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Concentration of sodium dodecyl sulfate used in occlusion body extraction affects *Spodoptera littoralis* nucleopolyhedrovirus biological activity*

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Abstract: Efficacy of a baculovirus as a biological control agent is evaluated according to biological parameters, median lethal dose (LD_{50}) of the virus, and median lethal time (LT_{50}) of infected larvae. Sodium dodecyl sulfate (SDS) is commonly used in concentrations of 0.1%–1% to extract infective units of the baculoviruses, occlusion bodies (OBs), from infected cadavers. Although several studies have shown disruption of OB structure by SDS, the effects of SDS concentrations on baculovirus biological parameters are not well known. Indeed, it is essential to detect the optimum SDS concentration for OB extraction to compare the activity of baculoviruses accurately. In our study, the effects of SDS (0.1%–2%) on baculoviral biological activity were examined using *Spodoptera littoralis* nucleopolyhedrovirus in third instar *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae. Use of 1% SDS in OB extraction caused the lowest LD_{50}, while higher concentrations than 1% significantly increased the LD_{50}. The differences between LT_{50} values at 0.1%–1% SDS were found to be insignificant; however, higher concentrations (>1.0%) caused significant loss of biological activity. Therefore, 0.1%–1.0% SDS could be used for OB extraction; however, evaluation of LD_{50} and LT_{50} values together suggests that 1% SDS provides the best biological activity. The mode of action of SDS on baculovirus biological activity is discussed further.

Key words: Sodium dodecyl sulfate, baculovirus, occlusion body, lethal dose, lethal time

1. Introduction

Baculoviruses are insect pathogenic viruses used as biological control agents. These viruses are unique because the virions, enveloped virus particles containing nucleocapsids, are occluded in polyhedral occlusion bodies (OBs) that protect the virus against environmental conditions for years. When an OB is ingested by larvae, it is dissolved in the alkaline environment of the midgut and the first phenotype of the virus, occlusion-derived virus (ODV), is released from the OB. ODVs bind to midgut epithelial cells, release the nucleocapsids into the cell, and initiate the infection. The second phenotype, budded virus (BV), is produced in the cell and disseminates the virus throughout the host.

The success of baculoviruses depends on their biological activity in the target pests. The biological activity of a baculovirus is determined by the effectiveness of initiating an infection within the host and producing virus progeny, which eventually leads to the death of the insect (1). Efficacy of a baculovirus as a biological control agent is based on its biological parameters, such as the median lethal dose (LD_{50}) of the virus and median lethal time (LT_{50}) of the infected larvae, obtained through bioassays. OBs must be extracted for such analysis, as well as electron microscopy and biochemical studies. Detergents such as sodium dodecyl sulfate (SDS), sodium deoxycholate, and Triton X are commonly used to extract OBs from baculovirus-infected dead larvae or infected cells (2,3). Although SDS is commonly used in many laboratories for OB extraction and is more efficient than other extraction reagents in providing cleaner OBs, several electron microscopy studies indicated that different concentrations of SDS (0.1%–1%) may cause serious damage to the OBs, which was demonstrated for *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV) (1) and *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) (4). However, the possible negative effects of SDS concentrations on viral efficacy in insects are poorly known. To our knowledge, the only study that examined the effect of different SDS concentrations on baculoviral activity was conducted by Nazli-Huda et al. (4). These researchers found that increasing the SDS concentration from 0.005% to 0.2% during OB extraction decreased mortality from 95% to 68% and increased LT_{50} values from 3.45 to 4.34 days in third instar *Spodoptera litura* larvae. Hence, it is essential to detect the optimum SDS concentration in OB extraction

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in order to compare the activity of different baculovirus isolates. Such information would also be critical to prevent the misinterpretation of biological data (LD_{50} and LT_{50}) in the comparison of efficacy of baculoviruses.

In this study, effects of SDS on baculoviral efficacy were examined using a local baculovirus isolate, *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV), in the Egyptian cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae). LD_{50} values were detected at 5 different SDS concentrations (0.1%, 0.5%, 1.0%, 1.5%, and 2.0%) and LT_{50} values were compared at biologically equivalent doses (LD_{90}). Here, we report that the efficacy of the virus isolate was best at 1% SDS.

2. Materials and methods

2.1. Insect and virus isolate

S. littoralis larvae were reared on lettuce leaves at 26 ± 0.5 °C, 75% humidity, and a 16:8 light:dark photoperiod. Leaves were sterilized with 1% NaOCl before being given to larvae. The baculovirus, SpliNPV-TR1, was previously described from nucleopolyhedrovirus (NPV)-infected *S. littoralis* larvae in Turkey (5).

