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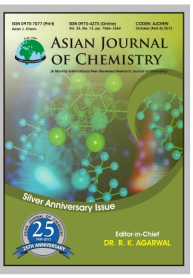

Adsorption Thermodynamic Characteristics of Cry1Ab Toxin from *Bacillus thuringiensis* in Chinese Latosol

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The adsorption thermodynamics of Cry1Ab toxin of *Bacillus thuringiensis* (Bt) in Chinese latosol was studied. The adsorption isotherms of Cry1 Ab toxin followed Langmuir equation ($R^2 > 0.98$) and the curves belonged to L type. The adsorption increased with the increased temperature and the maximum adsorption amount between 283 and 313 K ranged from 4.50×10^{-6} to 7.55×10^{-6} mol g^{-1}. The results show that the environmental risk of Cry1Ab toxin in Chinese latosol probably enhances if temperature increases. The adsorption of Cry1Ab toxin in Chinese latosol was a spontaneous, endothermic and entropy-increasing process. The separation factor ranged from 0.1865 to 0.4682, indicating that the adsorption of Cry1Ab toxin in Chinese latosol was favourable. The adsorption energy for Cry1Ab toxin ranged from 8 to 16 kJ mol^{-1}, indicating that it was an ion-exchange mechanism.

Key Words: *Bacillus thuringiensis*, Toxin, Latosol, Adsorption, Thermodynamics.

INTRODUCTION

Since GM crops first commercialized in 1996, *Bacillus thuringiensis* (Bt) crops became an integral component of pest management practices in many countries[1,2]. The planting of GM crops has consistently increased by 10 % or more each year worldwide. Transgenic plants encoding Cry1Ab toxins release these proteins into the soil through root exudates during the vegetative period[3,4] and through the incorporation of plant biomass into soil after harvest[5]. Cry1Ab toxins were easily adsorbed onto clay-sized fractions, humic acids and soils[6-9]. Main traits of adsorption to clay minerals are a rapid adsorption (0.5-3 h), with optimum pH between 6 and 8, mostly non reversible[10]. The adsorption affects protein mobility, bioavailability, degradability and persistence, therefore, some Cry proteins were shown to persist in soil for months following harvest and some to remain insecticidal in soils[5,11]. The toxin was not utilized as a source of carbon or carbon plus nitrogen when bound on montmorillonite and kaolinite homoionic to Na or clay-size fractions[12].

Previous studies showed that temperature had no significant influence on the adsorption of Cry1Ab toxin[13,14], therefore, this issue received little attention for a long time. However, recent studies reported that the adsorption of Bt toxin by montmorillonite was endothermic while the adsorption by kaolinite, goethite and silica was exothermic[15]. The characteristics that thermal effects of toxin adsorption changed with minerals attracted attention of researchers. In addition, although several reports investigated the thermodynamics of toxin adsorption on the surface of minerals, the thermodynamic behaviors of toxin adsorption in soil has received little attention. Adsorption thermodynamic behaviors are important factors for evaluating the environmental risk, as this process governs Bt toxin fate and bioavailability. Latosol is a zonal soil distributed in the tropical and subtropical areas. With the large scale cultivation of transgenic crops expressing Bt insecticidal proteins in this region, the problem of environmental safety caused by these Bt crops has received extensive attention[16]. The objectives of this study is to: (1) analyze the influencing laws of temperature on the adsorption of Cry1Ab toxin in Chinese latosol; (2) obtain the thermodynamic parameters (Gibbs standard free energy ΔG^0, standard enthalpy change ΔH^0, standard entropy change ΔS^0 and adsorption energy E). These results can extend our current knowledge on the fate and behavior of Cry1Ab toxin in the tropical and subtropical regions and benefit to evaluate its environmental risk.

EXPERIMENTAL

Preparation of the purified Cry1Ab toxin: A genetically modified strain *B. thuringiensis* subsp. *kurstaki* HD-1 provided by the State Key Laboratory of Huazhong Agricultural University. The purified Cry1Ab toxin was prepared as described by Helassa *et al.*[10]. The molecular weight of the toxin was 66 kDa as determined by polyacrylamide gel electrophoresis (SDS-PAGE).

