November 1987

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Ganapathy Muthukumar
University of Nebraska-Lincoln

Kenneth Nickerson
University of Nebraska-Lincoln, knickerson1@unl.edu

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1987, p. 2650–2655
0099-2240/87/112650-06$02.00/0

Vol. 53, No. 11

The Glycoprotein Toxin of *Bacillus thuringiensis* subsp. *israelensis* Indicates a Lectinlike Receptor in the Larval Mosquito Gut

GANAPATHY MUTHUKUMAR† AND KENNETH W. NICKERSON*

School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0118

Received 30 April 1987/Accepted 10 August 1987

The mosquito-active protein crystals produced by *Bacillus thuringiensis* subsp. *israelensis* contain covalently attached aminosugars which are critical for their larvicidal activity. The 50% lethal concentrations toward *Aedes aegypti* larvae were increased up to 10-fold by mild periodate treatment, up to 40-fold by forming the protein crystals in the presence of tunicamycin, and up to 7-fold by the presence during the mosquito bioassays of *N*-acetylglucosamine or its trimer, triacetylchitotriose. Periodate-treated crystals and crystals formed in the presence of tunicamycin had greatly reduced binding capacities for wheat germ agglutinin, an *N*-acetylglucosamine-specific lectin. These results suggest that the *B. thuringiensis* subsp. *israelensis* glycoprotein toxin binds to a lectinlike receptor in the larval mosquito gut. Furthermore, the distinct lectin-binding patterns exhibited by diptera-active versus lepidoptera-active *B. thuringiensis* crystals suggest that host specificity for the microbial insecticides is determined, in part, by the carbohydrate portion of their glycoprotein crystals.

The bacterium *Bacillus thuringiensis* subsp. *israelensis* produces a crystalline parasporal inclusion concomitant with sporulation that is toxic to the larval stage of many mosquito and blackfly species (6). Purified crystals are toxic to mosquito larvae at concentrations as low as 0.2 ng/ml (32, 42), but the toxin is not effective against lepidopteran larvae (42). The prospect of using *B. thuringiensis* subsp. *israelensis* as a biological control agent against mosquitoes has stimulated great interest in characterizing the crystal toxin and discovering the basis of its mode of action. The crystal is composed of multiple protein subunits (3, 15, 16, 32, 39, 47). Pfannenstiel et al. (32) found that both alkali-solubilized toxin and native crystals gave protein bands which fell into five groups: (i) a single broad band at 28 kilodaltons; (ii) a triplet at 38, 39, and 40 kilodaltons; (iii) a minor band at 53 kilodaltons; (iv) a doublet at 68 and 70 kilodaltons; and (v) a doublet at 135 and 140 kilodaltons. Qualitative agreement with these assignments has been provided by five other groups (3, 15, 16, 39, 47).

As part of a multipronged structure-function analysis of the *B. thuringiensis* subsp. *israelensis* crystal, we have been interested in the toxin as a glycoprotein (31). We recently found that *B. thuringiensis* subsp. *israelensis* crystals purified on NaBr gradients (2) contained 2.7% sugars consisting of 1.0% neutral sugars and 1.7% aminosugars (31). The aminosugars (70% glucosamine and 30% galactosamine) were evenly distributed among the major protein components of the crystal. Furthermore, the aminosugars were shown to be covalently attached to the crystal protein because they were still present after alkali solubilization, boiling in sodium dodecyl sulfate, separation by polyacrylamide gel electrophoresis, and transfer to nitrocellulose membranes (31). The present study demonstrates that these aminosugars are functionally significant for the larvicidal activity of the crystal. This realization has strong implications regarding the target of the *B. thuringiensis* subsp. *israelensis* toxin (lectinlike) and the molecular basis of host specificity among the microbial insecticides.

* Corresponding author.

† Present address: Department of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824.

MATERIALS AND METHODS

Abbreviations. FITC, Fluorescein isothiocyanate; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; kDa, kilodaltons; LC_{50}, 50% lethal concentration; NeuAc, *N*-acetylneuraminic acid; SBA, soybean agglutinin; and WGA, wheat germ agglutinin.

