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Binding affinity and adhesion force of organophosphate hydrolase enzyme with soil particles related to the isoelectric point of the enzyme

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ABSTRACT

The binding affinity of organophosphate hydrolase enzyme (OphB) with soil particles in relation to the isoelectric point (pI) was studied. Immobilization of OphB with soil particles was observed by confocal microscopy, Fourier transform infrared spectroscopy (FT-IR), and Atomic force microscopy (AFM). The calculated pI of OphB enzyme was increased from 8.69 to 8.89, 9.04 and 9.16 by the single, double and triple mutant of OphB enzyme, respectively through the replacement of negatively charged aspartate with positively charged histidine. Practically, the binding affinity was increased to 5.30%, 11.50%, and 16.80% for single, double and triple mutants, respectively. In contrast, enzyme activity of OphB did not change by the mutation of the enzyme. On the other hand, adhesion forces were gradually increased for wild type OphB enzyme (90 pN) to 96, 100 and 104 pN for single, double and triple mutants of OphB enzyme, respectively. There was an increasing trend of binding affinity and adhesion force by the increase of isoelectric point (pI) of OphB enzyme.

1. Introduction

Organophosphate (OP) pesticides are a group of highly toxic agricultural chemicals that are widely used to control a wide range of insect pests (Zhang et al., 2006). The organophosphate and carbamate pesticides methyl-parathion and carbaryl have a common action mechanism: they inhibit acetylcholinesterase enzyme by blocking the transmission of nerve impulses (Pérez-Legaspi et al., 2015). In agricultural areas worldwide, there is an increasing concern about watershed contamination due to the widespread use of pesticides (Xing et al., 2015). Papadakis et al. (2015) estimated risk quotient for the insecticides chlorpyrifos ethyl, diazinon and para-thion methyl and herbicide prometryn, and observed above acceptable risk values in the rivers and lakes of northern Greece. The pesticide poisoning is a serious problem for worldwide health hazard and responsible for poisonings of around three million people and 200,000 deaths annually (Yang et al., 2006). Although OP pesticides are banned in most of the developed countries, those are still widely applied in developing countries for the control of major agricultural pests (Zhang et al., 2005). After usage, the residual insecticides were found in the farm produces and aquatic products (Fu et al., 2004) and ecosystem as toxic substances (Tang et al., 2015). Bioremediation using enzymes has become an attractive approach for removing hazardous chemicals such as organophosphate pesticides from the environment (Gao et al., 2014).

Biodegradation is considered as the low cost and eco-friendly technique for the removal of pesticide contaminants from soil. Organophosphate hydrolase (Oph) enzyme exhibits broad substrate specificities and high hydrolytic activities against organophosphates (Yang et al., 2006; Barman et al., 2014). Various gene/enzyme systems involved in degradation of select organophosphates have been reported such as biodegradable *Phragmmites communis* for cypermethrin and chlorpyrifos (Feng et al., 2015), *opd* genes from methyl parathion-degrading bacteria (Zhang et al., 2006), and *opd* genes from fenitrothion-degradaing strain Burkholderia sp. FDS-1 (Zhang et al., 2006).

Degradation of the organophosphorus pesticide has been studied using the marine isolate, *Streptomyces venezuelae* ACT 1 (Naveena et al., 2013). However, if the microbial organophosphate hydrolase (Oph) enzyme binds with the soil particles it can be a good way for long-term biodegradation of pesticide from contaminated soil. Previously, binding affinity of organophosphate hydrolase (Oph) enzyme with soil particles has not yet been studied. Some researches detected protein molecules (enzyme) by using different instruments such as Mevskaya and Chirgadze (1976) determined α-helical structures of protein by infrared spectra (FT-IR), and Chen et al. (2004) and Kuznetsov et al. (2005) also

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detected single bio-molecules by the atomic force microscopy (AFM). Considering these facts, binding affinity of organophosphate hydrolase enzyme (OphB) with soil particles had been studied by the confocal microscopy, FT-IR and AFM.

