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Role of fungal laccase in iodide oxidation in soils

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ABSTRACT

Previously, we hypothesized that microbial laccase oxidizes iodide (I^-) in soils to molecular iodine (I_2) or hypoiodous acid (HIO), both of which are easily incorporated into natural soil organic matter, and thus plays a role in iodine sorption on soils. In this study, soil iodide oxidase activity was determined by a colorimetric assay to evaluate if laccase is responsible for iodide oxidation in soils. Three types of Japanese soil showed significant iodide oxidase activities (0.751–2.87 mU g soil^{-1}) at pH 4.0, which decreased with increasing pH, until it was no longer detected at pH 5.5. The activity was inhibited strongly by autoclaving or by the addition of common laccase inhibitors. Similar tendency of inhibition was observed in soil laccase activity, which was determined with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a substrate. Significant positive correlations (R^2 values of 0.855–0.896) between iodide oxidase activity and laccase activity were observed in two of three soils. Commercially available fungal laccases showed only very low iodide oxidase activities (4.68–18.0 mU mg^{-1}), but enhanced activities of 102–739 mU mg^{-1} were observed in the presence of redox mediators. Finally, we successfully isolated fungal strains with iodide-oxidizing phenotype in the presence of redox mediators. Polyacrylamide gel electrophoresis of the culture supernatant of *Scytalidium* sp. strain UMS and subsequent active stain revealed that the fungal laccase actually oxidized iodide in the presence of redox mediators. These results suggest that at least part of iodide in soils is oxidized by fungal laccase through the laccase-mediator system.

1. Introduction

Iodine is an essential trace element for vertebrates, and is a constituent of the thyroid hormones, *i.e.* thyroxine and triiodothyronine. Insufficient iodine in the diet can cause iodine deficiency disorders such as endemic goiter and cretinism (Hetzel and Mano, 1989). Iodine has two important radionuclides, ^{129}I (half life: 16 million years) and ^{131}I (half life: 8 days), both of which have been released into the environments by the action of human activity since the nuclear age. Of these, ^{131}I has the greater threat to human health due to its high specific activity, particularly immediately after nuclear accidents such as Chernobyl and Fukushima. In contrast, ^{129}I has much lower specific activity, but is problematic as a contaminant of nuclear waste disposal due to its very long half-life (Moran et al., 1999; Buraglio et al., 2001). Radioiodine is expected to behave similarly to stable iodine (^{127}I), and possibly accumulates in human thyroid gland (Vandecasteele et al., 2000). Therefore, it is necessary to obtain better information on the behavior of iodine in the environment for accurate safety assessments of ^{129}I.

The predominant chemical forms of iodine in terrestrial environments are iodate (IO_3^-; oxidation state: +5), iodide (I^-; oxidation state: −1) and organically bound iodine (Kaplan et al., 2014; Fuge and Johnson, 2015; Yeager et al., 2017). Iodate and iodide can bind to soils, with the former exhibiting a much higher partition coefficient (K_d) than the latter (Yoshida et al., 1995; Fukui et al., 1996). It is widely accepted that these inorganic iodine species are immobilized in soils in the form of organically bound iodine (Kaplan et al., 2014; Yeager et al., 2017). Both iodide oxidation and iodate reduction can result in the production of reactive intermediates, including molecular iodine (I_2; oxidation state:0) and hypoiodous acid (HOI; oxidation state: +1). These intermediates will readily interact with soil organic matter to form organically bound iodine through covalent attachments (iodination). For example, iodate is reduced to I_2 or HOI by humic substances in soils (Yamaguchi et al., 2006, 2010; Zhang et al., 2011), and these electrophilic iodine species react with aromatic rings in soil organic matter to form iodine-carbon covalent bond (Schlegel et al., 2006; Shimamoto et al., 2011; Xu et al., 2011b). On the other hand, abiotic oxidation of iodide to I_2 or HIO may not occur or occurs very slowly in soils, since iodide is stable under pH and Eh conditions generally found in soil environments (Luther et al., 1995).

