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Prediction of Lytic Segments from *Bacillus thuringiensis* var *israelensis* 130 kDa and 72 kDa Proteins

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The amino acid sequences of 130 kDa and 72 kDa proteins responsible for the larvicidal activity of *Bacillus thuringiensis* var *israelensis* (Bti) were analyzed by hydrophobic moment plots. A search for highly amphiphilic α-helices was made in these proteins using the helical hydrophobic moment as a criterion of amphiphilicity. The protein segments of the largest hydrophobic moments were analyzed. In the present communication we report the surface seeking helices in 130 kDa and 72 kDa proteins of *Bacillus thuringiensis* var *israelensis*. It is assumed that the surface seeking segments may participate in one of the membrane-related functions of *Bacillus thuringiensis*.

Keywords: Amphiphilicity, *Bacillus thuringiensis israelensis* (Bti), Helices, Hydrophobic moment, Lytic segments.

Introduction

A measure of the amphiphilicity of a α-helix is its helical hydrophobic moment. This quantity can be estimated for any amino acid sequence if it is assumed that the sequence is coiled as an α-helix and if some set of residue hydrophobicities are used.

Helices with large hydrophobic moments tend to seek the boundary between an apolar phase (air, the protein interior, or a membrane surface), and an aqueous phase (water or cytoplasm) by lying parallel to the interfacial boundary with their apolar phase and their polar charged side chains interacting with the strong dipoles of water. Some surface seeking helices, such as mellitin, are involved in membrane lysis. The rationale for this study was to search for α-helices that may have membrane lytic functions in *Bacillus thuringiensis* var *israelensis*.

Bacillus thuringiensis (Bt) is a gram-positive bacterium, which produces parasporal crystals during sporulation. (Aronson *et al.*, 1986). These crystals consist of one or more proteins of varying molecular masses that are toxic to insect larvae including lepidoptera, diptera and coleoptera (Hofte and Whiteley, 1989). The native δ-endotoxins are protoxins, which require solubilization and proteolytic activation in the larval midgut. The active toxin then binds to specific receptors on the midgut cells and is believed to insert into the epithelial cell membrane to form transmembrane pores, which destroy the cell by colloid osmotic lysis. (Knowles and Ellar, 1987). In our earlier investigation we reported the presence of Leucine rich repeats (LRR's) in various *Bacillus thuringiensis* sub-species. This sequence (LRR) was predicted to be involved in protein-protein interactions or receptor binding functions. (Suvarchala and Kaiser Jamil, 2000).

The C-terminal part of the protoxin is highly conserved among proteins, but this region is not important for the toxicity. In contrast, the N-terminal toxic fragment shows great diversity and is involved in toxicity (Chunjatupornchai *et al.*, 1988).

The three dimensional structure of cry III A toxin determined by X-ray crystallography (Li *et al.*, 1991) revealed that the Domain I, a seven helix bundle, was proposed to be implicated in pore formation within the insect membrane and lytic activities. However, the specific role of each segment is still unknown. In order to evaluate the region involved in pore formation, the 130 kDa and 72 kDa proteins of *Bacillus thuringiensis* var *israelensis* were analyzed by helical wheel diagrams and hydrophobic moment plots. The amino acid sequence of the 130 kDa protein was taken from Ward and Ellar (1987) and the amino acid sequence of the 72 kDa protein was obtained from Donovan *et al.* (1988).

Materials and Methods

Helical wheel diagrams were drawn for the 130 kDa and 72 kDa proteins using the PC gene. A helical wheel diagram consists of a projection of the side chain orientation of amino acids on to a plane perpendicular to the long axis of the helix. An 11 residue window was moved stepwise through each of the 130 kDa and 72 kDa amino acid sequences. The average hydrophobicity and

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Table 1. Constituent amino acid sequences of the N-terminal residues of 130 kDa Bti Protein. hydrophobicity <H> and hydrophobic moment <μH> values

Segment	Window Size	Sequence	Plot<H>	Co-ordinates<μH>
112-122	11	T W S D F I T Q T K N	-0.09	0.39
133-143	11	T Y I S N A N K I L N	-0.17	0.65
138-148	11	A N K I L N R S F N V	-0.05	0.67
139-149	11	N K I L N R S F N V I	0.01	0.68
235-245	11	R Q F D Y L E P L P T	-0.12	0.41
300-310	11	T T A V L D L V A L F	0.61	0.28
321-331	11	G V S E L T R E I Y Q	-0.08	0.65
322-332	11	V S E L T R E I Y Q V	-0.03	0.69
323-333	11	S E L T R E I Y Q V L	-0.03	0.67
324-334	11	E L T R E I Y Q V L N	-0.08	0.72
325-335	11	L T R E I Y Q V L N F	-0.09	0.64
440-450	11	I S K M D F F I T N G	0.25	0.33
495-505	11	D N Y S H I L S F I K	0.12	0.44
673-683	11	F L P I T R S I R E D	0.04	0.43
690-700	11	L E T V Q Q I I N T F	0.25	0.62