Besides, proteins/enzymes are macromolecules with large affinities for water-solid interfaces. Moreover, they are flexible polymeric chains with lateral groups having contrasting physicochemical properties such as hydrophilic or hydrophobic, negatively, neutrally, or positively charged lateral chains and they also have strong and complex interactions with the extensive mineral and organomineral surfaces in soil (Quiquampoix, 2000; Rigou et al., 2006). So, different factors such as types of soil (montmorillonite, kaolinite, and sea sand) had been studied on the soil binding affinity of OphB enzyme.

On the other hand, the electrostatic interaction between the protein and the negatively charged montmorillonite is most probably the origin of strong adsorption of protein (Rigou et al., 2006). Thus, in this study, surface negative charged amino acids (aspartate) from the opposite site of the active site of OphB enzyme had been replaced with positive charge amino acids (histidine) by site directed mutagenesis to increase isoelectric point of OphB enzyme to observe whether the isoelectric point of OphB has any role in the binding with soil particle or not. In this case, atomic force microscopy (AFM) has been used to detect binding force among OphB enzyme and soil particles. Because, atomic force microscopy (AFM) has been recognized as one of the powerful tools for not only the analysis of surface morphologies and properties but also the measurement of individual molecular interactions as a force spectroscopy (Kikkawa et al., 2008). Moreover, AFM has been utilized as an advanced method for the evaluation of affinity under *in vitro* conditions. By measuring a force curve, an adhesive force between a substrate and a tip on a cantilever can be detected as the bending amplitude of the cantilever in a separating motion of the tip from the substrate after approach and contact has been made (Okada et al., 2008). Some researchers have examined force curves and succeeded in evaluating interactions between one biomolecule immobilized on a cantilever tip and another biomolecule on the substrate (Rief et al., 1997; Schafer et al., 2007). Similarly, in this experiment, binding affinity and adhesion force of OphB wild type enzyme and OphB mutated enzymes (increased isoelectric point) on mica surface (properties like clay particle) were measured to determined relationship between isoelectric point (pI) and adhesion force or binding force by AFM.

2. Methods and methods

2.1. Cloning of ophB gene into pZsGreen vector and purification of OphB-GFP enzyme

To construct fusioned green fluorescent protein (GFP) with OphB, the PCR products were generated with the sense primer 2480F, 5′-ATTAAAGCTTATGCCCACCCACAAGGA-3′ (*Hin*dIII site is indicated by underline) and antisense primer 2481R, 5′-ATTGGTACCTTCTTGGGGTTGACGACCG −3′ (*Kpn*I site is indicated by underline). PCR was performed using *oph*B gene (accession number: HM191723) from the genomic DNA of *Pseudomonas* sp., Super-Therm DNA polymerase (JMR, Side Cup, Kent, UK), 1.5 mM $MgCl_2$, and 35 cycles of denaturation at 94 °C for 30 s, annealing at 66 °C for 30 s, and extension at 72 °C for 30 s followed by final incubation at 72 °C for 10 min. The PCR product of approximately 1030 bp was isolated from an agarose gel using a gel extraction kit (iNtRON Biotechnology, Suwon, Korea). The amplified fragments were ligated into pZsGreen vector (Clontech, CA, USA) using *Hin*dIII and *Kpn*I sites and transformed into *E. coli* DH5α. Expression of fusioned green florescent with OphB was observed in recombinant *E. coli* DH5α harboring pZsGreen/*oph*B by confocal microscopy (Fig. S1). For the purification of GFP and OphB enzymes, PCR amplification of *oph*B gene with *gfp* from the pZ*s*Green/*oph*B was conducted. The sense primer 2478 F 5′-TAATGGATCCGCCGCACCGCAGGT-3′(*Bam*HI site is indicated by underline) and antisense primer 2479 R 5′-ATTAAAGCTTTCAGGGCAATGCAGA-3′(*Hin*dIII sites are indicated by underline) were used and the PCR condition was same like previous. The PCR product of 1610 bp was isolated after agarose gel electrophoresis using a gel extraction kit (NucleoGen, Seoul, Korea). Amplified fragments were cloned into the expression vector pET-28a (+) (Novagen), resulting in the addition of a C-terminal $(His)_6$ tag. Purification of expressed His_6-tagged protein was carried out accordingly as previously described by Guo et al. (2005) and protein (OpdB-GFP) was eluted with 100 mM imidazole with 0.1% Triton X-100. The protein concentration was determined by the method of Bradford (1976) and set up concentration in 50 μg/ml.

