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In situ surface transfer process of Cry1Ac protein on SiO_2: The effect of biosurfactants for desorption

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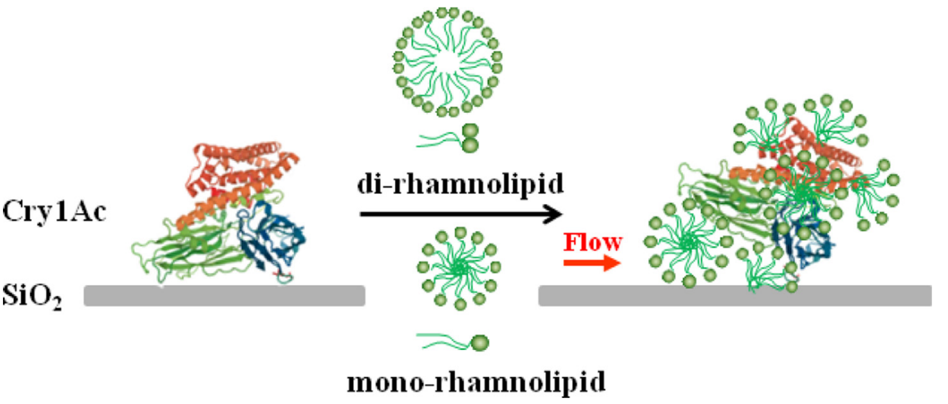
HIGHLIGHTS

- QCM-D for real-time monitoring of Cry1Ac adsorption and desorption kinetic.
- PCEA govern the adsorption of Cry1Ac to SiO_2 surface is demonstrated.
- Comparing mono-, di- and complex-rhamnolipid on the application of removing Cry1Ac.
- Rhamnolipids can rinse Cry1Ac rapidly and completely at ionic strength up to 50 mM.
- Cry1Ac can be desorbed during rhamnolipid solutions alkaline pH.

GRAPHICAL ABSTRACT



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ABSTRACT

Genetically modified *Bacillus thuringiensis* (*Bt*) crops, which have been widely used in agricultural transgenic plants, express insecticidal Cry proteins and release the toxin into soils. Taking into consideration the environmental risk of Cry proteins, biosurfactant—rhamnolipids were applied to desorb Cry proteins from soil environment, which has not been elucidated before. Quartz crystal microbalance with dissipation (QCM-D) was used in this article to investigate the adsorption and desorption behaviors of Cry1Ac on SiO_2 surface (model soil). Results showed that patch-controlled electrostatic attraction (PCEA) governed Cry1Ac adsorption to SiO_2, and the solution pH or ionic strength can affect PCEA. The adsorption kinetics could be fitted by the pseudo-second-order model, and the adsorption isotherm was fitted to Langmuir model with correlation coefficients higher than 0.999. The desorption characteristics of Cry1Ac from SiO_2 were assessed in the presence of mono-rhamnolipid, di-rhamnolipid or complex-rhamnolipid. Mono-rhamnolipid exhibited the most significant positive effect on desorption performance. With a complete removal of Cry1Ac reached when mono-rhamnolipid concentration was up to 50 mg L^{-1}. Additionally,

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the desorption was enhanced at alkaline pH range, and Cry1Ac can be completely and rapidly desorbed by rhamnolipids from SiO_2 at ionic strength of 5×10^{-2} M.

1. Introduction

In the agricultural production of many countries, the insecticidal crystalline (Cry) proteins of *Bacillus thuringiensis* (*Bt*) has been applied for the breeding of transgenic plants to bio-control of the invertebrate pests [1]. The *Bt* crops carry the trait of pest insect resistance by expressing one or more *Bt* gene that code for insecticidal Cry proteins (*Bt* toxins). It is possible that Cry proteins could enter into soil environment through secretion by crop roots, crop residues or deposition of pollens [2–6]. After that, Cry proteins could be quickly absorbed and deposited on soil particles surfaces [7]. In general, proteins are adsorbed on soil constituents strongly, particularly on clay mineral [8]. Thus, the mobility of Cry proteins is severely restricted in the natural environment. Cry proteins bound to soil could persist in soil for a long time [9], and maintain its insecticidal properties[10] due to high conformational stability of Cry proteins [11,12]. Cry proteins could also affect soil biodiversity due to their toxicity to microorganisms and invertebrates [13], and pose a long-term threat to the environment. Assessing the potential risks requires that the processes controlling the fate of Cry proteins in soil are understood. Adsorption and desorption behaviors of Cry proteins affect Cry proteins mobility, degradability and bioavailability, and, hence, accumulation and persistence. Actually, these subsequent behaviors depend largely on soil type (e.g., clay mineral composition, pH, other physicochemical characteristics), season (e.g., water tension, temperature) and other environmental factors [14].

