Cry1Ac, a Bacillus thuringiensis toxin, triggers extracellular Ca\(^{2+}\) influx and Ca\(^{2+}\) release from intracellular stores in Cf1 cells (Choristoneura fumiferana, Lepidoptera)

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Summary

Intracellular Ca\(^{2+}\) concentration was measured in single Cf1 cells (Choristoneura fumiferana, spruce budworm) loaded with Fura-2, a Ca\(^{2+}\)-sensitive fluorescent probe. Cf1 cells displayed Ca\(^{2+}\) surges in response to Cry1Ac and Cry1C proteins, two Cf1-toxic Bacillus thuringiensis products, but not to Cry1Aa and Cry3A, which are not toxic to Cf1 cells. In the presence of extracellular Ca\(^{2+}\), the toxin-induced Ca\(^{2+}\) response was insensitive to methoxyverapamil, a voltage-dependent Ca\(^{2+}\) channel blocker, but was abolished by lanthanum, a general inhibitor of Ca\(^{2+}\) transport. In the absence of external Ca\(^{2+}\), Cry1Ac induced a small intracellular Ca\(^{2+}\) transient which was inhibited by TMB-8, a blocker of Ca\(^{2+}\) release from inositol-1,4,5-trisphosphate-sensitive pools. Under these conditions, thapsigargin, which inhibits intracellular Ca\(^{2+}\)-ATPases, elicited a Ca\(^{2+}\) surge when applied alone. However, subsequent addition of Cry1Ac failed to induce a Ca\(^{2+}\) signal, indicating a depletion of intracellular Ca\(^{2+}\) pools. In Cf1 cells, therefore, bioactive B. thuringiensis toxins triggered intracellular Ca\(^{2+}\) surges which were mainly due to the influx of extracellular Ca\(^{2+}\) through toxin-made pores, as confirmed by planar lipid bilayer experiments. Furthermore, TMB-8- and thapsigargin-sensitive Ca\(^{2+}\) stores contributed to the Cry1Ac-induced Ca\(^{2+}\) signal.

Key words: intracellular Ca\(^{2+}\), Bacillus thuringiensis, lepidopteran cell line, ion channel, Ca\(^{2+}\) transport, Fura-2, planar lipid bilayer.

Introduction

The inclusion bodies produced during sporulation by Bacillus thuringiensis, a Gram-positive soil bacterium, are highly specific gut poisons causing insect death within a few hours of ingestion (Höfte and Whiteley, 1989; Gill et al. 1992). Several formulated products based on B. thuringiensis toxins are currently used as efficient tools for the control of agro-forestry insect pests (Cannon, 1996) and of the insect vectors of several human and animal diseases (Federici, 1995). The exact mechanism of action of B. thuringiensis toxins is not well understood (Gill et al. 1992; Knowles, 1994). Following ingestion and solubilisation by intestinal secretions in the insect midgut, the crystal proteins are cleaved by gut proteases. The resulting products are 60–65 kDa activated proteins which bind to specific sites of the brush-border membrane of the columnar cells lining the gut lumen. This triggers a cascade of poorly elucidated events leading to the death of the insect. It is believed that the pore-related increased permeabilisation of the target cells and the resulting cellular ionic and metabolite imbalance constitute the critical steps leading to cell disruption. With the recent elucidation of the atomic structures of Cry3A, a coleopteran-specific toxin (Li et al. 1991), and Cry1Aa, a lepidopteran-specific toxin (Grochulski et al. 1995), a better understanding of the molecular mode of action of these proteins should emerge. This will be essential to deal with insect resistance to B. thuringiensis insecticides, the most serious problem which these products will inevitably face (Tabashnik, 1994).

