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Cytolytic Activity and Immunological Similarity of the *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus thuringiensis* subsp. *morrisoni* Isolate PG-14 Toxins

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The parasporal bodies of the mosquitocidal isolates of *Bacillus thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* isolate PG-14 were compared with regard to their hemolytic and cytolytic activities and the immunological relatedness of the 28- and 65-kilodalton (kDa) proteins that occur in both subspecies. The alkali-solubilized parasporal bodies of *B. thuringiensis* subsp. *israelensis* caused 50% lysis of human erythrocytes at 1.14 μg/ml, whereas those of *B. thuringiensis* subsp. *morrisoni* caused similar lysis at 1.84 μg/ml. Preincubation of solubilized parasporal bodies with dioleolyl phosphatidylcholine significantly inhibited the hemolytic activity of both supspecies. In cytolytic assays against *Aedes albopictus* cells, the toxin concentrations causing 50% lysis for *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* were 1.87 and 11.98 μg/ml, respectively. Polyclonal antibodies raised separately against the 25-kDa protein (a tryptic digest of the 28-kDa protein) or the 65-kDa protein of *B. thuringiensis* subsp. *israelensis* cross-reacted, respectively, with the 28- and the 65-kDa proteins of *B. thuringiensis* subsp. *morrisoni*. However, neither of these antibodies cross-reacted with the 135-kDa protein of either subspecies. These results indicate that the mosquitocidal and hemolytic properties of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* isolate PG-14 are probably due to the biologically related proteins that are present in the parasporal bodies of both subspecies. The lower hemolytic activity of the *B. thuringiensis* subsp. *morrisoni* may be due to the presence of lower levels of the 28-kDa protein in that subspecies. However, the reason for the significantly lower cytolytic activity of *B. thuringiensis* subsp. *morrisoni* is not apparent. Potentially, the toxins that are responsible for hemolysis and cytolysis may not be identical, the mechanism of action by the toxins in the two cell types may differ, or both may be true.

Bacillus thuringiensis produces a proteinaceous parasporal body during sporulation which is highly toxic to insects when ingested (7). Although most subspecies of *B. thuringiensis* are active against lepidopterous insects, several *B. thuringiensis* subspecies including *israelensis*, *darmstadiensis* isolate 73-E-10-2, *kyushuensis*, and *morrisoni* isolate PG-14 are highly toxic to dipterans such as mosquitoes (8, 15–17).

Among these *B. thuringiensis* subspecies, the biological basis of toxic action has been established only for *B. thuringiensis* subsp. *israelensis*. The initial effect of parasporal bodies when they are fed to mosquito larvae is on the midgut epithelium. On exposure to toxins solubilized from the parasporal body by proteolytic alkaline gut juices, the cells swell, become vacuolated and disorganized, and lyse (4, 22). This cell lysis is thought to result primarily from the action of the toxins on membrane phospholipids (24; S. S. Gill, G. J. P. Singh, and J. M. Hornung, Infect. Immun., in press). In addition to its activity against midgut cells, the solubilized parasporal body of *B. thuringiensis* subsp. *israelensis* is also toxic and cytolytic to dipteran, lepidopteran, and vertebrate cells (2, 6, 21, 23).

In *B. thuringiensis* subsp. *israelensis* the parasporal body contains a series of proteins ranging from 28 to 230 kilodaltons (kDa). Much of the biological activity of this subspecies appears to be due to the 28-kDa protein which has been reported to be both cytolytic and mosquitocidal (2, 6, 23). Recently, however, there have been several reports indicating that the 65-, 135-, and 230-kDa proteins contribute to the insecticidal activity of *B. thuringiensis* subsp. *israelensis*, but not its cytolytic action (5, 9, 13, 18, 26), and that some of these proteins may potentiate the action of the 28-kDa toxin (10, 27).

