Analysis of insecticidal proteins from *Bacillus thuringiensis* and recombinant *Escherichia coli* by capillary electrokinetic chromatography

*Bacillus thuringiensis* and recombinant *Escherichia coli* proteinaceous protoxins were subject to proteolysis and analyzed by capillary electrokinetic chromatography. Three resulting toxins (65 kDa) were baseline-resolved within 22 min using a 10 mM borate, pH 11 separation buffer consisting of 25 mM sodium dodecyl sulfate (SDS) and 30 mM phytic acid. The toxins displayed differential interactions with the SDS and phytic acid phases to effect their separation. The ion-pairing interaction between the analyte and phytic acid was also useful in preventing adsorption to the capillary walls and thus enhanced separation resolution and efficiency. The use of electrokinetic chromatography allows achievement of the separation in a significantly shorter time than conventional high-performance liquid chromatography (HPLC) using a diethylaminoethyl (DEAE) weak-anion exchanger.

**Keywords**: *Bacillus thuringiensis* / Capillary electrophoresis / Phytic acid / Sodium dodecyl sulfate

DOI 10.1002/elps.200406046

1 Introduction

As widely used for the commercial production of microbial pesticides [1], *Bacillus thuringiensis* (Bt) toxins have proven to be potential alternatives to chemical insecticides for caterpillars, beetles, and mosquitoes [2]. During sporulation, Bt produces a number of different proteinaceous intracellular insecticidal crystal proteins as crystalline inclusions called ICPs [3]. For lepidopteran-specific Bt insecticidal strains, proteins are generally expressed as a large protoxin (120–135 kDa) [4]. After ingestion by susceptible insect larvae, the crystal is dissolved in the alkaline midgut juice and activated by trypsin-like gut enzymes to produce a protease-resistant core (~65 kDa). This toxin then binds to a specific surface receptor of the gut epithelial cell monolayer [5, 6] and penetrates into the cellular membrane to form an ion channel [7, 8]. An imbalance of the cellular ionic homeostasis ensues, causing cell death by an osmotic colloid lytic mechanism leading to a breach of gut integrity and eventual larval death.

*Bt* toxins, prepared from their protoxin (120–135 kDa) precursors, are only soluble at high pH and difficult to be resolved by capillary electrophoresis (CE) or other chromatographic techniques since their molecular masses (65 kDa), isoelectric points (6.5–6.6), and the amino acid sequences are very similar [9]. To date, a patented process for identifying proteinaceous protoxins expressed by *Bt* has been developed [10]. In this procedure, the protoxins were subject to proteolysis with a proteolytic enzyme in an aqueous suspension with a pH above 9.5. The resulting toxins were then separated by HPLC using a weak-anion exchanger at a constant pH in excess of 10 with an increasing salt gradient. The analysis time was over 70 min and the procedure required extensive sample pretreatment to prevent column fouling. More recently, matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) has been used to study crystal (Cry) toxins from different *Bt* strains [11].

To our knowledge, CE has not been developed as an analytical technique to resolve *Bt* toxins [12]. The low sample volume capability, high separation efficiency, simplicity, speed of analysis, and ease of operation make CE a powerful method for analytical and quality control applications. This study pertains capillary electrokinetic chromatography, using an anionic surfactant sodium dodecyl sulfate (SDS) and phytic acid, for analysis of the *Bt* toxins as a complementary technique to HPLC and MALDI-TOF-MS.
2 Materials and methods

2.1 Reagents

SDS, phytic acid (dodecascum salt), methyl-β-cyclohexylamino)-1-propanesulfonic acid (CAPS), 3-[(3-chased from Aldrich (Milwaukee, WI, USA). 3-(Cy-cholesterol)-1-propanesulfonic acid (CAPS), 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) (CHAPS), putrescine (1,4-diaminobutane), cadaverine (1,5-diaminopentane), and p-aminobenzamidine were obtained from Sigma (St. Louis, MO, USA). Sulfoxobutyl ether-β-cyclodextrin (ds = 4) and N-acetyl-β-galactosamine were purchased from CyDex (Overland Park, KS, USA), and ICN Biomedical (Aurora, OH, USA), respectively. Acetonitrile was HPLC-grade (Fisher, Nepean, ON, Canada) and water was purified using a Zenopure Quadra 90 filtration system (Zenon Environmental, Burlington, ON, Canada).