So far, only limited attention has been given to the interactions of B. thuringiensis toxin with physiological processes at the cell level, possibly because of the lack of appropriate cellular models. Only a few B. thuringiensis toxin-susceptible insect cell lines are available for physiological studies. While these cells are not the natural targets of the pathogens and their sensitivity to the crystal proteins is several orders of magnitude lower than that of the insects from which they originate, they allow appropriate detection of the entomocidal activity of activated B. thuringiensis products with reasonable species and interspecies selectivity (Johnson, 1994; McCarthy, 1994b). Several physiological mechanisms have been investigated in Sf9 cells from the fall armyworm (Spodoptera frugiperda, Lepidoptera) (Hu et al. 1994a,b), UCR-SE-1a cells from the beet armyworm (Spodoptera exigua, Lepidoptera) (Monette et al. 1994) and Cf1 cells from the spruce budworm (Choristoneura fumiferana, Lepidoptera)
The concept of a synergetic interaction between Ca\(_{2+}\) and toxins, provide simple and convenient models for studies of the mechanism of action of the toxin at the cellular level. Recently, the cellular effects of Cry1 toxins on Sf9 cells have been the object of intense scrutiny in our laboratories (Schwartz et al. 1991; Vachon et al. 1995a,b; Monette et al. 1997; Villalon et al. 1997). Cry1C induced cell swelling and caused the rapid diffusion and equilibration of K\(^+\), Na\(^+\) and H\(^+\) across the plasma membrane of Sf9 cells (Vachon et al. 1995b; Villalon et al. 1997). The toxin triggered an intracellular Ca\(^{2+}\) surge within seconds of toxin exposure and thereafter activated anion-selective channels in the cell membrane (Schwartz et al. 1991). We observed a similar Cry1C-mediated Ca\(^{2+}\) response in UCR-SE-1a cells (Monette 1991). We noticed a rapid, anion-selective conductance in the cell membrane (Schwartz et al. 1991). This response of the cell membrane to toxins was related to an increased concentration within seconds of toxin exposure and thereafter activated anion-selective channels in the cell membrane (Schwartz et al. 1991). We observed a similar Cry1C-mediated Ca\(^{2+}\) response in UCR-SE-1a cells (Monette et al. 1994). Furthermore, it was established that Cry1C toxicity to Sf9 cells was substantially stimulated by extracellular Ca\(^{2+}\) in a dose-dependent manner and that this effect was related to an increased concentration of intracellular Ca\(^{2+}\) (Monette et al. 1997). These data suggested that cellular Ca\(^{2+}\) changes related to toxin exposure represent an early step in the activity of the toxin and may be a general response of susceptible insect cells to the detection of B. thuringiensis toxins. Furthermore, they supported the concept of a synergetic interaction between Ca\(^{2+}\) and B. thuringiensis toxins, as demonstrated in several lepidopteran pests in vivo (for a review, see Dent, 1993) and by a recent in vivo study on the interaction of caffeine with B. thuringiensis toxin activity against the bertha armyworm (Mamestra configurata, Lepidoptera), suggesting that the augmented toxicity was mediated by the deregulation of cellular Ca\(^{2+}\) transport processes (Morris et al. 1994).

In this report, we used Fura-2, a Ca\(^{2+}\) fluorophore, to examine the effects of B. thuringiensis toxins on the intracellular Ca\(^{2+}\) concentration of Cf1 cells, a B. thuringiensis toxin-sensitive cell line (for a review, see McCarthy, 1994a). We demonstrated that active toxins triggered Ca\(^{2+}\) surges which were produced largely by the influx of extracellular Ca\(^{2+}\) through toxin-made membrane pores but also by a small component originating from the release of Ca\(^{2+}\) from intracellular stores. Furthermore, planar lipid bilayer experiments conducted in the present study demonstrated that the pores induced by Cry1Ac, which are permeable to K\(^+\) (Slatin et al. 1990; Schwartz et al. 1997), also allowed the passage of Ca\(^{2+}\).

**Materials and methods**

**Cells**

Cf1 cells are derived from trypsin-treated larval tissue of the spruce budworm Choristoneura fumiferana. They were obtained from S. Sohi (Natural Resources Canada, Sault Sainte Marie, Ontario, Canada) and grown in Grace’s medium supplemented with 0.25% (v/v) tryptose and 10% (v/v) heat-inactivated foetal bovine serum. Cultures were maintained in 25 cm\(^2\) plastic tissue culture flasks (Sarstedt Inc., Newton, North Carolina, USA) at 27°C and were subcultured every 3–4 days to a final concentration of 1.5×10\(^6\) to 2×10\(^6\) cells ml\(^{-1}\). In preparation for the experiments, 500 μl of cells from cultures at 80–90% confluence were deposited on glass coverslips (24 mm in diameter) in supplemented Grace’s medium at room temperature (20–22°C). Attachment to the coverslips occurred within 2 h to a final confluence of 80–90%.