In the highly mosquitocidal PG-14 isolate of *B. thuringiensis* subsp. *morrisoni* (17), the parasporal body has a profile similar to that of *B. thuringiensis* subsp. *israelensis* but, in addition, contains a 144-kDa protein (11). Other than its mosquitocidal activity, however, nothing is known about the biological activity of PG-14 in vitro or the immunological relationships between its proteins and those of *B. thuringiensis* subsp. *israelensis*. In this report we demonstrate that the parasporal body of isolate PG-14 has hemolytic and cytolytic activities similar to those of *B. thuringiensis* subsp. *israelensis* and show that the ca. 28- and 65-kDa proteins of PG-14 are immunologically similar to the 28- and 65-kDa proteins of *B. thuringiensis* subsp. *israelensis*.

MATERIALS AND METHODS

Isolates and growth of bacterial cultures. *B. thuringiensis* subsp. *israelensis* (serotype 14) was isolated from a commercial preparation (Bactimos, Biochem Products, Solvay Group, Brussels, Belgium), whereas *B. thuringiensis* subsp. *morrisoni* (serotype 8a:8b; isolate PG-14) was obtained from

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L. E. Padua, University of the Philippines, Los Banos, Manila. *B. thuringiensis* subsp. *israelensis* was grown in peptonized milk-yeast extract-glucose-salt (10 g of peptonized milk [BBL Microbiology Systems, Cockeysville, Md.]; 10 g of glucose [Difco Laboratories, Detroit, Mich.]; 2 g of yeast extract [Difco]; 0.3 g of $MgSO_4 \cdot 7H_2O$; and 20 mg each of $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, and $MnSO_4 \cdot H_2O$ per liter of medium) in 2.8-liter Fernbach flasks at 30°C, whereas *B. thuringiensis* subsp. *morrisoni* was grown similarly in modified glucose-yeast extract-salt solution (1 g of glucose, 5 g of yeast extract [Difco], 0.2 g of $MgSO_4$, 2 g of $(NH_4)_2SO_4$, 3 g of K_2HPO_4, 50 mg of $MnSO_4 \cdot H_2O$, and 60 mg of $CaCl_2$ per liter of medium) at 28°C. Autolysis of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis morrisoni* was complete by 96 and 48 h, respectively.

Isolation and solubilization of parasporal bodies. The spores, parasporal bodies, and cell debris were harvested by centrifugation (12,000 × *g*, 10 min), and the pellet was washed 3 times in cold 0.5 M NaBr. Parasporal bodies isolated on a linear discontinuous NaBr gradient (20 to 40%) (1) were collected, washed with cold double-distilled water, and stored at −20°C. For weight determinations parasporal body preparations were dried and weighed on a microbalance.

Freshly isolated parasporal bodies (2 mg/ml) were solubilized in 50 mM $Na_2CO_3 \cdot HCl$ (pH 10.5) for 1 h at 37°C (24). The insoluble material was removed by centrifugation (12,000 × *g*, 10 min). The supernatant was brought to a pH of 7.3 with 1 N HCl, concentrated with an ultrafiltration unit (Amicon Corp., Lexington; Mass.) with a 10,000-molecular-weight cutoff, and stored at −20°C until needed. Protein concentrations were determined by the method described by Lowry et al. (14).

SDS-PAGE and immunoblot. Molecular masses of the solubilized proteins and parasporal bodies were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 12%, 1.5 mm) as described previously (12). Proteins were visualized by Coomassie staining. For immunoblot analysis, the separated proteins were transferred electrophoretically to nitrocellulose (25). The electroblotted proteins were probed first with primary antibodies (immunoglobulin G [IgG] fractions) that were raised in rabbits against the 25-kDa protein (a protease-cleaved product of the 28-kDa protein) and the 65-kDa protein of *B. thuringiensis* subsp. *israelensis* and then with goat anti-rabbit IgG-alkaline phosphatase (3). IgG fractions were purified with Zetachrome 60 disks (AMF Laboratory Products). As the antigen used to raise antibodies against the 65-kDa protein had small impurities of the 28-kDa protein, the anti-65-kDa IgG fraction was passed through a Sepharose–25-kDa protein column to remove antibodies that cross-reacted with the 25- to 28-kDa proteins.

