Computational Modeling Deduced Three Dimensional Structure of *Cry1Ab16* Toxin from *Bacillus thuringiensis* AC11

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Abstract The first theoretical structural model of newly reported *Cry1Ab16* δ -endotoxin produced by *Bacillus thuringiensis* AC11 was predicted using homology modeling technique. *Cry1Ab16* resembles the *Cry1Aa* protein structure by sharing a common three domains structure responsible in pore forming and specificity determination along with few structural deviations. The main differences between the two is in the length of loops, absence of $\alpha 7b$, $\alpha 9a$, $\alpha 10b$, $\alpha 11a$ and presence of additional $\beta 12b$, $\alpha 13$ components while $\alpha 10a$ is spatially located at downstream position in *Cry1Ab16*. A better understanding of the 3D structure shall be helpful in the design of domain swapping and mutagenesis experiments aimed at improving toxicity.

Keywords Insecticidal crystal protein · *Cry* toxins · α -Helical bundle · Receptor insertion · Third-party annotation

Insecticidal crystal protein (ICP) produced by the soil bacterium *Bacillus thuringiensis* (Bt) belongs to a large toxin family with a target spectrum spanning insects, nematodes, flatworm, protozoa and until now is considered harmless to mammals [1]. The mode of action of *Cry* toxins is still a matter of hypotheses; the *Cry1A* series of toxins are produced as inactive protoxins within Bt sporangia and after ingestion by a susceptible insect larva,

protoxins are proteolytically cleaved to a core toxin fragment that binds to four different types of high affinity receptor sites on the insect gut membrane. Receptor binding phenomenon induces the conformational changes in the toxin necessary for its membrane insertion. The inserted toxin disturbs the cellular electrolyte balance by creating pores in the cell membrane, leading to cell lysis and finally to larval death [2, 3]. Crystal structures of the active toxins in solutions have been analyzed for *Cry1Aa* [4], *Cry2A* [5], *Cry3A* [6], *Cry3B* [7], *Cry4Ba* [8], and *Cry4Aa* [9] by X-ray diffraction crystallography and *Cry11Bb* [10], *Cry5Aa* [11], and *Cry5Ba* [12] has been predicted by homology modeling method. These reports have supported the three domains hypothesis [4] revealing domain I to be consisting of α -helical bundle, domain II of antiparallel β sheets and domain III made up of β sandwich. Hitherto *Cry1* toxins have extensively used in studies of lepidopteron insect control and have attracted less attention and fewer efforts have been focused on *Cry1Ab* member's structural studies. Since for a comprehensive understanding of mechanisms underlying toxicity it is imperative to have three-dimensional structural information of all the prevalent *Cry* family members. Here, a model for structure of the *Cry1Ab16* toxin based on the hypothesis of structural similarity [4] is reported. This model shall provide further initiation into the domain-mutagenesis experiments among *Cry* toxins for improving their toxicity.

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Materials and Methods

The model coordinates were developed essentially by generating alignments among target sequence (AAK 55546) with existing PDB entries. Alignment of those structure sequence that showed amino acid and secondary

structure similarity where directly used in homology modeling program MODELLER 9VB [13]. The resulted preliminary model was validated with PROCHECK [http://nihserver.mbi.ucla.edu/SAVES/Info.php], WHAATIF [http://swift.cmbi.ru.nl/servers/html/index.html] ProSA [https://prosa.services.came.sbg.ac.at] and RAMPAGE [http://mordred.bioc.cam.ac.uk/] servers. Possible outliers and side chains static constrain were minimized by refining the model with Summa's server [http://silvio.cs.uno.edu/proteinrefinementserver/]. Potential deviations were calculated with SUPERPOSE web server (http://wishart.biology.ualberta.ca/cgi-bin/) for root mean square deviation (RMSD).

Results and Discussion

The structural model of the *Cry1Ab16* core toxin was obtained comprising of 605 (68–673) amino acids out of 1155 long primary structure. As a general practice using multiple template alignment in modeling information is preferred to avoid biased folding and generation of atomic clashes and highly strained bond length and angles in target molecule. The secondary structure based alignment of each domain (I, II and III) found to be reliable and few manual corrections are to be incorporated within the possible limits of flanking domains to adjust the gaps and aligning the conserved block elements. The sequence identity for each template and their sequence alignment is shown in Table 1 and Figs. 1 and 2.

