Chinese Journal of Chemistry, **2009**, *27*, 2085—2089 **Full Paper**

Homology Modeling of Cyt2Ca1 of *Bacillus thuringiensis* and Its Molecular Docking with Inositol Monophosphate

ZHAO, Xinmin[a,b](赵新民) XIA, Liqiu*[,a](夏立秋) DING, Xuezhi[a](丁学知)
YU, Ziquan[a](余子全) LÜ, Yuan[a](吕媛) TAO, Wenna[a](陶文娜)

[a] *Key Laboratory for Microbial Molecular Biology of Hunan Province, College of Life Science, Hunan Normal University, Changsha, Hunan 410081, China*
[b] *Department of Chemistry and Environmental Engineering, Hunan City University, Yiyang, Hunan 413000, China*

Cyt2Ca1 is an insecticidal crystal protein produced by *Bacillus thuringiensis* ET29 during its stationary phase, and this δ-endotoxin demonstrates remarkable insecticidal activity against not only insects of the order *Coleoptera*, but also against fleas, and in particular the larvae of the cat flea, *Ctenocephalides felis*. The first theoretical model of the three-dimensional structure of Cyt2Ca1 was predicted and compared with Cyt2Aa, which is lethal to insect larvae. The three-dimensional structure of the Cyt2Ca1 was obtained by homology modeling on the structures of the Cyt2Aa protein. The deduced model resembles previously reported Cyt2Aa toxin. A binding mode of inositol monophosphate as a polar head group of the putative membrane phospholipid ligand to Cyt2Ca1 was presented using molecular docking. The residues of Leu9, Glu21, Tyr23 and Gln110 of the Cyt2Ca1 toxin are responsible for the interactions with inositol monophosphate via eight hydrogen bonds. Those residues could be important for receptor recognition. This binding simulation will be helpful for the design of mutagenesis experiments aimed at the improvement of toxicity, and lead to a deep understanding of the mechanism of action of Cyt toxins.

Keywords *Bacillus thuringiensis*, Cyt2Ca1, molecular docking, homology modeling, inositol monophosphate

Introduction

Bacillus thuringiensis (Bt) is a class of gram-positive spore-forming bacteria with entomopathogenic properties, which produce insecticidal proteins during the sporulation phase as parasporal crystals. These crystals are predominantly comprised of one or more proteins (Cry and Cyt toxins), also called *δ*-endotoxins.[1] Cyt proteins are parasporal inclusion proteins from *Bacillus thuringiensis* that exhibits hemolytic (cytolitic) activity or has obvious sequence similarity to a known Cyt toxin. These toxins are highly specific to their target insects, innocuous to humans, vertebrates and plants, and completely biodegradable. Therefore, Bt is a viable alternative for the control of insect pests in agriculture and of important human disease vectors.[2] Cry2Ca1 toxin is an insecticidal crystal protein produced by *Bacillus thuringiensis* ET29, and demonstrates remarkable insecticidal activity against not only insects of the order Coleoptera, but also against fleas, and in particular the larvae of cat flea, *Ctenocephalides felis*.[3]

There is no sequence or structure homology between the Cry family and Cyt family but they share common biochemical properties. To date, the only solved crystal structure of the Cyt family is that of Cyt2Aa, which is distinguished from the Cry structure and is composed of a single *α*-*β* domain comprising two outer layers of *α*-helix hairpins and a *β*-sheet in between.[4] More recently, the crystal structure of Cyt2Ba has been elucidated, which has a striking similarity to the protoxin form of Cyt2Aa, suggesting that the toxic monomer of these proteins has a similar mode of activity against cell membrane.[5]