Bioassays. The toxicity of purified parasporal bodies was determined by using 20 fourth instar larvae of *Aedes aegypti* (World Health Organization protocol VEC-SWG 5(18.3), 1981). The bioassay was performed 4 to 5 times by using larvae from different batches. The estimated concentrations of parasporal bodies that killed 50% of the larvae were determined by probit analysis (19).

Hemolytic and cytolytic activities. Determination of the hemolytic activity of the solubilized preparation was performed by monitoring hemoglobin release from fresh human erythrocytes (S. S. Gill and J. M. Hornung, J. Invertebr. Pathol., in press). The cytotoxicity of the solubilized parasporal preparations was monitored with *Aedes albopictus* C6/36 (obtained from D. Knudson, Yale University, New Haven, Conn.). Briefly, $Na_2{}^{51}CrO_4$ (Amersham Corp., Arlington Heights, Ill.)-labeled cells were added to 96-well microtiter plates (5×10^4 cells per 0.25 ml per well) and incubated with toxin for 1 h at 28°C. Subsequently, the plates were centrifuged (2,000 × *g*, 5 min) and the supernatants were analyzed in a gamma counter. The following equation was used to determine cytotoxicity: % cytotoxicity = [(experimental release − background release)/(maximum release − background release)] × 100, where experimental release, maximal release, and background release were measured in counts per minute.

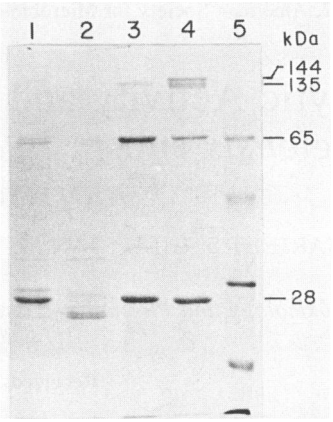

FIG. 1. Comparative analysis of the protein composition of parasporal bodies and alkali-solubilized parasporal bodies of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* isolate PG-14 by SDS-PAGE. Lane 1, solubilized *B. thuringiensis* subsp. *morrisoni* (20 μg); lane 2, solubilized *B. thuringiensis* subsp. *israelensis* (20 μg); parasporal bodies (2 mg/ml) were solubilized in 50 mM $Na_2CO_3 \cdot HCl$ buffer (pH 10.5) for 1 h at 37°C prior to analysis by SDS-PAGE; lane 3, parasporal bodies of *B. thuringiensis* subsp. *israelensis* (20 μg); lane 4, parasporal bodies of *B. thuringiensis* subsp. *morrisoni* (20 μg); lane 5, protein standards: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20.1 kDa), and lactalbumin (14.2 kDa).

Phospholipid treatment. Stock liposomal suspensions were prepared by ultrasonic treatment of lipids in 10 mM NH_4HCO_3. Solubilized parasporal bodies were then mixed with the desired phospholipid concentration to give various toxin:lipid (wt/wt) ratios (Table 1), and the mixture was incubated for 1 h at 37°C. The phospholipid-treated toxins were then tested for their hemolytic activity.

RESULTS

Parasporal bodies of both *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* had similar biological activities when assayed against larvae of *A. aegypti*. The estimated concentration of parasporal bodies that killed 50% of the larvae for *B. thuringiensis* subsp. *israelensis* was 7.32 ng/ml, with fiducial limits of 6.48 to 8.36 ng/ml at the 95% confidence level, whereas the value for *B. thuringiensis* subsp. *morrisoni* was 7.34 ng/ml, with fiducial limits of 6.61 to 8.18 ng/ml at the 95% confidence level.

