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THE INTERACTION OF THE δ-ENDOTOXIN PURIFIED FROM *BACILLUS THURINGIENSIS* SUBSP. *ENTOMOCIDUS* WITH LIPOSOMES AND MIDGUT EPITHELIUM MEMBRANES FROM TWO INSECT SPECIES

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ABSTRACT

The interaction between the δ-endotoxin purified from parasporal crystals of *Bacillus thuringiensis* subsp. *entomocidus* and insect membranes was studied wilh liposomes and midgut epithelium membranes from *Spodoptera littoralis* and *Schistocerca gregaria.* At the liposome level, the only membrane component that was associated with the toxin-induced impairment of membrane integrity was phosphatidylcholine. On the other hand, epithelial membrane from *Schistocerca gregaria* midgut showed resistance to the toxin action, compared to the susceptibility of *Spodoptera litloralis* midgut membrane. In both insects the major phospholipid is phosphatidylcholine. The data, therefore, indicate the involvement of other factors, such as the existence of a receptor for the δ-endotoxin, in the midgut epithelium membrane of susceptible insects.

INTRODUCTION

It is generally believed that the midgut epithelium membrane is the first site of interaction for the δ-endotoxin produced by various subspecies of *Bacillus thuringiensis* (Murphy et al., 1976; Fast et al., 1977). Yawetz et al. (1983) isolated from crystals of *Bacillus thuringiensis* subsp. *entomocidus (Bte)* a toxic fraction of molecular weigt (Mr) 64,000, which is characterized by specific hydrophobic properties.

The toxicity of the δ-endotoxin was evaluated using an "isolated Midgut System" (Yunovitz et al., 1986), The cytolytic activity was measured by recording glutathione s-transferase, released to the medium from epithelial cells that were ruptured by the toxin. The *Bte* δ-endotoxin tends to form high molecular weight aggregates (eluted with the void volume from a Sepharose 6B gel filtration column) in an aqueous medium due to hydrophobic interactions (Yawetz et al., 1983). The toxin maintained about 65% of its cytolytic activity at pH 7.5, which is the actual pH on the surface of the midgut epithelium of *Spodoptera littoralis* larvae (Yunovitz et al., 1987),

The δ-endotoxin induced release of [^{14}C]-sucrose from vesicles composed of phosphatidyl-choline and cholesterol, but not from vesicles where the phospholipidic component was phos-phatidylethanolamine, phosphatidylglycerol or sphingomyeline (Yunovitz and Yawetz, 1988). Organic anions such as glycerol phosphate, or acidic amino acids were inhibitory to the toxin action. Organic cations, apart from choline did not inhibit the δ-endotoxin activity. The results indicated that electrostatic and hydrophobic forces, as well as stabilizing hydrogen bonds, were the major factors of the toxin-membrane interaction.

In the present work we have tested the effect of the fluidity of the liposome membrane on the toxin induced release of entrapped radioactive marker. The specificity of the interaction of *Bte*

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δ-endotoxin with *Schistocerca gregaria* midgut epithelium was tested using the "isolated Midgut System."

MATERIALS AND METHODS

"Isolated Midgut System," isolation of larval midgut, and determination of reduced glutathione s-transferase activity were the same as described previously (Yunovitz et al., 1986). The insects used were fifth instar larvae of *Spodoptera littoralis* and adults of *Schistocera gregaria.*

The toxin fraction of 64,000 Mr was purified from *Bacillus thuringiensis* subsp. *entomocidus* δ-endotoxin crystals as described previously (Yawetz et al., 1983; Yunovitz et al., 1986). Detergent residue removed from the purified toxin by gel filtration on Sepharose 6B column previously equilibrated with 10 mM Tris-HCl, pH 8.5 (Yunovitz and Yawetz, 1988). In bioassays (Yunovitz et al., 1986) the purified toxin was highly insecticidal against 2nd instar larvae of *Spodoptera littoralis.*

Preparation of liposomes, toxin-induced release of [^{14}C]sucrose from reverse-phase vesicles composed of phosphatidylcholine and cholesterol (1:1 molar ratio), and the procedure to determine toxin-liposome interaction and activity were as previously described (Yunovitz and Yawetz, 1988), except that sonication and the second evaporation were carried out at 45°C.

Phosphatidylcholine dipalmitoyl and cholesterol were obtained from Sigma Chemical Company, St. Louis. [^{14}C]Sucrose (specific activity 0.56 Ci/mmol) was purchased from Amersham, Bucks, UK.

RESULTS

[^{14}C]Sucrose release from phosphatidylcholine dipalmitoyl vesicles induced by the *Bte* δ-endotoxin

Reversed phase vesicles composed of phosphatidylcholine dipalmitoyl and cholesterol were incubated for 15 min at 37°C and at 45°C. The sol-gel transition temperature of phosphatidylcholine dipalmitoyl is 42°C (Weinstein et al., 1981). Thus, at 37°C these vesicles exist in gel state while at 45°C they exist in a sol state with the membrane being fluid-like. As indicated by the data in Table 1, there is no significant difference in the toxin induced, dose-dependent release of [^{14}C]sucrose from the phosphatidylcholine dipalmitoyl and cholesterol vesicles, either above or below the sol-get transition point.