Toxin preparation. Both the lepidoptera-active *B. thuringiensis* subsp. *kurstaki* HD-1 (10) and a single-colony isolate of *B. thuringiensis* subsp. *israelensis*, taken from Bactimos powder, were grown on GGYS medium (29). After sporulation and cell lysis, the spores and crystals were harvested by centrifugation. Subsequently, the crystals were purified on 15 to 40% (wt/wt) gradients of NaBr (2), washed four times in deionized water, lyophilized, and stored at 4°C. Both intact and alkali-solubilized (29, 32) toxin preparations were used.

Lectin binding to intact crystals. Purified crystals (5 mg) were suspended in 1 ml of 10 mM sodium phosphate–150 mM NaCl–5 mM $CaCl_2$ (pH 7.2) in a 1.5-ml microcentrifuge tube and mixed with 10 μl of FITC-labeled lectin (1 mg/ml). The mixtures were incubated in the dark for 30 min at room temperature with occasional agitation. The crystals were collected by centrifugation (5 min at 15,000 × *g*), washed three times with 1 ml of buffer, and resuspended in 3 ml of buffer. Lectin binding was measured with a Perkin-Elmer 44 A fluorescence spectrophotometer with 490 nm as the excitation wavelength and 525 nm as the emission wavelength. The FITC-labeled lectins were from EY Laboratories, San Mateo, Calif.

Bioassays. Mosquito larvicidal activity was determined as described previously (27). Intact *B. thuringiensis* subsp. *israelensis* crystals were added to 10 baby food jars, each containing 20 ml of deionized water and 10 to 15 third-instar *Aedes aegypti* larvae. Final crystal concentrations ranged from 5 ng/ml to 1 g/ml. LC_{50}s were determined after 4 and 24 h at 22 to 24°C. Control larvae incubated in the absence of crystal toxin had no mortality in this time period. Experiments to determine the influence of sugars on toxicity replaced deionized water with 15 ml of the indicated carbohydrate solution and preincubated the larvae in that carbohydrate solution for 4 h before addition of the *B. thuringi-*

TABLE 1. Binding of FITC-labeled lectins by *B. thuringiensis* and *B. thuringiensis* subsp. *israelensis* crystals

Lectin[a]	Major sugar specificity	Fluorescence (arbitrary units)[b]	
		B. thuringiensis subsp. *israelensis* crystals	*B. thuringiensis* crystals
BPA	GalNAc	0.20	0.84
ConA	D-Mannose	0.20	0.06
DBA	GalNAc	0.20	0.28
GSI	D-Galactose	0.12	0.24
GSII	GlcNAc	0.38	ND[c]
MPA	D-Galactose	0.20	0.47
PNA	D-Galactose	0.20	0.56
SBA	GalNAc	0.47	1.94
UEAI	L-Fucose	0.18	0.60
WGA	GlcNAc	2.95	1.57

[a] BPA, *Bauhinia purpurea* agglutinin; ConA, concanavalin A; DBA, *Dolichosbiflorus* agglutinin; GSI and GSII, *G. simplicifolia* agglutinins I and II; MPA, *Maclura pomifera* agglutinin; PNA, peanut agglutinin; UEAI, *Ulex europaeus* agglutinin I.

[b] Background (no crystals) and turbidity (no lectins) corrections were subtracted from each value. The remaining low levels of fluorescence (i.e., 0.20 for *B. thuringiensis* subsp. *israelensis* crystals) may indicate nonspecific attachment. Values reported are the means of triplicate experiments.

[c] ND, Not determined.

ensis subsp. *israelensis* crystals. The preincubation was used because mosquito larvae are filter feeders; with simultaneous addition they would selectively concentrate the particulate toxin while excluding the sugars. None of the sugar controls caused larval mortality in the absence of crystal toxin. Finally, the larvicidal activity of solubilized *B. thuringiensis* subsp. *israelensis* crystal protein was determined after its attachment to 0.8-μm latex beads (34).

