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Degradation of the Potato Glycoalkaloids – α-Solanine and α-Chaconine in Groundwater

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Abstract The potato glycoalkaloids α-chaconine and α-solanine are produced in high amounts in potato plants from where release to soil takes place. Degradation of the compounds in groundwater was investigated, as their fate in the terrestrial environment is unknown. Abiotic and microbial degradation were followed in groundwater sampled from below a potato field and spiked with the glycoalkaloids (115 nmol/l). Degradation was primarily microbial and the glycoalkaloids were degraded within 21–42 days. The metabolites β_1-solanine, γ-solanine, and solanidine were formed from α-solanine, while β-chaconine, γ-chaconine and solanidine were detected from α-chaconine. Thus, indigenous groundwater microorganisms are capable of degrading the glycoalkaloids.

Keywords LC–MS · Metabolites · Natural toxin · *Solanum tuberosum* L.

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The important crop plant potato (*Solanum tuberosum* L.) grows on large areas all over the world (19 million ha in 2007 (FAO 2008)). All parts of the potato plant produce the two glycoalkaloids; α-chaconine and α-solanine (Fig. 1), which are toxic to e.g., man, fungi, insects, and snails (McKee 1959; Morris and Lee 1984; Fewell and Roddick 1993; Roddick 1996; Smith et al. 2001). For a detailed review of the toxicity please refer to Friedman (2006). The glycoalkaloids may be released to soil from the plant vegetative parts or from dead plant parts or potatoes left in the field after harvest. A risk for leaching to the groundwater may exist, as potatoes are often grown on sandy soils with high surplus precipitation. The glycoalkaloids are transformed by some potato pathogens, which overcome the toxicity by removing one or more of the carbohydrate units, thereby converting the glycoalkaloids into less toxic compounds (Morrissey and Osbourn 1999), but the fate of glycoalkaloids in the terrestrial environment is unknown. In a previous study, dissipation of α-solanine in a topsoil was shown to be slow at 5°C (Jensen et al. 2007), but it was not determined if dissipation was due to irreversible sorption or degradation.

The objective of this work is to investigate the dissipation of α-solanine and α-chaconine in groundwater where no sorption material is available. The type of degradation (abiotic or microbial) in groundwater is determined and formation of possible metabolites is followed to provide new insight to the degradation pathway.

Materials and Methods

Groundwater (pH 5.7) from 3 m below a sandy soil potato field at Fladerne Bæk in Denmark was sampled from an augered well using a peristaltic pump in October and stored