It is proposed that Cyt toxins do not bind to protein receptors but directly interact with membrane lipids inserting into the membrane and forming pores[4-7] or destroying the membrane by a detergent-like interaction.[8] However, the mechanism of action of Cyt toxins is still under controversy. According to one approach, the two outer layers of *α*-helix hairpins swing away from the *β*-sheet upon membrane contact, and the three last *β*-strands are allowed to insert into the membrane. Consequently, oligomerization with other monomers and formation of a *β*-barrel pore occur, resulting in a colloid osmotic lysis.[9,10] Another model suggests that the hydrophilic side of the helices interacts with residues from other monomers to form oligomer, which results in nonspecific aggregation of Cyt molecules on the surface of the lipid bilayer, leading to a detergent-like action and membrane disassembly.[8,11] The cytolysis of erythrocytes and mosquito cells is thought to be primarily due to the detergent-like action of the 25-kDa Cyt1Aa

* E-mail: xialq@hunnu.edu.cn; Fax: 0086-0731-8872298
Received February 7, 2009; revised May 4, 2009; accepted June 15, 2009.
Project supported by the National Natural Science Foundation of China (Nos. 30670052, 30570050), 863 Program of China (Nos. 2006AA02Z187, 2006AA10A212) and the Scientific Research Fund of Hunan Provincial Education Department (No. 08C203).

toxin on membrane phospholipids.[6] Incubation of the 24- and 25-kDa Cyt1Aa toxins with liposomes of phosphatidylinositol resulted in decreased activity of these toxins.[12] Phosphatidylinositol is an important lipid as a key membrane constituent. We use 1-L-myo-inositol-5-phosphate (PI) to represent the polar head group of the phospholipid as a ligand to study its interaction with Cyt2Ca1. This binding simulation will be helpful for the design of mutagenesis experiments aimed at the improvement of toxicity, and lead to a deep understanding of the mechanism of action of Cyt toxins.

Methods

An alignment of the amino acid sequences of Cyt1Aa (PDB entries 1CBY) and Cyt2Ca1 was produced with Clustalw program (http://www.ebi.ac.uk/c-lustalw/#). This alignment project file was submitted to Swiss-model in the ExPASy server (http://www.expasy.ch/spdbv/) and a preliminary model for Cyt2Ca1 was retrieved. The model was geometry optimized with calculation of the Hyperchem program[13] and recalculated by molecular dynamics at the same time. To perform the molecular mechanics geometry optimization and molecular dynamics, the following parameters were chosen. Dielectric: distance dependent, Scale factor: 1, both Electrostatic and van der Waals 1-4 scale factors: 0.5, Cutoffs: none, RMS gradient: 0.1, Heat time: 0.1 picosecond. Starting temperature: 100 K, Simulation temperature: 300 K, Temperature step: 30 K, and the default values for the other variables. The inositol monophosphate used for docking is 1-L-myo-inositol-5-phosphate. The structure file is from Klotho Biochemical Compounds Declarative Database (www.biocheminfo.org/klotho/, accession number: KLM0000001). Docking studies were performed using Molegro software.[14] This docking program is based on a new hybrid search algorithm, called guided differential evolution. The guided differential evolution algorithm combines the differential evolution optimization technique with a cavity prediction algorithm, which is dynamically used during the docking.

The Ramachandran plot of Cyt2Ca1 model was generated by the Swiss-model. The model was also validated with PROSA energy and ERRAT by submitting the coordinates to the online servers[15,16]. Sequence identities were calculated with ICM-Pro.[17] Ribbon representations and electrostatic potentials of Cyt2Ca1 were generated with Pymol program.[18] The final model was submitted to the PMDB database (http://www.caspur.it/PMDB/), and the PMDB identifier is PM0075609.

