Effects of recombinant baculovirus AcMNPV-*BmK* IT on the formation of early cables and nuclear polymerization of actin in Sf9 cells

Yuejun Fu · Taotao Lin · Aihua Liang ·
Fengyun Hu

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Abstract *Autographa californica* nuclearpolyhedrosis virus (AcMNPV) is one of the most important baculoviridae. However, the application of AcMNPV as a biocontrol agent has been limited. Previously, we engineered *Buthus martensii* Karsch insect toxin (*BmK* IT) gene into the genome of AcMNPV. The bioassay data indicated that the recombinant baculovirus AcMNPV-*BmK* IT significantly enhanced the anti-insect efficacy of the virus. The actin cytoskeleton is the major component beneath the surface of eukaryotic cells. In this report, the effects of AcMNPV-*BmK* IT on the formation of early cables of actin and nuclear filamentous-actin (F-actin) were studied. The results indicated that these baculovirus induced rearrangement of the actin cytoskeleton of host cells during infection and actin might participate in the transportation of baculovirus from cytoplasm to the nuclei. AcMNPV-*BmK* IT delayed the formation of early cables of actin and nuclear F-actin and accelerated the clearance of actin in the nuclei.

Keywords AcMNPV · *BmK* IT · Cables actin · Nuclear actin polymerization

Introduction

The actin cytoskeleton is the major component beneath the surface of eukaryotic cells. Many bacterial and viral pathogens induce rearrangement of the actin cytoskeleton of host cells during infection. This may be associated with viral genome replication, transcription, virus assembly, mobility, or budding (Cudmore et al. 1997). Several viruses have been shown to interact with the cytoskeleton or nuclear matrix in their replication, such varied activities as genome replication, protein synthesis and transport, and assembly, transport, and release of virions have been associated with these structures. A series of precisely controlled actin cytoskeleton rearrangements have been reported to occur during the baculovirus replication cycle: shortly after the release of viral nucleocapsids into the cytoplasm from endosomes, filamentous-actin (F-actin) cables are formed in the cytoplasm; then, after nucleocapsid penetration into the nucleus, F-actin cables are reorganized; later, granular-actin (G-actin) monomers are driven to accumulate in the nucleus and are polymerized into F-actin (Kasman and Volkman 2000; Ohkawa and Volkman 1999). The cytoplasmic F-actin cables are possibly involved in facilitating nucleocapsid penetration into the nucleus, whereas

Y. Fu (✉) · T. Lin · A. Liang
Key Laboratory of Chemical Biology and Molecular
Engineering of Ministry of Education, Institute of
Biotechnology, Shanxi University, Taiyuan 030006,
People's Republic of China
e-mail: yjfu@sxu.edu.cn

F. Hu (✉)
Department of Neurology, Shanxi Provincial People's
Hospital, Taiyuan 030012, People's Republic of China
e-mail: fengyun71@163.com

nuclear actin polymerization is essential for viral nucleocapsid morphogenesis (Li et al. 2010).

Scorpions use their venom as a weapon to hunt and for self-defense. The effective components of the venom are a group of proteins composed of 30–80 amino acids which possess neurotoxicity (Moskowitz et al. 1998). Scorpion venom contains phospholipase, mucopolysaccharides, hyaluronidase, low relative molecular mass molecules like histamine and serotonin, protease inhibitors, histamine releasers and polypeptidyl compounds and ion channels are the main targets for various neurotoxins (Cestele and Catterall 2000). The toxins class can be separated into two classes: toxins active on mammals (MaTx) and toxins active on insects (ITx; Gordon et al. 1998). Excitatory insect toxins act specifically on insects and induce a fast excitatory contraction paralysis upon injection. Depressant insect toxins induce a slow depressant and flaccid paralysis preceded by a short transient phase of contracture (Gurevitz et al. 1998). *Buthus martensii* Karsch (*BmK*) is a kind of scorpions which venom contains excitatory insect toxins (*BmK* IT). *BmK* IT are polypeptides of 69–72 amino acid residues, acting specifically on insects and induce a fast excitatory contraction paralysis upon injection (Goudet et al. 2002). The expressed *BmK* IT kills cotton bollworms at very high rates and considerably retards larval development (Hao et al. 2005) and the high toxicity and strict insect selectivity of this scorpion toxin make it a promising biological insecticide.

