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Effects of cetyltriethylammonium bromide on the replication of *Bombyx mori* nucleopolyhedrovirus

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Abstract

An experimental study was undertaken to quantify the effects of cetyltriethylammonium bromide (CTAB) on the replication of *Bombyx mori* nucleopolyhedrovirus (BmNPV) and the transcriptional activity of BmNPV *ie-1* promoter. The results demonstrated that the budded virus (BV) titer rose about 3.7-fold by adding CTAB to the culture media up to 0.1 μg ml^{-1} in infected Bm-N cells with a wild-type BmNPV. The transient expression level of luciferase driven by BmNPV *ie-1* promoter was enhanced by more than 3-fold in the presence of 0.1 μg ml^{-1} of CTAB in uninfected insect cells via a transient expression system. Contrary to the rise in BV titer, the polyhedra inside the nucleus of infected cells dropped linearly from 4.0×10^6 ml^{-1} down to 2.1×10^6 ml^{-1} within a range of CTAB concentrations from 0 to 0.25 μg ml^{-1}. The same trend in expression level of *β-galactosidase* or phytase was given when the Bm-N cells or fifth-instar silkworm larvae infected with a recombinant BmNPV containing the *β-galactosidase* or *phytase* reporter gene driven by the *polyhedrin* promoter. We deduced that CTAB appeared to affect the virus bi-phasic life cycle stages and production pathways, resulting in an enhancement in BV production and a suppression of occluded virus (OV) production and expression of foreign genes controlled by the *polyhedrin* promoter.

Introduction

Baculoviruses replicate in arthropods, specifically in lepidopteran insects. In the replication cycle, the baculovirus exhibits two types of morphology, budded virus (BV) phenotype and occluded virus (OV) particles that are enveloped nucleocapsids embedded within occlusion bodies (Blissard and Rohrmann, 1990; Faulkner, 1981; Granados and Williams, 1986). The BV causes cell to cell infection *in vivo* and *in vitro* and the OV causes host to host infection (Keddie and Volkman, 1985).

During infection, viral genes are expressed in a temporally regulated cascade fashion and are divided into four general classes based on their kinetics of expression (Friesen and Miller, 1986; Blissard and Rohrmann, 1990): immediate-early, delayed-early, late, and very late. The early and late phases of gene expression are separated by viral DNA replication. Late gene transcription initiates at or near the onset of viral DNA replication (Fuchs et al., 1983; Hun and Weaver, 1990). Early genes are transcribed by host RNA polymerase II; therefore no viral gene products are necessary for the expression of early genes (Fuchs et al., 1983; Hoopes and Rohrmann, 1991).

IE-1, the best-characterized transcriptional activator, encoded by an immediate-early gene of baculovirus, has been shown to function as a transcriptional regulator to activate the expression of some early viral genes, such as *39K* (capsid protein gene) and *p35* (apoptosis-inhibiting gene), as well as that of itself (Guarino and Summers, 1986, 1987; Kovaos et al., 1992; Carson et al., 1988). It also appears to be required for viral DNA replication (Kool et al., 1994; Lu

and Miller, 1995). The BmIE1, a protein product of immediate early-1 gene of *Bombyx mori* nucleopolyhedrovirus (BmNPV), can stimulate the promoter of the *39K* gene of *Autographa californica* nucleopolyhedrovirus (AcMNPV) (Huybrechts et al., 1992; Lu and Carstans, 1993). As a co-activator of the cytoplasmic actin gene promoter of the silkmoth *Bombyx mori* in transfected cells, the BmIE1 increased the level of transcription from this promoter by two orders of magnitude (Lu et al., 1996).