Results and discussion

Overall architecture

The final sequence and structural alignment of Cyt2Ca1 to Cyt2Aa (1CBY) show 57% sequence identity, which suggests this alignment is suitable for the construction of a reliable three-dimensional structure of Cyt2Ca1 (Figure 1). The three-dimensional structure of the model of Cyt2Ca1 is presented in Figure 2. The final model comprises 212 amino acid residues spanning amino acids 5 to 216 of the Cyt2Ca1 toxin. It is composed of a single domain of α/β architecture with a β-sheet surrounded by two α-helical layers representing a cytolysin fold. The sheet consists of six antiparallel β-strands ($\beta1$—$\beta6$) flanked by an α-helix layer composed of αA, αB and αF on one side and by a second α-helix layer composed of αC, αD and αE on the other. The four longest β-strands ($\beta2$, $\beta4$, $\beta5$ and $\beta6$) of the central β-sheet have a modified Greek-key topology (Figure 2). $\beta1$ and $\beta3$ are short, from then on the polypeptide chain forms a β-meander of $\beta4$, $\beta5$ and $\beta6$ to fill in the middle of the sheet, except a short helix αE inserted in the loop between $\beta5$ and $\beta6$.

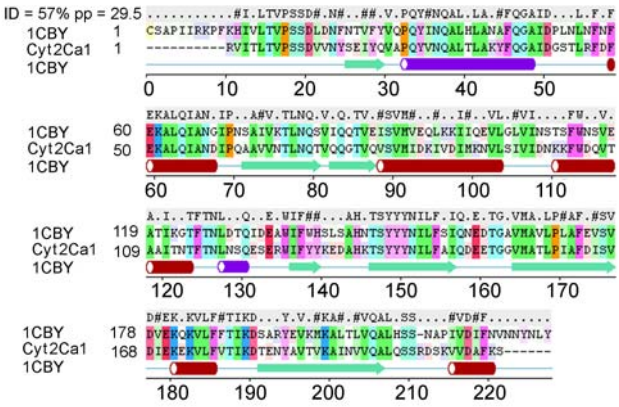


Figure 1 Alignment of the amino acid sequences between Cyt2Ca1 and Cyt2Aa (1CBY). Secondary structure elements of Cyt2Aa are indicated under the sequence. α-Helices are indicated by cylinders, and β-sheets by arrows. ID: sequence identity.

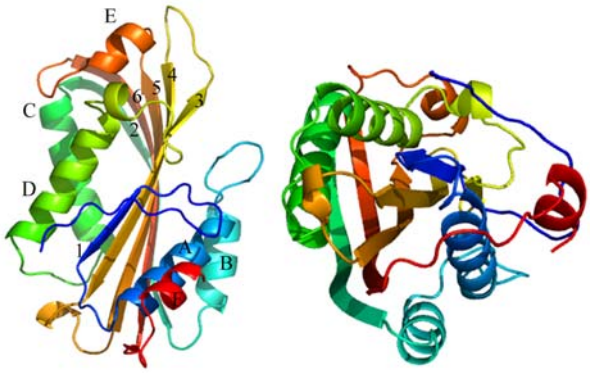


Figure 2 Ribbon representations of Cyt2Ca1 fold. Overall view of Cyt2Ca1 fold composed of one domain (left) and upper view of Cyt2Ca1 fold (right).

A striking similarity was observed between the structures of Cyt2Ca1 and Cyt2Aa (rmsd of 0.20 Å). The surface electrostatic potential distributions of Cyt2Ca1 and Cyt2Aa are not apparently different (Figures 3 and 4). We assume that the two toxins share the same mechanism of action.

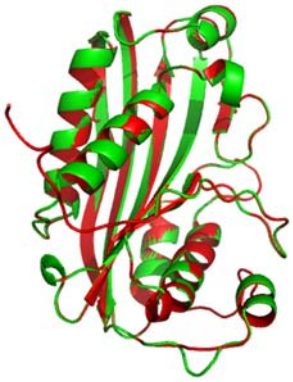


Figure 3 Superposition of the overall ribbon structures of Cyt2Ca1 (green) and Cyt2Aa (red).

