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Thermal deactivation of high-affinity H_2 uptake activity in soils

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ABSTRACT

Soils are the most important sink for atmospheric hydrogen, which is assumed to be oxidized by abiontic soil hydrogenases or by putative high-affinity hydrogenases of microbial origin. The activity of soil hydrogenases has been found to change with soil temperature as it changes during the day and the season. However, it is unclear whether and to which extent the soil hydrogenases are deactivated by increased temperature. Therefore, we incubated soils from different climates and different ecosystems (forest, agricultural, arid, hyper-arid, paddy, peat) at elevated temperature and measured the residual activity at 25 °C after different incubation times. We found that at least part of the soil hydrogenase is deactivated irreversibly already at relatively low temperatures (>30 °C) and short exposure times (>10 min) and that the deactivation was more pronounced in soil from cold versus hot climate. The deactivation kinetics could be fitted to a biexponential model, but were complex with respect to soil type and deactivation temperature. The results show that new hydrogenase activity has to be generated in the soil to compensate for activity loss by diurnal and seasonally increased temperature.

1. Introduction

The biological soil uptake has been identified as the largest sink for tropospheric H_2. Although uncertainties exist, contribution of the soil sink has been predicted to be about 75–92% (Novelli et al., 1999; Rhee et al., 2006; Ehhalt and Rohrer, 2009). The enzymatic nature and the kinetic characteristics of such activity have been investigated by analyzing uptake of different headspace H_2 concentrations by soil samples (Conrad et al., 1983; Schuler and Conrad, 1990; Häring and Conrad, 1994). Two different H_2-oxidation activities have been detected in soils and are differentiated on the basis of their affinity for H_2 (Schuler and Conrad, 1990). Microorganisms having a low affinity for H_2 ($K_m \sim 1000$ ppmv) have been ascribed to aerobic H_2-oxidizing bacteria (so called Knallgas bacteria), whereas the high-affinity activity ($K_m < 100$ ppmv) is currently attributed to hypothetical abiontic soil hydrogenases (Conrad et al., 1983; Häring et al., 1994; Guo and Conrad, 2008) or possibly to putative high-affinity hydrogenases of *Streptomyces* species (Constant et al., 2008, 2010). Attempts to purify the soil hydrogenases have yielded partially purified soil extracts which showed H_2-oxidation activity at ambient concentrations and typical biphasic kinetics (Guo and Conrad, 2008). However the yield of such hydrogenases was quite low, possibly because they are bound to soil organic matter or the mineral matrix, hindering their extraction.

One factor that can affect H_2-oxidation kinetics is temperature, which can have a complex effect on enzyme activity. The rate of enzyme-catalyzed reactions usually increases with increasing temperature until a certain temperature then the rate begins to decrease due to deactivation of the enzyme. The thermal deactivation of enzymes is classically described as a 'one step-two states' process where the native (active) form is transformed into the denatured (inactive) form. The mechanism of enzyme deactivation, however, can be complex. Aymard and Belarbi (2000) summarized several possible deactivation schemes including (1) a simple 'one step – two states' model, (2) 'parallel' deactivation assuming two enzymatic species, (3) 'series' deactivation with consecutive reactions, (4) 'competitive' deactivation resulting in different inactive products, and (5) mixed schemes with 'consecutive' and 'competitive' reactions. The 'one step – two states' is basically a uniform irreversible first-order deactivation reaction. The 'parallel' model has been proposed for a mixture of at least two active enzyme forms (isoforms, isoenzymes) with different heat sensitivities, each following its own irreversible first-order deactivation reaction. The 'series model' has been used for an deactivation reaction in which the initial active form first produces inactive form in a reversible reaction, which then is irreversibly converted into the ultimate inactive form. The 'competitive' model has been used for an active enzyme being converted into two inactive forms, one via

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a reversible and the other via an irreversible reaction. Aymard and Belarbi (2000) showed that all reaction schemes can be described by a 3-parameters biexponential equation:

$$v_t/v_0 = A\exp(-\alpha t) + (1-A)\exp(-\beta t) \quad (1)$$

where the kinetics of enzyme activity is expressed as the ratio of the measured enzyme activity at time t and time zero (v_t/v_0) of heat treatment, and A is the fraction that reacts according to deactivation constant α and $(1-A)$ is the fraction reacting according to deactivation constant β. The constants in the exponential arguments are complex expressions of rate constants.

The effect of temperature on soil H_2 uptake has been studied both in the laboratory and the field. Different temperature optima, usually between 20 °C and 40 °C, have been found (Liebl and Seiler, 1976; Fallon, 1982; Förstel, 1986; Schuler and Conrad, 1991; Smith-Downey et al., 2006; Guo and Conrad, 2008) and even more than one optimum has been reported (Schuler and Conrad, 1991; Guo and Conrad, 2008). Under field conditions, temperature affects not only the H_2-consuming biological activity, but also the diffusivity of H_2 within the soil column (Conrad and Seiler, 1985; Yonemura et al., 2000a,b; Smith-Downey et al., 2006, 2008; Lallo et al., 2008; Schmitt et al., 2009; Hammer and Levin, 2009). However, results of the different field studies were not always comparable and in several cases the relationship between soil temperature and H_2 flux was not well established (Smith-Downey et al., 2008). Irrespective of the origin, it is well understood that the soil uptake of tropospheric H_2 is an enzyme mediated process but it is unclear to which extent changes in temperature result in a reversible change of H_2 uptake activity or cause an irreversible deactivation. The knowledge of thermal deactivation kinetics is important, as soils undergo large changes in temperature during the day and over the season.