Enhancins, a group of proteins first identified in granuloviruses (GV), have the ability to enhance nucleopolyhedrovirus (NPV) infection. It was confirmed that the enhancin, a protein with 104 kDa encoded by a viral enhancing factor gene, purified from *Trichoplusia ni*. granuloviruses (TnGV), is a metalloprotease (Hashimoto et al., 1991; Lepore et al., 1996). It affects viral infection by altering the structural integrity of the peritrophic membranes lined the midgut prior to, during, and immediately after ecdysis in fourth- and fifth-instar larvae of *Trichoplusia ni* (Wang et al., 1994; Wang and Granados, 1998). In addition, lecithin (phosphatidyl choline) and some cationic detergents are synergistic to NPV (Yamamoto and Tanada, 1978; Tuan and Hou, 1988). Cheng and Hou (1992) reported that infection of silkworm cell line (Bm-N) with BmNPV was enhanced by 3.2 times in the presence of 0.1 μg ml^{-1} of cetyltriethylammonium bromide (CTAB). Raicu et al. (1998) investigated the dielectric properties of yeast cells in the absence and presence of CTAB. They reported that the conductivity of the cytoplasm and the vacuole interior decreased drastically after treating the cells with surfactant. The apparent capacitance of the plasma membrane increased systematically from 0.65 μF cm^{-2}, for untreated cells, up to about 0.75 μF cm^{-2}, at 0.3 mmol l^{-1} of CTAB. This rise was ascribed to the increase in the folding of the membrane surface associated with the surfactant-induced cell shrinkage. However, there are few reports on the correlation between the BV titer and the polyhedra inside the cell nucleus in infected Bm-N cells with BmNPV in the presence of CTAB, or the effects of CTAB on the infection of silkworm larvae with recombinant BmNPV (rBmNPV), or the effects of CTAB on the transcriptional activity of immediate-early gene (*ie-1*) promoter in uninfected cells. It is uncertain whether applying CTAB onto the host influences the transcriptional activity of *ie-1* promoter, then sets off a chain reaction of other genes depending on the presence of *ie-1* product.

In the present study, we investigated the synergistic effects of CTAB on the viral replication and gene expression with a wild-type BmNPV or a rBmNPV containing the *β-galactosidase* or *phytase* reporter gene under the control of the *polyhedrin* promoter in infected Bm-N cells or fifth-instar larvae. Meanwhile, we examined the effect of CTAB on the transcriptional activity of BmNPV *ie-1* promoter with a recombinant plasmid with the BmNPV *ie-1* promoter driving the expression of *luciferase* gene by using a transient expression assay system in uninfected insect cells.

Materials and methods

Reagents and chemicals

The reagents and chemicals used throughout this study were purchased from Life Technologies (Gaithersburg, MD, U.S.A.) and Sigma Chemical (St. Louis, MO, U.S.A.) except where a special indication was given.

Cell line and cell culture

The *Bombyx mori* cell line (Bm-N) and the *Spodoptera frugiperda* cell line (Sf-21), maintained in Key Laboratory of Silkworm Biotechnology, Ministry of Agriculture, Peop. Rep. China, were cultured with TC-100 medium supplemented with 10% fetal bovine serum, antibiotics, and other chemicals. The cells were incubated at 27 °C and subcultured every 3–5 days using a split ratio of 1:2 or 1:3. The details for cell culture were referred to Summers and Smith's (1987).

Virus

The BmNPV-ZJ8, a wild-type BmNPV, and the BmBacPAK6, a recombinant virus containing *β-galactosidase* reporter gene driven by *polyhedrin* promoter (Wu et al., 1998) were kindly provided by Professor Xiangfu Wu (Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Peop. Rep. China). The rBmNPV with the *phytase* gene replacing the *polyhedrin* gene under the control of the *polyhedrin* promoter was previously constructed in our laboratory (Wang, 1999). The methods for construction of expression vectors, viral propagation, viral titer, and viral maintenance were undertaken as Summers and Smith's (1987), Kitts and Possee's (1993), and Molecular Cloning by Sambrook et al. (1989).

Construction of the recombinant plasmid with the BmNPV ie-1 promoter

Two primers, 5′-TAGAATTCATCCCAACGGCGC-AGTGTA-3′ and 5′-ATGGATCCAATAGTCGTTTG-GTTCACG-3′, were used to amplify the *ie-1* promoter region from viral genomic DNA of BmNPV-ZJ8. The amplified product of 360-bp fragment of *ie-1* promoter region was cloned into the *Eco*RI/*Bam*HI sites of the pGEM3Z(+). The *luciferase* fragment containing an entire *luciferase* gene (1.8-kb) (Lei et al., 1994) digested with *Bam*HI was then placed under the control of the *ie-1* promoter in the right orientation.