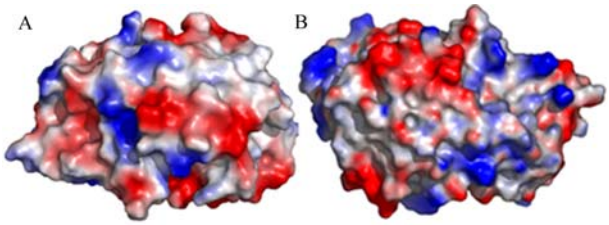


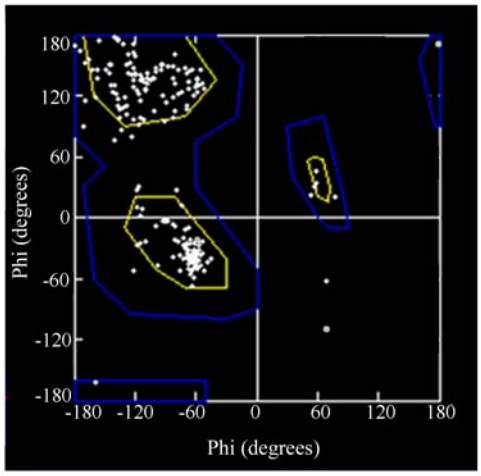
Figure 4 Surface representations of the electrostatic potential of Cyt2Ca1 (A) and Cry2Aa (B). Positive electrostatic potentials are blue, and negative electrostatic potentials are red.

Validation of the model

The Ramachandran plot indicats that most (99.5%) of residues have φ and ψ angles in the core and allowed regions (Figure 5). PROSA and ERRAT were also used to evaluate the homology model.[15,16] PROSA is a diagnostic tool that is based on the statistical analysis of all available protein structures. The potentials of mean force compiled from the data base provide a statistical average over the known structures. PROSA calculates an overall quality score for a specific input structure. If this score is outside a range characteristic for native proteins the structure probably contains errors. PROSA energy Z-score of Cyt2Ca1 is -6.57, which is within the range of scores found for all the experimentally determined protein chains. ERRAT is so called over all quality factor for non bonded atomic interactions and higher scores mean higher quality. The normally accepted range is >50 for a high quality model. In the current case, the ERRAT score of Cyt2Ca1 is 87.437. The backbone conformation and non bonded interactions of the homology model are all within a normal range. Therefore, all the evaluations indicate that the homology mode structure is very reasonable.

Molecular docking

The binding site in Cyt2Ca1 was predicted by Molegro program. It is located in the *N*-terminal arm and against the β-sheet. Leu9, Glu21, Tyr23 and Gln110 of the Cyt2Ca1 toxin are responsible for the interactions



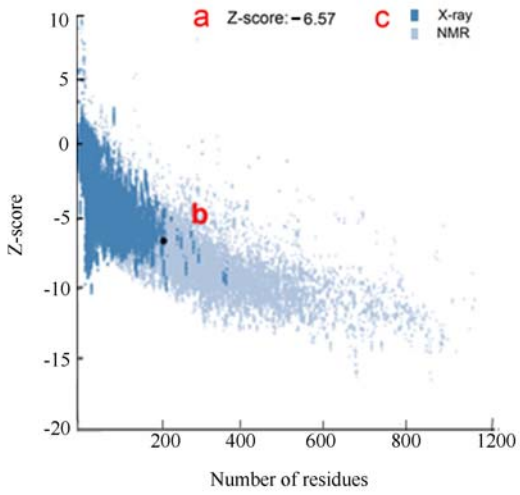


Figure 5 Ramachandran plot of ψ and φ and PROSA energy of Cyt2Ca1. The Z-score of PROSA energy indicates overall model quality, its value is displayed in a plot that contains the *Z*-scores of all experimentally determined protein chains in current PDB. In this plot, groups of structures from different sources (X-ray, NMR) are distinguished by blue and gray colors. It can be used to check whether the Z-score of the input structure is within the range of scores typically found for native proteins of similar size.

with inositol monophosphate via eight hydrogen bonds, five of which were related to the hydroxyls of inositol group, and the other three were related to hydroxyls of phosphate acid.