2.2. Confocal microscopy of OphB bound soil samples

The binding affinity of OphB enzyme with montmorillonite soil, kaolinite soil and sea sand was observed by confocal microscope according to Islam et al. (2010). The fusioned protein of GFP with OphB was incubated separately with montmorillonite soil, kaolinite soil and sea sand suspensions (50 mg/ml, pH 7.0). The protein soil mixtures were shaken at 180 rpm for 1 h at 25 °C. Enzyme bound soil was washed by distilled water with shaking (at 180 rpm) to remove unbound protein from soil suspension for 20 min at 25 °C. Incubated soil samples with bound protein were examined with a laser scanning confocal microscope (Olympus FluoView™ FV1000) set at 496–506 nm, with control (only soil samples).

2.3. Fourier transform infrared spectroscopy of ophb bound soil sample

To study the FT-IR adsorption spectra of OphB enzyme bound with soil particles, the soil suspension (50 mg/ml montmorillonite soil, pH 7.0) was shaken (at 180 rpm) with OphB enzyme (1 mg/ml) for 1 h at 25 °C. Enzyme bound soil was washed by distilled water with shaking (at 180 rpm) to remove unbound enzyme from soil suspension for 20 min at 25 °C. The enzyme bound soil (30 mg) was mixed with 10 mg of anhydrous KBr. The mixture was pressed into a 13 mm diameter pellet. FT-IR spectra were obtained with a vertex 80 v (Bruker Optics) fourier transform infrared spectrometer in vacuum state (Fu et al., 1999; Lee et al., 2010). The cell containing the samples was flushed with N_2 gas for 10 min before scanning to remove atmospheric water vapor and CO_2 from the interferometer. The data were analyzed by Grams/32 software (Galactic Industries, Salem, NH, USA).

2.4. Atomic force microscopy (AFM) of OphB bound soil samples

The binding affinity of OphB enzyme with montmorillonite soil, kaolinite soil and sea sand was observed by AFM according to previous research (Naidja et al., 2002; Cheung and Walker, 2008). For OphB enzymes binding with montmorillonite soil, kaolinite soil and sea sand suspensions (50 mg/ml, pH 7.0), the enzyme soil mixtures were shaken at 180 rpm for 1 h at 25 °C. Enzyme bound soil was washed by distilled water with shaking (at 180 rpm) to remove unbound protein from soil suspension for 20 min at 25 °C. Incubated soil samples with bound enzyme were observed by AFM with control (only soil samples) on mica surface. Moreover, OphB enzyme structure also observed on the mica surface to detect similar enzyme structure on the soil particles. The used atomic force microscope (AFM) was XE-100 (PSIA Corp., Korea). The spatial and vertical resolutions are less than 1 nm and the field is between 5 μm and 10 μm. The images were taken at high resolution (256 × 256 pixels) by using an intermittent-contact mode (called Tapping Mode™) coupled with phase detection imaging (PDI). The probe is in silicon (Si) chip of the NSC36 series (PSIA, Suwon, Korea) with a round tip of 0.4 mm. It has features such as 30° of full tip cone angle, a range of 20–25 μm of tip height, typical tip curvature radius of uncoated probe of < 10 nm, and the reflective side coated with Al. The resonant frequency, the stiffness and the amplitude of cantilever was