Table 2. Constituent amino acid sequences of the 72 kDa Protein of Bti. hydrophobicity <H> and hydrophobic moment <μH> values

Segment	Window Size	Sequence	Plot<H>	Co-ordinates<μH>
50-60	11	A A K A A F S K V L S	0.22	0.33
98-108	11	Y R G I I E V S D V F	0.22	0.58
99-109	11	R G I I E V S D V F D	0.12	0.6
179-189	11	D V D S F I K L F N Q	0.07	0.58
200-210	11	R M Y T E E F G R L C	-0.25	0.56
424-434	11	Y I R A I S A C P R G	-0.01	0.6
425-435	11	I R A I S AC P R G V	0.06	0.68
426-436	11	R A I S AC P R G V S	-0.08	0.69
427-437	11	A I S A C P R G V S L	0.25	0.41
428-438	11	I S A C P R G V S L A	0.25	0.42
510-520	11	A N P E W V D F V T D	-0.01	0.39
580-590	11	D Q A T D G S I K F A	0.13	0.34

hydrophobic moments were calculated from these helical wheel diagrams. (Eisenberg *et al.*, 1984).

A Plot of both hydrophobic moments and average hydrophobicity values for every n-residue long segment in a protein was plotted. This plot, often referred to as the Eisenberg Plot, enables the detection of different kinds of helices as they cluster into specific regions of the plot. Since the surface seeking segments were involved in membrane lytic functions, attention was focused on these segments.

Results

The segments selected for large hydrophobic moments of 130 kDa and 72 kDa proteins, as described in Methods, are listed in Tables 1and 2. Eisenberg and Wesson (1990) observed that lytic peptides were characterized by large helical hydrophobic moments and plot near the 'surface' region of the plot. Protein segments that have large estimated hydrophobic moments are often actually in the α-helical conformation. Several protein segments that are lytic themselves, or are segments from a lytic protein, have large hydrophobic moments and in several cases are known to be α-helices (Eisenberg *et al.*, 1984b, Cornette *et al.*, 1987). The hydrophobic moment plots that were drawn for 130 kDa and 72 kDa are shown in Fig. 1 and Fig. 2 respectively. Using this plot, we identified certain segments near or on the surface region.

The 130 kDa proteins appear to contain 10 putative helical regions. Out of the 10 putative helical segments of 130 kDa proteins, the first four of them are found to be α helices as revealed by X-ray crystallography of cry IIIA δ-endotoxin, i.e. *Bacillus thuringiensis tenebrionis*. These segments appear as amphiphilic when plotted as helical wheel diagrams. The regions from 112-122, 300-310, 495-505, 670-683 are also found to α helices as predicted by the Chou-Fasman Method.

Similarly the apparent/predicted segments near or on the surface seeking region of 72 kDa protein are listed in Table 2.

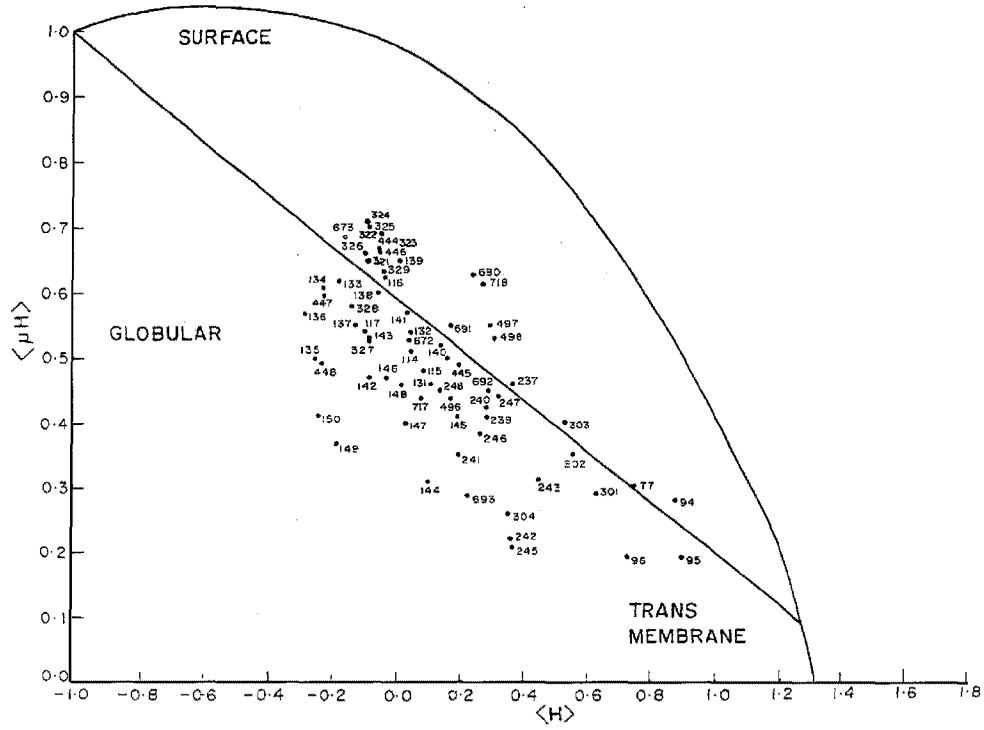

Fig. 1. Hydrophobic moment plot of 130 kDa protein of Bti, showing the largest hydrophobic moments, represented by filled circles. The horizontal coordinate gives the mean hydrophobicity of the segment. The vertical coordinate gives the hydrophobic moment of the segment.

