Colloids and Surfaces B: Biointerfaces 74 (2009) 279–283

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Conformation, activity and proteolytic stability of acid phosphatase on clay minerals and soil colloids from an Alfisol

Qiaoyun Huang[a,b,*], Jun Zhu[b], Xueqing Qiao[b], Peng Cai[b], Xingmin Rong[a], Wei Liang[b], Wenli Chen[a]

[a] State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070,China
[b] Key Laboratory of Subtropical Agriculture and Environment of Ministry of Agriculture, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 30 June 2009
Received in revised form 16 July 2009
Accepted 22 July 2009
Available online 30 July 2009

Keywords:
Acid phosphatase
Soil colloid
Clay mineral
Conformation
Activity
Stability

ABSTRACT

The present study was carried out to investigate the conformation, enzymatic activity and proteolytic stability of acid phosphatase on montmorillonite, kaolinite and soil colloids from an Alfisol by means of circular dichroism (CD) spectroscopy, isothermal titration microcalorimetry (ITC) and biochemical assay, respectively. The results showed that the secondary structure of phosphatase was changed from disordered type to ordered form during adsorption/desorption cycle, organic substance and 2:1-clay mineral in Brown Soil benefited the formation of ordered structure. Enzymatic activity of phosphatase was inhibited while the proteolytic stability was promoted after the interaction with active particles from permanent charge soil. The decrease of enzymatic activity and the increase of proteolytic stability resulted by montmorillonite and organic colloid were both greater than that by kaolinite and inorganic colloid, which was in consistent with the extent of structural change induced by different colloid particles. Thus, one of the most significant factors responsible for the variation of enzymatic activity and proteolytic stability might be the hiding or even damage of active sites and the irrecognition of cleavage sites in enzyme molecules induced by the formation of ordered structure. The information obtained in this study is of crucial significance for the understanding of the behavior and fate of extracellular enzymes in soils with permanent charges.

1. Introduction

Soil enzymes play critical roles in maintaining soil productivity and improving soil quality. The activities of soil enzymes have been used as indices of soil fertility, soil quality or soil pollution [1]. The extracellular biomolecules excreted from plant roots and soil microorganisms or faunas are tend to be physically or chemically immobilized on the surface of various soil active particles [2]. The activity and stability of enzymes might be promoted, inhibited or maintained after immobilization [3–9].

The structure of enzymes adsorbed on soil colloidal particles has been investigated by a number of studies. FTIR spectroscopy analysis revealed that the adsorption of ovPrP on montmorillonite induced an increase in β-structures and a loss in α-helices at pHs higher than 4.0 [10]. However, reduced amounts of β-sheet structures were observed for the adsorption of α-chymotrypsin on montmorillonite as the result of water diffusion inside the protein [11].

Structural variations in enzyme molecules interacted with various organic or inorganic soil components or complexes may result in changes in their enzymatic activity and hydrolytic stability by proteinase [12–14]. The activity and stability of enzymes affected by the interaction with natural soil components or clay minerals have been extensively examined. However, few studies paid attention to the conformational changes of enzymes induced by the interaction with soil colloidal particles and the subsequent consequences in activity and stability of enzymes. Acid phosphatase plays an important role in phosphorus cycles of soils. The aim of the present study was to investigate the conformation, enzymatic activity and proteolytic stability of acid phosphatase influenced by its interaction with the active colloidal particles separated from an Alfisol which is a typical permanent charge soil by means of circular dichroism (CD) spectroscopy, isothermal titration microcalorimetry (ITC) and biochemical assay, respectively.

2. Materials and methods

2.1. Enzyme

Acid phosphatase (EC3.1.3.2 Type II, 1.0 units mg^{-1} from potato) was purchased from Sigma Chemical Co., St. Louis, MO.

* Corresponding author at: State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China.
E-mail address: qyhuang@mail.hzau.edu.cn (Q. Huang).

0927-7765/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2009.07.031

Table 1
Basic properties of soil colloids and minerals studied.

Soil colloid and mineral	O.M.* ($g\,kg^{-1}$)	SESA ($m^2\,g^{-1}$)	CEC ($cmol\,kg^{-1}$)	PZC	Clay mineral composition
Organic soil colloid	67.6	31.7	46.2	2.1	Hydromica (45%), Vermiculite(35%), Kaolinite(20%)
Inorganic soil colloid	5.9	37.5	41.2	3.3	Hydromica(45%), vermiculite(35%), Kaolinite(20%)
Montmorillonite	–	73.6	90.2	2.5	Montmorillonite
Kaolinite	–	23.9	7.1	3.6	Kaolinite

* Organic matter (O.M.), specific external surface area (SESA), cation exchange capacity (CEC) and point of zero charge (PZC) were analyzed by $K_2Cr_2O_7$ digestion, N_2 adsorption method (Beijing Analytical Instrument Company), acid-base potentiometric titration, NH_4AcO method [26], respectively. Clay mineral was determined by X-ray diffraction analysis.