Preparation of soil: The Chinese latosol was sampled from the 0-17 cm layer of a cultivated land in Leizhou, Guangdong province, China. The air-dried samples were homogenized, crushed and passed through a 100-mesh sieve[17]. Organic matter and cation exchange capacity the specific surface area were determined by $K_2Cr_2O_7$ digestion and extraction with NH_4-acetate, respectively[18]. The specific area was determined by the N_2 adsorption method using the ST-2000/ST-08A instrument (Beijing Analytical Instrument Company). Some properties of soil are listed in Table-1.

TABLE-1
SOME PROPERTIES OF THE TROPICAL SOIL USED

Soil	OM (g kg^{-1})	SSA (m^2 g^{-1})	CEC (cmol kg^{-1})
Latosol	21.6	35.1	18.9

Adsorption thermodynamics: The adsorption isotherms were measured in the range of toxin concentrations from 4.85 × 10^{-6} to 1.82 × 10^{-5} mol L^{-1} and soil concentration of 1.0 mg mL^{-1} at pH 7.0. The soil-toxin mixtures were shaken at 300 rpm at 5 ± 1 ºC, 25 ± 1 ºC and 45 ± 1 ºC, respectively. After 3 h, the suspension was centrifuged at 20,000 g for 20 min and the absorbance of supernatant was measured as indicated previously. The data obtained from the adsorption process are fitted into Langmuir equation[19].

$$q_e = \frac{q_{max} bC_e}{1 + bC_e} \quad (1)$$

where q_e is the amount of adsorption at equilibrium, mol g^{-1}; q_{max} is the maximum adsorption capacity, mol g^{-1}; C_e is the equilibrium concentration of toxin in the bulk solution, mol L^{-1}; b is the constant related to the energy of adsorption (L mol^{-1}).

The essential feature of the Langmuir isotherm can be expressed by means of R_L, a dimensionless constant referred to as the separation factor or the equilibrium parameter. R_L is calculated using the following equation[20]:

$$R_L = \frac{1}{1 + bC_0} \quad (2)$$

where C_0 is the initial Cry1Ab toxin concentration, mol L^{-1}.

Thermodynamic parameters such as Gibbs free energy (ΔG^0), standard enthalpy change (ΔH^0) and standard entropy change (ΔS^0) for the process were calculated using the equations[21,22]:

$$\Delta G^0 = -RT \ln K_{ads} = -RT \ln (55.5b) \quad (3)$$

$$\ln K_{ads} = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (4)$$

where ΔG^0 is the standard free energy changes of the adsorption processes, J mol^{-1}; R is the universal gas constant, 8.314 J mol^{-1} K^{-1}; T is the absolute temperature, K; K_{ads} is equilibrium constant of the adsorption processes, dimensionless; 55.5 is the molecular weight of water, mol L^{-1}; ΔH_0 is the standard enthalpy changes of the adsorption processes, J mol^{-1}; ΔS_0 is the standard entropy changes of the adsorption processes, J mol^{-1} K^{-1}.

Energy of adsorption: The mean energy of Cry1Ab toxin adsorption can be calculated by eqn. (5)[23]:

$$E = \frac{1}{\sqrt{-2K_E}} \quad (5)$$

where E is the mean energy of adsorption, J mol^{-1}; K_E (negative value) is the parameter related to the adsorption energy, mol^2J^{-2}, it can be calculated by the linear form of Dubinin-Radushkevic (D-R) equation:

$$\ln q_e = \ln q_{max} - K_E\varepsilon^2 \quad (6)$$

where ε is the Polanyi potential, $\varepsilon = RT\ln(1+1/C_e)$, J mol^{-1}.

Analysis of transmission electron microscope: Before and after adsorption of Cry1Ab toxin, particles of latosol were dispersed in the water. Soil suspension was coated on the copper wire. After air drying, the particles of latosol were detected by TEM (H-600, Hitachi Ltd., Japan).