Cry proteins are specifically toxic to the insect orders *Lepidoptera*, *Coleoptera*, *Hymenoptera* and *Diptera*, and also to nematodes.[19] In contrast, Cyt toxins are mostly found in Bt strains active against *Diptera*. The attachment of Cry proteins to the larval intestinal membrane is mediated through specific receptors,[2] whereas Cyt proteins attach nonspecifically and are mediated by nonsaturated phospholipids such as phosphatidylcholine, phosphatidylethanolamine and sphingomyelin.[6] Further studies demonstrated that the determinants important for toxin binding were the nature of the lipid polar head group.[20]

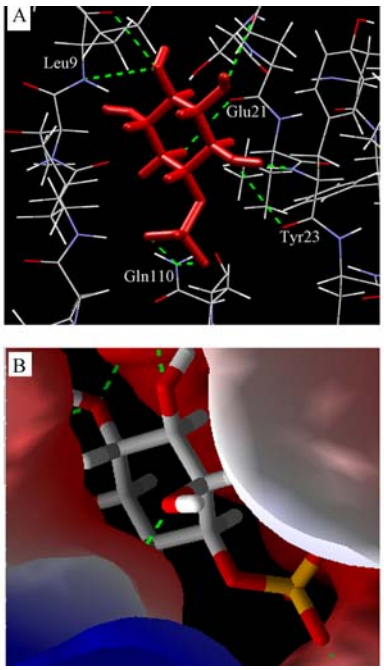


Figure 6 Visualization of the molecular docking of inositol monophosphate with Cyt2Ca1. The toxin is presented in wire style and hydrogen bonds are presented in green dotted lines (A), and in electrostatic protein surface (B).

Here, our study supports this hypothesis. A strong interaction of Cyt2Ca1 with the inositol monophosphate of membrane phospholipids was found, which is in clear contrast with the receptor-mediated insecticidal action of the genus specific Cry toxins.

As the polar head groups are presented in all the phospholipids, the phosphate acid group of the phospholipids must be very important not only as a constituent of the phospholipids, but also as a binding determinant. Our docking results show that the hydroxyls of each phosphate acid have interaction to Gln110 with two hydrogen bonds. Thus, the cytolytic toxin apparently did not act as a nonspecific detergent, but rather interacted with phospholipid receptors on the cell membrane. Such an interaction of the toxin with phospholipids probably results in the increased cell permeability, thereby causing cell lysis.

It is presently uncertain whether all the Bt toxins constitute human health risks. It should be noted, however, that (i) proteolysis under alkaline conditions is essential to activate the proteins,[21] (ii) Bt subsp. *israelensis*-based bioinsecticides are not harmful to humans,[22] although they contain the Cyt proteins highly toxic for a wide range of human cells, and (iii) there have been no reports of food-mediated human illness caused by Bt. Several investigators have reported that strong natural inhibitors aganist Cyt1 and Cyt2 proteins are commonly contained in vertebrate sera.[23,24]

Bt subsp. *israelensis* exhibits the most potent bacteria-derived mosquito larvicidal activity known so far. This stems from synergy between four toxins of Cry family (Cry4Aa, Cry4Ba, Cry10Aa, and Cry11Aa) and three toxins of Cyt family (Cyt1Aa, Cyt2Ba, and Cyt1Ca).[25] Bt subsp. *israelensis* has been used for nearly 30 years in large-scale mosquito and black fly control programs and resistance has not been detected in mosquito populations in nature that have been subjected to selection from Bt subsp. *israelensis* toxins.[26] The lack of resistance to Bt subsp. *israelensis* is due to the presence of the Cyt1Aa toxin in the crystal.[27] However, many Bt-resistant field populations of diamondback moth have been found in Asia and North America. Laboratory selection has produced Bt-resistant strains of more than a dozen other pests, demonstrating that the genetic potential to evolve resistance to Bt is not limited to diamondback moth.[28] Owing to their different mechanism of action in comparison to Cry toxins, Cyt toxins might be useful in managing resistance to Cry toxins used in microbial insecticides and transgenic plants.

Acknowledgement

We thank Dr. You-min Zhang (Gene Bridge GmbH, Dresden, Germany) for a critical reading of the manuscript.