Periodate oxidation and deglycosylation. Intact crystals were suspended in 0.2 M acetate (pH 4.5) at a concentration of 2 mg/ml. Sodium metaperiodate was added to final concentrations of 0, 10, 25, 50, and 100 mM, and the mixtures were incubated at 4°C in the dark for times ranging from 0.5 to 48 h. Reactions were stopped by the addition of ethylene glycol to a final concentration of 200 mM. These conditions were recommended to minimize nonspecific reactions (14).

Similarly, intact and alkali-solubilized crystals (2 mg/ml) were incubated with potential deglycosylating enzymes (100 μg/ml) in 50 mM citrate (pH 5.0) at room temperature for periods up to 3 weeks (22). The *N*-acetylglucosaminidases (jackbean and *Aspergillus niger*) and neuraminidase (*Clostridium perfringens*) were purchased from Sigma Chemical Co., St. Louis, Mo. The periodate-treated crystals and the enzyme-treated crystals were washed three times in deionized water and tested for their larval toxicity, electrophoretic protein pattern, and FITC-WGA-binding capacity.

Crystal formation in the presence of tunicamycin. *B. thuringiensis* subsp. *israelensis* cells in GGYS were partially synchronized by three transfers with an exponential-phase inoculum equal to 10% of the culture volume. Tunicamycin (0.5 to 2.5 μg/ml) was added just before sporulation. The tunicamycin (Sigma T7765) contained isomers A, B, C, and D. After sporulation and cell lysis, the crystals were purified and analyzed as already described.

RESULTS

Lectin binding by purified crystals. Ten FITC-labeled lectins were tested for their ability to bind NaBr-purified protein crystals (Table 1). Both lepidoptera-active *B. thuringiensis* crystals and diptera-active *B. thuringiensis* subsp. *israelensis* crystals were examined. For the *B. thuringiensis* subsp. *israelensis* crystals, elevated fluorescence levels (Table 1) indicated strong binding by WGA and weak binding by SBA and *Griffonia simplicifolia* agglutinin II. The binding of WGA was completely prevented by the presence of 1 mM GlcNAc. Since the monosaccharide specificities of WGA and SBA are for GlcNAc and GalNAc, respectively, these lectin binding data are in excellent agreement with our chemical analyses which indicated that the aminosugars in purified *B. thuringiensis* subsp. *israelensis* crystals consisted of 70% glucosamine and 30% galactosamine (31). The specificity of the binding data is illustrated by the distinctively different lectin-binding spectrum exhibited by purified *B. thuringiensis* crystals. These crystals bound SBA and WGA strongly and four other lectins weakly (Table 1). Note that the binding of WGA indicates that the lectin recognizes the crystal rather than vice versa (i.e., the toxin is also a lectin) because WGA, unlike most lectins (23), is not itself a glycoprotein (1, 26).

Lectin binding did not influence the biological activity of *B. thuringiensis* subsp. *israelensis* crystals. LC_{50}s for third-instar *A. aegypti* larvae were 5 ng/ml in the presence and absence of nonfluorescent WGA. There is, of course, no assurance that the lectin-toxin complex would remain attached in the highly proteolytic (20) and alkaline (5) larval gut. For instance, concanavalin A binding does not occur above pH 9 (9), while the mosquito larval midgut is normally pH 10 to 10.5 (5).

Larval toxicity in the presence of added sugars. If the aminosugar residues of the *B. thuringiensis* subsp. *israelensis* glycoprotein toxin are necessary for specific binding to host receptors, then the corresponding free sugars should compete for those binding sites and this competition should increase the concentration of toxin necessary for lethality. Accordingly, LC_{50}s were determined for purified *B. thuringiensis* subsp. *israelensis* crystals in the presence of 26 added carbohydrates. Five of these carbohydrates caused a significant reduction of crystal toxicity (Table 2), and each of them was structurally related to GlcNAc. The following carbohydrates did not influence the toxicity of *B. thuringiensis* subsp. *israelensis* crystals to *A. aegypti* larvae: GalNAc, *N*-acetyl-D-mannosamine, D-arabinose, D-galactosamine, D-galactose, galactouronic acid, D-glucose, glucouronic acid, lactose, levulose, maltose, D-mannose, melibiose, α-methyl-D-glucoside, raffinose, L-rhamnose, sucrose, trehalose, and D-xylose (all 10 mM) and pectic acid and pectin (10 mg/ml).

