2014

ISSN : 0974 - 7435 Volume 10 Issue 22

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(22), 2014 [13894-13898]

Structural and functional analysis of domain Ⅲ of cry1ie toxin

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ABSTRACT

Cry toxins are insecticidal proteins encoded by *cry* gene in *Bacillus thuringiensis*. *Cry1I* gene is silent in Bt, but can be over expressed as Cry1Ie toxin protein in *E.coli*. Its active fragment, IE648, can bind with insect peritrophic membrane, which is an important barrier that Cry toxin must pass through before binding with midgut epithelium. Research of our team indicated that domain Ⅲ of Cry1Ie seems to take a part in the interaction. In this study structural features of domain Ⅲ protein has been analyzed, its function was predicted. It has a β-sandwich structure composed of two bundles of antiparallel β sheets, structurally resemble the carbonhydrate binding proteins, may play a role in binding with polysaccharide like chitin of peritrophic membrane.

KEYWORDS

Bacillus thuringiensis; Cry1Ie; Domain Ⅲ; Structure; Function.

INTRODUCTION

Bacillus thuringiensis (Bt) can synthesize cry δ-endotoxins during sporulation phase, which are has highly specific insecticidal activity to lepidopteran larvae. Bt therefore has been widely used as biopesticide[1]. *Cry1I* gene was silent in Bt, but can be over expressed in *E.coli*, with a Cry1I protein of 719 amino acids as a result[2,3]. Its active fragment, IE648, corresponding to its first 648 amino acids from N-terminal, has higher insecticidal activity. It doesn't compete for Cry1Ac binding sites in the brush border membrane vesicles (BBMV) of the Asian corn borer (ACB), indicating that IE648 may be a good candidate for a multiple-toxin strategy in potential control of ACB insect pest resistant to Cry1Ac[4]. So far among 3D Cry toxins the three dimensional structure of seven different 3D-Cry toxins have been solved, Cry1Aa, Cry2Aa, Cry3Aa, Cry3Ba, Cry4Aa, Cry4Ba and Cry8Ea[5-11]. 3D structure of Cry1Ie or IE648 has not yet.

Peritrophic membrane (PM) is a semipermeable matrix that protects the insect gut epithelial cells from injury and infection. Before binding to midgut cells Cry toxins must cross it[12]. It is composed of chitin and protein-glycojugates[12]. IE648 was reported to be able to bind with PM. But the accurate binding site and mechanism remain unclear[4]. According to some results of our lab that has not been published, domain Ⅲ can bind with PM specially.

In the present study, the domain Ⅲ of Cry1Ie was characterized. The protein was subjected to several online and desktop based bioinformatics tools to study physicochemical properties. The structure reflects function, so the 3D structure of the protein was established using homology modeling approach. Some structural features was analyzed. The study will give us a direction to future research about function of domain Ⅲ.

METHODS

Amino acid sequence of the target protein was retrieved from NCBI Protein Database in FASTA format, The physicochemical parameters of the protein sequence that includes amino-acid and atomic compositions, molecular weight and isoelectric point (pI) were computed by Protparam program available at ExPaSY (www.expasy.org). SWISS-MODEL[13-16] was used to obtain the 3D structural model; Swiss PDBViewer was used for structural magic fit.

RESULT AND DISSCUSS

Homology-modelling

Amino acid sequence of Cry1Ie was got from NCBI protein database, its accession is AAG43526. 3D structure model of domain Ⅲ of Cry1Ie was modeled by Swiss-Model. The tool website automatically take Cry 1Aa (PDB ID 1CIY) as its template. Domain Ⅲ is a β-sandwich structure, composed of two bundles of β sheets, each bundle has four or five antiparallel β sheets (Figure 1.).

