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Mineralization of benzyl glucosinolate and its hydrolysis product the biofumigant benzyl isothiocyanate in soil

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Abstract

Plants produce many secondary metabolites that are used as a defense against herbivores, pests and pathogens. Among them are the glucosinolates (GSLs) that are produced by plants of the *Brassicales* order. Upon enzymatic hydrolysis, GSLs are transformed to primarily isothiocyanates (ITCs) and nitriles. ITCs are toxic compounds with the potential to be used as biofumigants for the control of soil-borne pests. In this study, mineralization kinetics of benzyl GSL and benzyl ITC in a sandy and a clayey soil at 8–9 °C were investigated. Mineralization of ^{14}C-benzyl GSL, which was *de novo* synthesized and purified from transgenic *Arabidopsis thaliana* after administering L-[U-^{14}C]phenylalanine, showed that 40–50% was mineralized after 60 days. Mineralization of ^{14}C-benzyl ITC produced by enzymatic hydrolysis of the ^{14}C-benzyl GSL resulted in mineralization of 35–50% after 45 days. The ITC or a metabolite of ITC was rate limiting for mineralization of ITC during the first 10–15 days in incubations with subsoils. This effect may be attributed to toxicity of the ITC or an ITC metabolite, or that a lag phase was required for the relevant degraders to grow. Although the compounds are of natural origin and thus "known" to microbial degraders the extent of mineralization was not higher than for synthetic compounds like pesticides. After termination of the mineralization experiments, the soils were extracted with water, ethyl acetate and 4 M NaOH, respectively. These extractions indicated that a large amount of the applied ^{14}C-labeled benzyl GSL and benzyl ITC was incorporated in organic fractions with low bioavailability.

Keywords: Mineralization; Kinetics; Glucosinolate; Isothiocyanate; Biofumigation

1. Introduction

Many pests such as nematodes, fungi and bacteria inhabit soils, from where they can cause serious damage to crops with economic loss as a consequence. Traditionally, such pests have been controlled by soil fumigation with methyl bromide or methyl isothiocyanate (ITC), but currently there is much interest in substituting these synthetic compounds with ITCs of natural origin (Kirkegaard and Matthiessen, 2004; Matthiessen and Kirkegaard, 2006). Natural ITCs are hydrolysis products of sulfur-rich secondary metabolites called glucosinolates (GSLs) (Fig. 1), which are produced by plants of the *Brassicales* order, including oilseed rape (*Brassica napus*) and the well-known *Brassica* vegetable crops such as broccoli (*Brassica oleracea*) (Bones and Rossiter, 1996; Mithen, 2001). ITCs are the major hydrolysis products, but other products such as nitriles and thiocyanates may be formed depending on the specific GSL, the presence of specific proteins such as epithiospecifier proteins, and environmental factors like pH and the presence of iron(II) (Mithen, 2001). The hydrolysis is catalyzed by a β-thioglucosidase called myrosinase (EC 3.2.3.1), which is present in all GSL-producing plants. In addition, myrosinase activity has been found to be present in soil (Mithen, 2001; Al-Turki and Dick, 2003; Gimsing et al., 2006). In the intact plant, myrosinase is separated from the GSLs. Upon tissue disruption, myrosinase and GSLs come in contact with each other and ITCs are formed (Mithen, 2001). ITCs are toxic to a wide range of organisms including nematodes,

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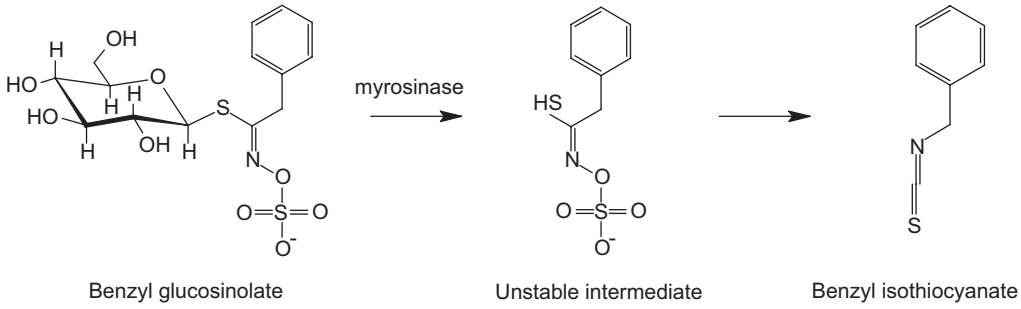