A large number of studies have shown that iodide sorption on soils is influenced by soil microbial activity. Decreased sorption of iodide by autoclaving, fumigation, air-drying, γ-irradiation, anaerobic treatment

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(N_2 gas flushing), heat treatment, and addition of antibiotics or reducing agents has been reported (Behrens, 1982; Bors and Martens, 1992; Bird and Schwartz, 1996; Seki et al., 2013). Inoculation of sterilized soil with fresh soil was shown to restore iodide sorption (Muramatsu and Yoshida, 1999). These results strongly suggest that soil microorganisms or microbial enzymes are involved in the sorption of iodide, probably through the oxidation of iodide to I_2/HIO and subsequent iodination of soil organic matter. Laccases (kinds of multicopper oxidases) are commonly produced by soil fungi and bacteria, and can oxidize various compounds such as methoxyphenols, diphenols, and aromatic diamines (Hoegger et al., 2006; Morozova et al., 2007). In addition, several fungal and bacterial laccases have been reported to possess capacities for iodide oxidation. Xu first found that *Myceliophthora thermophila* laccase can catalyze the oxidation of iodide, and that the catalysis was enhanced by the addition of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a redox mediator (Xu, 1996). Kulys et al. also reported iodide oxidation by *Polyporus pinsitus*, *M. thermophila*, *Coprinus cinereus*, and *Rhizoctonia solani* laccases in the presence of methyl syringate as a redox mediator (Kulys et al., 2005). Recently, iodide oxidation by marine bacterial laccase-like enzyme (IoxA) has also been reported (Suzuki et al., 2012; Shiroyama et al., 2015).

Recently, Seki et al. determined iodide sorption and laccase activity in two types of Japanese soil under various experimental conditions to evaluate possible involvement of this enzyme in the sorption of iodide (Seki et al., 2013). Batch sorption experiment using radioactive iodide tracer ($^{125}I^-$) revealed that the sorption was significantly inhibited by autoclaving, heat treatment, γ-irradiation, N_2 gas flushing, and the addition of reducing agents and general laccase inhibitors. Interestingly, very similar tendency of inhibition was observed in soil laccase activity, which was determined with ABTS as a substrate. The partition coefficient (mL g^{-1}) for iodide and specific activity of laccase in soils (U g^{-1}) showed significant positive correlations (R^2 values of 0.786–0.879) in both soils. Furthermore, addition of a bacterial laccase (IoxA) with an iodide-oxidizing activity to the soils strongly enhanced the sorption of iodide. K-edge XANES spectra of the adsorbed iodine revealed that 100% of iodine was in the form of organically bound iodine. These results suggest that microbial laccase is involved in iodide sorption on soils probably through the oxidation of iodide to I_2/HIO, and that these unstable intermediates interact with soil organic matter to form organically bound iodine (Seki et al., 2013). However, it is still unclear whether soil microbial laccase actually catalyzes the oxidation of iodide, since iodide-oxidizing activity (iodide oxidase activity) was not determined in the above-mentioned study due to very low level of iodide in soils.

The aim of this study was to correlate iodide oxidase activity in soils with laccase activity. To increase detection sensitivity of iodide oxidase activity in soils, we added very high concentration of iodide (50 mM) to soil slurries, and determined the activity colorimetrically based on the traditional “iodine-starch reaction”. Effect of various treatments, which are known to affect soil laccase activity (Seki et al., 2013), on iodide oxidase activity was tested to evaluate involvement of this enzyme in iodide oxidation. In addition, iodide oxidase activity of commercially available fungal laccases was determined in the presence or absence of redox mediators. Finally, fungal strains with an iodide-oxidizing phenotype were screened from soils to demonstrate direct involvement of their laccase in iodide oxidation.