Considering that the physical properties of the soil and the microbial community could play an important role in the high-affinity H_2 uptake activity, in the present work we hypothesized that the thermal deactivation kinetics of the high-affinity hydrogenase activity may be different in soils from different geographical origin and different ecosystems. High-affinity hydrogenases from soils which undergo large diurnal or seasonal variation in temperatures were expected to show a higher tolerance to temperature or a gradual thermal deactivation pattern in comparison to others from soils with moderate or minimal variation, showing a rapid deactivation pattern at elevated temperatures.

2. Materials and methods

The soils were from different locations and ecosystems. Their main characteristics are described in Table 1. The soils were stored at room temperature in the same state as they were collected, but the peat soil was stored at 4 °C for up to 2 months.

The kinetics of a thermal deactivation of the soil hydrogenase was studied by incubating 0.1 g of soil at a defined temperature. Soil incubated at room temperature (25 °C) was considered as control. Routinely, we tested each day the effect of one temperature together with the control temperature (25 °C) for one type of soil. The repeated measurement of hydrogenase activity in the control resulted in a constant hydrogenase activity over several days. The deactivation of soil hydrogenase was studied at different temperatures between 30 °C and 100 °C, one temperature run per day. 0.1 g of soil was incubated in Eppendorf tubes using a heating block and samples were taken every 10 min for 1 h. Then, the hydrogenase activity was measured by suspending the soil in 10 ml of sodium pyrophosphate buffer (pH 7.3) at 25 °C and incubating it in 120-ml serum bottles flushed with synthetic air (20% O_2 + 80% N_2). We used a suspension of soil for the measurement of H_2-oxidation activity to guarantee uniform assay conditions for all the different soils. It has been shown that under such conditions the H_2 consumption is proportional to the mass of soil and not limited by the transfer of H_2 from the headspace to the aqueous phase (Häring and Conrad, 1994). The H_2 mixing ratio in the headspace was adjusted to approximately 1.8 ppmv and the consumption of H_2 (under shaking conditions) was followed with time using a gas chromatograph (Shimadzu, GC-8A) with a 5A molecular sieve-filled stainless steel column (length 3 m, internal diameter 1/800) and a HgO-to-Hg conversion detector (Trace Analytical, RGD2) (Schuler and Conrad, 1990). For routine assay, 7 consecutive gas samples (0.5 ml each) were taken from the headspace over a time period of 180 min. The hydrogenase activity was determined as rate constant (h^{-1}) from the logarithmic decrease of H_2 with time, determined by linear regression (Origin 6.1). The standard error of the slope was given by the regression program.

The hydrogenase activities determined after different times of heat exposure were evaluated according to equation (1) using curve fitting by direct least-squares curvilinear regression. Curve fitting was done with MATLAB program using the Levenburg–Marquardt algorithm. The regression model used time as regressor and the relative residual activity of the soil hydrogenase activity as the regressand, i.e., the ratio of the measured activity v_t at time t of heat treatment to the initial (control) activity v_o.

3. Results

Our study showed that storage of the forest soil and agricultural soil at 25 °C did not result in loss of activity. Also paddy soil, which is normally under flooded conditions for long periods of time, did not lose activity when stored in dry state for several months at room temperature. The peat soil from Finland showed only weak H_2-oxidation activity in comparison to other types of soils, but it exhibited stable activity when stored at 4 °C for at least two months after collection and resulted in similar values as reported previously (Lallo et al., 2008). As the peat soil had a low pH (Table 1), the H_2-oxidation activity in this soil was also examined as suspension in citric acid-sodium citrate buffer (pH 3.4), but the activity was

Table 1
Characteristics of the soils used in this study.

Soil	Location	Site	Annual mean T (°C)	Nitrogen (%)	Carbon (%)	pH	Water content (%)	Rate constant at 25 °C, $v_o \pm$ SE (h^{-1})
Forest	Mainz, Germany	50°00′15″N, 8°10′23″E	10.1	0.1	1.33	5.94	16	0.042 ± 0.004
Agricultural	Heidelberg, Germany	49°24′59″N, 8°36′31″E	11	0.1	0.87	7.27	12	0.030 ± 0.002
Arid	Avdat, Israel	30°47′57″N, 34°45′57″E	22	0.012	0.33	8.56	2.4	0.024 ± 0.002
Hyper-arid	Arava, Israel	29°58′44″N, 35°05′16″E	25	BLD	0.06	8.40	BLD	0.012 ± 0.001
Rice paddy	Fuyang, China	30°04′37″N, 119°54′37″E	16.6	0.27	2.81	5.80	2.8	0.018 ± 0.001
Peat	Loppi, Finland	60°36′49″N, 24°21′8″E	4	1.11	44.0	3.50	88	0.012 ± 0.001

BLD: Below levels of detection.