The concentrations reported in Table 2 are those necessary for a 50% reduction in toxicity. As examples of the individual experiments used to generate Table 2, GlcNAc (20

TABLE 2. Reduction of *B. thuringiensis* subsp. *israelensis* larvicidal activity by carbohydrates

Carbohydrate	Concn required for a 50% increase in LC_{50}	
	4-h bioassay	24-h bioassay
D-(+)-Glucosamine	50 mM	105 mM
GlcNAc	10 mM[a]	More than 20 mM
N,N',N''-triacetylchitotriose	0.5 mM	1 mM
Chitosan[b]	200 μg/ml	400 μg/ml
Chitin[b]	200 μg/ml	400 μg/ml

[a] 10 mM GlcNAc = 2,212 μg/ml.

[b] Chitosan and chitin are variable-length polymers of glucosamine and GlcNAc, respectively.

mM) increased the 4-h LC_{50} from 7.5 to 42 ng/ml, while 1 mM *N,N',N''*-triacetylchitotriose (the trimer of GlcNAc) increased the 4-h LC_{50} from 7.5 to 37.5 ng/ml. The greater effectiveness of the GlcNAc trimer was in accordance with the known binding affinities of lectins such as WGA and potato lectin, $GlcNAc_3 > GlcNAc_2 > GlcNAc$ (1, 13, 23). Neither GlcNAc (100 mM) nor $GlcNAc_3$ (1 mM) inhibited larval feeding as determined by microscopic observation of larvae ingesting 0.8-μm latex beads. None of the 26 carbohydrates tested affected larval viability for at least 48 h. GlcNAc did not inactivate the *B. thuringiensis* subsp. *israelensis* toxin directly. Crystals incubated in GlcNAc (100 mM) for 4 h and then washed twice to remove the sugars were equally as toxic to *A. aegypti* larvae as control crystals which had not been incubated with GlcNAc.

Treatment of *B. thuringiensis* subsp. *israelensis* crystals with periodate. A complementary method for demonstrating the importance of sugar residues for larvicidal activity is to remove or inactivate those sugars before bioassay. Accordingly, *B. thuringiensis* subsp. *israelensis* toxin was incubated with three deglycosylating enzymes, two β-*N*-acetylglucosaminidases and a neuraminidase. However, in no case did the enzymes remove the aminosugar residues from the crystals; their WGA-binding capacities remained constant, and no changes in their protein subunit spectrum were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. No other deglycosylating enzymes were tested.

Instead, we used sodium metaperiodate to modify the sugar residues. This procedure opens the sugar rings by cleaving the bonds between adjacent hydroxyl groups (14); it does not release free sugars. Periodate treatment of *B. thuringiensis* subsp. *israelensis* crystals abolished the binding of fluorescent WGA (data not shown) and increased the LC_{50}s toward *A. aegypti* larvae (Table 3). Even brief exposure to mild periodate (10 mM for 0.5 h) increased the LC_{50} threefold. Higher concentrations and longer exposures made the *B. thuringiensis* subsp. *israelensis* crystals progressively less toxic. For both 25 and 100 mM periodate, virtually identical results were obtained when the periodate treatment was conducted in the presence of 100 mM L-lysine. This control was necessary because periodate modification of sugars may release formaldehyde (14). Aldehydes may derivatize free amino groups, while modification of the lysine side chains of the *B. thuringiensis* subsp. *israelensis* toxin is known to reduce larvicidal activity (29). The added L-lysine was intended to react with any formaldehyde produced before it could inactivate the protein toxin.