TABLE 1
The effect of temperature on the release of entrapped [^{14}C]sucrose from liposomes composed of phosphatidylcholine and cholesterol following incubation with the δ-endotoxin

Toxin concentration (μM)	Percentage increase in [^{14}C]sucrose released from liposomes at the indicated incubation temperature	
	37°C	45°C
1.0	43.5	44.9
1.5	67.3	67.9
2.5	73.8	86.3
5.0	81.8	86.3
10.0	84.7	86.8

The incubation was carried out for 15 min in 0.8 mM phosphate buffer, at pH 7.5

The activity of the δ-endotoxin on midgut epithelium cells from *Spodoptera littoralis* and *Schistocerca gregaria*

The only component that was found to be associated with the toxin induced disintegration of liposomes was phosphatidylcholine. Nevertheless, one cannot exclude the possible involvement of components other than phosphatidylcholine during the δ-endotoxin action at the cell membrane level. The susceptibility of midgut epithelial membrane from *Spodoptera littoralis* to the δ-endotoxin action was compared to that of midgut epithelial membrane of *Schistocerca gregaria.* In both insects the major component in membrane phospholipid composition is phosphatidylcholine (Fast, 1966).

The results presented by Table 2 indicate that a significant release of the enzyme, reduced glutathione s-transferase, occurred following the incubation of *Spodoptera littoralis* midgut epithelium cells with the δ-endotoxin. The incubation was carried out using the "Isolated Midgut System." Release of the transferase, which is an unbound cytosolic enzyme, is correlated to midgut epithelial cell rupture induced by the δ-endotoxin (Yunovitz et al., 1986).

With *Schistocera gregaria* no such transferase release could be observed, indicating resistance of the *Schistocerca gregaria* midgut epithelial membrane to the toxin action.

TABLE 2
The effect of the δ-endotoxin on the release of reduced glutathione s-transferase from ruptured midgut epitheliol cells of *Spodoptera littoralis* and *Schistocerca gregaria*

Species	δ-endotoxin (50 μg/ml)	Reduced glutathione S-transferase activity[a]
Spodoptera littoralis	absent	0.13 ± 0.014
	present	0.47 ± 0.57[b]
Schistocerca gregaria	absent	0.11 ± 0.04
	present	0.14 ± 0.06

The "Isolated Midgut System" employed was described in detail, together with the procedure, and the results for *Spodoptera littoralis* (Yunovitz et al., 1986).

[a]nanomoles 1-Chloro 2,,4-dinitrobenzene conjugated per min per 50 μl of medium from the cell ("Luminal cell") attached to the luminal side of the midgut. The pH in the "Luminal cell" was 7.5.

[b]Significantly different ($P < 0.05$) from control. The results are means ± SD of ten determinations.

DISCUSSION

The ability of the *Bte* δ-endotoxin to rupture vesicles in a gel state differentiate this endotoxin from bacterial toxins such as Streptolysin-s (Duncan and Buckingham, 1981) which is active only with liposomes in a gel state. Likewise, the activity of the *Bte* δ-endotoxin towards phosphatidylcholine dipalmitoyl liposomes indicate on indifference to the presence of the double bond in the fatty acyl tail, in contrast to *Bacillus thuringiensis* subsp. *israelensis* δ-endotoxin which binds to such liposomes (Thomas and Ellar, 1983).

Although there was no need to incorporate receptor to the phosphatidylcholine and cholesterol vesicles (Yunovitz and Yawetz, 1988), the data presented here indicate that phosphatidylcholine is not the only determinant of the toxin activity at the epithelial cell membrane level. An additional factor (or factors) in the membrane must be present in order to explain the resistance of the midgut epithelium of *Schistocerca gregaria* compared to the susceptibility of *Spodoptera littoralis.*

As the binding of the δ-endotoxin to cell membranes and its cytolytic activity are likely separate

processes (Hofman and Luthy, 1986), the toxin action may very well be biphasic, i.e., a very specific binding step is followed by a non-specific membrane disintegration process. The binding phase serves as an initial step that determines species toxicity or specificity. Upon binding of the toxin molecule with the phosphatidylcholine component of the membrane the membrane disintegrates.

An increasing line of evidence has been accumulated recently, indicating the existence of specific receptors for the *Bacillus thuringiensis* subsp. *kurstaki* δ-endotoxin (Knowles et al., 1984; Knowles and Ellar, 1986; Knowles et al., 1986). Binding is probably dependent on the presence of phosphatidylcholine at the membrane surface. The active site necessary for interaction with membrane phosphatidylcholine within the toxin molecule has been suggested as a hydrophobic amino acid residue adjacent ao an acidic amino acid residue in the toxin sequence (Yunovitz and Yawetz, 1988).

The interaction of the active site with the phosphatidylcholine polar head group is postulated to include hydrophobic attraction between the hydrophobic amino acid residue and the methyl groups of the choline, and at the same time electrostatic interaction between the acidic amino acid residue and the quaternary ammonium. As active sites may be present along the toxin molecule, the impact of such interaction on the membrane may be vibrations that will initiate its disingtegration process. The tendency of the δ-endotoxin to form high molecular weight aggregates (Yawetz et al., 1983) should enhance the membrane disintegration process.

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