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Metabolite profiling of Arabidopsis seedlings in response to exogenous sinalbin and sulfur deficiency

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ABSTRACT

In order to determine how plant uptake of a sulfur-rich secondary metabolite, sinalbin, affects the metabolic profile of sulfur-deficient plants, gas chromatography time-of-flight mass spectrometry (GC–TOF-MS), in combination with liquid chromatography–mass spectrometry (LC–MS), was used to survey the metabolome of Arabidopsis seedlings grown in nutrient media under different sulfur conditions. The growth media had either sufficient inorganic sulfur for normal plant growth or insufficient inorganic sulfur in the presence or absence of supplementation with organic sulfur in the form of sinalbin (*p*-hydroxybenzylglucosinolate). A total of 90 metabolites were identified by GC–TOF-MS and their levels were compared across the three treatments. Of the identified compounds, 21 showed similar responses in plants that were either sulfur deficient or sinalbin supplemented compared to sulfur-sufficient plants, while 12 metabolites differed in abundance only in sulfur-deficient plants. Twelve metabolites accumulated to higher levels in sinalbin-supplemented than in the sulfur-sufficient plants. Secondary metabolites such as flavonol conjugates, sinapinic acid esters and glucosinolates, were identified by LC–MS and their corresponding mass fragmentation patterns were determined. Under sinalbin-supplemented conditions, sinalbin was taken up by Arabidopsis and contributed to the endogenous formation of glucosinolates. Additionally, levels of flavonol glycosides and sinapinic acid esters increased while levels of flavonol diglycosides with glucose attached to the 3-position were reduced. The exogenously administered sinalbin resulted in inhibition of root and hypocotyl growth and markedly influenced metabolite profiles, compared to control and sulfur-deficient plants. These results indicate that, under sulfur deficient conditions, glucosinolates can be a sulfur source for plants. This investigation defines an opportunity to elucidate the mechanism of glucosinolate degradation in vivo.

1. Introduction

Sulfur is one of the essential macroelements in plants and is assimilated by the roots as sulfate from the soil (Leustek et al., 2000). In planta, sulfate is first reduced to sulfide and then converted into cysteine, with the latter serving as the principal substrate for the biosynthesis of various other sulfur-containing organic compounds which, in crucifer plants, include glucosinolates. One approach to understand sulfur metabolism has been to compare plant metabolite differences between sulfur-depleted and normal plants (Nikiforova et al., 2005). Under sulfur-deficient conditions, it was anticipated that plants employ adaptive mechanisms that compensate for nutrient deficiency. Interestingly, integrative metabolomic and transcriptomic approaches have identified aspects of the global responses of Arabidopsis plants to sulfur deficiencies that include changes in glucosinolate metabolism (Hirai et al., 2004; Nikiforova et al., 2005).

Glucosinolates are plant secondary metabolites whose basic skeleton consists of a β-thioglucose, an *N*-hydroxyiminosulfate moiety, and a variable side-chain (R) (Halkier and Gershenzon, 2006) (Fig. 1). Glucosinolates are biochemically stable and non-toxic compounds that accumulate in crucifers, yet they function as chemical defense compounds. Tissue damage associated with plant pest activity releases glucosinolates, which are hydrolyzed by myrosinase yielding glucose, sulfate, and a variety of other potentially cytotoxic products such as isothiocyanates, nitriles and thiocyanates (Halkier and Gershenzon, 2006). Sulfatase is the only other enzyme known to use all glucosinolates as substrates converting them to their desulfated derivatives (desulfoglucosinolates) (Fig. 1). The enzyme is found in the guts of *Plutella xylostella* larvae and the snail *Helix pomatia*, and modifies the ingested glucosinolates resulting in the inactivation of the glucosinolate–myrosinase defense system (Ratzka et al., 2002). Sinalbin (**1**)

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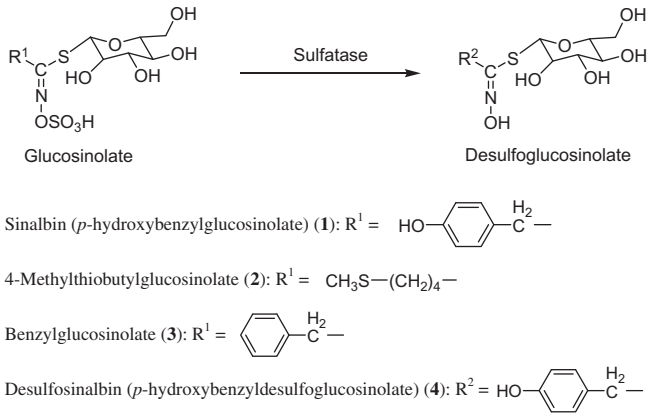

Fig. 1. Structures of glucosinolates and desulfoglucosinolates.

(*p*-hydroxybenzylglucosinolate) is found in seeds of white mustard (Thies, 1988). The variable side-chain of the sinalbin structure (**1**) is a *p*-hydroxybenzyl moiety derived from the amino acid tyrosine (Fig. 1). Because glucosinolates contain at least two sulfur atoms in their structure, they potentially have an important role in sulfur storage. Maruyama-Nakashita et al. (2003) regard the glucosinolates accumulating in root tissues as an alternative source for sulfur assimilation under sulfur-deficient conditions. Glucosinolates, such as sinalbin (**1**), that have been purified and administered to plants, are taken up, transported, and have been observed in the phloem exudates of Arabidopsis petioles (Chen et al., 2001). However, the effect of exogenous glucosinolate on the metabolite profile of sulfur-deficient plants is still unknown, as in the case when glucosinolates are broken down in intact plants through the activity of myrosinase or other unknown factors.

