds (Dürr et al., 2000; Perloff et al., 2001). For example, in *in vitro* experiments with cell cultures, the administration of hypericin extracted from St. John's wort elevates P-gp expression by 700% and significantly decreases the absorption of P-gp substrates (Perloff et al., 2001). The object of that study, as well as the others, was to identify compounds that interfere with the absorption of pharmaceuticals. Thus, the amount of activators applied may be far greater than the natural concentration of these compounds in plant tissues. Whether P-gp-activating compounds have synergistic effects on cooccurring secondary compounds at natural concentrations in plants when consumed by herbivores has not been examined. In theory, P-gp activators should

reduce the bioavailability of other coingested secondary compounds that are P-gp substrates. The adaptive significance to the plant of P-gp-activating compounds is not obvious. However, if P-gp activating compounds are produced at active concentrations in plants, the possibility exists that generalist herbivores take advantage of such compounds to reduce the absorption of secondary compounds from other plants in their diet. Thus, foraging behavior of herbivores may ultimately depend on the presence or absence of PSMs that are P-gp substrates, as well as on the presence of P-gp inhibitors and activators.

Broader Implications

Exploring how PSMs interact with transporter proteins and the relative role of these interactions in PSM tolerance in herbivores will not only contribute to understanding plant–herbivore interactions, but will likely have farther reaching applications. For example, the identification of PSMs that inhibit transporter proteins may contribute to a more efficient drug therapy. For example, a major complication in cancer therapy is due to multidrug resistance in cancer cells afforded by P-gp (Bellamy, 1996; Ambudkar et al., 2003). There is a pressing need to identify natural products and synthesize drugs that directly inhibit P-gp without concomitant toxicity to normal cells. Integration of knowledge from chemical ecologists who can predict which plants are most likely to possess P-gp inhibitors, and knowledge from pharmacologists who can predict which molecules are most likely to inhibit P-gp could facilitate the identification of compounds that enhance chemotherapy. Specifically, ecological knowledge of plant defenses and those acted on by active transporters may advance drug discovery. For example, assuming that P-gp is important in regulating the absorption of potent PSMs and that P-gp may be more important in specialist herbivores than generalists (Green et al., 2004; Sorensen et al., 2004), we can predict that leaves fed on by specialists contain compounds that are P-gp substrates, but are also likely to be potent drugs for humans. Moreover, we propose that individual plants or leaves within a host plant that are avoided by specialists may contain potency enhancers, compounds that inhibit the function of P-gp. We suggest that by applying the relationship between foraging strategies of herbivores and regulated absorption, a model can be used to efficiently target plants that contain potent compounds or potency enhancers. Further research into this area of plant–herbivore interactions could reveal the bioprospecting potential of herbivores.

Identifying active transporters in herbivores and PSMs that block the function of transporters could also be important in controlling both plant and animal pest species. Several pesticides, herbicides, anthelmintics, acaricides, and amebicides are P-gp substrates (Abu-Qare et al., 2003; Schinkel and Jonker, 2003). In addition, the absorption of many of these pesticides is limited by P-gp-like activity in insects (Lanning et al. 1996a,b; Buss et al., 2002; Srinivas et al., 2004) and plants (Windsor et al., 2003). The prevalence of P-gp may, therefore, help explain insect and plant resistance to pesticides and herbicides. Manipulation of transporters that regulate toxin absorption could be applied to development of more effective pest control agents. For example, pesticides that are P-gp substrates might be more effective if they were administered along with known P-gp inhibitors. This practice could result

in higher quantities of pesticides absorbed per unit exposure, thereby reducing the amount of pesticide used and/or increasing the susceptibility of insects to pesticides.

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