Transient expression and CTAB treatment

Cells were seeded into 15 cm^2 flasks at a density of 5×10^5 cells ml^{-1} (3 ml per flask) and cultured for 48 h then transfected with 60 μl transfection solution containing 5 μl lipofectin and 1 μg plasmid DNA in 1 ml of serum-free medium for 4–6 h. To generate a cell line transiently expressing luciferase, cells were incubated for another 48 h at 27 °C by replacing serum-free medium with 3 ml of conditioned medium containing 0.1 μg ml^{-1} of CTAB. Then, transfected cells were collected and ready for luciferase activity assay. The treatment without CTAB was made as a control. Each treatment was separately repeated three times.

Infection with virus and CTAB treatment in vitro

To examine the effects of CTAB on viral infection, a series of 15 cm^2 flasks were seeded at an appropriate density and cells were allowed to attach for 12 h. After the cells were attached, the medium was removed and 1 ml serum-free medium containing appropriate amount of virus was added (MOI = 0.1) for infection. The cells were incubated at 27 °C for 1 h. Then the inoculum was removed and the cells were washed once with 1 ml medium and then 3 ml medium was added. The cells were incubated at 27 °C for 3 days. The treatment without CTAB was made as the control. The treatment with CTAB by adding it to the infection solution (1 ml serum-free medium containing virus) was defined as pre-treatment. The treatment with CTAB by adding to both the infection solution and the 3 ml medium was defined as treatment. The supernatant was collected for determination of the $TCID_{50}$ value and the infected cells were used for measurement of polyhedra or β-galactosidase.

Infection with virus and CTAB treatment in vivo

The variety of silkworm used throughout this work was JY1 held by our laboratory. Three separate groups (selecting 15 larvae with about the same weight as one group) were taken as one treatment. Each treatment was repeated at least three times. CTAB at different concentrations was fed by spreading it onto the mulberry leaves or directly injected into the fifth-instar larvae (2 days after molting) grown at 25–26 °C at 12 h before or after infection with virus (5 μl solution containing about 1.0×10^5 pfu viruses diluted in serum-free medium per *os*), respectively. The treatment with sterile water was made as the control. The sick larvae were weighed and the hemolymph was collected and frozen at –20 °C for the phytase activity assay.

Estimation of the luciferase activity

The cell extracts were prepared with a kit from Promega (Cat. E4030). The harvested cells were washed twice by resuspended in phosphate buffered saline (PBS), then centrifuged at $5000 \times g$ for 4 min at 4 °C. After washing, the cells were lysed by a single freeze-thaw cycle with the Kit. The lysate was centrifuged at 4 °C to remove cell debris and supernatant in an ice-bath was ready for measurement. Measurements (Idahl et al., 1986) on three separate experiments were taken in triplicate using a liquid scintillation spectrometer (Beckman LS6000 Series, U.S.A.).

Estimation of the specific activity of β-galactosidase

The cells infected with the BmBacPAK6 for 3 days were collected and washed once with PBS and then lysed with the kit from Promega (Cat. E4030) by a single freeze-thaw cycle. The lysate was centrifuged at $5000 \times g$ for 4 min to remove cell debris and the supernatant in an ice-bath was ready for measurement. Measurements (Miller, 1992) were taken in triplicate using a spectrophotometer (UV-260, Shimadzu Co., Japan).

Virus titer and polyhedra measurement

The virus titer was measured using an end-point dilution assay (Summers and Smith, 1987). When titering the BmBacPAK6, X-gal was added to the wells of the 96-well microplate. This made the scoring of the wells easier because of the formation of the indigo blue color in the positive wells. The polyhedra inside cell

nuclei were measured microscopically using a hemocytometer (BOECO, Germany) after sonication by a supersonic oscillator (SONIPREP 150, England) in an ice-bath for 10 min.