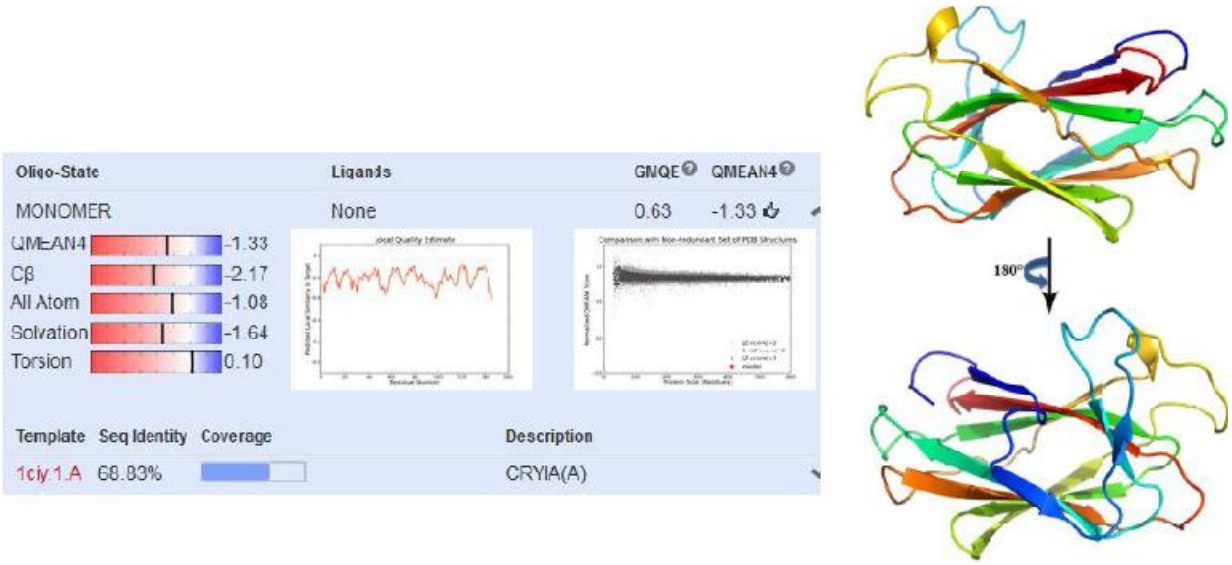

Figure 1 : 3D structure model of domain Ⅲ of Cry1Ie

Structure fit

A fitting database was built with the 3D similarities of Cry1Aa in Protein Data Bank (PDB), the template of modeling. Kicking those Cry toxins out, there are 54 proteins in this fitting database. Carry out the magic fit through Swiss PDBViewer between the model of domain Ⅲ and the proteins in the database one by one, record the involved atoms and calculate the RMS value as TABLE 1.

TABLE 1 : Fitting database

No.	Involved atoms	RMS	Protein	No.	Involved atoms	RMS	Protein
1	132	12.43	PDP:4BJ0Aa	28	41	2.51	PDP:2W47Aa
2	103	21.65	PDP:3AFGAa	29	40	4.62	PDP:2ZEXAa
3	86	8.95	d1gwma_	30	40	5.08	PDP:2W5FAa
4	77	14.53	PDP:4C91Ad	31	40	13.14	PDP:3MX0Aa
5	75	15.83	d2o14a1	32	39	10.63	d1w9sa_
6	71	3.36	PDP:3ZM8Aa	33	37	5.46	PDP:3JQWAa
7	70	15.86	PDP:3ECQAh	34	36	9.3	PDP:2Y8KAb
8	68	7.02	PDP:2WZ8Aa	35	35	8.59	PDP:2WAOAa
9	63	9.38	PDP:2QUOAa	36	34	11.64	PDP:4JDNAa
10	62	3.47	PDP:4QB6Aa	37	34	11.89	PDP:4P02Ba
11	62	6.08	PDP:2W87Aa	38	32	7.87	PDP:4QHWAa
12	58	13.22	PDP:4DEVAa	39	32	10.08	PDP:4D0QAa
13	56	11.68	d1gu3a_	40	32	11.66	PDP:2WAAAa
14	56	23.38	PDP:4KWUAe	41	31	11.35	PDP:1WCUAa
15	55	8.37	PDP:4QPWAa	42	30	4.08	PDP:3HWJAa
16	54	12	d1v0aa1	43	30	7.76	PDP:2XOMAa
17	52	3.76	PDP:3WNOAc	44	30	8.28	PDP:4QAWAc
18	52	15.91	PDP:4JDOAa	45	29	9.01	d1uxza_
19	51	6.21	PDP:2ZXQAd	46	26	8.36	d1od3a_
20	51	11.49	PDP:4CE8Aa	47	22	7.45	PDP:3NQHAb
21	49	11.43	PDP:4AW7Ab	48	20	8.58	d1tvga_
22	48	3.61	PDP:2VZPAa	49	18	3.99	PDP:3C7FAb
23	43	11.71	d2id4a1	50	16	3.7	PDP:2Q1FAa
24	43	19.27	PDP:3ZIWAb	51	14	7.56	d1gnya_
25	43	10.03	PDP:2BGOAa	52	11	1.97	PDP:2DCKAb
26	42	11.12	d1uy4a_	53	11	4.64	PDP:2YC2Aa
27	42	14.15	PDP:2CDOAa	54	9	0.76	d1w0na_

In order to select several proteins with higher similarity with domain Ⅲ, one selecting condition was set: the number of involved atoms above 60, RMS below 5. There are two proteins fulfilled the condition.

One is GH26 endo-β-1,4-mannanase from *Podospora anserine* (No. 6, PDB ID 3ZM8) as shown in Figure 2, the molecular can catalyze the random hydrolysis of 1,4-β-D-mannosidic linkages in mannans, galactomannans, glucomannans and galactoglucomannans[17].

