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New insights into the induction of the heat shock proteins in baculovirus infected insect cells

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ABSTRACT

Eight members of the HSP/HSC70 family were identified in *Spodoptera frugiperda* Sf9 cells infected with *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) by 2D electrophoresis followed by mass spectrometry (MALDI/TOF) and a Mascot search. The family includes five HSP70s induced by AcMNPV-infection and three constitutive cognate HSC70s that remained abundant in infected cells. Confocal microscopy revealed dynamic changes in subcellular localization of HSP/HSC70s in the course of infection. At the early stages (4 to 10 hpi), a fraction of HSPs is localized in distinct speckles in cytoplasm. The speckles contained ubiquitinylated proteins suggesting that they may be aggresomes where proteins targeted by ubiquitin are sequestered or processed for proteolysis. *S. frugiperda* HSP90 was identified in the 2D gels by Western blotting. Its amount was unchanged during infection. A selective inhibitor of HSP90, 17-AAG, decreased the rate of viral DNA synthesis in infected cells suggesting a supportive role of HSP90 in virus replication.

Introduction

Baculoviruses contain a circular double-stranded DNA genome of 80 to 180 kbp and infect insects of orders Diptera, Hymenoptera and Lepidoptera. The best studied baculovirus, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), possesses a 134-kbp genome that encodes about 150 proteins including factors essential for viral DNA synthesis. During infection, baculoviruses interrupt cell cycle, down-regulate most host genes while inducing synthesis of viral products necessary for replication of viral genomes and for the assembly of viral particles. In infected cells, baculoviruses produce two types of progeny viruses, budded virions (BV) and occlusion derived virions (ODV), and in permissive infections eventually kill the infected cells and their host (for review see Rohrmann, 2011). Baculovirus infection is recognized as a stress factor in host cells and this can lead to the activation of various cellular pathways including apoptosis. In order to prevent premature death of infected cells and ensure productive infection, baculoviruses use a dual strategy. First, baculoviruses inhibit the apoptotic pathway by expressing viral factors, such as p35 and other anti-apoptotic proteins (Clem, 2007; Clem et al., 1991). Second, baculoviruses activate prosurvival pathways mediated by the signal kinases such as MAPKs, ERK, and JNK, and the phosphatidylinositol 3-kinase (PI3K)–Akt pathway. The inhibition of these pathways significantly reduces virus production in infected cells (Chen et al., 2009; Katsuma et al., 2007; Schultz and Friesen, 2009; Xiao et al., 2009). A hallmark of universal cellular defense reactions to various environmental and pharmacological stresses is the activation of the heat shock response (HSR). It is characterized by drastic up-regulation of members of the ubiquitous chaperone family of heat shock proteins (HSPs) with molecular masses of approximately 70 kDa. HSP70s and the cognate heat shock proteins (HSC70s) play a central role in protein homeostasis and protection against proteotoxic stresses by preventing protein misfolding and aggregation, or by directing damaged proteins to the ubiquitin–proteasome system for degradation. The ATP-dependent chaperoning activity of HSP/HSC70s and another key chaperone, HSP90, is regulated by a battery of co-chaperones, which specify the function of HSPs and regulate their interaction with client proteins. HSP70s and HSP90s are involved in the replicative cycles of various viruses regulating gene expression *via* interaction with viral proteins and participating in capsid assembly and disassembly (Burch and Weller, 2004, 2005; Couturier et al., 2010; Dutta et al., 2009; Lahaye et al., 2009; Livingston et al.,

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2008, 2009; Mayer, 2005; Nagy et al., 2011; Song et al., 2010; Weeks et al., 2010; Yu et al., 2009; Zhao et al., 2009).

Little is known about function of HSPs in the infection cycle of baculoviruses. The cognate HSC70 was found associated with occlusion derived virus (ODV) of the *Bombyx mori* NPV (BmNPV) (Liu et al., 2008) suggesting the possible involvement of HSPs in the assembly of virions. Although expression of the HSP genes has been analyzed during the course of baculovirus infection by several researchers, different approaches and diverse viral and cellular strains used in these experiments prevent the formulation of a unified understanding of their role during infection. In the case of *Spodoptera frugiperda* cell lines commonly used for propagation of AcMNPV, the analysis was further hampered by the absence of the host cell genome sequence. In an early report, Ooi and Miller demonstrated by Northern blot analysis the substantial reduction of mRNA levels of actin, histone, and HSP70 from 12 to 18 h following infection of Sf21 cells with AcMNPV (Ooi and Miller, 1988). Nobiron and coworkers used a differential display approach and confirmed the down regulation of several selected Sf9 genes from 12 h post infection with AcMNPV. One transiently up-regulated host transcript was encoded by the *hsc70* gene. Its mRNA level peaked at 6 hpi and dropped markedly from 12 to 24 hpi (Nobiron et al., 2003). Expression of the silkworm *B. mori hsc70* ortholog was increased by 1.6-fold in NIAS-Bm-oyanagi2 cells 24 hpi after infection with BmNPV, but no increase was evident at 12 hpi (Sagisaka et al., 2010). A microarray assay based on ESTs validated by qRT-PCR was used by Salem et al. (2011) for the host transcriptome analysis of Sf21 cells during AcMNPV infection. In contrast to the majority of host genes that were down regulated in the course of infection, a few genes were up regulated. Among the most highly up-regulated genes were two representatives of the *hsp70* family.