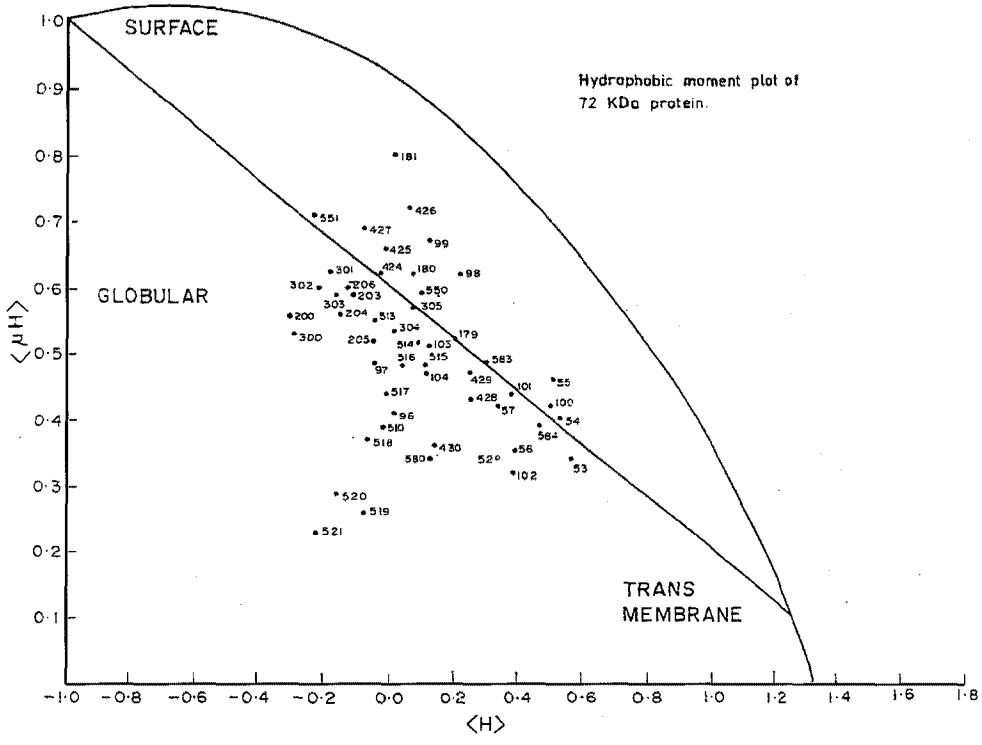

Fig. 2. Hydrophobic moment plot of 72 kDa protein of Bti, showing the largest hydrophobic moments, represented by filled circles. The horizontal coordinate gives the mean hydrophobicity of the segment. The vertical coordinate gives the hydrophobic moment of the segment.

The first above two sequences of the 72 kDa protein form α-helices according to the Chou Fasman Method.

Amphiphilic secondary structures were important for many biologically active peptides and proteins (Kaiser and Kezdy,

1984). The functional properties suggested for amphipathic helices include the lipid association membrane perturbation in the form of fusion, or lysis and the transmembrane helical bundle formation etc. (Segrest *et al.*, 1990). Some surface seeking helices, such as mellitin from Bee-venom, function in the process of membrane lysis and fusion (Terwilliger *et al.*, 1982).

Discussion

Using the hydrophobic moment plot, the segments that fall near the surface region, are identified and compared with other lytic segments. Similar to mellitin, meganin, cecropin etc, all of these toxins have a high α helical content in hydrophobic environments. In all of the above cases the hydrophobic characteristics fall into the surface region of the plot. The amino acids from the 133-149, 321-334 regions of 130 kDa have very high μH values of 0.68 comparable to other lytic segments. These regions have consecutive windows of 4 segments. Although the crystal structure of Bt tenebroinis is known, the seven-helix bundle of domain 1 does not have potential surface regions. The identified surface regions of the 130 kDa protein of Bti may have more lytic properties. Similarly the amino acids from 424-428 of 72 kDa have 4 consecutive windows in the surface region. This is also a potential lytic segment.

Gazit and Shaw synthesized 2 putative helical structures of the 130 kDa protein of Bti helix 1from amino acids; 50-71 helix 2 from amino acids 110-131. He has shown that helix 2 permeates phospholipid vesicles with a potency similar to that of naturally occurring pore forming peptides. Thus, the results support a role for helices 1 and 2 in the assembly and in the pore-formation by the Bti 130 kDa protein. It was proposed that the helices might aggregate on the membrane surface to form a pore.

It can be concluded that the large hydrophobic moments of segment and the position of segment within the protein sequence may suggest a membrane-related function. The protein segments analyzed using the hydrophobic moment plot may have a similar "pore forming" function. Further studies are warranted on these segments.

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