The baculoviridae is a large family of enveloped, rod-shaped viruses with circular, double stranded, supercoiled DNA genomes ranging from 100 to 180 kb (Volkman et al. 1995). *Autographa californica* nuclear polyhedrosis virus (AcMNPV) is one of the most important baculoviridae. But as for biocontrol agents, the use of AcMNPV has been limited. Their use as pesticides has been less successful, one reason is the time required for an infected insect to cease feeding. Previously, we engineered *BmK* IT gene into the genome of AcMNPV. The bioassay data indicated that the recombinant baculovirus AcMNPV-*BmK* IT significantly enhanced the anti-insect efficacy of the virus (Fan et al. 2008).

In this study, the effects of AcMNPV-*BmK* IT on the changes in the distribution of microfilaments and nuclear polymerization of actin during infection of *Sf9* (*Spodoptera frugiperda*) cells were examined to analyze its anti-insect mechanism.

Materials and methods

Cell culture and virus

Spodoptera frugiperda IPLB-Sf21-AE colonial isolate 9 (Sf9) cells were maintained in TNM-FH Insect medium (Sigma, St. Louis, MO, USA) with a supplement of 10 % fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) at 27 °C. The baculovirus was the second passage of AcMNPV and AcMNPV-*BmK* IT from culture medium 48 h post inoculation (p.i.).

Antibodies

The antibody of rabbit-anti β -actin was purchased from Boster Biological Technology (Wuhan, China). Horseradish peroxidase-conjugated goat anti-rabbit IgG and the antibody against rabbit-anti glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Sangon Biotech (Shanghai, China).

Chemicals

All chemicals were reagent grade and purchased from Sangon Biotech (Shanghai, China). Fixative solution: 4 % formaldehyde in 0.1 M PBS (pH 7.0). Permeabilization buffer: 0.5 % Triton X-100 in PBS. Rhodamine phalloidin stock solution (Cytoskeleton, Inc., Denver, CO, USA): the lyophilized product was reconstituted to a 14 μ M solution with 500 μ M of 100 % methanol. DAPI working stock: 100 mM DAPI in PBS. RIPA buffer: 10 mM Tris-HCl (pH 8.0), 140 mM NaCl, 1 % Triton X-100, 0.1 % SDS, 1 % deoxycholic acid, and 1.5 mM Phenylmethanesulfonyl fluoride (Sangon Biotech Corp, Shanghai, China). Buffer A: 20 mM HEPES (pH 7.5), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, and 1.5 mM Phenylmethanesulfonyl fluoride (Sangon Biotech Corp, Shanghai, China). Nonidet-P40 (NP40) buffer: 150 mM sodium chloride, 1.0 % NP-40, 50 mM Tris (pH 8.0). NE buffer: 20 mM Tris (pH 8.0), 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 25 % glycerol, and 1.5 mM Phenylmethanesulfonyl fluoride (Sangon Biotech Corp, Shanghai, China). 5 $\times$ SDS-PAGE loading buffer: 250 mM Tris-HCl (pH 6.8), 10 % SDS, 0.5 % BPB, 10 % glycerol and 5 % 2-mercaptoethanol. BCA Protein Assay Kit was purchased from Beyotime Biotechnology Corp (Haimen, Jiangsu, China). 1 $\times$ Tris-glycine buffer:

25 mM Tris base, 190 mM Glycine, 0.1 % SDS, pH 8.3. Wet transfer buffer: 39 mM Glycine, 48 mM Tris, 0.037 % SDS, 20 % methanol. TBST buffer: 20 mM Tris (pH 8.0), 150 mM NaCl and 0.1 % Tween 20. Blocking buffer: 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1 % Tween 20, 10 % powdered milk.