The validation of model for artifacts and errors is evaluated with webs based PROCHECK and WHATIF. Another program, ProSA evaluates the model by parsing its coördinates and energy using a distance based pair potential by capturing the solvent exposed protein residues. This program consists of sufficiently large database of natural structures, which it deploys in the validation of experimental and computational models [14]. The results are displayed in form of Z-score and plot of residues energy. The Z-score shows overall model quality and provides deviations from the random conformation. It also checks whether the Z-score of the protein is within the range of

similar proteins (NMR and X-ray derived structures). Result in Fig. 3a shows the plot location of the Z-score. The value -10.09 is among native conformation and overall residues energy of developed structure is found to be largely negative. The Ramachandran plot showed that most of the residues ($\sim 91.2\%$) have Φ and Ψ angles in the favored regions and additionally $\sim 7.1\%$ is in allowed region, still some proline and glycine residues (1.7%) fall in outlier region (Fig. 3b). Most bond lengths, bond angles, and torsion angles were among values expect for a naturally folded protein. Structural comparison of the *Cry1Aa* toxin with the *Cry1Ab16* model shows correspondence with the general *Cry* toxin model (a $\alpha + \beta$ structure with three domains, Fig. 3a) and their superimposed C^α backbone traces showed low RMSD ($1.14\text{\AA}$). This low value show that final developed structure is similar to *Cry1Aa* (Fig. 4a). This condition is anticipated since both the sequence has a high homology. The few differences observed between them were in length of the loops of domains II and III absence of $\alpha 7b$, $\alpha 9a$, $\alpha 10b$, $\alpha 11a$ and presence of additional $\beta 12b$, $\alpha 13$ components. The component $\alpha 10a$ is located spatially at different position (Table 2). This RMSD after optimal superposition between model and the native structure is frequently used as a means of correctness determination, In general the accuracy of the backbone trace of structure model has a profound impact on the ability to accurately predict near natural state of protein structure, including the side chain placement and later refining it into natural state like protein structure [15].

Domain I is composed of N terminal 234 (70–304) amino acid residues folded into a bundle of amphipathic helices and consist of nine α -helices and two small β -strands (Fig. 4b). This feature is believed to be highly conserved among the *Cry* toxins [4] and it has been proposed that it is involved in 'pore-formation' analogy with the helical bundle pore forming structures of colicin A toxin [16] and diphtheria toxin [17]. Evidence from several studies has shown that the central helix ($\alpha 5$) is specifically involved in the 'pore' formation process [18, 19].

As like other *Cry* toxins, domain II of the *Cry1Ab16* toxin, consists of three Greek key anti parallel β sheets

Table 1 The percentage identity score of *Cry1Ab16* sequence verses *Cry1Aa* (1ciy:A); *Cry3Aa* (1dlc:A); *Cry8ea1* (2qkg:A). The comparative scores were read out of secondary structure alignments using SAS software (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/sas)

Sl no.	Smith–Waterman Score	% identity	a.a. overlap	Seq. length	Z-score	E value	PDB code	Protein name
1.	–	–	–	606	–	–	–	<i>Cry 1Ab16</i>
2.	3337	87.8	580	577	4015.0	7.9e-217	1ciy:A	<i>Cry 1Ab1</i>
3.	1254	36.4	563	584	1500.3	9.2e-77	1dlc:A	<i>Cry 3Aa</i>
4.	1305	38.0	600	589	1438.8	2.5e-73	2qkg:A	<i>Cry8ea1</i>
5.	1150	34.7	585	589	1296.6	2e-65	1ji6:A	<i>Cry3bb1</i>