2.2 Instrumentation

Separation was performed on a P/ACE 5500 capillary electrophoresis system (Beckman, Fullerton, CA, USA). The capillary (50 μm ID and 360 μm OD; Polymicro Technologies, Phoenix, AZ, USA) was installed in a capillary cartridge. The overall length was 57 or 47 cm with an effective capillary length of 50 or 40 cm, respectively. The capillary temperature was regulated at 25 °C using a liquid coolant in the sealed cartridge. On-column UV detection was performed with a Beckman modular UV detector operated at 214 nm, to provide maximal sensitivity for the Bt proteins. Data acquisition and analysis were facilitated by using the P/ACE Station software (Version 1.0, Beck- man). Sample injection was performed by applying pressure (0.5 psi, 3.44 kPa) at the inlet of the capillary for 5 s. The separation voltage (25 or 20 kV) was applied over a 1 min ramp to prevent any possible current breakdown.

2.3 Capillary electrophoretic separation

Stock solutions of SDS, phytic acid, and putrescine were prepared in water at 500 mM, 150 mM, and 50 mM, respectively. Separation buffers were prepared by diluting appropriately the various buffer additives with the stock borate buffers (150 mM, pH 9.25 and 100 mM, pH 11). All solutions were filtered through 0.45 μm Milllex HV filters (Millipore, Bedford, MA, USA). New capillaries were conditioned with 1 M HCl followed by 1 M NaOH for about 10 min each and then extensively washed with water and the running buffer. Before each analysis, the capillary was preconditioned with 1 M HCl (1 min), 1 M NaOH (2 min), 0.1 M NaOH (1 min), water (1 min), and the separation buffer (2 min). The number of theoretical plates (N) for each peak in the electropherograms was calculated as 5.54 (tR/w½)² where tR and w½ represent the migration time of the analyte and the full peak width at half-maximum, respectively [13]. The resolution (R) of the peak from the preceding peak was calculated as 1.18(1/2−1/tR)/(w½), where tR and w½ are defined as above for the two peaks [13]. Peak areas were expressed as absorbance units (AU)-s.

2.4 Preparation of Cry1A toxins

Three Cry1A protoxins, Cry1Aa, Cry1Ab, and Cry1Ac, were expressed in Escherichia coli and isolated individually as insoluble inclusion bodies. The inclusion bodies were then solubilized and activated by incubation at 37°C for 2 h with 50 mM carbonate, pH 10.7, containing 0.1% w/v trypsin. The insoluble materials were removed by ultracentrifugation at 200 000 × g for 2 h. The activated 65 kDa toxin was then purified by ion-exchange liquid chromatography using the 650E Advanced Protein Purifica- tion System (Waters, Milford, MA, USA) with a Q-sepharose strong anion exchange resin (Pharmacia, Uppsala, Sweden). The bound 65 kDa trypsin resistant toxin was eluted from the column with a 50–500 mM NaCl gradient in 50 mM carbonate, pH 10.7, at 1.0 mL/min. The fractions corresponding to the protein peak at 350–400 mM NaCl were pooled and dialyzed against four changes of distilled water until the toxin proteins were precipitated. The precipitated, activated toxin was then washed with HPLC-grade deionized water several times. The toxin was subsequently resolubilized in 50 mM carbonate, pH 10.7, containing 0.5 mM NaCl. The final concentration was adjusted to 1 mg/mL as determined by the dye-binding method of Bradford [14], as well as by SDS-PAGE [15] with BSA as standard. The samples were aliquoted and stored frozen at −20°C until needed. Equal volumes of these three individual forms were pooled to make a sample (0.33 mg/mL of each toxin) to be used for preliminary optimization studies. Cry1A toxins were also expressed in Bt and isolated as crystal inclusions. The sample of an HD-1 Bt strain (containing all three Cry1A protoxins) was obtained from the Bacterial Genet- ics Stock Center (BGSC) of The Ohio State University. The purification protocol for the crystal inclusions was the same as that used for the inclusion bodies from E. coli. The purified sample (1 mg/mL) contains ~0.24 mg/mL Cry1Aa, 0.43 mg/mL Cry1Ab, and 0.33 mg/mL Cry1Ac, based on the proportions known for HD-1. The augmentation in peak height from run to run after spiking with the three individual E. coli standards was attempted.
However, due to problems encountered with the E. coli standards, Cry1A standards from available Bt strains were used as alternative standards in spiking experiments for peak identification. The Bt strain HD-73 was provided by the Canadian Forestry Service (Sault St. Marie, ON, Canada) and used as a source of Cry1Ac. The Bt strain BM-1 was a DIPELÔ (kurstaki HD-1) strain, which has lost the Cry1Ab gene, and was provided by Dr. Bill Moar (Auburn University, AL) and used as a source of Cry1Aa and Cry1Ac. Cry1Aa was identified by comparing electropherograms of BM-1 and HD-73. The pAB-4 Bt strain was a crystal (−) plasmid cured derivative of HD-73 transformed with a Cry1Ab bearing plasmid for identification of Cry1Ab.