**Solutions**

The normal bath solution (NBS) was a simplified Grace’s solution containing 50 mmol l\(^{-1}\) KCl, 21 mmol l\(^{-1}\) NaCl, 6.8 mmol l\(^{-1}\) CaCl\(_2\), 14 mmol l\(^{-1}\) MgCl\(_2\), 11 mmol l\(^{-1}\) MgSO\(_4\), 3.9 mmol l\(^{-1}\) d-glucose and 20 mmol l\(^{-1}\) Pipes. Osmolarity was adjusted to 380 mosmol l\(^{-1}\) with sucrose, and pH was set to 6.4 with NaOH. Ca\(^{2+}\)-free NBS (0NBS) was obtained by replacing CaCl\(_2\) with 5 mmol l\(^{-1}\) EGTA. Concentrated stock solutions of test agents were prepared in NBS, 0NBS or, when required, dimethylsulphoxide (DMSO). Working solutions were obtained by dilution in NBS or 0NBS. Fura-2/AM (Fura-2 acetoxyethyl ester) stock solution (1 mmol l\(^{-1}\)) was prepared in DMSO and was used at a final concentration of 2 μmol l\(^{-1}\) in NBS.

**Cell Ca\(^{2+}\) measurements**

Intracellular Ca\(^{2+}\) concentration in single cells or small groups of cells was determined using Fura-2 as described previously (Schwartz et al. 1991). Briefly, cells were loaded with Fura-2/AM, the Ca\(^{2+}\)-insensitive, membrane-permeant form of Fura-2, a fluorescent Ca\(^{2+}\) indicator, in NBS for 1 h at room temperature. Cells were washed three times and incubated for 10 min in NBS to achieve intracellular Fura-2/AM hydrolysis by cellular esterases into Fura-2, which remained trapped intracellularly. Upon binding to Ca\(^{2+}\) (135 nmol l\(^{-1}\) dissociation constant at 20°C), the excitation spectrum of Fura-2 undergoes a dose-dependent shift towards lower wavelengths with no change in emission peak (505 nm). This spectral property of Fura-2 was used for high-sensitivity, largely artefact-free determination of cellular Ca\(^{2+}\) concentration, which can be derived from the ratio of Fura-2 fluorescence intensities measured at 340 nm and 380 nm excitation (Gryniewicz et al. 1985). Coverslips were mounted in a custom-made experimental chamber containing 0.5 ml of NBS or 0NBS and located on the stage of an IMT-2 inverted fluorescence microscope (Olympus Optical Co., Tokyo, Japan) equipped with a 40×, 0.85 NA epifluorescence objective and attached to a dual-excitation photometric instrument (Photon Technology Instrument, Monmouth Junction, New Jersey, USA). Test compounds were added to the chamber after a 2 min control period. Diluted working solutions of B. thuringiensis toxins were prepared in NBS or 0NBS and added to the chamber to a final concentration of 0.35 μmol l\(^{-1}\). For calibration, the maximum and minimum fluorescence ratios were determined at the end of each experiment by the sequential addition of 20 μmol l\(^{-1}\) ionomycin and 20 μmol l\(^{-1}\) EGTA. All experiments were performed at room temperature.

Auto fluorescence of Cf1 cells at either wavelength was at least 10 times lower than that of Fura-2 loaded cells and, therefore, fluorescence levels were not corrected for
background. The fluorophore was uniformly distributed in the cell cytosol, with no sign of compartmentalisation in the nucleus or cytoplasmic organelles. The basal level of intracellular Ca\(^{2+}\) in NBS was 82±14 nmol l\(^{-1}\) (mean ± S.E.M., N=23).