Estimation of the phytase activity

The activity of phytase was measured (Nelson, 1967; Yamada et al., 1968) using a spectrophotometer (UV-260, Shimadzu Co., Japan). The data from averages of at least three separate experiments were analyzed by Statistical Analysis System (SAS, version 6.12). One hundred microliter of dilute hemolymph was added to 900 μl incubation mixture containing 0.25 mol l^{-1} of sodium acetate buffer (pH 5.5) and 1 mmol l^{-1} of sodium phytate. The resulting mixture was incubated for 30 min at 37 °C. The reaction was stopped by the addition of 1 ml 10% TCA (trichloroacetic acid). After the reaction had terminated, 2 ml reagent (3.66 g of $FeSO_4 \cdot 7H_2O$ in 50 ml ammonium molybdate solution) was added. The OD value was measured spectrophotometrically at 750 nm. The measurements were indicative of the quantity of phosphate released in relation to a calibration curve of phosphate in the range from 0 to 1 mmol l^{-1}. One unit of enzyme is defined as the amount of enzyme that is able to liberate 1 μmol of inorganic phosphate from substrate per min under the assay conditions described above.

Results

Effects of CTAB on the replication of BmNPV

Effects of CTAB on the replication of a wild-type BmNPV

The variation of the BV titer and the number of polyhedra in the presence of CTAB at different concentrations was investigated in infected Bm-N cells with the BmNPV-ZJ8 as shown in Table I and Figure 1.

From Table I, the $TCID_{50}$ was increased from 1.12×10^8 ml^{-1}, for untreated cells, up to 4.18×10^8 ml^{-1} in the presence of 0.05 μg ml^{-1} or 0.10 μg ml^{-1} of CTAB. The enhancement was as high as about 3.7-fold compared with the control. At the concentration of 0.25 μg ml^{-1} of CTAB, the $TCID_{50}$ decreased to 1.10×10^8 ml^{-1}. This is in agreement with the studies conducted by Cheng and Hou (1992).

On the contrary, we found that the polyhedra number inside the nucleus of infected cells decreased systematically from 4.0×10^6 ml^{-1} down to 2.1×10^6 ml^{-1} within the range of 0 to 0.25 μg ml^{-1} of CTAB. Figure 1 revealed that the index (Polyhedra) dropped linearly as 100.00, 88.75, 75.00, and 52.50% corresponding to the CTAB concentrations of 0, 0.05, 0.10, and 0.25 μg ml^{-1}, respectively.

The pre-treatment of cells with 0.1 μg ml^{-1} of CTAB (see the Materials and Methods section) gave about 2.4-fold increase in BV titer and 10% decrease in the polyhedra number (Table II). However, they are lower than the 3.6-fold increase in BV titer and the 25% decrease in polyhedra with 0.1 μg ml^{-1} of CTAB treatment. This suggests that the presence of CTAB continues to affect the secondary cell to cell infection of virus after the course of primary infection of virus.

Effects of CTAB on the replication and expression of a rBmNPV

To evaluate whether there exists the similar effect of CTAB on the *polyhedrin*-negative rBmNPV, we took the *β-galactosidase* and *phytase* gene as the reporter genes that were under the control of the *polyhedrin* promoter to examine the effects of treatment with CTAB on the specific activity of β-galactosidase, in infected Bm-N cells, or phytase in infected fifth-instar silkworm larvae.

When the Bm-N cells were infected with the Bm-BacPAK6, the variation of BV titer and the expression level of the β-galactosidase showed the same trend as that with the BmNPV-ZJ8. $TCID_{50}$ increased from 1.71×10^8 ml^{-1}, without CTAB treatment, up to 3.37×10^8 ml^{-1} with about 2-fold enhancement at the concentration of 0.05 μg ml^{-1}, then dropped systematically to 0.36×10^8 ml^{-1} at the concentration of 0.25 μg ml^{-1} of CTAB. Similar to polyhedra, the specific activity of β-galactosidase decreased systematically from 17970 units down to 6730 units per mg protein extracted from infected Bm-N cells within the investigating range of CTAB concentrations. The index (Galactosidase) dropped systematically as 100.00, 87.20, 71.80, and 37.45% corresponding to the CTAB concentrations of 0, 0.05, 0.1 and 0.25 μg ml^{-1}, respectively (Figure 1). This suggested that CTAB functioned as the same role either with a wild-type BmNPV or with a rBmNPV, resulting in an enhancement in BV titer and a decrease in enzymatic activity. These results showed that CTAB suppressed the expression of *β-galactosidase* gene under the control of *polyhedrin* promoter.