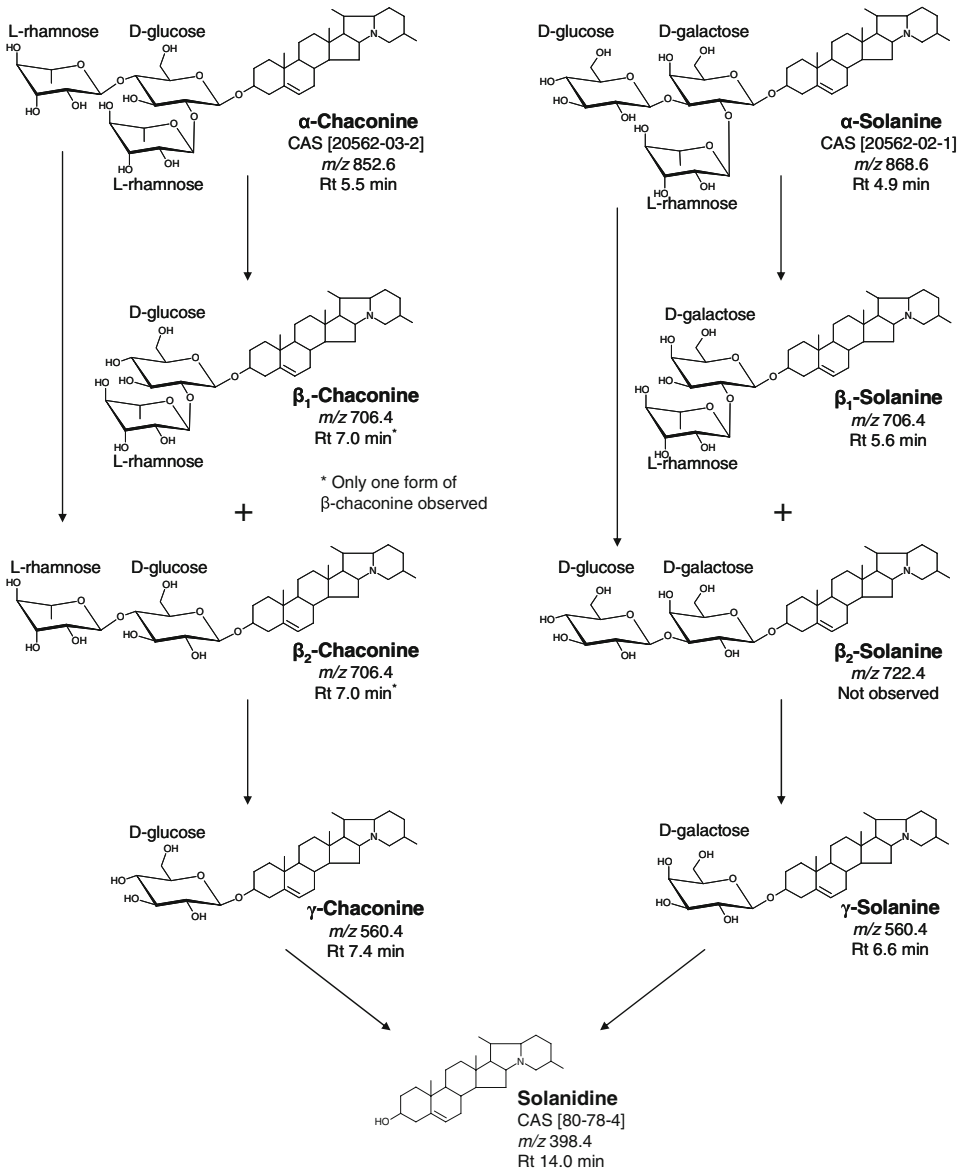

Fig. 1 Structures of α-chaconine and α-solanine and possible metabolites. LC retention times (*Rt*) for the observed compounds and the *m/z* values used for MS quantification are given

at 5°C until use. Groundwater conductivity was 31 mS/m and the concentrations of cat- and anions were; sodium 24 mg/l, potassium 25 mg/l, magnesium 3.1 mg/l, calcium 20 mg/l, nitrate 34 mg/l, phosphate <0.05 mg/l, chloride 28 mg/l, and sulphate 63 mg/l. The flask of groundwater was shortly shaken to obtain a proper mixing before the groundwater was divided into 100 ml incubation flasks (40 ml groundwater each). Duplicates were spiked with either α-chaconine or α-solanine to obtain a total initial concentration of 115 nmol/l. Microbial activity was inhibited in one set of microcosms with α-chaconine by addition of sodium azide (0.02%); the concentration was previously tested to be sufficient for inhibition of microbial activity. Similar microcosms without glycoalkaloids were prepared for control. Microcosms were incubated in darkness at 20°C at 80 rpm on a rotating shaking table. Sub-samples from the microcosms were taken 17 times during 6 weeks, filtered through 0.20 μm regenerated cellulose filter using a glass syringe and subsequently analysed by liquid chromatography–mass spectrometry (LC–MS). Controls without glycoalkaloids were analysed three times during the experiment and showed no traces of glycoalkaloids or metabolites.

Four times during the experiment, bacterial colony forming units (CFU) were counted in diluted sub-samples using Petrifilm aerobic count plates (3 M, St Paul, MN, USA). One mL of the diluted sample was placed on the Petrifilm and the films were incubated at 20°C in dark before CFU was counted after 3 days.

The glycoalkaloids were separated by LC (Waters model 2690, Milford, MA, USA) using a C18 Xterra column (Waters) and an acetonitrile-water (3 mM ammonium

acetate) gradient (Jensen et al. 2008). Detection was made on a triple quadrupole MS equipped with electrospray ionisation (Quattro Ultima, Micromass, Manchester, UK). The following parameters were used; desolation gas (N_2) flow 800 L/h, cone gas (N_2) flow 80 L/h, capillary voltage 3 kV, sample cone voltage 100 V, desolation temperature and source temperature 300 and 110°C, respectively. Positive ionisation mode was used and the analytes were quantified by selected ion monitoring. The ion traces (*m/z* values) recorded are shown in Fig. 1. External standards were used for quantification of α-chaconine, α-solanine, and solanidine, while the metabolites were quantified from the α-solanine $[M^+ H]^+$ response as no standards for these compounds were available.