**Planar lipid bilayer**

Reconstitution of *B. thuringiensis* toxins in planar lipid bilayers has been described in detail elsewhere (Schwartz et al. 1993). Briefly, phospholipid membranes were formed from a 7:2:1 lipid mixture of phosphatidylethanolamine, phosphatidylcholine and cholesterol painted on a 250\(\mu\)m circular aperture in a Delrin wall separating two low-volume chambers (4 ml *trans*, 3.5 ml *cis*). Under the experimental conditions used in the present study, membranes had a capacitance of approximately 150–200 pF and remained stable for hours. Channel activity, following injection of 0.3 \(\mu\)mol l\(^{-1}\) activated protein near the membrane in the *cis* chamber, was monitored by step changes in the current recorded when test voltages were applied to the planar lipid bilayer. All experiments were performed at room temperature in solutions containing either 50 or 450 mmol l\(^{-1}\) CaCl\(_2\) and buffered with 10 mmol l\(^{-1}\) Tris, pH 9.0. Single-channel currents were recorded with an Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, California, USA). Analysis was performed on a personal computer using pClamp and Axotape software (Axon Instruments).

**Toxins and chemicals**

Toxins were produced, activated, purified and tested for purity as described previously (Masson et al. 1989). They were stored in lyophilised form and reconstituted to a final concentration of 1 mg ml\(^{-1}\) in high-purity water with 10 mmol l\(^{-1}\) Tris, pH 10.0. Grace’s insect cell culture medium was purchased from Gibco BRL (Life Technologies, Burlington, Ontario, Canada). Foetal bovine serum was purchased from Gibco BRL (Life Technologies, Grand Island, New York, USA). Anhydrous DMSO was obtained from Aldrich Chemicals, Milwaukee, Wisconsin, USA. Fura-2/AM was purchased from Molecular Probes, Eugene, Oregon, USA. Lipids were obtained from Avanti Polar Lipids, Alabaster, Alabama, USA.

**Results**

*Effects of B. thuringiensis toxins*

The spruce budworm is susceptible to several Cry toxins (Van Frankenhuyzen et al. 1991, 1993), some of which also show *in vitro* activity against Cf1 cells (Schwartz et al. 1993; McCarthy, 1994a), as summarised in Table 1. Cell Ca\(^{2+}\) measurements were conducted with 0.35 \(\mu\)mol l\(^{-1}\) Cry1Aa, Cry1Ab, Cry1Ac and Cry1C, four lepidopteran-specific toxins, and 0.35 \(\mu\)mol l\(^{-1}\) Cry3A, a coleopteran-specific toxin. In NBS, i.e. in the presence of 6.8 mmol l\(^{-1}\) extracellular Ca\(^{2+}\), neither Cry1Aa (N=9) nor Cry3A (N=6) elicited a Ca\(^{2+}\) surge in Cf1 cells (results not shown). However, the cells responded to Cry1Ac, Cry1Ab and Cry1C exposure (Fig. 1). Cry1Ac triggered a large, sustained Ca\(^{2+}\) surge. A 260 % increase in fluorescence ratio was observed. The signal reached 90 % of its maximum amplitude after 132±95 s (mean ± S.E.M., N=35). Cry1C also induced a sustained Ca\(^{2+}\) surge, but the fluorescence ratio was only 20–60 % greater than the basal level and it took 405±110 s (mean ± S.E.M., N=13) to reach 90 % of the final level. The response to Cry1Ab was transient. Its peak amplitude was between those of Cry1Ac and Cry1C (a 130 % increase) and the time to 90 % peak was 221±123 s (mean ± S.E.M., N=18).

**Fig. 1.** Ca\(^{2+}\) surges in single, Fura-2 loaded Cf1 cells in response to exposure to Cry toxin. The cells were bathed in a Ca\(^{2+}\)-rich physiological saline solution (NBS). Cry1Ab, Cry1Ac or Cry1C (0.35 \(\mu\)mol l\(^{-1}\)) was added to the bath at the time indicated by the arrows. Traces are representative of 35 experiments with Cry1Ac, 18 with Cry1Ab and 13 with Cry1C.