The effects of CTAB on the infection of fifth-instar silkworm larvae with the rBmNPV containing *phytase* gene are also presented in Figure 1. The analysis of

Table 1. The variation of $TCID_{50}$ with different CTAB concentrations in infected Bm-N cells. The virus titer measured by an end-point dilution assay was presented as $TCID_{50}$ per ml medium in Bm-N cells infected with the BmNPV-ZJ8. The comparison in titer affected by different concentrations of CTAB was presented as enhancement index (%) over the control without CTAB treatment that was arbitrarily set as a 100. The results represented averages from three sets of separate experiments

CTAB concentrations (μg ml^{-1})	0.00	0.05	0.10	0.25
$TCID_{50} \times 10^8$ ml^{-1}	1.12±0.07	4.18± 0.25	4.18±0.31	1.10±0.09
Enhancement index (%)	100.00±6.25	373.21±22.32	373.21±27.68	98.21±8.04

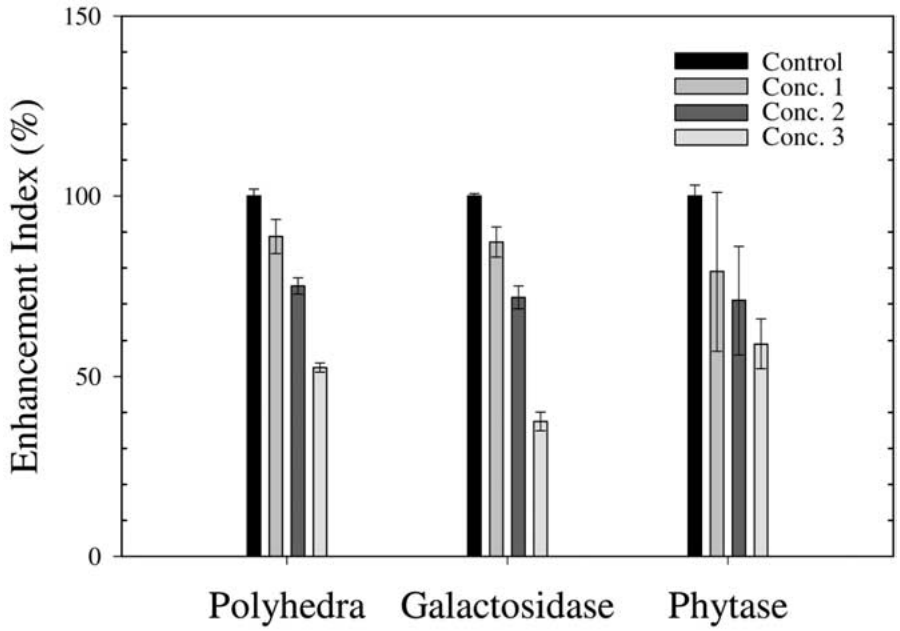

Figure 1. Effects of different concentrations of CTAB on the polyhedra and products of foreign gene expression in Bm-N cells or fifth-instar larvae. The variation of polyhedra, *β-galactosidase*, and phytase activity was indicated on Y axis as enhancement index (%) over the control without CTAB treatment that was arbitrarily set as a 100. Polyhedra and Galactosidase on X axis, by a mean from three separate experiments, presented the effects of different CTAB concentrations ranging from 0 to 0.25 μg ml^{-1} on the polyhedra number and *β-galactosidase* activity in Bm-N cells infected with the BmNPV-ZJ8 or BmBacPAK6, respectively. Phytase on X axis, by a mean from four separate experiments, presented the effects of different CTAB concentrations ranging from 0 to 0.75 μg per *os* on the phytase activity in fifth-instar larvae infected with a rBmNPV containing *phytase* gene driven by *polyhedrin* promoter. Conc. 1, 2, 3 presented the CTAB concentration of 0.05, 0.1, and 0.25 μg ml^{-1} in Bm-N cells, or 0.25, 0.5, and 0.75 μg per *os* in fifth-instar larvae, respectively.