2.2. Isolation of OBs

In the isolation of OBs, cadavers were treated with 0.1%, 0.5%, 1%, 1.5%, and 2% SDS concentrations (1 mL per cadaver) for 1 night at 4 °C and filtered through 5 layers of cheesecloth. OBs were pelleted by centrifugation at 3600 × *g* for 10 min at room temperature in 50-mL centrifuge tubes. The pellets were resuspended in the same concentrations of SDS used for each treatment. Samples were then spun at 3600 × *g* and the pellet was resuspended in 0.3 mL of NaCl, followed by a final spin at 3600 × *g*. SDS treatment–spinning in NaCl was repeated 3 times. The final pellets were washed in 3 volumes of distilled water, then centrifuged at 3600 × *g* for 20 min at 4 °C and resuspended in double-distilled water. The OBs were stored at –20 °C.

2.3. Bioassays

Third instar *S. littoralis* larvae were bioassayed to determine their dose-response or time-response to the NPV using a leaf disk assay. Inocula were supplied from the stock OBs extracted at the 5 different SDS concentrations mentioned above. For dose–mortality bioassays, 6 doses (5, 25, 100, 250, 500, and 1000 OBs) were applied on 5-cm leaf disks with 5% blue food coloring dye and 1% sucrose. Larvae that consumed the entire leaf disk within 4 h were transferred to 16-well insect rearing plates and supplied with leaves when needed. Mortality was recorded daily until all larvae had either pupated or died.

In lethal time bioassays, larvae were inoculated with biologically equivalent doses (LD_{90}) according to the method described above. Mortality was recorded every 8 h from the first larval death until 304 h.

All bioassays were conducted at 26 ± 0.5 °C, and 16 larvae were used for each assay. Each assay was repeated 3 times and sucrose solution (1% in 5% food coloring) without virus was used as a control.

2.4. Statistical analysis

LD_{50} and LT_{50} data were evaluated with the probit analysis method (6). Nonoverlapping of the 95% confidence limits was considered as evidence of significant statistical differences among the values being evaluated.

3. Results

LD_{50} values of the virus extracted at 5 different SDS concentrations are shown in Table 1. The lowest LD_{50} was obtained from the OB stock extracted at 1% SDS (Table 1). LD_{50} values obtained from the stock extracted with 0.1% and 0.5% SDS are not significantly different. SDS concentrations higher than 1% caused a significant increase in LD_{50} values (Table 1).

LT_{50} values for larvae inoculated with OBs extracted at 5 different SDS concentrations are shown in Table 2. Inocula from different SDS concentrations at 0.1%–1%

Table 1. Dose–mortality response of third instar *Spodoptera littoralis*[i] larvae against SpliNPV-TR1 isolate extracted at 5 different SDS concentrations (LD_{50}: OBs/larva; CL: confidence limits).

Treatment	LD_{50}[ii] (95% CL)	Slope (±SE)	χ^2	Heterogenicity
0.1% SDS	86.0 bc (54.8–142.1)	0.83 ± 0.13	6.08	0.41
0.5% SDS	57.7 b (38.8–85.3)	0.99 ± 0.14	7.75	0.52
1.0% SDS	21.5 a (12.0–33.3)	0.90 ± 0.13	9.96	0.62
1.5% SDS	233.2 cd (118.0–703.5)	0.53 ± 0.11	4.07	0.25
2.0% SDS	955.0 d (393.7–6047.9	0.55 ± 0.12	9.74	0.61

i. Sixteen larvae were used per virus dose in each SDS concentration and each assay was repeated 3 times.
ii. Different letters (a, b, c, d) in LD_{50} values indicate significant differences.

Table 2. LT_{50} values for third instar *Spodoptera littoralis*[i] larvae inoculated at the LD_{90} dose of SpliNPV-TR1 isolate extracted at 5 different concentrations of SDS (LT_{50}: hour; CL: confidence limits).

Treatment	LT_{50}[ii] (h) (95% CL)	Slope (±SE)	χ^2	Heterogenicity
0.1% SDS	197 a (189–205)	–6.74 (±0.48)	20.91	1.23
0.5% SDS	191 ab (177–214)	–7.39 (±0.54)	85.02	5.67
1.0% SDS	211 ab (201–223)	–7.08 (±0.52)	31.38	1.85
1.5% SDS	213 b (206–220)	–7.92 (±0.40)	42.58	1.77
2.0% SDS	232 b (212–260)	–5.40 (±0.31)	148.46	5.50

i. Sixteen larvae were used per virus dose in each SDS concentration and each assay was repeated 3 times.
ii. Different letters (a, b, c, d) in LT_{50} values indicate significant differences.

revealed no difference in LT_{50} values, based on overlapping confidence limits. However, the LT_{50} obtained from virus stock extracted with 1.5% and 2% SDS was significantly higher than that extracted with 0.1% SDS.

It is also important to emphasize that deaths ended at 232–256 h after inoculation with stocks extracted with 1% or lower concentrations of SDS; however, mortality ended 288–304 h after inoculation with the stocks extracted with 1.5% and 2% SDS.