Results and Discussion

Complete dissipation of α-solanine was observed in the spiked groundwater microcosms within 21 days (Fig. 2), and half life was observed to be 7–10 days. Three of the four recorded α-solanine metabolites were detected (β_1-solanine, γ-solanine, and solanidine), while no traces of β_2-solanine were seen. β_1-Solanine and γ-solanine occurred at the same time, while solanidine appeared a few days later, indicating formation of solanidine to proceed from β_1- or γ-solanine.

In the α-chaconine spiked microcosms, a complete dissipation of α-chaconine was observed within 42 days, while no degradation and hence no metabolites were observed in the azide treated microcosms within 36 days (Fig. 3a, b). In the microcosms without azide, three metabolites: β-chaconine, γ-chaconine, and solanidine were found above the limit of detection (2–5 nmol/l) (Fig. 3c, d). Only one form of β-chaconine was observed, either β_1 or β_2. In duplicate A, small amounts of β-chaconine and γ-chaconine could only be detected at day 14, when degradation of α-chaconine was most rapid. Solanidine was found from day 7 to 14. In duplicate B, where the initial degradation of α-chaconine was slower, the concentrations of metabolites were relatively higher and present for a longer time; β-chaconine appeared at day 14 and was detectable until day 36, and similar solanidine was present from day 10 until the end of the experiment. γ-Chaconine was not detected in duplicate B.

In three of the four microcosms without azide, bacterial growth was observed during the experiment (Table 1), while in duplicate B spiked with α-chaconine no bacterial growth was seen, and the number of CFU did not exceed the number in the control.

As dissipation of α-chaconine and formation of the metabolites were exclusively observed in the untreated microcosms for 36 days, it can be concluded that microbial degradation is the major transformation process in this groundwater. This conclusion is also likely for α-solanine due to its chemical similarity to α-chaconine.

The pools of metabolites are a function of both formation and degradation rates, and as only small pools of both β- and γ-compounds were observed, this shows the following degradation steps to solanidine to proceed fast. Further degradation of solanidine did also proceed relatively fast, but slower than the previous steps. Lower concentrations of metabolites were observed in the fastest degrading microcosms (Figs. 2a, 3c), which is in agreement with the higher microbial growth (CFU) in these microcosms. The bacterial growth shows that the degraded glycoalkaloids are partly converted into bacterial biomass. An exception was observed in duplicate B spiked with α-chaconine, where no bacterial growth was observed. One possible explanation for the difference in bacterial growth between the microcosms can be an uneven initial bacterial distribution between the microcosms due to the relatively low number of bacteria in the groundwater.

Fungal transformation of α-chaconine or α-solanine into less toxic metabolites by removing one or more of the carbohydrate units has previously been reported (McKee 1959; Weltring et al. 1997; Oda et al. 2002). The removal of carbohydrate units may occur in a specific order, where only one of the two possible β-compounds is produced. *Gibberella pilicaris* did only form β_2-chaconine (Weltring et al. 1997) while filamentous fungi isolated from potato sprouts produced only β_1-chaconine (Oda et al. 2002).

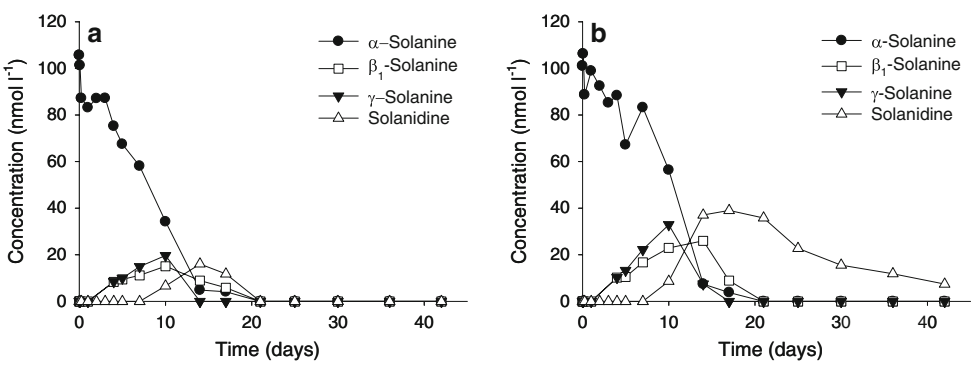

Fig. 2 Dissipation of α-solanine and formation of metabolites in groundwater samples (**a:** Duplicate A, **b** Duplicate B)