Immunofluorescence analysis of the formation of early cables and nuclear polymerization of actin in AcMNPV and AcMNPV-*BmK* IT infected cells

Glass coverslips (22 mm²) were soaked over night in 5 % K-bichromate –50 % H₂SO₄, rinsed 10 times with double-distilled water, baked at 65 °C for 1 h, and allowed to cool. Sterile coverslips were placed in six-well tissue culture plates. A suspension of 5×10^5 cells in 500 µl of medium with a supplement of 10 % fetal bovine serum was applied to each coverslip, and cells were cultured on the coverslips and allowed to adhere overnight. The cells were then infected with a multiplicity of ten infectious particles per cell in 250 µl of medium.

At 2–46 h p.i., cells were processed for microscopy analysis. 100 mM working stock of rhodamine phalloidin was prepared by diluting 3.5 µl of 14 µM rhodamine phalloidin stock solution. For the localization of cellular actin, infected cells were fixed for 15 min in fixative solution at room temperature, washed thrice with PBS for 30 s. After that, cells were permeabilized in permeabilization buffer for 15 min at room temperature, washed once with PBS at room temperature for 30 s. Then coverslips were moved to a piece of parafilm in a humid chamber and add 50 µl of 100 mM rhodamine phalloidin, incubated at room temperature protected from light for 30 min with slow rotation on an orbital shaker. Following three times gentle wash with PBS at room temperature, cells were stained with 100 mM DAPI working stock in dark place. Finally, coverslips were rinsed with PBS three times and inverted on a drop of glycerol on a glass slide. The excess glycerol was gently removed and each side was sealed with nail polish. Digital images were captured using the Delta Vision RT.

Cell harvesting and fractionation

Cells were grown in 75 cm² tissue culture flasks to 4×10^6 cells. Cells were infected with a multiplicity of ten infectious particles per cell in 10 ml of medium

for 6–46 h p.i and harvested by centrifugation and washed with ice-cold PBS. One-fifth cells were used to make whole-cell lysates by centrifugation and lysis in 100 µl of RIPA buffer. The remaining cells were pelleted by centrifugation, resuspended in 350 µl of buffer A, and incubated on ice for 15 min. Then NP-40 buffer was added to a final concentration of 1 % (v/v) and vortexed for 2 min, and nuclei were pelleted by centrifugation for 10 min at 2,100 rpm at 4 °C. The supernatant cytosolic fraction was transferred to a new tube, and the nuclear pellet was washed one time with 400 µl of buffer A, pelleted for 10 min at 2,100 rpm at 4 °C and solubilized by addition of one pellet volume of NE buffer. After that, one-fourth pellet volume of 5 M NaCl and one pellet volume of NE buffer were added and then vortexed (Everly et al. 2004).

Western blot analysis of nuclear polymerization of actin in AcMNPV and AcMNPV-*BmK* IT infected cells

Cell lysates were clarified by centrifugation and the protein concentration was determined by BCA Protein Assay Kit (Beyotime, China). The protein samples were boiled in $5 \times$ SDS–PAGE loading buffer and indicated amounts of proteins were separated using 12 % acrylamide SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to PVDF membranes (Millipore, Billerica, MA, USA) for western blot analysis, then blocked for 1 h in blocking buffer with slow rotation on an orbital shaker. PVDF membranes incubated with primary anti-β-actin or anti-GAPDH antibody overnight at 4 °C, washed six times with PBST for 5 min. Blots were incubated with species specific secondary (horseradish peroxidase-conjugated goat anti-rabbit IgG, diluted at 1:4,000) for 2 h with slow rotation on an orbital shaker, washed six times with PBST for 5 min and then developed using ECL kit (Amersham, Pittsburgh, PA, USA).

Results

AcMNPV-*BmK* IT decelerated the formation of early cables of actin in Sf9 cells

Immunofluorescence analysis was performed to observe the formation of early actin cables in the AcMNPV and AcMNPV-*BmK* IT infected Sf9 cells.

