The role of glucosinolates and their hydrolysis in pathogens, herbivores, and humans

A Review

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Colorado State University
Jessica V Eilers
Abstract

Glucosinolates are characteristic in the Brassicaceae family of plants. In the past they have been implicated as toxic feeding deterrents for many herbivorous insects, but currently there is abundant research on their roles in health promotion in humans. Some insects are deterred by these plant-produced compounds, while others benefit from them. For example, Pieris butterflies are stimulated by glucosinolates for feeding purposes, but deterred from the same plant with cues from another chemical. In the case of humans, some perceive glucosinolates as a bitter taste, while other humans cannot perceive the bitterness in vegetables containing glucosinolates, including kale, cabbage, broccoli, arugula, and cauliflower. This can be problematic for health companies promoting food with these compounds, because genetics may be a significant element in the rejection of bitter glucosinolates by some humans. Glucosinolate bitterness perception is mainly studied in humans since their reactions to the taste can be communicated. In some studies, the variation in bitter perception of glucosinolates has been correlated to different allelic combinations of a particular bitter taste receptor gene, called TAS2R38. However, other studies have not found support for these correlations. The sensation of glucosinolates is most likely transduced as bitter perception as an adaptation to avoid toxic foods. However, these compounds are not toxic to humans if consumed at a reasonable concentration. This review article is intended to bring together current research on the health benefits of glucosinolate consumption in humans and how the perception of these compounds varies throughout human populations and how pathogens, insects, or other animals can be deterred by or attracted to glucosinolate compounds in plants.


**Introduction**

Glucosinolates are found in the *Brassicaceae* family of plants, which includes kale, cabbage, arugula, broccoli and cauliflower (Araki *et al*., 2013; Drewnowski & Gomez-Carneros, 2000). Recent studies have characterized the genes and proteins required for the synthesis pathway of glucosinolates (Araki *et al*., 2013; Vo *et al*., 2013; Halkier & Gershenzon, 2006). Glucosinolates differ in their amino acid composition, and whether they have a long or short carbon chain, which collectively make up the variable R group (Halkier & Gershenzon, 2006). When hydrolyzed, the resulting compounds can cause damage to the pathogen or herbivore that threatens the plant. However, glucosinolates are not hydrolyzed until they come into contact with the enzyme myrosinase, which is either spatially segregated from glucosinolates or inactive until induced by tissue wounding (Halkier & Gershenzon, 2006). Although it is widely accepted that glucosinolates function as a defense mechanism for *Brassicaceae* plants, some studies have found evidence that glucosinolates and their metabolites may act as kairomones for particular species (van Loon & Schoonhoven, 1999). Furthermore, recent evidence has revealed that the human consumption of glucosinolate containing plants may have significant health benefits, including anti-cancer, anti-inflammatory, and antioxidant effects (Castro-Torrez *et al*., 2014; Araki *et al*., 2013; Vo *et al*., 2013; Schreiner *et al*., 2012; Gorovic *et al*., 2011; Halkier & Gershenzon, 2006). Although that is an exciting health benefit for humans, humans are variable in their perception of glucosinolates. Some people are very sensitive to the bitterness of these compounds, while others are not bothered by it. Some studies have found correlations between allelic differences in a specific bitter taste receptor gene, TAS2R38, and the human sensitivity to these bitter glucosinolates; others have found no correlations (Lipchock *et al*., 2013; Gorovic *et al*., 2011). Despite the lack of supporting evidence in few studies, others are excited about the
idea of up-regulating the production of glucosinolates in *Brassicaceae* plants for human consumption in order to increase the health benefits, although some people find the taste of glucosinolates aversive. In the following review the chemistry and production of glucosinolate compounds in *Brassicaceae* will be overviewed, and then the role of glucosinolates and their metabolites as either allomones or kairomones will be discussed. The health benefits to humans will also be included. Lastly, this article will touch on the important implications that manipulations to glucosinolate content in crops can be beneficial.

**Discussion**

**Glucosinolates in Plants**

Recent research has identified the genes responsible for the production of glucosinolates in select plants. These plants include *Arabidopsis thaliana* and *Brassica oleracea*, which is better known as kale (Araki *et al.*, 2013). Many of these genes were first identified in *A. thaliana* because of its fast generation time and ease of transformation (Halkier & Gershenzon, 2006). Homologous genes in kale and *A. thaliana* have been identified by comparisons between the two genomes (Araki *et al.*, 2013). Glucosinolates come in many forms, but the underlying structure includes a glucose sugar, nitrogen, sulfur, hydroximinosulfate ester, and an R group that consists of a variable carbon chain length and one of the eight amino acids that are found in glucosinolates (Halkier & Gershenzon, 2006). The different types of glucosinolates are classified based on their R group composition. The eight amino acids that can be found in glucosinolates are alanine, leucine, isoleucine, methionine, valine, (those 5 make up aliphatic glucosinolates); tryptophan, (which makes up indole glucosinolates); and phenylalanine and tyrosine, (which make up aromatic glucosinolates) (Halkier & Gershenzon, 2006).
Glucosinolates are found throughout the plant, although they are most abundant in younger tissue and reproductive tissue such as the siliques and seeds, which contributes the most to fitness (Halkier & Gershenzon, 2006). Some glucosinolates are constitutively present in the plant tissue, while the synthesis of others is induced after receiving a cue, which is most often tissue wounding. Glucosinolates can exhibit long-distance travel through the plant’s phloem (Halkier & Gershenzon, 2006). The plant physiology relating to glucosinolate movement, expression, and abundance ensures that the compounds will be released if defense is necessary. However, intact glucosinolates are not the actual toxic compounds used for defense by the plant. The hydrolyzed products that form after cleavage by the enzyme myrosinase are the defensive toxic compounds. Myrosinase and glucosinolates do not come into contact with each other in the plant until damage occurs, either because they are spatially segregated or myrosinase is inactive until it receives a cue (Halkier & Gershenzon, 2006).