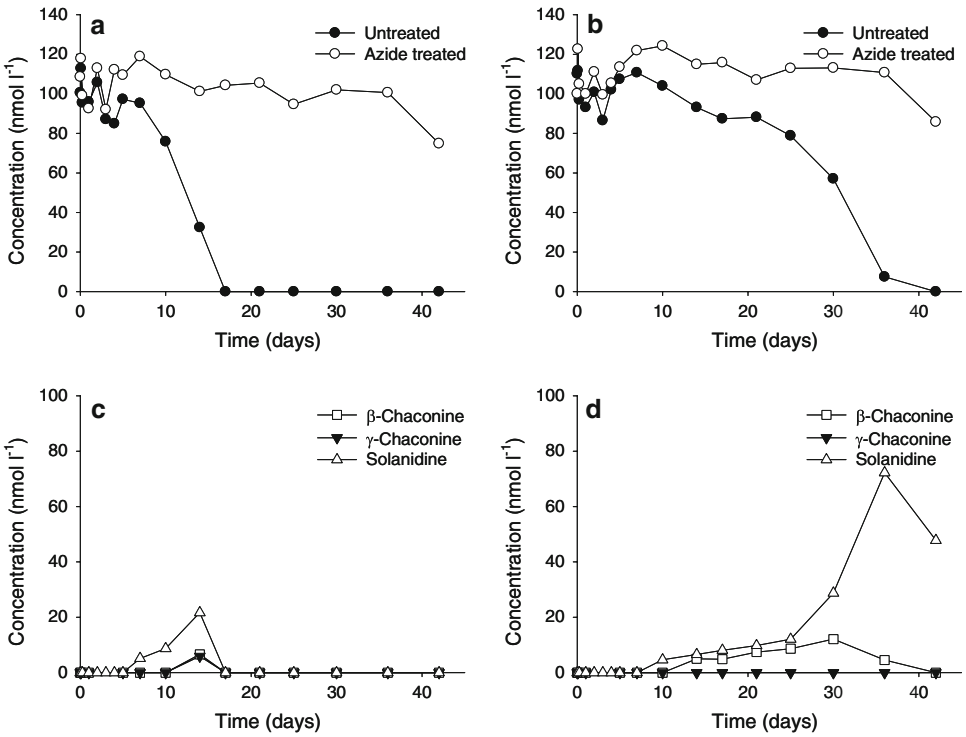

Fig. 3 Dissipation of α-chaconine in untreated and azide treated groundwater samples (**a** Duplicate A, **b** Duplicate B). Formation of metabolites in the untreated groundwater samples (**c:** Duplicate A, **d** Duplicate B)

Table 1 Number of CFU/ml (3 days incubation) in the untreated microcosms

	Control[a]	α-Chaconine		α-Solanine	
		Duplicate A	Duplicate B	Duplicate A	Duplicate B
Day 0	<3,000	<3,000	<3,000	<3,000	<3,000
Day 4	15,000	16,000	ND[b]	60,000	ND[b]
Day 17	<30,000	150,000	<30,000	2–300,000	115,000
Day 39	<30,000	240,000	<30,000	ND[b]	ND[b]

[a] No glycoalkaloids were added to the control

[b] Not determined

Similarly, we observed only one form of β-chaconine, too. The most commonly found β-form of β-solanine has been β_2-solanine. Fungal degradation (Weltring et al. 1997), potato enzymatic degradation (Guseva and Paseshnichenko 1957; Swain et al. 1978), as well as acid catalyzed hydrolysis (Friedman et al. 1993) almost exclusively leads to the formation of β_2-solanine. In contrast, the first degradation step in our experiment is a removal of the glucose unit forming β_1-solanine solely. Hence, the groundwater microorganisms too have a preference for removing the carbohydrate units in a specific order, and for α-solanine this order is different from what was previously observed for potato pathogens and enzymes. In our study, both α-chaconine and α-solanine were transformed by the groundwater microorganisms, whereas some fungi did solely convert α-chaconine and not α-solanine (Weltring et al. 1997; Oda et al. 2002).

In conclusion, it has been shown that groundwater microorganisms are capable of degrading glycoalkaloids and that the predominating dissipation process for glycoalkaloids in groundwater is microbial degradation. Three degradation products for α-solanine and α-chaconine have been detected, respectively. The carbohydrate units are being removed where after the aglycone solanidine is degraded further. In perspective, the results show that if potato glycoalkaloids are leached to the groundwater, the present microorganisms have a potential for degrading them.

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