Fig. 1 Formation of early cables of actin in AcMNPV and AcMNPV-*BmK* IT infected Sf9 cells.

a Uninfected cells as a mock were fixed in 4 % paraformaldehyde. Then, the actin filaments were stained with the F-actin-specific stain rhodamine-phalloidin and the cellular DNA in the nuclei was stained with DAPI. **b**, **c** Cells were infected with AcMNPV and AcMNPV-*BmK* IT with a multiplicity of ten infectious particles per cell in 250 μ l of medium and fixed in 4 % paraformaldehyde at 2 and 3 h p.i., respectively, and then stained with rhodamine-phalloidin and DAPI. The actin cytoskeleton was visualized with Delta Vision RT. Scale bar 50 μ m

As shown in Fig. 1, cells were fixed and the actin filaments were stained with the F-actin-specific stain rhodamine-phalloidin and the cellular DNA in the nuclei was stained with DAPI at 2 and 3 h p.i. In Fig. 1a, TRITC-phalloidin labeling of uninfected cells showed a fine homogeneous network of microfilaments with occasional small F-actin aggregates in the cytoplasm as well as microfilaments at the plasma membrane and in microspikes and no microfilaments appeared in nuclei.

However, in Fig. 1b, cells infected with AcMNPV and AcMNPV-*BmK* IT for 2 h had a dramatic different configuration of microfilaments compared with uninfected group (Fig. 1a). Coarse actin cables appeared traversing the cytoplasm, along the surface of the cell, or in microspikes and the cell shape was similar to that of uninfected cells (Fig. 1a). In

AcMNPV-*BmK* IT treatment group, the coarse actin cables were less than AcMNPV treatment group. As shown in Fig. 1c, cells infected with AcMNPV and AcMNPV-*BmK* IT for 3 h. The coarse actin cables increased compared with 2 h infected cells in both of these treatment groups. Moreover, the coarse actin cable formation in AcMNPV-*BmK* IT treatment group was still less than that in AcMNPV treatment group. These findings showed that the actin cables were formed in AcMNPV-*BmK* IT infected cells more slowly than that in AcMNPV infected cells.

Effects of AcMNPV-*BmK* IT on the formation of nuclear F-actin in infected Sf9 cells

Fluorescence microscopy analysis was performed to observe the nuclear polymerization of actin in

Fig. 2 Formation of nuclear F-actin in AcMNPV and AcMNPV-*BmK* IT infected Sf9 cells. **a–g** Cells were infected with AcMNPV and AcMNPV-*BmK* IT with a multiplicity of ten infectious particles per cell in 250 μ l of medium and fixed in

4 % paraformaldehyde at 6, 12, 21, 25, 31, 37 and 46 h p.i., respectively, then stained with rhodamine-phalloidin and DAPI. The actin cytoskeleton was visualized with Delta Vision RT. Scale bar 50 μ m

AcMNPV and AcMNPV-*BmK* IT infected Sf9 cells. At 6, 12, 21, 25, 31, 37, 46 h p.i., cells were fixed and the actin filaments were stained with the F-actin-specific stain rhodamine-phalloidin; and the cellular DNA in the nuclei was stained with DAPI. As shown in Fig. 2a, cells infected with AcMNPV and AcMNPV-*BmK* IT for 6 h, microfilaments were formed on the ventral surface of the cells both in these treatment groups, but little microfilaments were formed in nuclei. As shown in Fig. 2b, cells infected

with AcMNPV and AcMNPV-*BmK* IT for 12 h, microfilaments were formed increasingly on the ventral surface of the cell both in AcMNPV-*BmK* IT and AcMNPV treatment group. In the AcMNPV-*BmK* IT treatment group, some actin filaments appeared in the nuclei, and the microfilaments were less than those in the AcMNPV treatment group. In Fig. 2c, d, cells were infected with AcMNPV and AcMNPV-*BmK* IT for 21 h and 25 h, respectively. Microfilaments were formed increasingly on the ventral surface of the cell