The protein compositions of the parasporal bodies were very similar (Fig. 1). The principal proteins in *B. thuringiensis* subsp. *israelensis* were of 28, 65, 126 (not visible in Fig. 1, lane 3), and 135 kDa, whereas *B. thuringiensis* subsp. *morrisoni* contained all of the proteins listed above and, in addition, a 144-kDa protein (Fig. 1, lane 4). However, the

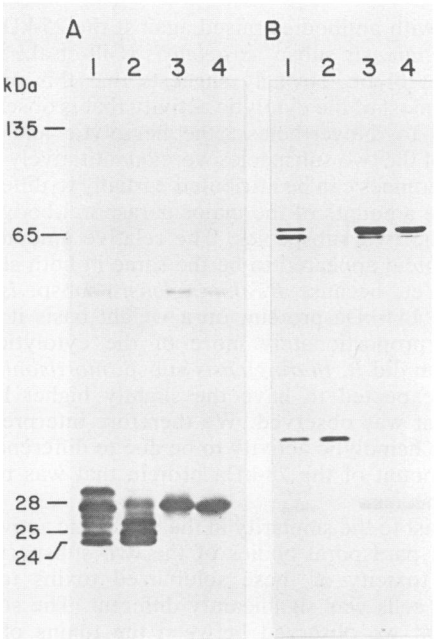

FIG. 2. Immunoblot of the parasporal bodies and alkali-solubilized parasporal bodies of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni*. (A) Probed with antibodies raised against the 25-kDa protein of *B. thuringiensis* subsp. *israelensis*. (B) Probed with antibodies raised against the 65-kDa protein of *B. thuringiensis* subsp. *israelensis*. Subsequent analysis was performed as described in the text. Lanes 1, Na_2CO_3 · HCl-solubilized parasporal bodies of *B. thuringiensis* subsp. *morrisoni* (2 μg); lanes 2, solubilized parasporal bodies of *B. thuringiensis* subsp. *israelensis* (2 μg); lanes 3, parasporal bodies of *B. thuringiensis* subsp. *israelensis* (2 μg); lanes 4, parasporal bodies of *B. thuringiensis* subsp. *morrisoni* (2 μg).

28-kDa protein from *B. thuringiensis* subsp. *morrisoni* consistently showed a marginally greater mobility, viz. a molecular mass of ca. 28 kDa. On solubilization in Na_2CO_3 · HCl buffer, however, the 28-kDa proteins of the two subspecies showed different SDS-PAGE protein patterns. *B. thuringiensis* subsp. *israelensis* showed two major bands at ca. 25 kDa (Fig. 1, lane 2); however, *B. thuringiensis* subsp. *morrisoni* gave a major band at ca. 28 kDa with minor bands at 25 kDa (Fig. 1, lane 1). This different protein pattern was not as evident when the proteins were probed with antibody raised against the 25-kDa protein of *B. thuringiensis* subsp. *israelensis* (Fig. 2A, lanes 1 and 2). The ability of the antibody raised against the 25-kDa protein of *B. thuringiensis* subsp. *israelensis* to easily detect proteins from *B. thuringiensis* subsp. *morrisoni* shows that the two proteins are immunologically very similar. The 65-kDa proteins in the parasporal bodies of the two subspecies were also shown to be immunologically similar (Fig. 2B, lanes 3 and 4). On alkali solubilization these 65-kDa proteins were cleaved to proteins of 36 kDa (Fig. 2B, lanes 1 and 2). Neither of these antibodies, however, cross-reacted with the 126- to 144-kDa proteins of *B. thuringiensis* subsp. *morrisoni* or the 126- to 135-kDa proteins of *B. thuringiensis* subsp. *israelensis* (Fig. 2).

The Na_2CO_3-solubilized fractions of both subspecies were highly hemolytic, with the activity of the two subspecies being quite similar (Fig. 3). The toxin concentrations causing 50% hemolysis for *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* were 1.14 and 1.84 μg/ml, respectively. There apparently was no difference in the rate at which the solubilized toxins exerted their hemolytic activity because both toxins showed a rather rapid lysis of human erythrocytes. In addition, the shape of the curve for the two solubilized toxins was similar.