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1Ab16	-----YTPIDISLSLTQFLLESEF-VPGAG-----FVLGL----VDI IWGIFGPSQ---WDAFLV	46
1ciy:A	-----YTPIDISLSLTQFLLESEF-VPGAG-----FVLGL----VDI IWGIFGPSQ---WDAFLV	46
1dlc:A	TTKDV IQKGISVVGDLLGVVGFFFG-GALVS-----FYTNF-----LNTIWPS EDP-----WKAFME	51
2qkg:A	-----ISERDAVKTAISLVGTILGKLGVPLVG-----PIVSLYSTLDIVLWP G-KGSQ---WEIFME	53
1ji6:A	-----DAVG TGISVVGQILGVVG-VFAGALT SFQYSF-----LNTIW-----PSSADPWKAFA	49
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	QIEQLINQR IE EFARNQAISRLEGLSNLYQ IYAESFREWEADPT--N-PALREEMRIQ---FNMD	105
1dlc:A	QVEALMDQKIADYAKNKALAE LQGLQNNVEDYVSALSSWQKNPVSSRN- PHSQGRIREL--FSQA	113
2qkg:A	QVEALINQKIAEYARAKALAELEGLGNNYQLYLTALEE WQENPS--S-TRVLRDVRNR--FEIL	112
1ji6:A	QVEVLIDKKIEEYAKSKALAE LQGLQNNFEDYVNALNSWKKTPL--SLRSKRSQDRIRELFSQA	111
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	NSALT TAIPLF AV QNYRVPLL SVYVQAVNLHLSVL RDVL VFG QRWGFDAAATINSRYNDLTRIGN	170
1dlc:A	NSALT TAIPLLAV QNYRVPLL SVYVQAVNLHLSVL RDVSVFG QRWGFDAAATINSRYNDLTRIGN	170
2qkg:A	ESHFRNSMP SFAISGYEVFLFTTYAQ AANTHLFLKDQAQ IYGEEWG EKEDI AE FYKRQLKLTQE	178
1ji6:A	DSLF TQYMP SF RV TGYEV PLLSVYAQAANL HLLL K DAS IF GEEWG FSTTAIN NYNRQM SLIAQ	177
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	YTDHAVRWYNTGLERVWGPDSRDWRIRYNQFRRELTLTVLDIVSLFPNYDSRTYP IR TVSQLTREI	235
1dlc:A	YTDYAVRWYNTGLERVWGPDSRDWRIRYNQFRRELTLTVLDIVALEF SNYDSRRYP IR TVSQLTREI	235
2qkg:A	YTDHCVCVNWVGLDKRLGSSYESWNFNRYRREM TLTVLDIALFPLYDL RLYPKTELTREDV	243
1ji6:A	YSDHCQWYRTGLDRLGKSNAGQVEYNRFREMTLSVLDIMTFELPMYDMRTYPMETKAQLTRE V	242
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	YTNPVL--E-NFDGSGFRGAQQ--IEGS-IRSPHLM DILNSITIYT--DAH RGEY-----Y--	283
1dlc:A	LTDPIGVGN- NLRG-YGTTFSN--IENY-IRKPHLFDYLHRIQFHT--RFQP GYYG NDSFNY-	298
2qkg:A	YTDPIG--AIGAQS WYDSAPSFNTLES TFIRGHKLF DPITRLSYTG RSSFSASN--YLK	299
1ji6:A	FTDPIF--SLNTL QEY GPTFLS--IENS-IRKPHLFDY LQGIEFHT--RLQPG YFGKDSFNY--	296
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	-WSGHQIMASPVGFS GPETF PFLYGT M-GNAAPQQRIVAQLGQGVYRTL SST-LYRRP---FNI	341
1dlc:A	-WSGHQITASPVGFS GPETF AFPLFGNA-GNAAPPV-LVSLTGLGIFRTL SSP-LYRRII--LGS	341
2qkg:A	-WSGNYVSTRPSIGSNDII TS PFYGNK--SSEPQNLFN-GEKVYRANVTNAVWP--SAVY	353
1ji6:A	KWIGHQISSQFIGGS--IQTOTYGTSSGVIAQTQIGFTGF DVYKTLSTA-GVLFA--YTS	356
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	GINNQQLSVL-DG-----TEFAYGT-SSNLPSAVYRKSGTV--DSLDEIPPQNNNVPRQ	392
1dlc:A	SGVTKEVFSGYNDQ-----TDEA--S-TQTYDSK--RNVGAVSW-DSIDLQLP PETTDEPLEK	407
2qkg:A	KYYGVSKVVF-DAIYPD NKYKTTFTYNP-GSEGIGAQEK--DSEVELPPETLDPQNFYE:	400
1ji6:A	-GVTKVDFSQY-DD-----QK--NE-TSTQTYDSKRNGHVSAQSDSIDQLPETTDEPLEK	407
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	GFSHRLSHVSMFRSGFSNSSVSIIRAMPFSWIHRSAEFNNIIPSSQITQIPLTKSTNLGSGTSVV	457
1dlc:A	GFSHRLSHVM TMSQ--AAGAVYTLRAPTF SWQHRSAEFNNIIPSSQITQIPLTKSTNLGSGTSVV	456
2qkg:A	GYSHQLNYVMCFMLQGSRG TI-----PVLTWTHRSVDFFNMIDSKKITQLPLVKAYKLQSGASVV	467
1ji6:A	AYSHRLNYVFTFIR--NPDV--PVFSWTHRSADR TNVYSDKITQIPLVVKASD-GPKPASN	455
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	KGPGFTGGDI LR-RTSPGQISTLRVNI-TAPLSQR YRVRIRYASTNLQFH TSIDGRP INQGNF	518
1dlc:A	AGPFRFTGGDIQ-CTENGSAATIYVTP-DVSY SQKYRARIRHYASTSQITQLFSLDGAFPNQYFF	529
2qkg:A	EYVGHILGGDPIS-FNSSGSTGVIRLNI- NSPLSQKYRVRIRYCSSVD FDDL DVVRGGT TVNNGRFN	518
1ji6:A	EGPGETGGNLLFLFKESNSIAKFYVTLNSAALLQRYRVRIRYASTNLRLRFVQNSNND FLVIYI	531
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1Ab16	-----+-----+-----+-----+-----+-----+-----+	
1ciy:A	SATMSGSGSNLQSGSFRTVGFTTFPFNFSGSSVFTLSAHVFNS--GNEVYIDRIEFVPAEVTFE	580
1dlc:A	DKTINKGDDLTYSNFLASFSTPFELS GN--LQIGVTGLSA--GDKVYIDKIEFI PVN---	584
2qkg:A	KSA PNWQSL KYENFKFASFSTPF TFNQADTLKISVRNFSSIVG SVYIDRIELIPVN---	579
1ji6:A	NKTMNKDDDLTYQTFDLATTSNMGFS GDKNELIIGAESFVS--NEKIYIDKIEFI PQVL---	589