Resistance to Pathogens and Herbivores

In many cases, glucosinolates act as a mode of defense, through the jasmonic acid mediated pathway, to many generalist pathogens and herbivores that choose to exploit glucosinolate-containing plants (Falk et al., 2014; Markovich et al., 2013; Witzel et al., 2013; Halkier & Gershenzon, 2006). Upon plant wounding by the pathogen or herbivore, myrosinase comes into

Figure 1: Basic structure of a glucosinolate. Taken from http://www.google.com/patents/US20120122685.
contact with the glucosinolates and hydrolyzes the glucosinolates. The products are commonly nitriles, isothiocyanates, and others, which can act as allomones to herbivores and pathogens (Halkier & Gershenzon, 2006).

*Verticillium longisporum* is a soil-borne fungus that is suppressed by *A. thaliana* plants after spreading through the plant via the xylem (Witzel *et al.*, 2013). Growth is suppressed mostly by volatile compound products of hydrolyzed glucosinolates in the leaves. The volatiles from the roots did not suppress *V. longisporum* as effectively as volatiles from the leaves (Witzel *et al.*, 2013). The glucosinolate metabolite that contributed the most to fungal inhibition was 2-prop-isothiocyanate. Although it is now known which glucosinolate-derived compound is responsible for inhibition of this particular fungus, the mechanism of inhibition within the fungus is not known. However, crops managers that are experiencing fungal issues may find the application of 2-prop-glucosinolates, which form 2-prop-isothiocyanates when hydrolyzed by myrosinase, to be helpful in suppressing further fungal growth in the crops (Witzel *et al.*, 2013).

Some generalist insects are negatively impacted by glucosinolate metabolites (Markovich *et al.*, 2013). *Bemsia tabaci*, an insect that feeds from the plant’s phloem, was found to have decreased plant selectivity and decreased oviposition preference on *A. thaliana* plants that had been transformed to produce higher levels of glucosinolates (Markovich *et al.*, 2013). Phloem-feeding insects usually present minimal damage to the plants, so this study concluded that either glucosinolates are directly present in the phloem, or hydrolyzed products are released following damage to the phloem sieve element, which is the feeding location of *B. tabaci* (Markovich *et al.*, 2013). Either way, this is a prime example of how generalist insect herbivores can be harmed by the plant’s toxic hydrolyzed glucosinolate products.
Arion lusitanicus is a slug that is found to be deterred by hydrolyzed glucosinolates in A. thaliana (Falk et al., 2013). This slug feeds on the plant leaves, and spreads a slime residue in its trail. The slugs feed at night, which is consistent with the finding that the plants produce higher levels of jasmonic acid and aliphatic glucosinolates at night (Falk et al., 2013). Plants that produced lower levels of the defensive compounds had more wounded tissue from slug feeding, and plants that were transformed to produce higher levels of jasmonic acid and aliphatic glucosinolates had higher success rates of deterring the slugs. Slugs consuming higher levels of glucosinolates from transformed plants lost weight at first, showing the initial toxicity effects of the hydrolyzed products of glucosinolates. However, in time they gained the weight back, so they may have a mechanism for detoxifying the compounds (Falk et al., 2013). It is clear from many studies that the hydrolyzed products of glucosinolates act as allomones to different generalist exploiters such as fungi, insects, and even mollusks. However, as is often seen in plant specialists, some animals are attracted to glucosinolate-producing plants.