As with any reagent causing covalent modification of proteins, we need to be concerned with specificity of periodate. Indeed, the 166-fold-decreased toxicity after 48-h exposure to 50 or 100 mM periodate and the lysine protection

TABLE 3. Larvicidal activity of periodate-treated *B. thuringiensis* subsp. *israelensis* crystals

Periodate concn (mM)	LC_{50} (ng/ml)[a] at an incubation time (h) of:					
	0.5	1	2	4	24	48
0	12	12	12	12	12	12
10	40	75	55	60	132	300
25	22	37	50	60	134	333
50	20	28	38	66	422	>2,000
100	22	50	267	244	875	>2,000
100 (with lysine)	22	59	244	268	500	ND[b]

[a] Based on a 4-h bioassay. Similar data were obtained in a 24-h bioassay, with the actual LC_{50}s being somewhat lower owing to the longer assay.
[b] ND, Not determined.

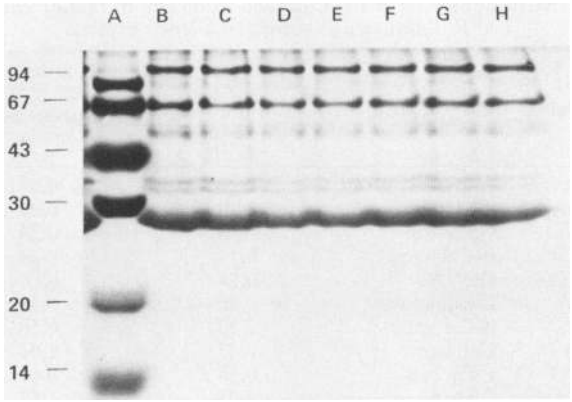

FIG. 1. Protein composition of periodate-treated *B. thuringiensis* subsp. *israelensis* crystals. Crystal toxin (20 μg per lane) was subjected to gel (12.5%) electrophoresis in sodium dodecyl sulfate (21) followed by Coomassie blue staining. Lane A contains molecular weight standards whose size in kilodaltons is indicated at the left. Lanes B through F contain crystal treated for 4 h with 0, 10, 25, 50, and 100 mM sodium metaperiodate, respectively. Lanes G and H contain crystals treated for 24 h with 50 and 100 mM sodium metaperiodate, respectively.

evident by 24 h (Table 3) probably reflect noncarbohydrate modifications. However, the conditions chosen were those recommended to minimize side reactions (14), and periodate treatment was not accompanied by detectable changes in the apparent molecular weight of any of the crystal proteins (Fig. 1). Furthermore, no peptide degradation products were evident (Fig. 1).

Crystal formation in the presence of tunicamycin. Our final approach indicating the importance of larvicidal toxin as a glycoprotein was to prevent sugar attachment during crystal formation. The antibiotic tunicamycin is frequently used to prevent carbohydrate attachment to eucaryotic glycoproteins (12). However, even though tunicamycin is inhibitory toward gram-positive bacteria (12), the number of glycoproteins known to be produced by procaryotic organisms is so low (31) there can be no a priori assurance tunicamycin will have a similar mode of action in bacteria. Tunicamycin has been reported to inhibit the synthesis of bacterial cell wall polymers (45), and tunicamycin-resistant mutants of *Bacillus amyloliquefaciens* (8) were found to be highly pleiotropic. With the hope that pleiotropism indicated a similar mode of action in procaryotes as in eucaryotes, we added tunicamycin (0, 0.5, 1.0, and 2.5 μg/ml) to *B. thuringiensis* subsp. *israelensis* cultures just before the onset of sporulation. Each culture completed sporulation within the same time frame and produced spores and crystals of normal morphology. However, when the spore-crystal mixtures were used in a 24-h bioassay with *A. aegypti* larvae, they gave LC_{50}s of 8, 69, 90, and 112 ng/ml, respectively.