YTPIDISLSLTQFLLSEFVPGAGFVLGLVDI IWGIFGPSQWDAFLVQIEQLINQRIEEFA

 68 75 80 85 90 95 100 105 110 115 120 125

 RNQAI SRLEGLSNLYQIYAESFREWEADPTNPALREEMRIQFNDMNSALTTAIPLFAVQN

 128 135 140 145 150 155 160 165 170 175 180 185

 YRVPLLSVYVQAVNLHLSVLRDVLVFGQRWGFDAATINSRYNDLTRLIGNYTDHAVRWYN

 188 195 200 205 210 215 220 225 230 235 240 245

 TGLERWGPDSRDWIRYNQFRRELTLTVLDIVSLFPNYDSRTYPIRTVSQTLTREIYTNPV

 248 255 260 265 270 275 280 285 290 295 300 305

 LENFDGSFRGSAQGLEGSIRS PHLMIDLNSITITYTDAHRGEYYWSGHQIMASPVGFSGPPE

 308 315 320 325 330 335 340 345 350 355 360 365

 FTFPLYGTMGNAAPQQRIVAQLQGQGVYRTLSSTLYRRPFNIGINNQQLSVLDGTEFAYGT

 368 375 380 385 390 395 400 405 410 415 420 425

 SSNLP S A V Y R K S G T V D S L D E I P P Q N N N V P P R Q G F S H R L S H V S M F R S G F S N S S V S I I R A P M

 428 435 440 445 450 455 460 465 470 475 480 485

 F S W I H R S A E F N N I I P S S Q I T Q I P L T K S T N L G S G T S V V K G P G F T G G D I L R R T S P Q G I S T L R

 488 495 500 505 510 515 520 525 530 535 540 545

 V N I T A P L S Q R Y R V R I R Y A S T T N L Q F H T S I D G R P I N Q G N F S A T M S S G S N L Q S G S F R T V G F T

 548 555 560 565 570 575 580 585 590 595 600 605

 T P F N F S N G S S V F T L S A H V F N S G N E V Y I D R I E F V P A E V T F E A E Y D L E R A Q K A V N E L F T S S N

 608 615 620 625 630 635 640 645 650 655 660 665

 Q I G L K T

 668

Domain III composed of residues 522–658 and showed highly conserved residues. Calculation of amphiphilicity