Statistics: Data are expressed as the means ± the standard errors of the means (±SEMs). Unless indicated otherwise, the SEMs are within the dimensions of the figure symbols.

RESULTS AND DISCUSSION

Adsorption isotherms: The adsorption isotherm is the equilibrium relationship between the concentration in solution and concentration in the adsorbent surface at a given temperature. As shown in Fig. 1, the adsorption isotherms of Cry1Ab toxin followed Langmuir equation (R^2> 0.98), the curves is of the L-type with an initial steep rise in uptake followed by a gradual increase to a more or less flat plateau at 283, 298 and 313 K. The adsorption rose faster at high temperature than at low temperature. The maximum adsorption amount q_{max} of the toxin by latosol at three temperatures was 4.50 × 10^{-6}, 5.99 × 10^{-6} and 7.55 × 10^{-6} mol g^{-1}, respectively (Table-2).

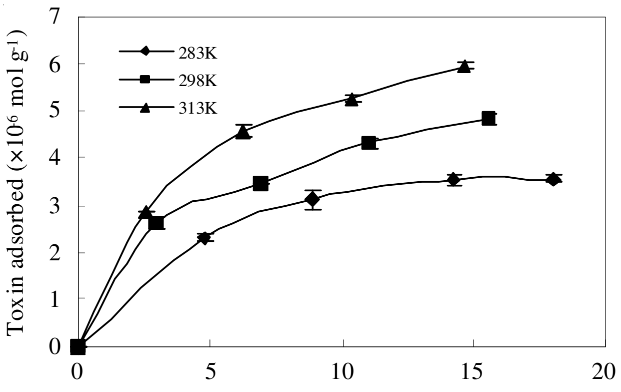


Fig. 1. Adsorption isotherm of the Cry1Ab toxin by latosol

TABLE- 2
LANGMUIR PARAMETERS FOR THE ADSORPTION OF THE Cry1Ab TOXIN

Temperature (K)	b (L mol^{-1})	K_{ads}	q_{max} (mol g^{-1})	R^2
283	2.35×10^5	1.31×10^7	4.50 ×10^{-6}	0.9970
298	2.34×10^5	1.30×10^7	5.99×10^{-6}	0.9891
313	2.40×10^5	1.33×10^7	7.55×10^{-6}	0.9987

The effect of the isotherm shape has been discussed with a view to predict whether an adsorption system is favorable or unfavorable. The value of R_L indicates the type of the isotherm to be either unfavorable ($R_L > 1$), ($R_L = 0$). Values of R_L were shown in Table-3.

TABLE-3
SEPARATION FACTOR CONSTANT OF Cry1Ab TOXIN ADSORBED BY LATOSOL

Temperature (K)	Initial concentration of Bt toxin (mol L^{-1})	Separation factor (R_L)
283	4.848×10^{-6}	0.4672
	9.091×10^{-6}	0.3187
	14.39×10^{-6}	0.2280
	18.18×10^{-6}	0.1895
298	4.848×10^{-6}	0.4682
	9.091×10^{-6}	0.3095
	14.39×10^{-6}	0.2287
	18.18×10^{-6}	0.1901
313	4.848×10^{-6}	0.4623
	9.091×10^{-6}	0.3144
	14.39×10^{-6}	0.2246
	18.18×10^{-6}	0.1865

The variations of the separation factor (R_L) of Cry1Ab toxin adsorption by latosol ranged from 0.1865 to 0.4682 (Table-3), which indicated that the Cry1Ab toxin was favourably adsorbed by tropic soil. The R_L values decreased as the initial concentration of Bt toxin increased, but minor changes of R_L were observed between 283 and 313K at a fixed toxin initial concentration.

Adsorption thermodynamic parameters: The thermodynamic parameters of Cry1 Ab toxin adsorbed by latosol are shown in Table-4.