**Table 1.** Ca\(^{2+}\) response to, and toxicity of, Cry toxins

<table>
<thead>
<tr>
<th>Activated Bacillus thuringiensis toxin</th>
<th>In vitro change in [Ca(^{2+})] in response to 0.35 (\mu)mol l(^{-1}) toxin</th>
<th>In vitro toxicity: effective dose(^{a}) (ng)</th>
<th>In vivo toxicity: frass failure dose(^c) (ng larva(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cry1Aa</td>
<td>No change</td>
<td>Non-toxic</td>
<td>12.6</td>
</tr>
<tr>
<td>Cry1Ab</td>
<td>+++</td>
<td>500</td>
<td>13.2</td>
</tr>
<tr>
<td>Cry1Ac</td>
<td>+++</td>
<td>0.1</td>
<td>27.9</td>
</tr>
<tr>
<td>Cry1C</td>
<td>+</td>
<td>6.0</td>
<td>23.6</td>
</tr>
<tr>
<td>Cry3A</td>
<td>No change</td>
<td>Non-toxic</td>
<td>Non-toxic</td>
</tr>
</tbody>
</table>

\(^{a}\)+++ large surge, short delay; ++, smaller surge, longer delay; +, smallest surge, longest delay.

\(^{b}\)Lawn assay toxicity threshold (Schwartz et al. 1993).

In the absence of extracellular Ca\(^{2+}\), Cry1Ac triggered a small, but significant, Ca\(^{2+}\) surge (Fig. 2). The response was sustained over the measurement period. Its amplitude was 20\% greater than the basal fluorescence ratio ($N=7$). When tested in 0NBS, Cry1Aa, Cry1Ab, Cry1C and Cry3A did not affect the intracellular Ca\(^{2+}\) concentration of the cells.

**Effects of Ca\(^{2+}\) transport modulators on Cry1Ac-induced Ca\(^{2+}\) surges**

**Ca\(^{2+}\) transport through the cell membrane**

Ca\(^{2+}\) entry through the plasma membrane was investigated using D600, Co\(^{2+}\), Ni\(^{2+}\) and La\(^{3+}\). D600 is an efficient blocker of voltage-dependent Ca\(^{2+}\) channels (Triggle, 1990). Co\(^{2+}\), Ni\(^{2+}\) and La\(^{3+}\) are general inhibitors of Ca\(^{2+}\) transport across cell membranes (Tsien, 1990). The addition of 50 mmol l\(^{-1}\) D600, either before or after Cry1Ac application, had no effect on the sustained [Ca\(^{2+}\)] elevation observed in the presence of extracellular Ca\(^{2+}\) ($N=11$, results not shown).

Intracellular Ca\(^{2+}\) concentration was unaffected by 5 mmol l\(^{-1}\) Co\(^{2+}\) or 5 mmol l\(^{-1}\) Ni\(^{2+}\) when added to NBS in the absence of Cry1Ac toxin. However, following Cry1Ac application in Co\(^{2+}\)- or Ni\(^{2+}\)-containing NBS, both 340 nm and 380 nm fluorescence intensities decreased (results not shown). It was verified in control experiments that the ions alone did not induce cell swelling or dye leakage. Thus, it appeared that, in the presence of Cry1Ac, Ni\(^{2+}\) and Co\(^{2+}\) entered the cells and quenched the Ca\(^{2+}\) fluorophore. In fact, it has been reported that Ni\(^{2+}\) and Co\(^{2+}\) can enter melanotrophs through Ca\(^{2+}\) channels and that Fura-2 is indeed quenched by these ions (Shibuya and Douglas, 1992).

When 5 mmol l\(^{-1}\) LaCl\(_3\) was added to NBS before the toxin, Cf1 cell Ca\(^{2+}\) concentration was unaffected by exposure to Cry1Ac (Fig. 3). When LaCl\(_3\) was added during the plateau phase of the Ca\(^{2+}\) response to Cry1Ac, the surge was immediately interrupted, but the intracellular Ca\(^{2+}\) concentration did not return completely to the baseline.
Bacillus thuringiensis and cell Ca\(^{2+}\) 1855