3 Results and discussion

3.1 General characteristics of the Bt toxins

Preliminary experiments utilized the three Cry1A toxins prepared by trypsin digestion of the protoxins obtained from recombinant E. coli. The samples were most stable when prepared in carbonate/NaCl buffer, pH 10.7, the buffer used during the column chromatography for purification of the toxins after the trypsin digestion. Samples (especially Cry1Ab) prepared in CAPS at a similar pH precipitated, whereas samples in borate changed with time as their peaks in the electropherograms shifted around indicating sample instability. Samples obtained directly from the columns were not sufficiently concentrated for CE analysis, therefore it was necessary to dialyze these samples to form the precipitate, which was then reconstituted at 1 mg/mL. Proteins are polyelectrolytes and adsorption often occurs because of Coulombic attractions between the negatively charged capillary wall and the positive charges on the protein molecule. Therefore, separation was performed at high pH, above the isoelectric point of the toxins to minimize their adsorption onto the capillary surface, mainly due to electrostatic interaction. Above the isoelectric point of the protein, the protein molecule would be repelled from the negatively charged capillary surface due to its net charge (at pH above 2).

3.2 Electrokinetic chromatography using SDS

A pooled mixture of the three Bt toxins prepared from E. coli migrated at a similar time (4.2–4.5 min) when the separation was conducted using 50 mM borate, pH 9.25 (Fig. 1, curve a). Cry1Aa and Cry1Ab comigrated shortly after the electroosmotic flow (4.1 min). At 35 mM borate, pH 11, separation was not significantly improved and the three-peak cluster was observed at about 5.5 min (Fig. 1, curve b). CAPS buffer (50 mM, pH 10) gave an unsatisfactory separation, likely due to the low current developed (20 μA) (Fig. 1, curve c).

Various surfactants, including SDS, deoxycholic acid, CHAPS, and CTAB, were attempted to resolve the three Bt peaks. SDS offered better resolution and shorter migration time, therefore, this surfactant was selected and optimized for the three Bt toxins. In brief, the addition of SDS (25 mM) to the separation buffer (50 mM borate, pH 9.25) shifted the migration time of the toxins to about 11–12 min while increasing the current from 38 μA to 43 μA (Fig. 2, curve a). Borate was superior to either phosphate or sodium carbonate in the presence of 25 mM SDS. For...
example, with phosphate (50 and 100 mM, pH 7), the poorly resolved peaks emerged in 10–20 min with a resultant current of 65 μA and 110 μA, respectively. Although the toxins were better resolved in 50 mM, pH 12 sodium carbonate, the baseline became much more noisy in comparison to borate buffer. Sodium carbonate was attempted since the Bt samples were reconstituted in this buffer after the dialysis step in the purification protocol.