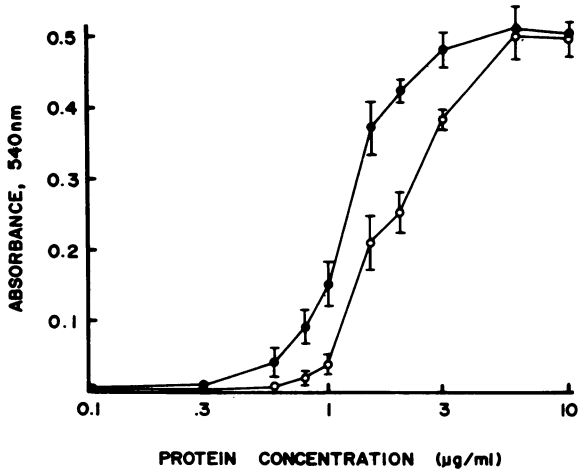

FIG. 3. Hemolytic activity of solubilized parasporal bodies of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* monitored by lysis of human erythrocytes. Symbols: ●, *B. thuringiensis* subsp. *israelensis*; ○, *B. thuringiensis* subsp. *morrisoni*. The data illustrated are expressed as means ± standard errors of four to five assays, each of which was performed in duplicate.

The hemolysis that was observed with solubilized parasporal bodies of both subspecies was inhibited by unsaturated phospholipids (Table 1). Preincubation of the solubilized parasporal bodies from *B. thuringiensis* subsp. *israelensis* with dioleolyl phosphatidylcholine at 10 times the toxin concentration reduced erythrocyte lysis to near background levels. Similar results were obtained with solubilized parasporal inclusions from *B. thuringiensis* subsp. *morrisoni*. Preincubation of the solubilized toxins from either subspecies with disteroyl phosphatidylcholine, however, did not have any effect on their hemolytic activities (Table 1).

The solubilized parasporal bodies from both subspecies

TABLE 1. Effect of phosphatidylcholine on the hemolytic activity of alkali-solubilized parasporal inclusions of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni*

Toxin (concn [μg/ml])	Lipid treatment	Toxin: lipid ratio (wt/wt)	A_{540}[a]	% Change
B. thuringiensis subsp. *israelensis* (1.5)	None		0.427 ± 0.006	
	Dioleolyl	1:5	0.049 ± 0.012[b]	−88.5
	Dioleolyl	1:10	0.010 ± 0.002[b]	−97.6
	Disteroyl	1:5	0.410 ± 0.011	−2.0
	Disteroyl	1:10	0.413 ± 0.009	−3.2
B. thuringiensis subsp. *morrisoni* (3.0)	None		0.433 ± 0.009	
	Dioleolyl	1:5	0.081 ± 0.006[b]	−81.2
	Dioleolyl	1:10	0.008 ± 0.004[b]	−98.0
	Disteroyl	1:5	0.444 ± 0.005	2.5
	Disteroyl	1:10	0.424 ± 0.011	−2.0

[a] Mean ± standard error of four assays, each of which was performed in triplicate.

[b] Significantly different from untreated controls ($P < 0.01$).