TABLE- 4
THERMODYNAMIC PARAMETERS OF CRY1AB TOXIN ADSORBED BY TROPICAL SOIL

Temperature (K)	ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1})
283	-38.55	0.4740	137.9
298	-40.58		
313	-42.69		

Gibbs free energy (ΔG^0) of the toxin adsorbed by latosol ranged from -38.55 to -42.69 kJ mol^{-1}. In general, the adsorption belongs to chemical process when the ΔG^0 is more than 40 kJ mol^{-1}. The negative values of the adsorption ΔG^0 by latosol indicated that Cry1Ab toxin adsorption occurred *via* a spontaneous process, implying that changes in the protein structure favor the adsorption process[24]. The values of ΔG^0 increased with the increase of temperature, indicating that the adsorption had higher spontaneous trend at high than low temperature. The Langmuir isotherm equation has been widely used in adsorption process. However, the theoretical sense of the concentration of adsorbate in the Langmuir equation is usually paid little attention. Liu[25] pointed out that the concentration of adsorbate used in Langmuir isotherm equation must be expressed as its molar concentration, otherwise, it would eventually leads to misapplication of the Langmuir isotherm sequation in calculation of the change in standard free energy (ΔG^0) of adsorption. In our studies, the concentration of Cry1Ab toxin was mol L^{-1} and the exchange adsorption between adsorbate and dissolvent was also considered.

The adsorption of protein onto adsorbent-solution interface can be denoted as a substitutional adsorption process between the protein molecules in the aqueous solution (Protein(sol)) and the water molecules on the adsorbent surface $[H_2O_{(ads)}]$[21,22,26]:

$$\text{Protein}_{(sol)} + XH_2O_{(ads)} \rightleftharpoons \text{Protein}_{(ads)} + XH_2O_{(sol)} \quad (7)$$

where $\text{Protein}_{(sol)}$ and $\text{Protein}_{(ads)}$ are the protein molecules in the aqueous solution and adsorbed on the mineral surface, respectively; $H_2O_{(sol)}$ and $H_2O_{(ads)}$ are the water molecules in the aqueous solution and adsorbed on the mineral surface, respectively; X is the size ratio representing the number of water molecules replaced by one molecule of protein.

The equilibrium constant K_{ads} (dimensionless) is not equal to the parameter 'b' from Langmuir equation. The 'b' in Langmuir equation is not a dimensionless parameter where as its dimension is the reciprocal of solute concentration. According to the previsions of IUPAC (International Union of Pure and Applied Chemistry), the standard equilibrium constant for calculating the ΔG^0 must be dimensionless[27].

The relationship between K_{ads} and 'b' for the liquid-solid adsorption process in dilute solution is expressed as follows[27,28]:

$$K_{ads} = b \times a_{H_2O}^{sol} \quad (8)$$

where $a_{H_2O}^{sol}$ is the water activity in solution. If the solution is dilute and the adsorbed phase is an ideal solution, the activity of water ($a_{H_2O}^{sol}$) in solution can be replaced by its mole concentration (55.5 mol/L)[21,22,29]. Thus

$$K_{ads} = 55.5b \quad (9)$$

The ΔH^0 of Cry1Ab toxin adsorbed by tropical soil was 0.4740 kJ mol^{-1} (Table-4). The positive sign of ΔH^0 indicated that the adsorption of toxin on tropical soil was endothermic, favorable adsorption would occur at higher temperature. Fu[15] reported that the adsorption of Cry1Ab toxin by montmorillonite was endothermic (ΔH^0 = 9.0 kJ mol^{-1}) while by kaolinite, goethite and silica was exothermic. Zhou *et al.*[26] verified that the adsorption of Cry1Ab toxin by montmorillonite, kaolinite and silica was exothermic and the adsorption amount increased with the decreasing temperature. Helassa *et al.*[30], however, observed that the adsorption affinity of Cry 1Ab protein in soil was less at lower temperature, which was consistent with our results. The interaction between the toxin and soils probably ascribed to hydrophobic affinity[30,31], clay minerals are usually considered to be hydrophilic surfaces and soil organic matter may considerably enhance hydrophobicity leading to macroscopic changes in soil properties, therefore, Cry1Ab toxin variety, soil type and soil organic matter may affect the adsorption of Cry1Ab toxin in soil.