It is a great challenge to obtain the whole metabolome using a single analytical technique. GC–MS gives possibilities of analyzing different classes of compounds, including organic and amino acids, sugars, sugar alcohols, sterols, phosphorylated and lipophilic compounds (Wagner et al., 2003; Lisec et al., 2006). However, LC–MS is also a powerful technology to separate and analyze semi-polar secondary metabolites that can comprise large and unique groups of compounds in plants (Brown et al., 2003; von Roepenack-Lahaye et al., 2004; Stobiecki et al., 2006; de Vos et al., 2007). Therefore, the combination of GC–MS and LC–MS allows for a broad view of metabolic differences when comparing plants grown on different nutrient media. In the present study, GC–TOF-MS and LC–MS were used as profiling methods to study whether exogenously administered glucosinolate, such as sinalbin (**1**), could be metabolized in sulfur-deficient plants as a sulfur source and how such sulfur-containing organic compounds affect the metabolic profiles under sulfur-deficient conditions. Also, the effect of sinalbin (**1**) on the content of endogenous glucosinolate formation was studied in detail.

2. Results and discussion

2.1. Comparison of symptoms

In contrast to the morphology of 13-day-old Arabidopsis plants grown under control conditions (+S medium) (Fig. 2A), plants grown on inorganic sulfur-deficient (−S) medium supplemented with 846 μM sinalbin (**1**) were characterized by root and hypocotyl growth inhibition (Fig. 2B). The culturing experiments were replicated six times with consistent results. Arabidopsis seedlings grown in the presence of either other intact or desulfated glucosinolates, such as 4-methylthiobutylglucosinolate (**2**), benzylglucosinolate (**3**) and *p*-hydroxybenzyldesulfoglucosinolate (**4**) gave the same results as plants supplemented with sinalbin (**1**) (data not shown). Up to now, however little was known about the influence of exogenous glucosinolates on plant morphology. A report by Gijzen et al. (1989) found that the addition of 2-propenylglucosinolate to a growth medium containing rapeseed embryos resulted in a decline in embryo fr. wt. In the present study, Arabidopsis plants grown on sulfur-deficient media showed typical symptoms of sulfur deficiency such as stunted growth, leaf chlorosis and enhanced root growth (Fig. 2C). Enhanced root growth is a developmental response of plants to nutrient deficiency (Forde and Lorenzo, 2001; Kutz et al., 2002).

2.2. Comparison of metabolite profiles obtained with GC–TOF-MS

To investigate differences in the primary metabolite levels of samples prepared from plants grown on different nutrient media, whole seedlings (eight biological replicates) were harvested and extracted, and the extract samples derivatized and analyzed. The average relative concentration of a metabolite was compared by calculating the response ratio ($R_{-S/+S}$ or $R_{+sinalbin/+s}$) of the average relative concentration on −S medium or +sinalbin medium to that on +S medium as a control. When the relative ratio ($R_{-S/+S}$ or $R_{+sinalbin/+s}$) was more than 2.0 or less than 0.5 with $P < 0.05$, the relative concentration of metabolites was considered to be significantly altered, which was similar to the criterion designated by Nikiforova et al. (2005). The differences in the metabolite levels obtained with GC–TOF-MS are listed in Table 1. A total of 90 metabolites (**1, 5–92**) were identified, but the levels of some sulfur-containing compounds (e.g., cysteine and methionine) were below limits of detection.

Twenty-one metabolites (**5–25**) had similar responses (increased or decreased abundance) to both sulfur-deficient and sinalbin-supplemented nutrient conditions relative to the sulfur-sufficient

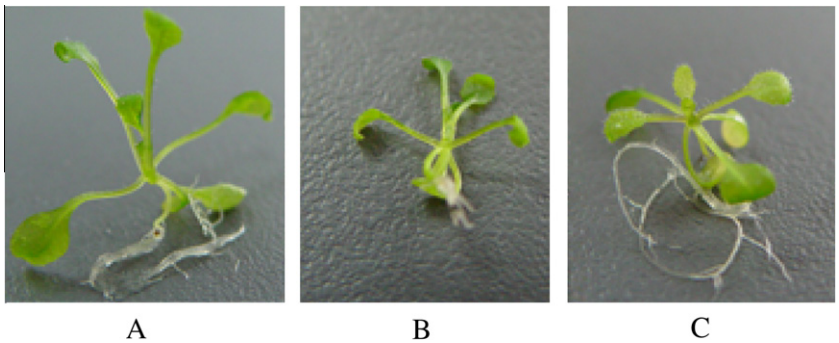

Fig. 2. The morphological appearance of 13-day-old Arabidopsis grown on Murashige and Skoog media with the sole sulfur source as (A) standard 846 μM sulfate (+S medium, control), (B) 846 μM sinalbin (**1**) (+sinalbin medium) or (C) no sulfur (−S medium).