2.2. Soil colloids and minerals

Brown soil (Alfisol, pH 7.0) was sampled at the depth of 0–17 cm from Taishan, Shandong province, China. After removal of organic residue, the soil was rinsed in deionized water and the pH value of suspension was adjusted to 7–8 by the addition of 0.01 mol L^{-1} NaOH or HCl solution. The suspension was dispersed by sonication for 30 min and the <2 μm colloidal fraction was separated by sedimentation method. Part of separated colloidal fraction was oxidized by H_2O_2 to remove organic matter. After flocculation by the addition of $CaCl_2$ solution, the colloid was washed with deionized water and ethanol until no more Cl^- and Ca^{2+} ions were detected. The colloid was finally air-dried and ground to pass a 100 mesh (0.154 mm) sieve.

Montmorillonite and kaolinite (CP grade) were purchased from Henan Xinyang montmorillonite company and Shanghai Wusi Chemical Reagent Company, respectively. The <2 μm fraction was separated by sedimentation method after the dispersion by sonication and the adjustment of pH value by 0.01 mol L^{-1} NaOH or HCl solution.

Some basic properties of soil colloids and minerals were listed in Table 1.

2.3. Preparation of desorbed and immobilized phosphatase

In a 10 ml centrifuge tube, 3 mg of soil colloid or mineral was mixed with 3 ml of 10 mmol L^{-1} Tris buffer (pH 7.0) containing 900 μg of phosphatase. The mixture was gently shaken at 25 °C and 250 r min^{-1} for 2 h and then centrifuged at 20,000 × *g* for 15 min. The supernatant in first centrifugation was collected, the phosphatase adsorbed on the residue was then desorbed twice with 1.5 mL of 10 mmol L^{-1} Tris buffer. The desorbed phosphatase was also collected by centrifugation and the final residue was stored. The amount of enzyme in first supernatant and the desorbed phosphatase was determined at 280 nm by UV-spectrophotometer using BSA as the standard. The surplus of desorbed phosphatase was stored at 4 °C for subsequent spectral measurements. The final residue was the complex of immobilized phosphatase and soil active particles, the complex was kept for following enzymatic activity tests and proteolytic stability assays. The amount of immobilized phosphatase was calculated by subtracting the quality of phosphatase in first supernatant and desorbed phosphatase from enzyme added primely.

2.4. Circular dichroism (CD) measurements

The CD spectra were used to investigate the conformational change of phosphatase during adsorption/desorption process. Native phosphatase (dissolved in 10 mmol L^{-1} Tris buffer) and desorbed phosphatase from active soil particles were measured over the wavelength range of 200–260 nm using a Jacob J-810 spectropolarimeter with a 10 mm quartz cylindrical cuvette. Each sample was scanned for three times at a velocity of 200 nm min^{-1} and the average was calculated as the final result. The spectral analysis was performed by calculating the proportion of secondary structures including α-helix, β-sheet, β-turn and random in native and desorbed enzyme molecules as described by Norde and Giacomelli [15].

2.5. Microcalorimetric tests

A TAM III thermal activity monitor (Thermometric AB, Sweden) controlled by ANOVA software was used to test the catalytic reaction heat of free and immobilized phosphatase. The determination was performed under the isothermal titration mode at 25 °C with a 4 ml ampoule and a 250 μl syringe. The complexes of immobilized phosphatase were resuspended in 3 ml of 10 mmol L^{-1} Tris buffer. Freshly prepared *p*-nitrophenyl phosphate (pNPP) solution (100 mmol L^{-1}) was used as the substrate. During the determination, 800 μl of free or immobilized enzyme (0.1 mg ml^{-1}) suspension was introduced to the ampoule and was then dispersed by gentle shaking at 90 rpm. The titration of pNPP solution from the syringe to the ampoule was started after the thermal equilibrium between ampoule and the heat sink was attained. For control experiment, soil particle suspension with Tris buffer (1 mg ml^{-1}) was also titrated by pNPP solution. The titration for each sample was repeated twice and lasted for 10 min. The total heat produced by each sample was obtained after the deduction of dilution and adsorption heat from the control curve. The net catalytic heat for free phosphatase was calculated by subtracting the heat of Tris buffer (dilution heat) from the total heat of free enzyme. All the data was tested for the significance at $p \leq 0.05$.

2.6. Proteolytic stability assays

One hundred microliter of free and immobilized phosphatase was suspended in 3 ml of 10 mmol L^{-1} Tris buffer and was then mixed with 900 μl of proteinase K-Tris buffer containing 20 μg of in a 10 ml centrifuge tube, after thorough dispersion, the mixture was incubated at 37 °C for 1, 2 and 24 h, respectively. One millilitre of Tris buffer containing 6 μmol pNPP was then pipetted into the tube. After incubation again at 37 °C for 1 h, the enzymatic reaction was terminated immediately by the addition of 1 mL of 1 mol L^{-1} NaOH solution. The control was performed in the absence of proteinase K. The concentration of enzymatic product *p*-nitrophenol was determined directly at 405 nm spectrophotometrically. The specific activity of free and immobilized phosphatase in the absence and presence of proteinase K was defined as micro mole *p*-nitrophenol released by the catalysis of 1 mg phosphatase within 1 min. Proteolytic stability of enzyme was calculated according to the following expression: Proteolytic stability(%) = (specific activity of phosphatase in the presence of proteinase K/specific activity of phosphatase in the absence of proteinase K) × 100.