2. Experimental methods

2.1. Soil samples

Three types of Japanese soil collected from surface layer (0–5 cm) of Matsudo city, Chiba (soils A and B) and Kaminokawa-town, Tochigi (soil C) were used. Soils A and B were classified as light-colored andosol, while soil C was gray lowland soil. The soils were passed through a 2-mm sieve under field moist condition and stored in a refrigerator at 7 °C until use.

2.2. Iodide oxidase activity in soils

Soil (2.0 g wet weight) was transferred to a 100-mL Erlenmeyer flask and mixed with 40 mL of 100 mM MES buffer (pH4.0). To increase detection sensitivity of iodide oxidase activity in soils, potassium iodide (KI) was added to the soil slurry at a final concentration of 50 mM. After static incubation at 30 °C, a portion of the slurry was centrifuged (18,000 × *g*, 4 °C for 3 min). In the presence of excess iodide, I_2 forms triiodide ion (I_3^-), which then easily forms a purple complex with starch.

$$I_2 + I^- \rightarrow I_3^-$$

Thus, after the centrifugation, 500 μL of supernatant was removed and mixed with 100 μL of 1% soluble starch, and the absorbance of the mixture was determined at 525 nm. The amount of I_2 was calculated based on a standard curve, where 0–500 μM of I_3^- was mixed with soluble starch. In most cases, iodide oxidase activity in soil was expressed as amount of I_2 produced per gram wet soil (μmol I_2 produced g $soil^{-1}$). In some cases, specific activity of iodide oxidase in soil is expressed as units per gram wet soil (U g $soil^{-1}$). One unit of iodide oxidase activity was defined as the formation of 1 μmol of I_2 per min. Specific activity was calculated as the maximal I_2-formation rate observed over any 24-h period during the 3-day incubation. The detection limit of iodide oxidase activity in soils was approximately 0.035 μmol I_2 produced g $soil^{-1}$ or 0.012 mU g $soil^{-1}$.

2.3. Laccase activity in soils

Soil (2.0 g wet weight) was transferred to a 100-mL Erlenmeyer flask and mixed with 40 mL of 100 mM MES buffer (pH4.0). ABTS was directly added to the slurry at a final concentration of 1.5 mM (Seki et al., 2013). After static incubation at 30 °C, a portion of the slurry was centrifuged (18,000 × *g*, 4 °C for 3 min), and the absorbance of the supernatant was determined at 420 nm. Substrate oxidation was followed by using the molar absorption coefficient (ε_{420}) of 36.0 mM^{-1} cm^{-1} for ABTS. In most cases, laccase activity in soil was expressed as amount of ABTS oxidized per gram wet soil (μmol ABTS oxidized g $soil^{-1}$). In some cases, specific activity of laccase in soil is expressed as units per gram wet soil (U g $soil^{-1}$). One unit of laccase activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of the product (ABTS cation radical) per min. Specific activity was calculated based on the ABTS-oxidizing rate at appropriate 24-h period, where specific activity of iodide oxidase has been calculated. The detection limit of laccase activity in soils was approximately 0.040 μmol ABTS oxidized g $soil^{-1}$ or 0.014 mU g $soil^{-1}$.

2.4. Effect of various treatments on soil enzyme activity

Soil slurries were subjected to various treatments that potentially repress or inhibit iodide oxidase and laccase activities (Seki et al., 2013). For sterilization, the slurry was autoclaved at 121 °C for 20 min. The pH of the slurries was adjusted at 4.5, 5.0, and 5.5 with NaOH. General laccase inhibitors including sodium azide (NaN_3), potassium cyanide (KCN), EDTA, and *o*-phenanthroline were added at a final concentration of 10 mM.

2.5. Iodide oxidation by fungal laccases

Trametes versicolor laccase (TvL), *Pleurotus osteatus* laccase (PoL), and *Agaricus bisporus* laccase (AbL) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Iodide oxidase activity by these laccases was determined similarly as described above. The reaction mixture (total 1.5 mL) contained 20 mM sodium acetate buffer (pH4.0), 100 μg