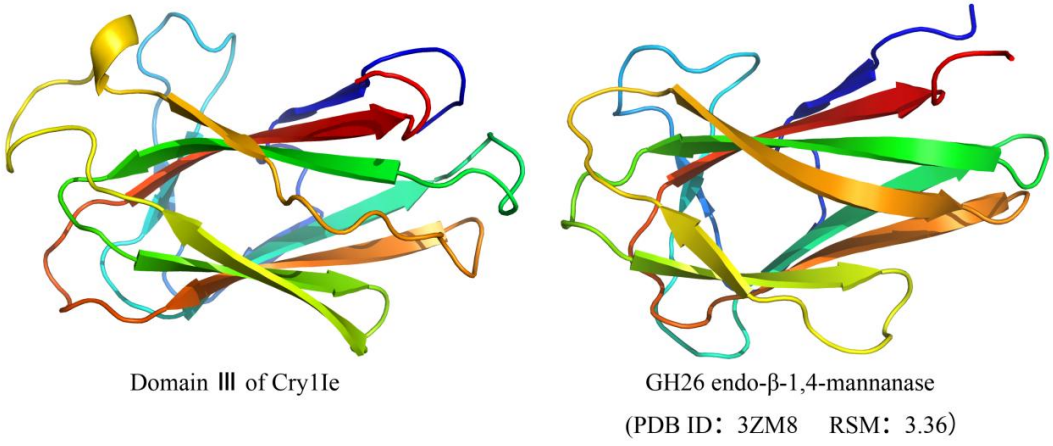

Figure 2 : Structure Fit of domain Ⅲ and GH26 endo-β-1,4-mannanase

The other one is glucuronoxylanase Xyn30D (No. 10, PDB ID 4QB6), it is a modular enzyme containing a family 30 glycoside hydrolase catalytic domain and an attached carbohydrate binding module of the CBM35 family[18].

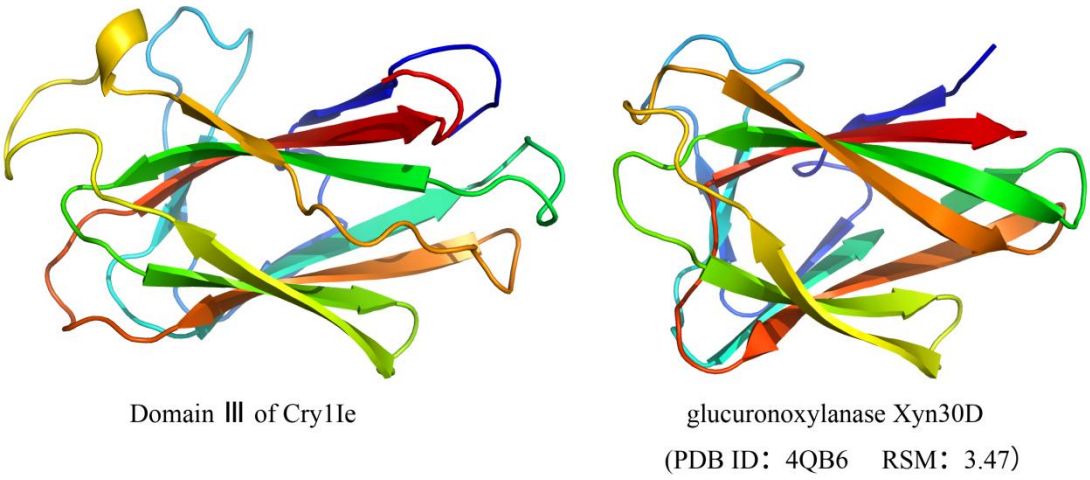

Figure 3 : Structure Fit of domain Ⅲ and glucuronoxylanase Xyn30D

The two proteins are all carbohydrate binding proteins, many of other proteins in the fitting database also have carbohydrate binding ability, similarity between domain Ⅲ and them suggest that domain Ⅲ may also have this ability. It can bind with peritrophic membrane of Asian corn borer, which is composed of chitin and proteins. Based on this analysis, domain Ⅲ probably bind with PM through binding chitin in it. More experiments are needed to conform this. Interaction between Cry toxin and chitin has never been reported before.

CONCLUSIONS

Domain Ⅲ has a β-sandwich structure composed of two bundles of antiparallel β-sheets, to a great excent it structurally resemble carbonhydrate binding proteins, indicating that it may also have similar function and play a role in the interaction between Cry1Ie toxin and carbonhydrate.

ACKNOWLEDGEMENT

This work was supported by the Natural Science Foundation of China (*grant* no. 31171911).

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