Ca\(^{2+}\) in the bath indicated that the Ca\(^{2+}\) surge observed in response to Cry1Ac exposure was probably due to the influx of the divalent ion through toxin-made pores. However, a Ca\(^{2+}\) signal was also recorded when the toxin was applied to the cells in the absence of extracellular Ca\(^{2+}\), suggesting that Ca\(^{2+}\) was released from intracellular pools into the cytosol. In an attempt to identify the origin of the Cry1Ac-induced signal recorded in 0NBS, experiments were conducted using thapsigargin and TMB-8. These compounds modulate Ca\(^{2+}\) transport across the membranes of Ca\(^{2+}\) stores. Thapsigargin inhibits the Ca\(^{2+}\)-ATPase of Ca\(^{2+}\) pools, thus increasing the level of cytosolic Ca\(^{2+}\) by preventing Ca\(^{2+}\) uptake into cellular stores (Thastrup et al. 1990). TMB-8 is an antagonist of Ca\(^{2+}\) release from the endoplasmic reticulum (Chiou and Malagodi, 1975). Fig. 5 shows the effect of thapsigargin. In NBS, 100 nmol l\(^{-1}\) thapsigargin elicited a large Ca\(^{2+}\) transient. Subsequent addition of Cry1Ac resulted in a sustained elevation of cell [Ca\(^{2+}\)] similar to that observed with the toxin alone. In 0NBS, thapsigargin triggered a transient small rise in [Ca\(^{2+}\)]. Subsequent addition of Cry1Ac had no effect on the intracellular Ca\(^{2+}\) concentration, which remained at the basal level. The same experimental protocol was used with TMB-8.
The results of this study clearly show that the Cry1Ac-induced Ca$^{2+}$ surge had two components: a large Ca$^{2+}$ surge was recorded in the presence of Ca$^{2+}$ in the bath, and a small transient Ca$^{2+}$ signal took place in a Ca$^{2+}$-free environment. Our data indicate that the large surge was due to the influx of Ca$^{2+}$ into the cell. La$^{3+}$, which is known to inhibit the cell Ca$^{2+}$ extrusion mechanisms (Triggle, 1990), had no effect on cell [Ca$^{2+}$] in the absence of Cry1Ac. However, La$^{3+}$ prevented the Ca$^{2+}$ surge when added to the bath before the toxin and terminated the toxin-induced response when applied after the toxin. This suggests that the trivalent ion blocked the pathway used by Ca$^{2+}$ to enter the cells. Experiments with D600, a general inhibitor of voltage-dependent Ca$^{2+}$ channels (Tsien, 1990), showed that such channels were not involved in toxin-induced Ca$^{2+}$ influx, either because Cf1 cells do not possess voltage-dependent Ca$^{2+}$ channels or because, under our experimental conditions, the cells were fully depolarised and the channels were inactivated. Indeed, preliminary whole-cell patch-clamp experiments in our laboratory and elsewhere (B. Escriche, N. De Decker and E. Van Kerkhove, personal communication) indicated that the resting potential of Cf1 cells was close to 0 mV. While other Ca$^{2+}$-permeable channels that could be activated by Cry1Ac and inhibited by La$^{3+}$ may exist in Cf1 cells, the more likely explanation for the large rise in Ca$^{2+}$ response to toxin exposure is that Ca$^{2+}$ entered the cells through the pores formed by the insertion of toxin into the cell membrane. Several studies have shown that Cry toxins formed ion channels in planar lipid bilayers (Slatin et al. 1990; Schwartz et al. 1993, 1997; English et al. 1991; Von Tersch et al. 1994; Grochulski et al. 1995; Lorence et al. 1995) and single insect cells (Schwartz et al. 1991; Monette et al. 1994). The toxins also permeabilised liposomes (Yunovitz and Yawetz, 1988; Haider and Ellar, 1989; English et al. 1991; Butko et al. 1994), midgut brush-border membranes vesicles (Sacchi et al. 1986; Hendrickx et al. 1989; Woltersberger, 1989; Uemura et al. 1992; Carroll and Ellar, 1993; Lorence et al. 1995; Martin and Woltersberger, 1995) and isolated midguts (Harvey and Woltersberger, 1979; Liebig et al. 1995; Peyronnet et al. 1997). In the present study, we have provided evidence that divalent ions entered toxin-exposed cells, most probably through the toxin pores, as demonstrated by Fura-2 quenching by Ni$^{2+}$ and Co$^{2+}$. Furthermore, using planar lipid bilayers, we have demonstrated that Cry1Ac channels are indeed permeable to Ca$^{2+}$.

