1 (C 1)

H L , *, 1, J , 1, , F , M , H ,
H , F H

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ABSTRACT

A ,
, .
H , fl , , C 1 A
, *Apis cerana*. fl (λ = 332) C 1
fi . D , $\Delta H > 0$, $\Delta S > 0$,
C 1 .
fl fl C 1 ,
. M -
.
C (CD) α- C 1
C 1 . I , C 1 fl β-
50% (K_A) 0.28−2.53 /

N , , 15 . A - - , 16 , fi . F , (AO) LD_{50} 72 69.68 / 17 . fi , , , ( ) 18 . / , fl 19,20 . C , - *CSP8* , *Plutella xylostella*, 21 , *CSP1* *CSP2* , *Bombyx mori*, 22 , *CSP1* fl , *Bemisia tabaci*, 23 . C - , C , . A , *Apis cerana*, fl , 24 . I , C 1 , fi β- . I C 1 *A. cerana* 25 . A , , 26–28 , , - fl , , fi - C 1 C 1 . , *A. cerana*, .

2. Materials and methods

2.1. Chemicals and reagents

I (>97%) (F .1(A)) β- (>98%) CI . J , (, A) 1.0 × 10 $^{-3}$ L^{-1} , 4 °C . M - (18.2 MΩ, M ,) , .

2.2. Preparation of recombinant CSP1 protein

CSP1 fi - C , MD18- 29 . *CSP1* E -32 (+) BL21 (DE3) C 1 . A 1 L^{-1} I G, C 1 - fi fi I ™ N $^{2+}$-N A , B (H 7.4) 72 4 °C C 1 . fi C 1 B , fi 1 μ L^{-1}, −20 °C .

2.3. Multiple fluorescence spectra of CSP1 protein with imidacloprid

(1) F . F F-5301 fl (, J). fl - 281 , fl 5 , 290–500 . C 1 1 μ L^{-1} B (H 7.4), 1 . C 1 1 L^{-1} , fl fl . 284 K 294 K, .

(2) fl (F) . F C 1 Δλ (λ - λ) = 15 60 , . C 1 .

(3) ( ) -1800 - (, J). C 1 C 1 190–400 .

2.4. Circular dichroism (CD) spectra

C 1 , CD 200–250 . 1: 0, 1: 0.5 1: 4, . C 1 CD ELCON3 30 .

2.5. Molecular docking analysis

31 . - C 1 *Mamestra brassicae* C M A6 (DB , 1 8) 32 I -MODEL 33 . 3D NCBI (: 638014). B M D (M D) 4.2 ( ), C 1 . M D O M D , 34 .

2.6. Functional inhibition of CSP1 by imidacloprid

1 μ L^{-1} C 1 10 L^{-1} β- 2.3-(1) . fl fl

C 1 . C 1/ (1:0, 1:1, 1:5, 1:9, 1:13 μ L^{-1}) 10 L^{-1} β- , . β- C 1 .

3. Results and discussion

3.1. Expression of recombinant CSP1 protein and fluorescence quenching spectra

E -32 (+)/C 1 BL21 (*DE3*) *E. coli*. A I G, fi N^{2+}-N A , C 1 B . D - AGE fi F . 1, L 1 2 E 32-C 1 - 1 L^{-1} I G. L 3 fi C 1 . C 1 28 KD, 95%. C 1 , F . 2(A), fl C 1 - 332 , . I fl C 1 . , C 1.

3.2. Synchronous fluorescence spectra

I - fl ( ) C 1 , fl $\Delta\lambda = 15$ 60 , . B fl - fl , 35 . I ,

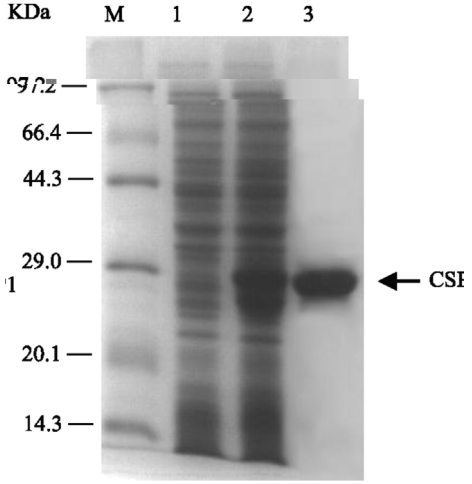


Fig. 1. SDS-PAGE of induced and purified recombinant CSP1 protein. M . L 1 2 E 32-C 1 1 L^{-1} I G, . L 3 fi C 1 , fi .

A F . 2(B C), fl fi , fl - C 1 . , fl , . I , F . 2(C), 36 . I , (F . 2(B)). - C 1 - , 37 , 38 , - 39 , .

3.3. Fluorescence quenching mechanism

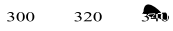
F 40 . D - , - . fl 41 . D - 42 :

$$\frac{F_0}{F} = 1 + K_q\tau_o[Q] = 1 + K_{sv}[Q] \quad (1)$$

F_0 fl fl ; F fl fl Q ; K_q fl ; ($\tau_0 = 10^{-8}$); K_{sv} - 43 . fl 284 294 K , . A F . 3(A) 1, K_{sv} , - . - , , 44 . , , 45 . A F . 3(B), C 1 - (1 μ L^{-1}), C 1 , fi . I , C 1 - A 2 *A. cerana* 46 , - .