Fig. 3 Western blot analysis of polymerization of β -actin in the nuclei. **a** The distributing level of β -actin in the nuclei at 6, 12, 21, 25, 31, 37 and 46 h p.i., respectively, in the AcMNPV and AcMNPV-BmK IT treatment groups; **b** The expression level of

GAPDH in total protein of Sf9 cells at 6, 12, 21, 25, 31, 37 and 46 h p.i., respectively, in the AcMNPV and AcMNPV-BmK IT treatment groups

in AcMNPV-BmK IT and AcMNPV treatment group with the time went by, and the early cables disappeared in AcMNPV treatment group (Fig. 2d). In succession, actin filaments appeared in nuclei in both AcMNPV-BmK IT and AcMNPV treatment group. As shown in Fig. 2e, f, cells were infected with AcMNPV and AcMNPV-BmK IT for 27 and 31 h, respectively. Both in AcMNPV-BmK IT and AcMNPV treatment groups, more and more actin were polymerized in nuclei. Moreover, the nuclear polymerization of actin in the AcMNPV treatment group were more than that in the AcMNPV-BmK IT treatment group at 27 and 31 h post infection. In Fig. 2g, cells infected with AcMNPV and AcMNPV-BmK IT for 46 h, nuclear actin decreased in the AcMNPV-BmK IT treatment group. These finding showed that, in the AcMNPV-BmK IT treatment group, the nuclear F-actin was formed more slowly and disappeared faster than that in the AcMNPV treatment group.

Effects of AcMNPV-BmK IT on nuclear polymerization of actin in infected Sf9 cells

Western blot analysis of nuclear polymerization of actin was performed when cells were infected with AcMNPV-BmK IT at 6–46 h p.i., respectively. As shown in Fig. 3a, in the AcMNPV-BmK IT treatment group the nuclear polymerization of actin appeared at 21 h post infection which had a delayed time of 9 h compared with that in the AcMNPV treatment group. At 46 h post infection, the level of the nuclear polymerization of actin decreased in AcMNPV-BmK IT treatment group but no change was observed in the AcMNPV treatment group. The results indicated that the polymerization of

actin in the nuclei in the AcMNPV-BmK IT treatment group appeared more slowly and disappeared sooner than that in the AcMNPV treatment group.

Discussion

Actin exists in two forms in the cell: monomeric G-actin and filamentous F-actin. To accomplish body functions such as morphogenesis, migration of immune cells, formation of the neural network, endocytosis, exocytosis and cytokinesis, cells must reorganize cell surface structures through mobilization of the actin remodeling machinery (Watanabe 2010). The sequential actin rearrangement during baculovirus infection suggested specific interactions of the viruses with the actin cytoskeleton. How the different steps of actin rearrangement contribute to viral infection, however, is still speculative (Dreschers et al. 2001). The thick microfilament cables were formed without protein synthesis indicating that a component of the viral inoculum induced this first rearrangement of microfilaments from preexisting cellular actin. One of the unique features of baculovirus propagation in insect cells is nuclear F-actin formation in virus-infected cells (Goley et al. 2006). In this report, we found that both the formation of early cables of actin and the formation of nuclear F-actin in AcMNPV-BmK IT treatment group were delayed compared with that in AcMNPV treatment group. We inferred this delay was due to the interaction between BmK IT and its receptor-sodium channel on the cell surface and the changes of microenvironment in the cells caused by this interaction. Moreover, the special

phenotype is a consequence of nuclear recruitment and subsequent protein–protein interactions of several host and viral factors, including but not limited to P78/83, G-actin, the Arp2/3 complex, C42 (Wang et al. 2007, 2008). So, the relationship between AcMNPV-*BmK* IT and those proteins should be analyzed in the future work.