Interestingly, in the absence of extracellular Ca$^{2+}$, Cry1Ac induced a small Ca$^{2+}$ transient in Cf1 cells, a clear indication that the effect of the toxin on intracellular [Ca$^{2+}$] could not be solely attributed to the influx of Ca$^{2+}$ through toxin-made pores. Such a response when Ca$^{2+}$ was omitted from the bath was also observed in a previous study on the effects of Cry1C on UCR-SE-1a cells (Monette et al. 1994). It has been reported that, in both the Cf1 cell line and in a Mamestra brassica (cabbage moth) cell line, adenylate cyclase was activated by B. thuringiensis proteins that are toxic to these cells (Knowles and Farndale, 1988). However, in the M. brassica cells, this effect could not be related to the cytolytic mechanism, and it

Discussion

This study demonstrates that cytotoxic B. thuringiensis proteins triggered Ca$^{2+}$ surges in Cf1 cells, and the results are similar to previous studies in our laboratory in which we reported comparable Ca$^{2+}$ responses in Sf9 cells (Schwartz et al. 1991) and UCR-SE-1a cells (Monette et al. 1994) exposed to Cry1C toxin, to which both cell lines are susceptible (McCarthy, 1994a,b). Table 1, which summarises Cf1 toxicity data (Van Frankenhuyzen et al. 1991; Schwartz et al. 1993), and the results of the present study show that non-cytotoxic proteins (Cry1Aa and Cry3A) failed to elicit a change in Cf1 intracellular Ca$^{2+}$ concentration, whereas Cry1Ab, Cry1Ac and Cry1C, which are active against the cell line, induced Ca$^{2+}$ surges in the cells. The correlation between toxicity and the extent of intracellular Ca$^{2+}$ activity was not perfect: Cry1C, which is more toxic than Cry1Ab, induced smaller Ca$^{2+}$ surges in the cells. However, of the three proteins, Cry1Ac, the most toxic to Cf1 cells, triggered the largest Ca$^{2+}$ response, suggesting that the increase in cell [Ca$^{2+}$] participates in the mode of action of the toxin. This is consistent with our previous work on Sf9 cells, which demonstrated that Cry1C toxicity was related to extracellular Ca$^{2+}$ concentration in a dose-dependent manner and that this effect was influenced by several Ca$^{2+}$ transport modulators, implying that changes in intracellular [Ca$^{2+}$] may be related to cytotoxicity (Monette et al. 1997).
was suggested that the toxins interacted with cell membrane components, possibly lipids, thus affecting adenylate cyclase activity. In Cf1 cells, hormone-mediated receptor responses include the activation of adenylate cyclase and phospholipase C (Orr et al. 1988). Adenylate cyclase, which produces cyclic AMP, is sensitive to intracellular Ca^{2+} concentration and protein kinase C, a Ca^{2+}-activated, phospholipid-dependent enzyme. This protein is stimulated by diacylglycerol, one of the second messengers produced by phosphoinositide metabolism (Rasmussen and Barrett, 1984). Protein kinase C has recently been characterised in Cf1 cells (Gupta and Downer, 1993). Our data on Bacillus thuringiensis-induced Ca^{2+} mobilisation from thapsigargin- and TM-B-sensitive stores indicate that the inositol trisphosphate second messenger may also be produced in Cf1 cells. The mechanism by which Ca^{2+} is released from organelles in response to toxin exposure has yet to be investigated. It is tempting to speculate that, upon binding to a specific, as yet poorly identified, surface receptor (Knowles and Ellar, 1986), which may be coupled to both adenylate cyclase and phospholipase C, Bacillus thuringiensis toxins induce intracellular Ca^{2+} signalling and the production of protein kinase C, which in turn modulates cyclic AMP levels, as observed by Knowles and Farndale (1988). Further studies are needed to examine the role that such signals may play in the mode of action of the Bacillus thuringiensis protein.

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