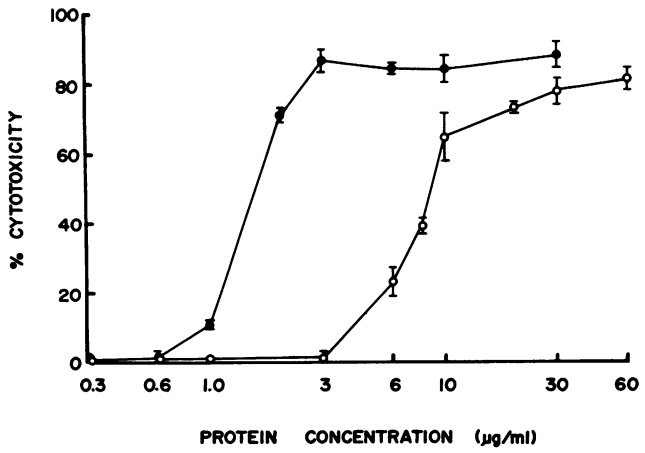

FIG. 4. Toxicity of solubilized parasporal bodies of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* to *A. albopictus* cells. symbols: ●, *B. thuringiensis* subsp. *israelensis*; ○, *B. thuringiensis* subsp. *morrisoni*. The data illustrated are expressed as means ± standard errors of three assays, each of which was performed in duplicate.

were also cytolytic to *A. albopictus* cells (Fig. 4). However, the solubilized parasporal bodies from *B. thuringiensis* subsp. *israelensis* were significantly more cytolytic than those from *B. thuringiensis* subsp. *morrisoni*. The toxin concentration causing 50% cytotoxicity for *B. thuringiensis* subsp. *israelensis* was 1.87 μg/ml, whereas that for *B. thuringiensis* subsp. *morrisoni* was 11.98 μg/ml. The cytotoxicity assays with *A. albopictus* and human erythrocytes were performed from the same solubilized parasporal bodies and at the same time.

DISCUSSION

The protein pattern of the parasporal bodies of both subspecies determined by SDS-PAGE was essentially as that reported earlier (11). On alkali solubilization, however, there were significant differences, particularly in the region of 24 to 29 kDa, probably because of differences in the bacterial proteases between the two subspecies and because the ca. 28-kDa protein of *B. thuringiensis* subsp. *morrisoni* was not identical to the 28-kDa protein of *B. thuringiensis* subsp. *israelensis*, as observed by differences in their mobility on SDS-PAGE. With *B. thuringiensis* subsp. *morrisoni* a band which had a slightly higher molecular mass than that of the 28-kDa protein was routinely obtained. As this band cross-reacted strongly with antibodies raised against the 25-kDa protein of *B. thuringiensis* subsp. *israelensis* (Gill et al., in press), it was probably derived from the 28-kDa protein or a larger protein which had epitopes similar to those of the 28-kDa protein of the *B. thuringiensis* subsp. *israelensis* protein.

The contribution that each parasporal body protein makes to mosquito toxicity in *B. thuringiensis* subsp. *israelensis* remains controversial (2, 5, 6, 9, 10, 13, 18, 20, 24, 26, 27), although there is general agreement that the 28-kDa protein accounts for the cytolytic properties that the solubilized parasporal body has against a variety of vertebrate and insect cells in vitro (2,6; Gill and Hornung, in press). Although we did not purify and assay the 28-kDa protein from *B. thuringiensis* subsp. *morrisoni*, its high cross-reactivity with antibodies raised against the 25-kDa protein of *B. thuringiensis* subsp. *israelensis* (Gill et al., in press), which is cytolytic, strongly suggests that this protein accounts for most of the cytolytic activity that is observed with isolate PG-14. Nevertheless, the hemolytic and cytolytic activities of the two subspecies were quantitatively different. These differences can be attributed partially to differences in the relative amounts of the major parasporal body proteins between the two subspecies. The relative amounts of the 28-kDa protein appeared to be the same in both subspecies (Fig. 1). Yet, because *B. thuringiensis* subsp. *israelensis* lacked the 144-kDa protein, on a weight basis it probably contained proportionately more of the cytolytic 28-kDa protein than did *B. thuringiensis* subsp. *morrisoni* and thus could be expected to have the slightly higher hemolytic activity that was observed. We therefore interpret the differences in hemolytic activity to be due to differences in the relative amount of the 28-kDa protein that was present in each subspecies.