Recently, several studies have investigated the adsorption of Cry proteins onto silica, humic acids, clay minerals, mica and natural soils [15–21]. However, only a few studies of the desorption behavior of Cry proteins adsorbed on soils or soil minerals have been carried out. The limited literatures indicated that only a small proportion of adsorbed toxin were removed after washing with either water or buffer in most cases [20–24]. In view of the environmental risk of *Bt* toxin, a more effective method to desorb it from soil environment should be sought out. Soil washing with extracting solutions has been presented as a promising technique for contaminated soil remediation in recent years. The washing solutions include different types of chemical agents, such as acids, surfactants, and chelating agents [25]. Currently, a number of studies paid close attention to the application of surfactants in enhancing the desorption and mobilization of contaminants in soil. Surfactants could facilitate the wetting, emulsification, and solubilization of various types of inorganic and organic contaminants [26]. Surfactants are commonly composed of both hydrophobic and hydrophilic portions, which could form micelle aggregates in aqueous phase to promote the solubilization of contaminants and their transport from soil to aqueous phase [27]. Significantly, surfactants could be adsorbed on soils, which represents a major constraint for their application in remediation [28]. Chemical surfactants such as sodium dodecyl sulfate (SDS), Tween 80 and Triton X-100 were widely applied in environmental remediation in previous studies [29–31]. However, some synthetic surfactants are toxic and cause a potential risk for human health and environment. Accepted as a kind of less toxic and more prominent alternative, biosurfactants, such as rhamnolipids, possess several distinct advantages in comparison with their chemically synthesized equivalents: such as less toxicity, higher biodegradability, better foaming properties, higher selectivity [32], and tolerance to extreme temperatures, pH, and salinity [33]. In addition, rhamnolipids show relatively low adsorption tendency on soil, as compared with synthetic surfactants [34]. Rhamnolipids have been often considered for environmental contaminants remediation [35], such as the recovery of heavy metal [36–39], PAHs [27,28], and other various of contaminants in previous studies. However, a good understanding of various factors involved in rhamnolipids-enhanced remediation is still a challenge in many respects. Previous investigations focused on the effect of rhamnolipids dosage, soil composition and environmental factors such as pH and ionic strength on the removal of contaminants in the soil-water system [27,35]. More importantly, changes in solution pH and ionic strength would significantly affect the morphology of rhamnolipids [40–42]. However, few studies have explored the effect of rhamnolipid structure. The structure of rhamnolipids was an important parameter for the mobilization of contaminants in soil, as different structures may have different micelle and surface properties, which in turn influence the efficiency of solubility enhancement and the transport of contaminants.

Some soil washing technologies have been used to study the environmental behaviors of Cry proteins in soil. N.Helassa et al. elucidated the adsorption and the desorbability of Cry1Aa in contact with two clays by the batch equilibrium method [8]. Saxena et al. studied the vertical movement of Cry1Ab in repacked soil columns [43]. These methods determine the amount of adsorbed/desorbed Cry proteins commonly by using a commercial ELISA (enzyme-linked immunosorbent assay) kit. However, the quantitative method of the ELISA kit have many operating steps and have strict requirements for experimental operation. In contrast, the *in situ* technique, QCM-D, is a simple, precise and fast method for protein quantification [44], and can measure minute changes in mass adsorbed layer on the solid surface. Moreover, the method can provide information on the structure of the adsorbed layer [45,46], thereby exploring the interaction between Cry proteins and soil components at the molecular level.

To our best knowledge, no studies have focused on *Bt* toxin desorption by rhamnolipids. Moreover, the studies that integrate the process of adsorption with desorption of *Bt* toxin has also not been reported. In this study, Cry1Ac was chosen because of its expression in several commercially important *Bt* crops. SiO_2 with a ubiquitous mineral of relatively simple structure, was the predominant constituent of sand and silt fraction in many soils. Y. Asci et al. chose the quartz as soil components to explore the adsorption-desorption of heavy metal on the soil [37]. The adsorption-desorption behaviors of Cry1Ac by rhamnolipids on SiO_2 were systematically investigated. An *in situ* surface technique, quartz crystal microbalance with dissipation monitoring (QCM-D) was employed in quantifying adsorption of Cry1Ac and the effect of different rhamnolipids on Cry1Ac desorption in SiO_2-water systems, as well as evaluating the effects of solution pH, ionic strength on desorption. The results show important implications in soil washing technologies for protein-contaminated soil remediation.

2. Materials and methods

2.1. Protein, biosurfactant and chemicals

High purity Cry1Ac (lyophilized) (>85%, molecular weight (MW_{Cry1Ac}) = 64 kDa) was from Envirologix Inc, USA (details in