In our previous report, five members of the HSP/HSC70 family were detected in Sf9 cells infected with AcMNPV by using Western blotting and monoclonal antibody to Drosophila HSP70s (Lyupina et al., 2010). Three of HSP70s were induced or highly stimulated by virus infection. The cellular HSP/HSC70 content increased moderately in AcMNPV-infected cells under standard conditions, whereas the infection markedly potentiated the response to heat shock boosting the HSP/HSC70s content in infected cells in comparison with uninfected cells. The inhibition of the HSR by KNK437 decreased markedly the rate of viral DNA synthesis in infected cells indicating the importance of HSR for baculovirus replication. In this report we describe a continuation of the study of HSPs in Sf9 cells infected with AcMNPV. The proteome analysis enabled the identification of eight members of the HSP/HSC70 family and suggested possible roles that they may play during the course of baculovirus infection. The possible cooperation of HSP/HSC70s with the ubiquitin–proteasome system in infected cells was revealed by confocal microscopy.

Results

S. frugiperda HSP/HSP70s

In order to identify members of the HSP/HSC70 family in Sf9 cells, proteomic analysis was applied to a fraction of cellular proteins that were recognized by a monoclonal antibody and to several other proteins having molecular masses and pI values similar to HSP70s. Proteins for the analysis were obtained from uninfected cells and from AcMNPV-infected cells heated at 37 °C for 2 h at 22 hpi. As shown in the previous report, moderate heating markedly augments expression of HSP70s induced by AcMNPV (Lyupina et al., 2010). Proteins were subjected to 2D electrophoresis as previously described (Lyupina et al., 2010). One gel for each sample was stained with Coomassie blue (Figs. 1A and B), and the other was processed for Western-blot analysis with the anti-HSP/HSC70 antibody 7.10.3 and with anti-Drosophila HSP90 antibody 16F1. The blot derived from mock infected cells is shown in Fig. 1C, whereas Figs. 1D1 and D2 are from infected cells and show different exposures of an area delimited by the large arrows. The areas of the gel

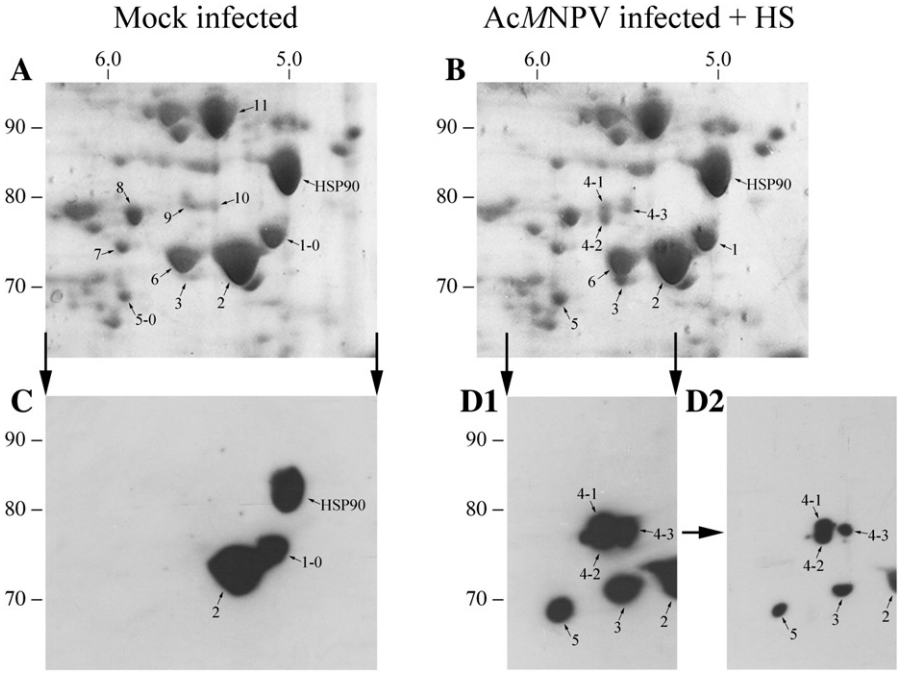

Fig. 1. Changes in the HSP/HSC70 pattern in Sf9 cells after infection with AcMNPV and heating analyzed by two-dimensional PAGE followed by Coomassie staining and Western blotting. Extracts were prepared from uninfected cells (A, C) and cells infected with AcMNPV and heated at 37 °C for 2 h at 22 hpi (B, D1 and D2). Electrophoresis was performed by the isoelectric focusing in the first dimension (horizontal) and then by SDS-8% PAGE in the second dimension (vertical). Each sample was analyzed in duplicate. One gel was stained with Coomassie blue (A, B), and another one was blotted onto a membrane for antigen visualization (C, D1, and D2). The membranes were probed sequentially with the rat mAb 7.10.3 to HSP/HSC70s and then with the rat anti-HSP90 mAb. Panels D1 and D2 show different exposures (long and short, respectively) of a portion of the membrane corresponding to the gel area delimited by the large arrows. Protein spots indicated by small arrows and numbers were cut from the Coomassie stained gels and subjected to mass spectrometry (MALDI/TOF) followed by Mascot software analysis.