The three Bt toxins were postulated to acquire a certain degree of separation through their differential association with the charged SDS micelles which act as a moving stationary phase. The improved resolution was possibly due to an ion-pairing mechanism and/or hydrophobic interaction between the surfactant tail and the protein. The result was somewhat surprising since SDS is generally not useful for protein separations because of its strong binding to the protein molecules. Approximately, 1.4 g SDS is adsorbed onto each gram of protein [16]. The longer migration times implied a strong attraction between the toxins and SDS, which emerged more slowly. The detection sensitivity was also improved, likely due to an improved solubility of the toxins. Compared to pH 9.5, better resolution ($R_s = 1.2$ and 0.92) was achieved at 35 mM borate buffer, pH 11 (Fig. 2, curve b). Three discernable peaks with the theoretical plates ranging from 56 000/m to 103 000/m were obtained within 22–24 min. The migration order for the Bt protein toxins was Cry1Ab (peak 1) followed by Cry1Aa (peak 2) and finally Cry1Ac (peak 3) as confirmed by spiking experiments of the pooled mixture with the individual Cry1A toxins. This behavior was attributed as an ionic strength phenomenon rather than a pH effect, since the pH 11 borate buffer was prepared by the addition of NaOH. For instance, increasing the borate concentration at pH 9.25 from 50 to 100 mM or simply adding 50 mM NaCl resulted in improved results as the currents were now in the range of 70–90 μA. At pH 9.25, the best result was obtained at 125 mM borate/25 mM SDS, whereby the three peaks were almost discernible by 29–32 min ($R_s = 1.3$ and 0.82) with a resultant current of 96 μA (Fig. 2, curve c). A small extra peak after Cry1Ac comes from an impurity in the Cry1Ab standard. Decreasing SDS to 10 mM had an adverse effect on the separation as the peaks emerged less resolved at about 23–25 min, whereas at 50 mM SDS the run time increased to 33–37 min without improving the separation significantly. As a result, 25 mM SDS was chosen for further optimization studies.

### 3.3 SDS-electrokinetic chromatography with organic modifiers

An initial attempt was carried out with N-acetyl-β-galactosamine (10 mM), with an anticipation that this sugar could selectively facilitate the separation of Cry1Ac from the other two toxins, since it has been known to bind partially competitively to the same receptor as Cry1Ac [17]. However, no improvement in separation was attained and a similar result was obtained by adding urea to the separation buffer. As Bt toxins contain large numbers of arginine residues, p-aminobenzenamide (10 mM) was added to the running buffer, since similar to m-aminobenzenamide this ligand displays high affinity for the amino acid arginine [18]. A reaction occurred in the inlet buffer vial as the solution turned brown, which might have been due to an oxidation caused by the potential applied. Acetonitrile and cupric sulfate were separately added to the running buffer and exhibited an adverse effect on the separation. Cupric sulfate was attempted since like Zn$^{2+}$, Cu$^{2+}$ can interact with N, O, or S atoms in proteins and thereby affect separation as described for analysis of histidine-containing peptides by addition of Zn$^{2+}$ to the separation buffer [19]. Adding methyl-β-cyclodextrin (5 mM) or negatively charged sulfobutyl ether-β-cyclodextrin (10 mM) to the SDS-containing running buffer exhibited no further beneficial effect. Notice that differential partitioning between neutral/charged cyclodextrins and negatively charged surfactants has often been shown to improve the separation of similar and neutral compounds such as polyaromatic hydrocarbons (PAHs) [20].