In contrast to the similarity in the hemolytic activity of the solubilized parasporal bodies of the two subspecies, however, the toxicity of these solubilized toxins toward *A. albopictus* cells was significantly different. The sixfold difference that we observed between the toxins of the two subspecies cannot be explained just by differences in the amounts of the 28-kDa protein. The reason for such a difference is not apparent in light of the minor differences in their hemolytic activities and because the hemolytic and cytotoxic assays were performed with the same solubilized preparations. Although we have no evidence, these differences could be explained if the toxins that were responsible for hemolysis and cytolysis were not identical, if the mechanisms of action of the solubilized toxins in the two cell types differed, or both. Potentially, these differences could also be due to a combination of the relative levels of the various toxins as well as specific cell receptors, if these are involved in cytotoxicity. The slight differences that were observed in the slope and shape of the curve (Fig. 4) also suggest that various toxin interactions may be responsible for the cytotoxic differences that were observed. Alternatively, bacterial proteases may be involved in cytotoxicity; and the higher level of proteases that was observed in *B. thuringiensis* subsp. *israelensis*, as indicated by a larger amount of cleaved 28-kDa protein (Fig. 1 and 2), could explain the differences that were observed. At this point we can only note that differences in hemolytic and cytolytic activities between the two subspecies appear to be real and demonstrate the need for further studies in which the role of each major protein in cell and mosquito toxicity will be clarified.

The similarity of the response of the solubilized parasporal bodies of both subspecies to dioleolyl and disteroyl phosphatidylcholine suggests that the hemolytic toxins of both subspecies likely act at very similar sites on the erythrocyte membrane. Moreover, as shown previously (24; Gill et al., in press) for *B. thuringiensis* subsp. *israelensis*, the acyl group of phosphotidylcholine plays an important role in neutralizing the hemolytic activity of *B. thuringiensis* subsp. *morrisoni*. It is also probable that the principal hemolytic toxin in *B. thuringiensis* subsp. *morrisoni* selectively binds to membrane phospholipids, as has been demonstrated for *B. thuringiensis* subsp. *israelensis* (Gill et al., in press).

With regard to the relatedness of the 65-kDa protein that occurs in both subspecies, results of our immunoblot analyses indicated that this protein is basically the same, if not

identical in both subspecies, and it likely has the same biological function. The 65-kDa protein is proteolytically cleaved to the 36-kDa protein (5, 10, 18, 28) because the 36-kDa protein cross-reacted with antibodies that were raised against the 65-kDa protein. Based on the relatedness of the 28- and 65-kDa proteins in the two subspecies noted above, we surmise that the 135-kDa proteins in the two subspecies are also highly related and have similar functions. Thus, the 28-, 65-, and 135-kDa proteins of both subspecies are immunologically distinct. Similar results have been reported recently (18) for *B. thuringiensis* subsp. *israelensis*, showing that the 28-, 65-, and 135-kDa proteins probably are products of different genes.

Thus, *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* isolate PG-14 have four of five major parasporal body proteins in common. Although this similarity is significant, there are differences between these subspecies. For example, the 28-kDa protein of *B. thuringiensis* subsp. *morrisoni* migrates in SDS-PAGE faster than its homolog in *B. thuringiensis* subsp. *israelensis* and is thus slightly smaller in size. Additionally, *B. thuringiensis* subsp. *morrisoni* does not appear to have the 75-MDa plasmid that encodes the 28-kDa protein in *B. thuringiensis*, subsp. *israelensis* (11), and thus, in the former subspecies the gene must be on another plasmid or chromosomal DNA.

In summary, we conclude that the biological basis for the high mosquitocidal and hemolytic activities that are characteristic of *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* subsp. *morrisoni* is due to the presence of similar proteins in parasporal bodies of both subspecies. Differences do occur between these subspecies, such as the presence of the 144-kDa protein in *B. thuringiensis* subsp. *morrisoni*, differences in the cleavage of the 28-kDa protein, as well as differences in the cytolytic activity of the solubilized parasporal bodies. Further study of these differences will prove to be important in understanding the mode of action of these interesting and potentially useful bacterial toxins.

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