On the basis of this indication of a dramatically reduced toxicity, we purified the crystals produced in the presence of 2.5 μg of tunicamycin per ml. However, these crystals constituted a heterogeneous population with various levels of glycosylation, and consequently, we decided the only way to gain a clear understanding of the significance of the sugar was to separate the total crystals into more uniform fractions. Six fractions (15 ml) were taken from the crystal portion of a NaBr density gradient. The crystals in each fraction were analyzed for their larvicidal activity and WGA-binding capacity (Table 4). The crystals from fractions of increasing density contained progressively less aminosugars

TABLE 4. Density fractionation of *B. thuringiensis* subsp. *israelensis* crystals formed in the presence of tunicamycin

Crystal fraction[a]	LC_{50} (ng/ml)		FITC-WGA binding[b]
	4-h bioassay	24-h bioassay	
Control	7.5	3.0	0.59
1	20	15	0.26
2	32	25	0.11
3	53	35	0.12
4	100	70	0.07
5	225	101	0.06
6	238	138	0.04

[a] Control crystals were synthesized without tunicamycin. Crystals synthesized in the presence of tunicamycin (2.5 μg/ml) were collected from NaBr gradients (2) in six fractions of 15 ml each covering the range from 1.27 to 1.28 g/cm^3.

[b] Fluorescence (arbitrary units) for crystal fractions (1 mg/ml). Note that this protein concentration is five times less than that used for Table 1.

(as measured by their ability to bind fluorescent WGA) accompanied by a progressive loss of toxicity (higher LC_{50}s). The fractions were from 5- to 46-fold less toxic than control crystals.

Crystals formed in the presence of tunicamycin were also examined with regard to their protein subunit patterns (Fig. 2). The major proteins present were similar (Fig. 2), but some differences were observed. In particular, the 68/70 ratio in the 68- to 70-kDa doublet exhibited a dramatic shift. The sugar-rich crystal fraction (Fig. 2, lane C) contained mostly the 70-kDa protein, whereas the sugar-poor crystal fractions (Fig. 2, lanes E and F) contained the 68-kDa protein exclusively. One explanation for this shift is that the 70-kDa protein is the glycosylated form of the 68-kDa protein and that tunicamycin prevents that glycosylation. The corollary of this explanation is that glycosylation of the 68-kDa protein is important for larvicidal activity.

DISCUSSION

Our data strongly suggest that the aminosugar portion of the *B. thuringiensis* subsp. *israelensis* glycoprotein crystals makes an important contribution to mosquito larvicidal activity. The LC_{50}s for *A. aegypti* larvae increased 3- to 46-fold whenever sugar attachment was reduced (via tunicamycin), the sugars were inactivated (via periodate), or free aminosugars were present during the bioassay. The carbohydrates found effective for toxicity reversal were all structurally related to GlcNAc. Throughout, our studies have focused on the presence and function of GlcNAc, even though WGA also binds NeuAc (sialic acid) because (i) glucosamine and galactosamine were detected chemically in *B. thuringiensis* subsp. *israelensis* crystals (31), whereas NeuAc was not (31); (ii) there was no decrease in toxicity when intact or solubilized toxin was treated with neuraminidase, an enzyme which removes NeuAc from complex carbohydrates; and (iii) fluorescent *Limulus polyphemus* agglutinin, specific for NeuAc, did not bind to purified *B. thuringiensis* subsp. *israelensis* crystals (31).

The amino acid sequences of several crystal proteins have recently been deduced from the DNA sequences of their cloned genes (36, 41, 43, 44). GlcNAc residues are most commonly attached to proteins at Asn-X-Ser and Asn-X-Thr sequences via N-glycosidic linkage to the amide N of asparagine (33). Confirmation that the aminosugars are N linked (as opposed to the alkali-labile O linked) is provided by the LC_{50}s of crystal toxin solubilized at increasingly alkaline pH values. Toxicity toward *A. aegypti* larvae was unchanged on increasing the solubilization pH from 10 to 12 (4). Thus, assuming Asn-X-Ser/Thr tripeptide attachment sites, it is easy to define the glycoprotein potential of these *B. thuringiensis* subsp. *israelensis* toxin sequences. For instance, an open reading frame specifying 675 amino acids (presumably the 68- to 70-kDa doublet) contains six Asn-X-Thr sequences and seven Asn-X-Ser sequences (41). Similarly, the lepidoptera-active 134-kDa protoxin from *B. thuringiensis* crystals consisting of 1,176 amino acids contains four Asn-X-Thr sites and three Asn-X-Ser sites (36). Significantly, five of these sites are in the amino-terminal 55% of the crystal protein which constitutes the lepidopteran toxin (35).