Putrescine (3 mM) was added to the 35 mM borate/25 mM SDS buffer, pH 11.0, and baseline separation was almost achieved with three well-defined toxin peaks between 30–35 min (Fig. 3A, curve a). Apparently, the amine competes with the proteins for adsorption sites on the capillary wall. Putrescine forms a dynamic coating, which passivates the capillary surface, resulting in enhanced resolution for various compounds including proteins, such as ovalbumin glycoforms [21] and transferrin sialoglycophorins with a few sialic acid residues also known as carbohydrate-deficient transferrin [22–24]. Cationic amines, such as 1,4-diaminobutane (putrescine) and 1,5-diaminopentane (cadaverine), are effective in suppressing protein-capillary interaction, and the larger the protein, the higher the concentration (up to 60 mM) must be of the cationic amine to obtain the desired effect. The resolution ($R_s$) was very much improved, 1.4 and 1.1, respectively, with the theoretical plates ranging from 53 000 to 69 000 plates/m. The peaks migrated 10 min slower compared to the case without putrescine (Fig. 2, curve b) with the resolution numbers greater than 1. Putrescine (3 mM) was also added to the 125 mM/25 mM SDS buffer, pH 9.25 (Fig. 3A, curve b) and baseline separation was almost achieved ($R_s = 1.7$ and 1.3) with better separation efficiency (59 000 to 81 000 plates/m) than at pH 11. Higher levels of putrescine (> 5 mM) did not improve the separation and in fact resulted in peak-broadening and an increased run time of 40–50 min. Similar results with
3.4 SDS-electrokinetic chromatography in the presence of phytic acid

The improved separation of proteins in the presence of phytic acid (5–20 mM) has been attributed to ion-ion pairing interactions between the positively charged proteins and the phytic acid poly-anionic species [25, 26]. Since these early studies, phytic acid has become increasingly popular for the improved resolution of numerous protein samples including albumin isofoms from *Lupinus* species [27], phycobiliproteins [28], and proteinases [29]. With 6 phosphate groups and $pK_a$ values in the range of 1.9–9.5, the charge of this acid can be manipulated over a wide pH range, such that 9–11 negative charges will exist at pH values close to 9. When added to the separation buffer, phytic acid reacts with cationic sites on the protein molecules, thus making it electrophoretically net negative charged. At the high pH, the overall charges of the toxins are negative in view of their estimated $pK_a$ values, however, the toxins still contain both negatively and positively charged groups.

As shown in Fig. 3B (curve a), the addition of phytic acid at low concentration (20 mM) improved the baseline separation of the toxins significantly ($R_s = 1.4$ and 1.7) and increased the separation time (26–30 min) due to an increase in the current to 106 mA. Phytic acid in water has a very high pH (11–12) and contributed substantially to the current due to its ionic strength. For ionic strength management, the borate concentration had to be decreased with an increase in the phytic acid concentration. Optimal conditions were achieved with 10 mM borate buffer, pH 11, containing 25 mM SDS and 30 mM phytic acid, with a resultant current of 125 mA (Fig. 3B, curve b).

The three toxin peaks were baseline-resolved ($R_s = 2.2$ and 2.7) between 33–38 min and, compared to putrescine (Fig. 3A), these resolution numbers were much higher. Due to the alkaline nature of the phytic acid it was not possible to use this additive at pH 9.25 borate buffer. Baseline separation obtained at a relatively low phytic acid concentration (30 mM) implied that the interaction between the analyte and phytic acid indeed played an important role in the separation mechanism. Apparently, phytic acid was able to differentially form an ion-pairing with the toxins by interacting with the cationic sites of the toxins, thus making these target analytes have different net negative charges. In view of the negative charges of SDS and the toxin-phytic acid complex, the addition of phytic acid to the separation buffer significantly reduced the hydrophobic interaction and/or ion-pairing between SDS and the analyte. The toxin-phytic acid complex was anticipated to spend more time in the aqueous phase with its own electrophoretic mobility, thereby increasing the separation window. Some parallels can be drawn be-
tween this technique and aqueous CE using two different phases such as SDS and a negatively charged \( \beta \)-cyclo-
dextrin derivative [30]. In this mixed-mode separation scheme, SDS and phytic acid displayed different mobili-
ties and selectivities toward the toxins in an electrical field. Hence, the optimal resolution of the mixed-mode
system could be manipulated by adjusting the concentra-
tion ratio of the two phases. On the basis of the obtained results, it might be tempting to suggest that the
ion-pairing interaction between the toxin and phytic acid was useful in preventing the adsorption of the \( Bt \) toxins
onto the walls of the capillary and greatly increased separation resolution and efficiency.