References

1 Schnepf, E.; Crickmore, N.; van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D. R.; Dean, D. H. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 772.
2 Bravo, A.; Gill, S. S.; Soberón, M. *Toxicon* **2007**, *49*, 423.
3 Rupar, M. J.; Donovan, W. P.; Tan, Y.; Slaney, A. C. *US 5354563*, **2000**.
4 Li, J.; Koni, P. A.; Ellar, D. J. *J. Mol. Biol.* **1996**, *257*, 129.
5 Cohen, S.; Dym, O.; Albeck, S.; Ben-Dov, E.; Cahan, R.; Firer, M.; Zaritsky, A. *J. Mol. Biol.* **2008**, *380*, 820.
6 Thomas, W. E.; Ellar, D. J. *FEBS Lett.* **1983**, *154*, 362.
7 Promdonkoy, B.; Ellar, D. J. *Biochem. J.* **2003**, *374*, 255.
8 Butko, P. *Appl. Environ. Microbiol.* **2003**, *69*, 2415.
9 Du, J.; Knowles, B. H.; Li, J.; Ellar, D. J. *Biochem. J.* **1999**, *338*, 185.
10 Promdonkoy, B.; Ellar, D. J. *Biochem. J.* **2000**, *350*, 275.
11 Manceva, S. D.; Pusztai-Carey, M.; Russo, P. S.; Butko, P. *Biochemistry* **2005**, *44*, 589.
12 Gill, S. S.; Singh, G. J. P.; Hornung, J. M. *Infect. Immun.* **1987**, *55*, 1300.
13 *Hyperchem Professional 8 Evaluation*, Hyperchem Inc, **2007**.
14 Thomsen, R.; Christensen, M. H. *J. Med. Chem.* **2006**, *49*, 3315.
15 Wiederstein, M.; Sippl, M. J. *Nucl. Acids Res.* **2007**, *35*, 407.
16 Colovos, C.; Yeates, T. O. *Protein Sci.* **1993**, *2*, 1511.
17 Abagyan, R. A.; Totrov, M. M.; Kuznetsov, D. A. *J. Comp. Chem.* **1994,** *15*, 488.
18 DeLano, W. L. *The PyMOL Mol. Graph. Syst.*, DeLano Scientific, San Carlos, CA, USA, **2002**.
19 Zhao, X. M.; Xia, L. Q.; Wang, F. X.; Ding, X. Z.; Zhang, Y. M. *Acta Chim. Sinica* **2008**, *66*, 108 (in Chinese).
20 Haider, M. Z.; Ellar, D. J. *Biochem. J.* **1987**, *248*, 197.

21 Mizuki, E.; Ohba, M.; Akao, T.; Yamashita, S.; Saitoh, H.; Park, Y. S. *J. Appl. Microbiol*. **1999**, *86*, 477.
22 Siegel, J. P.; Shadduck, J. A. *Safety of Microbial Insecticides*, Eds.: Laird, M.; Lacey, L. A.; Davidson, E. W., Chemical Rubber Company Press, Boca Raton, Florida, **1999**, p. 101.
23 Drobniewski, F. A. *Clin. Microbiol. Rev*. **1993**, *6*, 324.
24 Kallapur, V. L.; Mayes, M. E.; Edens, F. W. *Pestic. Biochem. Physiol*. **1992**, *44*, 208.
25 Berry, C.; O'Neil, S.; Ben-Dov, E.; Jones, A. F.; Murphy, L. *Appl. Environ. Microbiol.* **2002**, *68*, 5082.
26 Batra, C. P.; Mittal, P. K.; Adak, T. *J. Am. Mosq. Control Assoc*. **2000**, *16*, 321.
27 Wirth, M. C.; Park, H. W.; Walton, W. E.; Federici, B. A. *Appl. Environ. Microbiol.* **2005**, *71*, 185.
28 Federici, B. A.; Bauer, L. S. *Appl. Environ. Microbiol*. **1998**, *64*, 4368.

(E0902071 Lu, Y.)