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References

- Cestele S, Catterall WA (2000) Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. *Biochimie* 82:883–892
- Cudmore S, Reckmann I, Way M (1997) Viral manipulations of the actin cytoskeleton. *Trends Microbiol* 5:142–148
- Dreschers S, Roncarati R, Knebel-Mörsdor DF (2001) Actin rearrangement-inducing factor of baculoviruses is tyrosine phosphorylated and colocalizes to F-Actin at the plasma membrane. *J Virol* 75:3771–3778
- Everly DN Jr, Kusano S, Raab-Traub N (2004) Accumulation of cytoplasmic β -catenin and nuclear glycogen synthase kinase 3 β in Epstein-Barr virus-infected Cells. *J Virol* 78:11648–11655
- Fan XJ, Zheng B, Fu YJ, Sun Y, Liang AH (2008) Baculovirus-mediated expression of a Chinese scorpion neurotoxin improves insecticidal efficacy. *Chin Sci Bull* 53:1855–1860
- Goley ED, Ohkawa T, Mancuso J, Woodruff JB, Alessio JAD, Cande WZ, Volkman LE, Welch MD (2006) Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. *Science* 314:464–467
- Gordon D, Savarin P, Gurevitz M, Zinn-Justin S (1998) Functional anatomy of scorpion toxins affecting sodium channels. *J Toxicol-Toxin Rev* 17:131–159
- Goudet C, Chi CW, Tytgat J (2002) An overview of toxins and genes from the venom of the Asian scorpion *Buthus martensii* Karsch. *Toxicon* 40:1239–1258
- Gurevitz M, Froy O, Zilberberg N, Turkov M, Strugatsky D, Gershburg E, Lee D, Adams ME, Tugarinov V, Anglister J, Shaanan B, Loret E, Stankiewicz M, Pelhate M, Gordon D, Chejanovsky N (1998) Sodium channel modifiers from scorpion venom: structure-activity relationship, mode of action and application. *Toxicon* 36:1671–1682
- Hao CJ, Xu CG, Wang W, Chai BF, Liang AH (2005) Expression of an insect excitatory toxin, *BmK* IT, from the scorpion, *Buthus martensii* Karsch, and its biological activity. *Biotechnol Lett* 27:1929–1934
- Kasman LM, Volkman LE (2000) Filamentous actin is required for lepidopteran nucleopolyhedrovirus progeny production. *J Gen Virol* 81:1881–1888
- Li K, Wang Y, Bai H, Wang Q, Song J, Zhou Y, Wu C, Chen X (2010) The putative pocket protein binding site of *Autographa californica* nucleopolyhedrovirus BV/ODV-C42 is required for virus-induced nuclear actin polymerization. *J Virol* 84:7857–7868
- Moskowitz H, Hermann R, Jones AD, Hammock BD (1998) A depressant insect-selective toxin analog from the venom of the scorpion *Leiurus quinquestriatus* hebraeus. *Eur J Biochem* 254:44–49
- Ohkawa T, Volkman LE (1999) Nuclear F-actin is required for AcMNPV nucleocapsid morphogenesis. *Virology* 264:1–4
- Volkman LE, Blissard GW, Friesen P, Keddie BA, Possee R, Theilmann DA (1995) Family Baculoviridae. In: Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli GP, Mayo MA, Summers MD (eds) *Virus Taxonomy*. Springer, New York, pp 104–113
- Wang Q, Liang C, Song J, Chen X (2007) HA2 from the *Helicoverpa armigera* nucleopolyhedrovirus: a WASP-related protein that activates Arp2/3-induced actin filament formation. *Virus Res* 127:81–87
- Wang Y, Wang Q, Liang C, Song J, Li N, Shi H, Chen X (2008) *Autographa californica* multiple nucleopolyhedrovirus nucleocapsid protein BV/ODV-C42 mediates the nuclear entry of P78/83. *J Virol* 82:4554–4561
- Watanabe N (2010) Inside view of cell locomotion through single-molecule: fast F-/G-actin cycle and G-actin regulation of polymer restoration. *Proc Jpn Acad Ser B Phys Biol Sci* 86:62–83