3.5 Separation of toxins from recombinant
\( E. coli \) and \( Bacillus thuringiensis \)

Although repeated analyses of the same sample were similar, subsequent batches of the purified three toxins
often yielded different results (Fig. 4A). The migration
time, peak separation, and peak height of the three indi-
vidual peaks were not consistent from sample to sample,
and in addition peak shoulders were often observed. The
resolution and theoretical plate values also varied con-
siderably. Such a result could be due to the presence of contaminants associated with the proteins and the varia-
tion from batch to batch could well demonstrate the sens-
itivity of the CE technique to detect minor changes in the protein's environment and structure. The samples from \( E. coli \) prepared from inclusion bodies may contain a variety of minor contaminants including lipids, DNA, and other peptides. Therefore, to ensure reproducibility from batch to batch it was necessary to perform postcolumn treatments on the \( Bt \) standards derived from \( E. coli \) with DNAase, lipase, or trypsin. Indeed, treatment of certain samples with trypsin was found to alter the peak position of the toxins. Instead, to circumvent this problem of postcolumn treatment of the \( E. coli Bt \) standards, the use of toxins from the \( Bt \) crystal HD-1 might be less pro-
blematical, since they contain less conjugated lipid in comparison to the \( E. coli \) standards.

The crystal sample from \( Bt \) (HD-1) containing the three protoxins of Cry1A was treated with trypsin to form the three Cry1A toxins. Three well separated peaks (Fig. 4B) were observed (39–43 min) in a similar migration range as the \( E. coli \) standards with resolution numbers of 2.4 and 1.7, respectively. Contaminating peaks were not noticed and the electropherogram displayed a very stable base-
line. As in Fig. 4A, the absorbance baseline (typically 0.006–0.008) was adjusted for comparison. Different pre-
parations of HD-1 gave very similar electropherograms
(Fig. 4B), unlike the case of the \( E. coli \) standards. The samples changed after a few months storage time at

\(-20^\circ C\) as reflected in the electropherograms, therefore it was necessary to use fresh samples otherwise shoulders began to appear on the peaks, especially Cry1Aa and Cry1Ab (figure not shown).

Shortening the effective capillary length from 50 cm (Fig. 5, curve a) to 40 cm (Fig. 5, curve b) decreased the run time (32–36 min) without significantly affecting the separation of the three peaks \( R_s = 2.4 \) and 1.9 and \( N = 65,000 \) to 79,000 plates/m). Note that the applied potential was reduced from 25 kV to 20 kV to obtain a similar cur-
rent of 125 \( \mu \)A. However, at 25 kV, the current was 184 \( \mu \)A (which could cause the Joule heating effect) and the run time was shortened by almost 20 min (all three peaks emerged separately before 21 min) although the back-
ground baseline was somewhat higher (Fig. 5, curve c).

Compared with the 50 cm effective length, resolution was slightly reduced even though the peaks are still baseline-
separated \( R_s = 2.0 \) and 1.6). Therefore, depending on the degree of resolution required, the electrophoretic runs for the separation of the three toxins can be achieved in fewer than 22 min.
Figure 5. Effect of operating conditions on the separation of \(Bt\) toxins from HD-1 at 10 mM borate, 25 mM SDS, 30 mM phytic acid, pH 11. 57 cm column, applied potential of 25 kV, 122 \(\mu\)A (curve a) (peak 1: 58 000 plates/m; peak 2: 44 000 plates/m; \(R_s = 2.4\); peak 3: 47 000 plates/m, \(R_s = 1.7\)) 47 cm column, applied potential of 20 kV, 125 \(\mu\)A (curve b) (peak 1: 65 000 plates/m; peak 2: 79 000 plates/m; \(R_s = 2.4\); peak 3: 65 000 plates/m, \(R_s = 1.9\)); 47 cm column, applied potential of 25 kV, 184 \(\mu\)A (curve c) (peak 1: 52 000 plates/m; peak 2: 64 000 plates/m; \(R_s = 2.0\); peak 3: 60 000 plates/m, \(R_s = 1.6\)).