Fig. 1. Hydrolysis of benzyl GSL to produce benzyl ITC. The reaction is catalyzed by the enzyme myrosinase.

insects, bacteria and fungi, which suggests that they may be utilized as fumigants (biofumigants) for pest control in agriculture (Lazzeri et al., 1993; Brown and Morra, 1995, 1997; Borek et al., 1997; Manici et al., 2000; Smolinska et al., 2003; Kirkegaard and Matthiessen, 2004; Matthiessen and Shackleton, 2005). In biofumigation, *Brassica* plants are mulched and incorporated into the surface soil. When the ITCs are released, upon hydrolysis, they may potentially kill soil-borne pests (Kirkegaard and Matthiessen, 2004; Matthiessen and Kirkegaard, 2006).

One reason for the interest in biofumigants is that these naturally produced compounds are anticipated to be more environmentally safe than synthetic compounds. However, this cannot be taken for granted, and studies of the environmental fate of naturally produced compounds, like the ITCs, are just as important as that of synthetic pesticides. Studies of mineralization, the complete breakdown of the parent compound to water, carbon dioxide and salts, form an important part of fate studies. Mineralization kinetics and the extent of mineralization gives information about the rate of complete degradation, the degradation pathway, the response of the microbial degraders to the parent compounds and metabolites, and the likelihood of accumulation of residues in the soil environment. Mineralization experiments have been done with many pesticides like atrazine, glyphosate and 2,4-D (Gimsing et al., 2004; Farenhorst et al., 2006; Zablotowicz et al., 2006), but only few studies have been done with naturally produced compounds due to the difficulty in obtaining the required ^{14}C-labeled compounds.

The aim of this study is to investigate the mineralization kinetics of both benzyl GSL and benzyl ITC in two soil types by application of, respectively, ^{14}C-labeled benzyl GSL and benzyl ITC. The experiments were done at moisture contents present at the time of sampling at 8–9 °C with A- and B-horizons of a sandy and a clayey soil from Denmark.

2. Experimental section

2.1. Soils

A sandy and a clayey Danish soil were used in the experiments. Both soils were agricultural soils, and no GSL-containing crops had been grown on the soils for at least 5 years. The soils were chosen because of their different soil characteristics, and because they represent two of the most common soil types found in Denmark and Northern Europe.

The sandy soil was from Jyndevad in the southwestern part of Denmark. It was developed on coarse sandy glacifluvial material and has been classified as a Humic Psammentic Dystrudept (Soil Survey Staff, 1999). The clayey soil was from Sjællands Odde (Sj. Odde) in the northeastern part of Denmark. It was developed on calcareous clayey lodgement and melt-out till from the Weichselian glaciation and has been classified as a Typic Agriudoll (Soil Survey Staff, 1999). Selected properties of the soils are given in Table 1. The soil respiration rate was determined from measurements of the CO_2 production rate over a period of 2 h (Gimsing et al., 2004).

After the soils were sampled, they were stored at −18 °C at the moisture they had when sampled. Before use, the soils were thawed in a refrigerator (5 °C) and sieved through a 2 mm sieve. All experiments were done with the original moisture content.

2.2. Production of ^{14}C-labeled benzyl GSL and benzyl ITC

^{14}C-labeled benzyl GSL was produced by administering L-[U-^{14}C]phenylalanine to transgenic *Arabidopsis thaliana* genetically engineered to produce benzyl GSL. Transgenic CYP79A2 plants of *A. thaliana* L. cv. Columbia (Wittstock and Halkier, 2000) were grown in climate chamber with a temperature of 20 °C during day and night. The light period was 8 h with a light intensity around 80 μ Einstein. Humidity was 75%. Air exchange was 10,000 $m^3 h^{-1}$ with atmospheric CO_2 content. A modification of the method described by Chen and Halkier (2000) was used to synthesize and purify benzyl GSL: Young and healthy looking leaves from the transgenic *A. thaliana* were cut at the petiole and placed in open tubes with 50 μl L-[U-^{14}C]phenylalanine (specific activity 17.6 GBq $mmol^{-1}$, Amersham Life Science). Each batch consisted of 20 tubes with 8–10 leaves in each. The tubes were placed in a humid chamber under light for 24 h. When the amino acid solution was absorbed, 100 μl of water was added. After incubation, the leaves were pulverized in liquid nitrogen with a mortar and pestle. After pulverization, the tissue