Table 2. Effects of different CTAB treatment on the $TCID_{50}$ and polyhedra in infected Bm-N cells. The virus titer was presented as $TCID_{50}$ per ml medium in Bm-N cells infected with the BmNPV-ZJ8. The polyhedra inside the nucleus of infected cells was presented as number per ml medium. Control indicates the treatment without CTAB. Pre-treatment and treatment indicate the treatment with 0.1 μg ml^{-1} of CTAB described in the Materials and Methods section. The results represented averages from three sets of separate experiments

	BV Titer		Polyhedra	
	$TCID_{50}$ ($\times 10^8$ ml^{-1})	Enhancement Index (%)	Number ($\times 10^6$ ml^{-1})	Enhancement Index (%)
Control	1.38±0.07	100.00±5.07	2.8±0.16	100.00±5.71
Pre-treatment	3.37±0.51	244.20±36.96	2.5±0.11	89.29±3.93
Treatment	4.91±0.31	355.80±22.46	2.1±0.03	75.00±1.07

variance procedure by SAS showed that the mean expression level of phytase, similar to polyhedra, linearly dropped with significant differences from 1 66.0 units down to 97.7 units per ml hemolymph by directly injecting different dose of CTAB ranging from 0 to 0.75 μg per *os* at 12 h before infection with virus. The index (Phytase) dropped as 100.00, 79.00, 71.00, and 59.00% corresponding to the CTAB dose of 0, 0.25, 0.5, 0.75 μg per *os*, respectively. The treatments without virus infection had no effect on the growth and development of larvae. By feeding 4 μg per *os* of CTAB through spreading it onto the mulberry leaves or injection with 0.25, 0.5, and 0.75 μg per *os* into the larvae body respectively, the mean weight per *os* was not significantly different (data not shown). These suggested that CTAB treatment influenced the expression of *phytase* gene driven by very late gene, *polyhedrin* promoter, in Baculovirus-Silkworm Expression System (BSES).

Although the suppressing effect by injection with CTAB after infection with virus was weaker than that before infection with virus, it also showed a marked suppression on the expression of *phytase* gene with a same trend. The index dropped as 100.00, 82.82, 77.03, and 70.30% corresponding to the CTAB dose of 0, 0.25, 0.5, 0.75 μg per *os*, respectively.

However, by feeding different dose of CTAB ranging from 1 to 4 μg per *os* by spreading it onto the mulberry leaves either before or after virus infection, the phytase activity showed no significant differences over the control ($F = 0.0000$, $Pr > F = 0.9997$). This was possibly due to the inadequacy of CTAB penetrating in the hemolymph of larvae.

Effects of CTAB on the transcriptional activity of BmNPV ie-1 gene promoter

As mentioned in introduction, IE-1, a transcriptional activator, encoded by an immediate-early gene of baculovirus, has been shown to function as transcriptional regulator to transactivate some other immediate-early genes and several delayed-early genes. It could stimulate transcriptional activity, for the *helicase* gene promoter, by up to about 5-fold, and for *39K* gene promoter, up to approximate 17-fold (Lu and Carstans, 1993). Also, IE-1 appears to be involved in baculovirus DNA replication (Lu and Miller, 1995). In present work, the effects of CTAB on the transient expression activity of luciferase driven by BmNPV *ie-1* promoter, from average of three separate transfections, are shown in Table III.

In correspondence with the enhancement on BV titer in Bm-N cells infected with the BmNPV-ZJ8, the transient expression level of the luciferase was augmented more than 3-fold in both cell lines by treatment with 0.1 μg ml^{-1} of CTAB. In Bm-N cells, the activity of luciferase from 3 μg cell extracts rose from 16 321.3 counts per minute (cpm), without CTAB treatment, up to 51 575.3 cpm with CTAB treatment. In Sf-21 cells, the activity of luciferase from 3 μg cell extracts rose from 132 247.5 cpm, without CTAB treatment, up to 419 139.0 cpm with CTAB treatment. This indicated that CTAB treatment enhanced the transcription of *ie-1* gene promoter.

Discussion

Faulkner (1981) and Kelly (1982) reported that baculoviruses had a unique, bi-phasic replication cycle. After the infection of insect cells, BVs are produced and released into the medium by budding through the cell membrane, causing cell to cell secondary infection. Later in the infection stage this process switches to the occlusion of the OV particles in newly-synthesized polyhedra in the cell nucleus. Finally, the infected cells disintegrate, releasing the polyhedra. In the present study, CTAB, a cationic detergent, neutralized the negative charge on virion envelope and cell membrane (Ohba and Tanada, 1984; Raicu et al., 1998) and enhanced the infection of BmNPV to cells, increasing the BV titer which resulted in the decrease in polyhedra inside nucleus of infected cells either by pre-treatment or by treatment with CTAB. Thus, the CTAB appears to affect the bi-phasic replication cycle and the pathway for producing BVs and OVs during the infection of BmNPV *in vitro*.