As anticipated from Figs. 4A and B, if the crystal sample was spiked with the \(E.\ coli\) standards, the peaks did not match up, therefore spiking experiments were performed with Cry1A toxins isolated from different \(Bt\) strains. Using the 47 cm column at 20 kV, the peak order in the electropherogram was confirmed as Cry1Ab, Cry1Aa, and Cry1Ac as shown in Fig. 6. Spiking of an HD-1 (1 mg/mL) sample (containing all three \(Bt\) toxins) with an equal volume of HD-73 (1 mg/mL) identified the last peak as Cry1Ac (curve a compared to curve c). Spiking with an equal volume of BM-1 (1 mg/mL) identified the second peak as Cry1Aa (curve b compared to curves a and c). The first peak was confirmed as Cry1Ab after spiking of the sample from curve c with an equal volume of pAB-4 (1 mg/mL) (curve d versus curve c). The absorbance baseline (typically 0.006–0.008 AU) has been adjusted for the purpose of comparison. The peaks overlaid exactly indicating the reliability of the purification protocol (Fig. 6). Good reproducibility for both the migration time (1.9–2.0% at 95% confidence interval, \(n = 8\)) and the peak area (3.8–4.9% at 95% confidence interval, \(n = 8\)) was observed for repeated analyses of the same HD-1 sample (1 mg/mL) containing the three Cry1A toxins. The migration times for Cry1Ab, Cry1Aa, and Cry1Ac were 31.9 ± 0.6, 33.8 ± 0.6, and 35.2 ± 0.7 min, respectively, while the corresponding peak areas were 0.451 ± 0.006 AU-s, 0.236 ± 0.016 AU-s, and 0.356 ± 0.007 AU-s. The ratio of the peak areas for the three Cry1A toxins was also very consistent with the proportions expected (Cry1Ab – 43%, Cry1Aa – 23%, Cry1Ac – 34%, \(n = 8\)).

Using CE and the Cry1Ac (HD-73) sample, the linear concentration range for the detection of this toxin was from 25 \(\mu\)g/mL to 1 mg/mL (Fig. 7). Linearity was only observed in peak area mode since in peak height mode the peak widths vary as the concentrations increases. The sensitivity in peak area mode (Fig. 7, inset) was 1.19 ± 0.02 AU-s/µg/mL Cry1Ac, at a 95% confidence interval (\(n = 6\)). The value reported above for Cry1Ac (0.34 mg/mL) of the HD-1 sample (1 mg/mL) was 0.356 AU-s or about 1.05 AU-s for 1 mg/mL, in good agreement with the above value of Cry1Ac from HD-73. The correlation coefficient (\(R^2\)) was 0.999, and the detection limit (S/N = 3) was 10 \(\mu\)g/mL as determined from the background absorption noise of 0.00005 AU (the signal height for 25 \(\mu\)g/mL was 0.00040 AU). Similar results were obtained for Cry1Aa and Cry1Ab.

In brief, an electrokinetic chromatography technique using SDS and phytic acid was developed for identifying three similar protoxins expressed by a \(Bacillus thu-\)

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Figure 6. Identification of Cry1A peaks from HD-1 from spiking experiments run at 10 mM borate, 25 mM SDS, 30 mM phytic acid, pH 11 (curve a), HD-1 (0.5 mg/mL); (curve b) HD-1 with BM-1 (containing Cry1Aa and Cry1Ac); (curve c) HD-1 with HD-73 (containing Cry1Ac); (curve d) curve c diluted 1:1 with pAB-4 (containing Cry1Ab). 47 cm column, applied potential of 20 kV, 125 \(\mu\)A.
ringiensis gene. The resolution of the toxins was accomplished by differential association/interaction of the analyte between the SDS and phytic acid phases. The toxins complexed with SDS and migrated against the electrolyte between the SDS and phytic acid phases. The toxins also acted as an ion-pairing reagent with the phytic acid, effectively distinguishing the three closely related analytes. Phytic acid also acted as an ion-pairing reagent with the toxins to suppress protein adsorption to improve separation efficiency.

Received April 13, 2004

4 References