Fig. 3 Evaluation of Cry1Ab16 model **a** on ProSA server. The plot indicating nearness of developed model with the native structures. The Z-score of evaluated model is shown as large black dot. **b** Ramachandren plot analysis showing placement of its residues in deduced model. General plot statistics are: 551 (91.2%) residues in favored regions; 43 (7.2%) of residues were in allowed regions; the outlier residues totals to 10 (1.7%) only. The plot was generated using RAMPAGE web server (<http://mordred.bioc.cam.ac.uk/>)

with the Hoops and Woods value, indicated that $\alpha 1$, $\alpha 2$ (a & b), $\alpha 3$, $\alpha 6$, are well exposed and available for interaction, but helix $\alpha 1$ seems to be packed with domain II. This condition of $\alpha 1$ may cause it to show certain degree of displacement in post receptor binding phenomenon [21]. The possibility of regions outside domain II being involved in receptor recognition was evaluated by mutagenesis study in domain III loop of Cry1Ac [22].

The Coulombic surface charge distribution pattern in the model corresponds to negatively charged patch along $\beta 4$ and $\beta 13$ of domains II and III (Fig. 4c). While data of Gazit et al. [23] relates well with domain I, who initially suggested that $\alpha 4$ and $\alpha 5$ insert into the lumen receptor in an antiparallel helical hairpin manner. It is possible that according to the surface electrostatic potential of helices 4 and 5, there is a neutral region in the middle of the helices which shows, if the umbrella model is correct that both helices cross the membrane with their polar sides exposed to the solvent, as it has been suggested by the results of mutagenesis experiments in case of the Cry1Ac toxin [23].

Kumar and Aronson [24] demonstrated that mutation in the base of helix 3 and the loop between $\alpha 3$ and $\alpha 4$ that cause alterations on the balance of negative charged residues may cause loss of toxicity. Mutations in helices $\alpha 2$, $\alpha 6$ and the surface residues of $\alpha 3$ have no important effect on toxicity; meanwhile, helices $\alpha 4$ and $\alpha 5$ seem to be very sensitive to mutations. Helix $\alpha 1$ probably does not play an important part in toxin activity after cleavage of the protoxin. It is possible that mutations aimed to an increase in amphiphicity in these helices will improve the pore forming activity of Cry1Ab type of toxins. Also, chemical modifications of four Arg or seven Tyr residues resulted in greatly reduced toxicity and binding in Cry1A toxin [25].

It is presumed that additional and dislocated components may have some implications in specificity of the Cry1Ab16 toxin. Mutations in defined regions of the Cry1Aa toxin have been identified (equivalent to residues in the $\beta 6$ – $\beta 7$ loop of Cry1Ab16), as essential for binding to the membrane of midgut cells of *Bombyx mori* [20, 26]. In the Cry1Ab16 model, this region is longer than their counterparts. Loop $\beta 2$ – $\beta 3$ seems also to be able to modulate the toxicity and specificity in Cry1C [27]. The dual specificity of Cry2Aa for Lepidoptera and Diptera has been mapped to residues that correspond in the Cry1Ab16 theoretical model to α sheet 1, strand $\beta 6$, and loop between $\beta 6$ and $\beta 7$. This loop probably interacts with the receptor through both hydrophobic and electrostatic interactions; that probably also helps in receptor binding by providing more mobility to glycine and other like residues that may interact through salt bridges with the receptor.