3.4. Thermodynamic analysis

I , , - , 47 . - :



$$\Delta G = -RT \quad K = \Delta H - T\Delta S \tag{2}$$

$$\Delta H = \frac{RT_1T_2 \quad (K_{0,2}/K_{0,1})}{T_2 - T_1} \tag{3}$$

$$\Delta S = (\Delta H - \Delta G)/T \tag{4}$$

$\Delta G, \Delta H$ ΔS G , , . ΔH <0 ΔS <0, . $\Delta H > 0$ $\Delta S > 0$, . $\Delta H < 0$ $\Delta S > 0$, 48 . - C 1 1. $\Delta G < 0$, C 1 . $\Delta H > 0$ $\Delta S > 0$, . - A 2 46 , .

3.5. Circular dichroism (CD) spectra

CD 49 . 208 222 CD α- 50 . A F . 3(C), C 1 , , , , C 1 . A 2 , 46 .

3.6. Molecular docking

A C M A6 (DB ID, 1 8) *Mamestrabrassicae* 32 , - C 1 I -MODEL . C 1 C M A6 (1 8) F . 4(B). A M D (2), C 1 - F . 3(A). B , 8 C 1 (11, 43, 44, I 62), (G 63), 2 (A 9 A 40), (H 47) (F . 3(A)). F F . 3(A), (73 82) C 1, fl C 1 fl (F . 3(B)).

Table 2
I C 1 .

	N	E
	44	−24.1035
G	63	−16.0482
A	9	−9.4608
	43	−9.3838
A	40	−8.6277
H	47	−7.3377
I	62	−6.9446
	11	−4.0133

E :
.

3.7. Functional inhibition of CSP1 by imidacloprid

F C 1 , 10 L^{-1} β- 1 μ L^{-1} C 1 C 1 (C 1: 1:1, 1:5, 1:9 1:13,). 281 , fl fl 332 . K_A L 51 :

$$\frac{F_0 - F}{F} = K_A + n\ [Q] \quad (5)$$

F_0 fl fl ; F fl fl Q ; K_A ; n . A F . 4(C) 3,

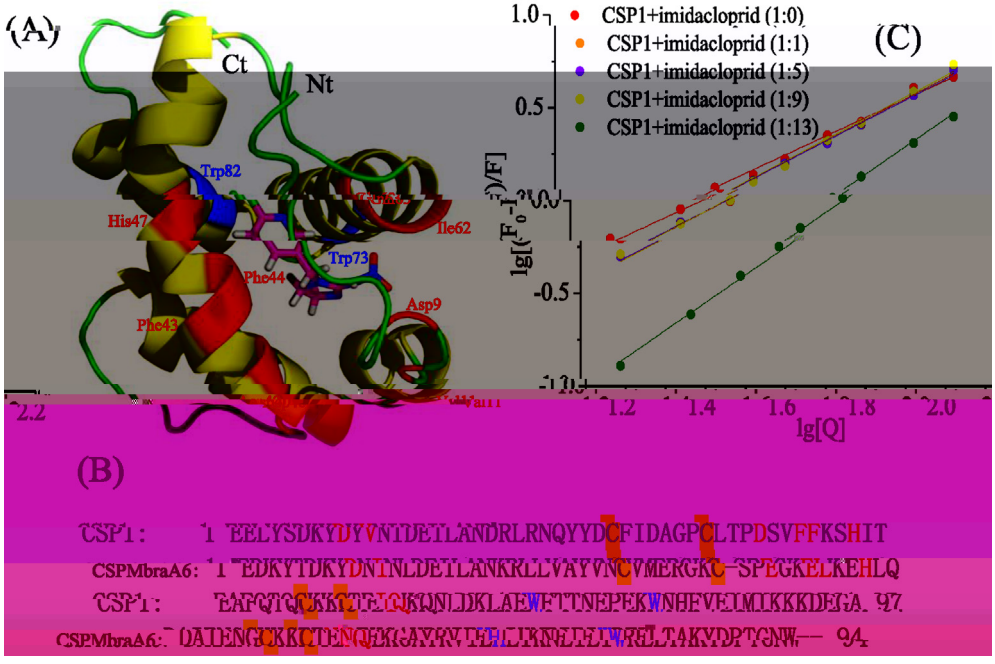


Fig. 4. Molecular docking of CSP1 with imidacloprid (A) and the Double-Log plots of the fluorescence of the mixture of CSP1 and imidacloprid quenching by β-ionone (C).
(A). I α- N- fl . . B . (B). A C 1 C M A6 (1 8). 8 C 1 . 4 . (C). C 1 1 μ L^{-1}, β- 10 L^{-1} (fi 0, 1, 5, 9, 13 μ L^{-1}, . fi β- 20, 30, 40, 50, 60, 76, 93, 125 157 μ L^{-1},). (F fi , .)

K_A (1: 0) β

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