The ΔS^0 of toxin adsorbed by latosol was 137.9 J mol^{-1} K^{-1} (Table-4). The positive sign of ΔS^0 indicated that the freedom increased when the toxin adsorbed by latosol, suggesting that the entropy was the driving forces for adsorption[32,33]. As the molecular volume of water is very small compared to Bt protein, the adsorption of one mole protein result in the desorption of dual mole water, therefore, mutual penetration of hydration layer causing disordering of the water, following the increase of entropy.

Energy of Cry1Ab toxin adsorption: The relationship between ln q_e and ε^2 during the adsorption of Cry1Ab toxin by tropical soil was shown in Fig. 2.

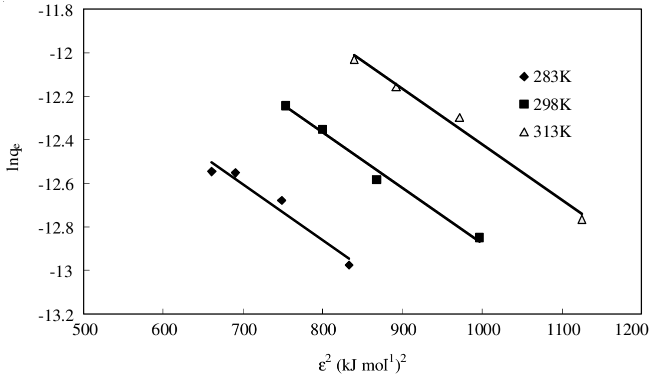
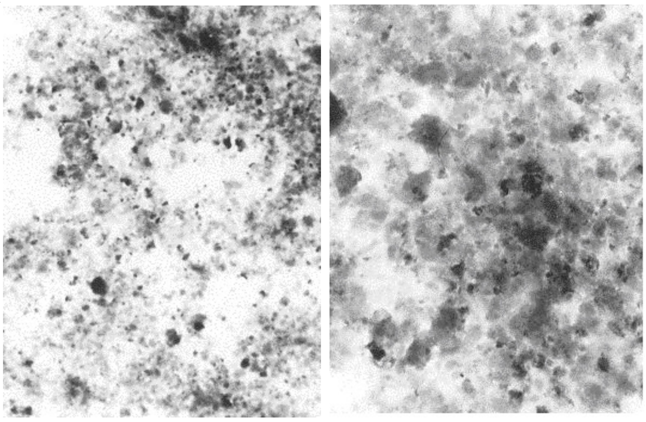


Fig. 2. Relationship between ln q_e and ε^2 during the adsorption of Cry1Ab toxin by latosol

The mean energy of Cry1Ab toxin adsorption at 283 K, 298 K and 313 K was 13.92, 14.04 and 13.98 kJ mol^{-1}, respectively. The magnitude of E is useful for estimating the type of sorption reaction, *i.e.*, an energy ranged from 8 to 16 kJ mol^{-1} indicated an ion-exchange reaction. The above results suggested that the adsorption of Cry1Ab toxin in latosol belong to ion-exchange process.

Analysis of TEM: The size of latosol particles which are fully dispersed in the water ranged from 100 to 400 nm (Fig. 3). There is no significant change before and after adsorption of Cry1Ab toxin.



(a)Before adsorption of toxin (b)After adsorption of toxin

Fig. 3. TEM images of latosol particles before and after adsorption of toxin

Conclusion

The adsorption isotherms of Cry1Ab toxin followed Langmuir equation ($R^2 > 0.98$) and the curves belonged to L type. The adsorption increased with the increased temperature between 283 and 313 K, suggesting that the environmental risk of Cry1Ab toxin in latosol probably enhances if temperature increases. The adsorption of the Cry1Ab toxin by latosol was a spontaneous, endothermic, entropy-increasing and favourable process. The separation factor R_L ranged from 0.1865 to 0.4682, indicating that the adsorption of Cry1Ab toxin in latosol was favourable. The adsorption energy for Cry1Ab toxin ranged from 8 to 16 kJ mol^{-1}, indicating that it was an ion-exchange mechanism.

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