Loop $\beta 4$ – $\beta 5$ is hydrophilic, and the charged residues at the tip of the loop are probably important determinants of insect specificity. Presence of aromatic amino acids within and adjoining vicinity of apical loops 2 and 3 of domain II has been postulated for protein–protein, protein–legend interactions and have been reported to tend to interact specifically with the outer envelope of lipid membrane [28] these residues were proposed to interact with hydrophobic lipids tails. The exposed loop architecture has structural affinity for binding to a glycoprotein receptors of the target insect membrane [29]. Several studies in Cry1Ac [4] and

Fig. 4 Complete protomer structure of the *Cry1Ab16* toxin. **a** Superposition C^{α} backbone of *Cry1Aa1* (blue) and *Cry1Ab16* (pink) molecules. Images are generated using UCF chimera ([http://](http://www.cgl.ucsf.edu/chimera)

www.cgl.ucsf.edu/chimera). **b** Ribbon diagram showing three-dimensional three domain oligomer structure. **c** Surface electrostatic potential representation of *Cry1Ab16* toxin

Table 2 The comparison among three domain structural components of *Cry1Aa* (1ciy:A) and *Cry1Ab16* toxin molecules

<i>Cry1Aa</i>		<i>Cry1Ab16</i>	<i>Cry1Aa</i>		<i>Cry1Ab16</i>	<i>Cry1Aa</i>		<i>Cry1Ab16</i>
Domain I			Domain II			Domain III		
$\alpha 1$	Pro35–Ser48	Pro70–Ser83	$\alpha 8a$	Pro271–Glu274	Pro306–Asn310	$\alpha 11a$	Leu475–Lys477	–
$\alpha 2a$	Aln54–Ile63	Aln89–Trp100	$\alpha 8b$	Ala284–Gln289	Ser318–Ser325	$\beta 13a$	Ser486–Val488	Ser522–Val524
$\alpha 2b$	Pro70–Ile84	Pro105–Gly119	$\beta 2$	Asp298–His310	Ile334–His341	$\beta 13b$	Ile498–Arg 501	Ile534–Arg 537
$\alpha 3$	Glu90–Ala119	Arg128–Ala154	$\beta 3$	Phe313–Trp316	Glu343–His345	$\beta 14$	Gly505–Asn513	Gly541–Asn549
$\alpha 4$	Pro124–Leu148	Pro159–Phe183	$\beta 4$	Gly318–Pro325	Glu348–Leu418	$\beta 15$	Tyr522–Ser530	Tyr558–Ser566
$\alpha 5$	Gln154–Trp182	Arg189–Trp217	$\alpha 9a$	Val326–Phe328	–	$\beta 16$	Leu534–Ile540	Leu570–Ile576
$\alpha 6$	Ala186–Val218	Ala221–Val253	$\beta 5$	Val348–Ser351	Gly353–Ser359	$\beta 17$	Arg543–Phe550	Arg579–Phe586
$\alpha 7a$	Ser223–Thr239	Ser258–Tyr285	$\beta 6$	Ile357–Arg367	Arg384–Ala387	$\alpha 12a$	Ser562–Ser564	Ser598–Ser600
$\alpha 7b$	Leu241–Tyr250	–	$\beta 7$	Leu380–Leu383	Tyr394–Leu401	$\beta 18$	Arg566–Gly569	Arg602–Gly605
$\beta 1a$	Glu266–Thr269	Ile302–Thr304	$\beta 8$	Gly385–Phe390	Val417–Ala424	$\beta 19$	Ser580–His588	Ser616–His624
			$\beta 9$	Thr400–Tyr402	Ala434–Tyr436	$\beta 20$	Val569–Pro605	Val632–Pro641
			$\alpha 10a$	Ser410–Asp412	<i>Ser441–Glu447^a</i>	$\alpha 13$	–	Leu652–Ala658
			$\alpha 10b$	Pro423–Gly426	–			
			$\beta 10$	His429–Val434	His463–Met470			
			$\beta 11$	Phe452–His456	Pro486–His492			
			$\beta 12a$	Thr471–Pro474	Thr507–Pro510			
			$\beta 12b$	–	Thr515–Leu517			

– Similar component not present

^a Components in italics are spatially present at downstream sites

Cry1Aa [30, 31] showed that mutation in conserved block residues led to decreased toxicity and alter channel properties. Similar alterations in case of *Cry1Ab16* of conserved block residues may also causes altered toxin properties.

Finally, evidence presented here, based on the identification of structural equivalent residues of *Cry1Ab16* toxin

through homology modeling show that, due to high amino acid homology, absence and presence of structural components few of which are at different positions, *Cry1Ab16* shares a common three-dimensional structure. This is the first model of a *Cry1Ab16* protein and major challenge still is for its crystallization and verification of predicted structural variations.

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