We have demonstrated that CTAB treatment enhanced the transcription of the *ie-1* promoter and BV titer *in vitro* and suppressed the expression of *polyhedrin* and *β-galactosidase* or *phytase* gene under the control of the *polyhedrin* promoter *in vitro* or *in vivo*. Besides the neutralization of negative charge on virion envelope and cell membrane, we suggest that CTAB, possibly, through immediate early gene products, for example, IE-1, a key factor in baculovirus cascade regulation and required for replication of viral DNA, interrupts the equilibrium between BV and OV production and alters the bi-phasic replication cycle of baculovirus and production pathway, which results in an increase in BV titer and a decrease in polyhedra. This was supported by the decrease in expression level

Table 3. Effects of CTAB on the activity of luciferase in uninfected insect cells. The luciferase activity was presented as counts per minute (cpm). The comparison in enhancing ability by 0.1 μg ml^{-1} of CTAB was presented as enhancement index (%) over the control without CTAB treatment that was arbitrarily set as a 100. Each reaction contained 3 μg protein extracted from Bm-N or Sf-21 cells. The results represented averages from three separate transfections

	Luciferase activity (cpm)		Enhancement index (%)	
	Bm-N	Sf-21	Bm-N	Sf-21
Control	16321.3±1835.3	132247.5±19987.2	100.00±11.25	100.00±15.11
CTAB treatment	51575.3±2794.6	419139.0±27792.1	316.00±17.12	316.94±21.02

of *β-galactosidase* in infected cells and phytase in infected silkworm larvae with rBmNPV.

Conventional baculovirus expression vectors are recombinant viruses that can express a foreign gene in insect cells under the control of the *polyhedrin* promoter, which provides high-level transcription during the very late phase of infection. Unlike the *polyhedrin* promoter, the *ie-1* promoter can be active in uninfected cells, in the absence of any other viral factors (Guarino and Summers, 1986, 1987). Therefore, the *ie-1* promoter based-constructs are able to be used to isolate genetically transformed insect cells. This nonlytic insect cell expression system provides a high-level expression of recombinant proteins under *ie-1* control without viral infection, with the additional advantage of continuous production in a cellular environment in contrast to that generated by a baculovirus infection (Vulsteke et al., 1993; Cartier *et al*., 1994). This implies that potential application with CTAB for foreign gene expression driven by *ie-1* promoter in stably transformed cell lines and in transgenic silkworm, *Bombyx mori*, is highlighted.

The *phytase* gene used in this experiment was original from *Aspergillus niger* 963 (Yao et al., 1998). Phytase (*myo*-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) hydrolyzes phytate to *myo*-inositol and inorganic phosphate, which is of great benefit to monogastric animals to enhance plant phosphorus utilization and to circumvent the deleterious effects of phytic acid in animal nutrition.

GP64, which is peculiar to BV, is a major virion envelope glycoprotein of some baculoviruses. It plays an important role in viral infection, mediating penetration of BV form into host cells through the endocytic pathway (Jarvis and Garcia, 1994). *gp64* early promoter could be transactivated by IE-1 (Blissard and Rohrmann, 1991). The mechanism by which CTAB affects the balance between BV and OV, and whether it influences the activity of *gp64* promoter, or it augments the BV titer through the transactivation of *gp64* by IE-1 needs addressing. We are currently examining these in greater detail.

Conclusion

The treatment of CTAB appeared to affect the virus bi-phasic life cycle stages and production pathways by enhancing BV production, while decreasing OV production and suppressing the expression of foreign genes controlled by *polyhedrin* promoter during the infection of BmNPV. Also, the treatment with CTAB could augment *ie-1* promoter transcription. Therefore, treatment with CTAB may provide a means for increasing the production of stock virus and heterologous proteins driven by the *ie-1* promoter in stable transformed insect cell lines.

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