

David K. Thumbi¹, Basil Arif², Eric Carstens³, Satish Deshpande⁴ and Peter Krell¹.

¹ Department of Microbiology, University of Guelph, Guelph, ON, N1G 2W1, ² Great Lakes Forestry Centre, Sault Ste Marie, ON, P6A 2E5, ³ Department of Microbiology and Immunology, Queen's University, Kingston, ON, K7L 3N6 and ⁴ SpectraDigital Corporation Inc. Guelph, ON, N1H 3B3

Introduction

Baculoviruses are ideal candidates for the biocontrol of insect pests particularly of the order Lepidoptera. Insights into the molecular basis of baculovirus DNA replication could help to enhance the rate of virus replication and hence result in earlier death or debilitation of the target pest. Several approaches to identify different antiviral compounds of potential use in baculovirus DNA replication studies were employed. 1. An initial screen was performed using a PTI fluorescence assay by monitoring the expression of an enhanced green fluorescent protein (EGFP) following infection of Sf 21 cells with a recombinant AcEGFP virus. Preliminary data showed that aphidicolin, camptothecin, phosphonoacetic acid (PAA) and (E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVdU), of the compounds analyzed, were effective inhibitors of AcEGFP virus replication. 2. Infected cells treated with different concentrations of camptothecin and PAA exhibited diagnostic light scattering profiles compared to untreated infected cells. 3. Dot blot analysis showed inhibitory effects on viral DNA replication by different compounds. 4. The inhibitors had varying effects on cell viability and virus production as determined by cytopathic effects and PIB production.

1. PTI assay: Effects of Antiviral compounds on the expression of EGFP in AcEGFP virus

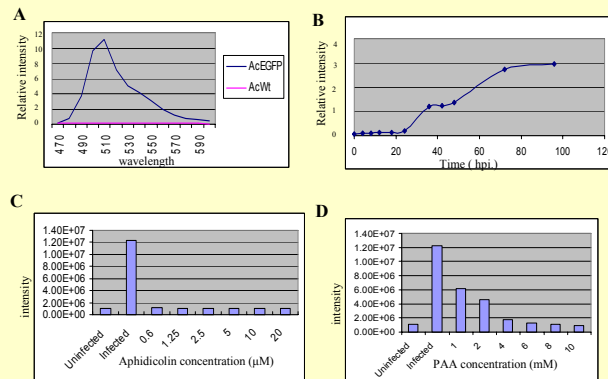


Figure 1. Sf 21 cells (1x10⁶ cells/ml) were infected with AcEGFP virus (MOI = 10). After 1 hr incubation at 27°C, medium containing unattached virus was removed and replaced with fresh medium and varying concentrations of antiviral compounds. Expression of EGFP was determined from the treated cells lysate at 36 hr p.i. (A) Emission scan for viral EGFP. (B) Time course for EGFP expression (hpi). (C) Effect of Aphidicolin on EGFP expression. (D) Effect of PAA on EGFP expression.

2. Dynamic light scattering analysis

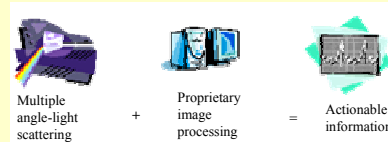


Figure 2. Suspension cultures of Sf-21 cells at 1 x 10⁵ cells/ml were incubated at 4°C for 1 hr. AcEGFP virus (MOI = 10) was added to the cells and left to attach for 1 hr then incubated at 27°C. Different concentrations of antiviral compounds were added to the infected cells and the inhibitory effects were determined based on changes in the spectral properties of the cells.

Effect of Camptothecin and PAA on spectral properties of Sf-21 cells treated at various concentrations

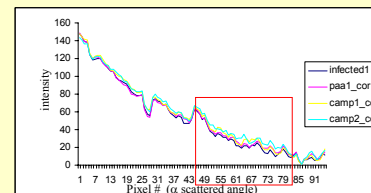


Figure 3. Light scattering of treated cells with different concentrations of camptothecin and PAA was determined at 72 hrs p.i. The area under the 'infected' curve depicts the light scattering profiles of AcEGFP Sf 21 infected cells.

3. Dot blot analysis: Effects of Antiviral compounds on AcEGFP viral DNA replication

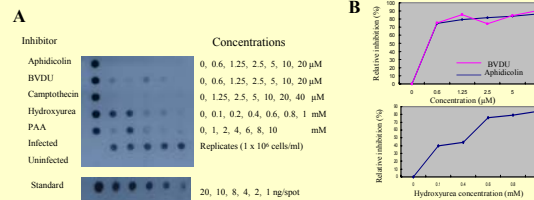


Figure 4. (A) Effect of antiviral compounds on AcEGFP viral DNA replication. Budded virus DNA was isolated from infected Sf 21 cells (1 X 10⁶ cells/ml) treated with different antiviral compounds at 24 hpi. DNA was bound to positively charged nylon membrane and probed with a virus specific probe. (B) Percent relative inhibition by BVdU, aphidicolin and hydroxyurea was determined from the integrated density value for each spot.

4. Screening for inhibitor by virus production and cytopathic effects (CPE)

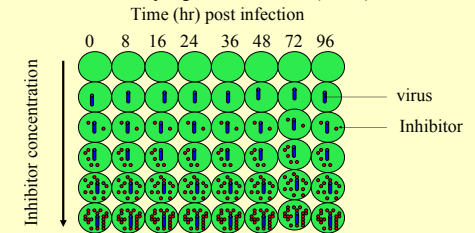


Figure 5. Sf 21 cells (1 X 10⁶ cells/ml) were infected with AcMNPV (MOI = 10) for 1 hr. Unattached virus was removed and the media was replaced with fresh medium containing varying concentrations of antiviral compounds. Cells were monitored for production of polyhedral inclusion bodies (PIBs) and cytopathic effects (CPE).

Table 1. Effect of antiviral compounds on virus production

Inhibitor	Concentration						Effect on
	0	2.5	5	10	20	40	
(E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVdU)	-	-	-	-	-	+	Cells PIBs
Aphidicolin	0	5	10	20	40	80	µM Cells PIBs
Camptothecin	0	2.5	5	10	20	40	µM Cells PIBs
Phosphono-acetic acid (PAA)	0	1	2	4	8	10	mM Cells PIBs

For cell (+) = decreased cell viability
For virus (+) = decreased viral replication

Conclusions

□ The antiviral compounds showed different inhibitory effects on budded virus and PIBs production.

□ The expression of EGFP in AcEGFP and DNA replication was diminished following treatment of Sf 21 infected cells with different antiviral compound.

□ Treatment with varying concentrations of camptothecin and PAA had resulted in different light scattering profiles of infected cells