Attraction to Exploiters
The hydrolyzed products of glucosinolates are toxic, but some species have developed ways to avoid the toxic effects, possibly through rapid excretion or detoxification by conversion of the compound into a different one (Halkier & Gershenzon, 2006). For example, Pieris rapae, a cabbage butterfly, has a specialized protein that redirects myrosinase to cleave the glucosinolates into the less toxic nitriles instead of the more toxic isothiocyanates, and the nitriles are then immediately excreted (Halkier & Gershenzon, 2006; van Loon & Schoonhoven, 1999). Once the glucosinolates are ingested by these specialists, some have the ability to sequester the compounds and use them for their own form of defense. Some species that are able to do this include Athalia rosae and Brevicoryne brassicae (Halkier & Gershenzon, 2006). B. brassicae
has its own myrosinase, and hydrolyzes the glucosinolates when this aphid is under attack (Halkier & Gershenson). In a different light, humans may be seen as exploiters of glucosinolate-containing plants. Humans have developed ways to avoid the toxic effects, and as recent research shows, humans receive immense benefits from consumption of glucosinolate metabolites. Humans have a metabolic pathway to detoxify the isothiocyanate products of glucosinolates (Falk et al., 2013). This may be the reason why humans can eat large amounts of *Brassicaceae* vegetables, yet do not have harmful effects from the toxin.

**Human Consumption and Perception of Glucosinolates**

Humans are variable in their ability to perceive a bitter taste in glucosinolate-containing vegetables, or *Brassicaceae* (Lipchock et al., 2013; Gorovic et al., 2011). Some people perceive these vegetables as very bitter. Other people cannot taste bitterness when consuming them, and there are still other people who are somewhere in-between. Many studies have concluded that human perception of bitter glucosinolates depends in part on allelic differences at a bitter taste receptor gene (Lipchock et al., 2013). Humans have 25 bitter taste receptors, called TAS2R’s. The bitter taste receptor TAS2R38 is implicated in variable perception of bitterness in glucosinolates. There are two common alleles that arise from single nucleotide polymorphisms (SNPs) in this gene, giving rise to a different combination of amino acids: PAV and AVI. Since humans have two alleles of each gene, they can be homozygous for one of these haplotypes or they can be heterozygous. The studies in support of TAS2R38 partly determining bitter perception of glucosinolates have linked people that are sensitive to the bitterness as individuals with at least one PAV haplotype, and people who do not taste the bitterness as having at least one AVI haplotype (Lipchock et al., 2013). However, it should be noted that another study using a completely different method of data collection does not support this (Gorovic et al., 2011).
Although humans are variable in whether they can or cannot perceive the bitterness of glucosinolates and therefore most likely dislike vegetables containing them, the consumption of glucosinolates has been linked with many health benefits including anti-cancer, antioxidant, and anti-inflammatory benefits (Castro-Torres et al., 2014; Vo et al., 2013; Schreiner et al., 2012). An example is the activity of sulforaphane, an isothiocyanate produced from hydrolyzed glucosinolates. Sulforaphane helps induce detoxification proteins which aid in blocking of the cell cycle and prevention of tumor proliferation (Halkier & Gershenzon, 2006). Humans are yet another species that benefit from glucosinolates, and have overcome the toxic effects (Falk et al., 2014). It is believed that humans have evolved to overcome the toxins resulting from glucosinolates, so they no longer need to perceive a bitter taste when consuming them, since bitter taste is thought to exist to warn the brain if a toxin is being consumed (Drewnowski & Gomez-Carneros, 2000). Although for humans it is not necessary to be warned of glucosinolates by eliciting a bitter perception since they are not harmed by the toxic metabolites, the PAV allele is still in the human gene pool. *Brassicaceae* vegetables are considered functional foods, because they have specific compounds that help protect against human diseases. Although it may not be agreed upon exactly why humans have variable perception to the bitterness of glucosinolates, recent research is revealing that glucosinolates are not only non-harmful to humans; they may actually be very beneficial. As a result, mechanisms are being looked into for the purpose of increasing the glucosinolate content in the foods humans eat, either by genetic engineering or even by application of UV-B radiation (Schreiner et al., 2014; Halkier & Gershenzon, 2006).
Implications of Glucosinolate Manipulation in Crops

The knowledge that glucosinolates are an effective defensive tactic to ward off predators is helpful when trying to grow crops that are negatively impacted by these pathogens or herbivores. Crops grown for human consumption are undergoing manipulation in response to recent findings. Glucosinolates are beneficial to human health, so it would be a benefit to increase the amount of glucosinolates in *Brassicaceae* vegetables that humans eat. For humans, this is an exciting idea; crops used for human consumption should have an increase in glucosinolates so that certain aspects of human health are positively impacted, and the crops may be less threatened by pathogens and other herbivores.

Conclusion

Glucosinolates are compounds found in the *Brassicaceae* family of plants. These compounds come in different forms which differ by their amino acids and subsequent R group. Depending on the type of glucosinolate, they are hydrolyzed by myrosinase to form different products, but only upon contact with pathogens and herbivores. Once hydrolyzed, the products exhibit different effects in different species. To many of these species which are generalists, the hydrolyzed products are detrimental to their health or growth. To other species which are specialists, the hydrolyzed products do not exhibit a toxic effect because of adaptations of the specialist. Humans may be an example of a specialist for glucosinolate containing plants because the hydrolyzed products are not harmful upon ingestion, and they even confer major health benefits. The knowledge of how glucosinolates and their hydrolyzed products affect other species when consumed may be very important when considering pest management and human health.
References