Realization that the *B. thuringiensis* subsp. *israelensis* toxin is a glycoprotein puts major constraints on the chemical nature of the target of the toxin in the larval midgut. We believe a lectinlike membrane receptor is indicated. However, our data per se indicate only that the aminosugars make a major contribution to mosquito larvicidal activity. Because most lectins are themselves glycoproteins (23), it is difficult to distinguish between two possible scenarios. The first possibility is that the insect receptor is a lectin which recognizes specific sugars on the bacterial toxin. Since the larval midgut is so alkaline (5), such a receptor would have to resemble the castor bean lectin (binding relatively pH insensitive [9]) rather than concanavalin A (no binding above pH 9 [9]). The second possibility is that the bacterial toxin is a lectin which recognizes specific sugars on the insect receptor and that the sugars of the toxin participate in the recognition process. This possibility ignores the generalization for lectins (23) that their carbohydrate moieties are not required for biological activity, e.g., chemically deglycosylated potato (7) and tomato (17) lectins retained their carbohydrate specificity. However, the second possibility must still be considered because of the significant precedent of bacterial protein toxins such as cholera toxin, *Shigella* toxin, tetanus toxin, botulinum toxin, and *Escherichia coli* heat-labile toxin

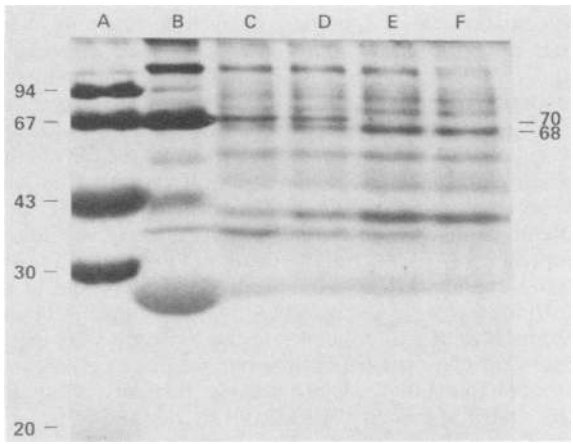

FIG. 2. Protein composition of *B. thuringiensis* subsp. *israelensis* crystals formed in the presence of tunicamycin. Crystal toxin was subjected to gel (12.5%) electrophoresis in sodium dodecyl sulfate (21) followed by Coomassie blue staining. Lane A contains molecular weight standards whose size in kilodaltons is indicated at the left. Lane B contains 50 μg of control crystals (no tunicamycin). Lanes C through F contain 20 μg each of crystal protein formed in the presence of 2.5 μg of tunicamycin per ml, fractions 1 through 4 (Table 4), respectively.

(11, 25) for which the oligosaccharide portion of a membrane ganglioside serves as the receptor (11, 25). Indeed, for the lepidoptera-specific *B. thuringiensis* toxin, Knowles et al. (19) followed this analogy by suggesting that the bacterial toxin is itself lectinlike and recognizes a terminal GalNAc residue on the plasma membrane of insect cells (18). However, their conclusion was based on an in vitro assay mixing activated *B. thuringiensis* toxin with an insect *Choristoneura fumiferana* cell line, and the sugar recognition process demonstrated could equally well indicate GalNAc on the toxin and a lectinlike receptor. Note (Table 1) that purified *B. thuringiensis* crystals strongly bind fluorescent SBA, a GalNAc-specific lectin. Resolution of this point awaits the availability of purified toxin and purified receptor in both the native and deglycosylated forms.

For *B. thuringiensis* subsp. *israelensis*, our working hypothesis is that a lectinlike receptor in the larval mosquito gut recognizes the aminosugar portion of the glycoprotein toxin. The aminosugars are covalently attached to the toxin (31), and we were unable to find any evidence that the toxin itself is a lectin. With regard to WGA, the binding is unambiguous; WGA is not a glycoprotein (1, 26) and the WGA-toxin binding can be reversed by GlcNAc. The lectinlike receptor model also provides a ready explanation for the data of Sekar and Carlton (37). They transferred the toxin genes from *B. thuringiensis* subsp. *israelensis* to *Bacillus megaterium*. During sporulation the *B. megaterium* transformant produced crystalline inclusions identical in appearance to those from *B. thuringiensis* subsp. *israelensis*. However, purified transformant crystals were found to be fivefold less toxic to *A. aegypti* larvae. Sekar and Carlton (37) suggested that the explanation for this reduced toxicity could be found in posttranslational modifications. We suggest that the *B. megaterium* cells possess a reduced capacity for glycosylation of the crystal protein. Similarly, *Escherichia coli* should be totally incapable of protein glycosylation, and yet *B. thuringiensis* subsp. *israelensis* genes cloned into *E. coli* can exhibit some larvicidal activity (24, 41). We suggest that the factor by which such activity is reduced constitutes another measure of the sugar enhancement of crystal toxicity.

The existence of a specific glycoprotein receptor in mosquitoes and blackflies would explain why *B. thuringiensis* subsp. *israelensis* crystals are not toxic to animals or lepidoptera insects (6, 42). Identification of the normal physiological function of this receptor would be of interest since it constitutes an apparently novel target for chemical and biological insecticides. One possibility is that the target is a receptor in an endocytosis system. Animal lectins frequently function to achieve intracellular translocation of glycoproteins (23, 28). Entry via endocytosis would provide a partial explanation of why the toxin is larvicidal at concentrations as low as 0.2 ng/ml (32, 42) since the endocytosis receptors function to concentrate the molecules being internalized (28). Confirmation of the importance of glycoprotein receptors in mosquitoes is provided by the work of Sundin et al. (38) on the specificity of LaCrosse virus, the causal agent of California encephalitis. The replication of arthropod-borne viruses is typically restricted to a very limited number of vector species. For the mosquito *Aedes triseriatus*, they found that a midgut receptor specific for a glycoprotein in the viral envelope determined the vector-virus interaction (38).

B. thuringiensis subsp. *israelensis* crystals exhibit two biological activities which occur at markedly different protein concentrations, a mosquito larvicidal activity by intact crystals (at ca. 0.2 to 5 ng/ml) and a general cytolytic activity by solubilized crystal proteins (at ca. 1 to 5 μg/ml). The general cytolytic activity is commonly measured via erythrocyte lysis (32, 39), but it also causes rapid lysis of insect and mammalian cells in vitro (39). The general cytolytic activity resides in the 28-kDa protein subunit (3, 30, 40, 46), but there is substantial disagreement on the identity of the larvicidal protein (3, 15, 16, 40). Our current view is that the larvicidal activity of intact crystals requires a synergistic combination of the 28-kDa protein and higher-molecular-weight proteins (30, 46). The distinction between the two biological activities of *B. thuringiensis* subsp. *israelensis* crystals is important when considering their mode of action. The present paper on the importance of the aminosugars concerns the larvicidal activity of the crystal. In contrast, the model of Thomas and Ellar (40) on in vitro cell lysis by the 28-kDa protein (via a detergentlike interaction with phospholipids containing unsaturated fatty acids) concerns the general cytolytic activity of the crystal.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grants AI 16538 (to K.W.N.) and AI 21021 (to Mary Ann Pfannenstiel) from the National Institutes of Health.

We thank Nancy Pfeiffer for her generous gift of FITC-labeled lectins and Graham A. Couche for testing larval feeding in the presence of GlcNAc.

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