4. Discussion

SDS is commonly used in the extraction of baculovirus OBs from cadavers. Several studies have revealed its damaging effect on OBs; however, its impact on baculovirus efficacy has not been well examined. Indeed, it is critical to elucidate this issue; thus, the loss of activity due to SDS may be misleading in the biological comparison of different baculovirus isolates. In our study, 1% SDS concentration revealed the lowest LD_{50}, while no significant difference was found in the LT_{50} values obtained from SDS concentrations of 0.1%–1.0%. Thus, OBs extracted at 1% SDS concentration provide the optimum biological activity; however, lower concentrations could also be used. SDS concentrations higher than 1% caused significantly higher LD_{50} and LT_{50} values, suggesting that these concentrations could cause deceptive results in the assessment of baculovirus biological data. In similar findings to those of our study, Nazli-Huda et al. (4) reported that increasing the SDS concentration from 0.005% to 0.2% in OB extraction decreased mortality from 95% to 68% and increased LT_{50} values from 3.45 to 4.34 days in third instar *S. litura* larvae. These researchers recommended that SDS of less than 0.1% should be used to obtain optimum biological activity.

The decreased biological activity of baculoviruses is likely to be related to the disruption of OBs due to SDS, as SDS-related damage of OBs was demonstrated in several studies. HaSNPV treated with 0.5% SDS caused 20% damaged OBs (1), while SpltNPV treated with 0.1% and 0.2% SDS caused 42% and 54% damaged OBs, respectively (4). SDS causes OBs to lose their polyhedron envelopes (PEs) (1,4), an electron-dense structure surrounding the OB that is vital to the stability and biological activity of baculoviruses (7,8). OBs without PEs could not keep virions, causing gaps within the OBs (1,4).

Mean time to death was compared at a biologically equivalent dose, LD_{90}, in our study, while an absolute dose (2.4×10^7 OBs) was used in the study by Nazli-Huda et al. (4). Both studies report an increase in LT_{50} by increasing the SDS. Since larvae had consumed the same amount of OBs treated with different concentrations of SDS in the study by Nazli-Huda et al. (4), any increase in the lethal time could be a direct consequence of the relatively lower ratio of intact (undamaged) OBs at higher SDS concentrations. In other words, finding lower LT_{50} values at lower SDS concentrations could be due to relatively higher doses taken by larvae. Indeed, it is commonly known that the higher viral doses decrease the lethal time (9,10). In order to evaluate the effect of a factor (different SDS concentrations in this case) on mean time to death of baculovirus-infected larvae, use of biologically equivalent dosages was recommended (11–14). Thus, the effect of different SDS concentrations was evaluated at the LD_{90} dose in the current study, and an increase in mean time to death by SDS concentration was still found, suggesting that a different mode of action of SDS may occur on baculovirus OBs other than just damaging the OBs.

One possibility for the decreased baculoviral activity by higher SDS concentrations (>1%) could be the effect of remnant SDS on the insect-originated alkaline proteases associated with the OBs (15–19) or midgut serine proteases (20–22) that may both affect the baculovirus infection process. Both proteases are active in alkaline conditions and are likely to contribute to the disruption of the OB and

PE for virion release (23). Activity of an alkaline protease in the SpliNPV virions (17) and *Trichoplusia ni* granulovirus (TnGV) OBs (24) was inhibited by SDS, suggesting that SDS may decrease the baculovirus biological activity through inhibiting these alkaline proteases. In contrast to the inhibitory effect of SDS, several studies reported an SDS-induced activity for several midgut serine proteases with antiviral features (20,22). Thus, inducement of antiviral midgut serine proteases by higher concentrations of SDS (>1%) could also contribute to the loss of baculoviral activity.

Ionic interactions between SDS and OBs could also affect the baculoviral biological activity. As indicated above, SDS is an anionic detergent and baculovirus OBs are negatively charged (25). For instance, SpliNPV, the baculovirus isolate used in this study, was reported to have a greater charge in comparison to TnGV and *Trichoplusia ni* nucleopolyhedrovirus, suggesting it has a greater number of ionizable groups (25). In addition to this, treatment of SpliNPV OBs with 1% SDS reduced the mobility of OBs significantly in cell electrophoresis (25). This finding was attributed to the adsorption of the SDS to the OB surface, which exposes positively charged sites by releasing negatively charged surface components (25). The disruption or reorientation in the charge complex of an infective OB may affect the regular release of the embedded virions from the OBs during dissolution. As a result of this, movement of the virions through the midgut epithelium may be delayed. It is noteworthy that the effect of detergents on OB charge composition is complicated (25) and needs to be clarified.

Another potential factor that may interact with SDS could be the per os infectivity factor (PIF) proteins required during the ODV infection process. PIF proteins have been shown to be essential for per os infection of the larvae (23) and involved in binding of the ODVs into midgut epithelial cells (26). These proteins are associated with the ODVs and have been shown to form a stable protein complex containing at least 6 PIFs, other than acting individually (27,28). PIF proteins have been identified in several baculoviruses including SpliNPVs (29), the baculovirus species used in the current study. Although in vitro treatment of the ODVs with 2% SDS-5% β-mercaptoethanol at 50 °C revealed no effect on the formation and stability of the PIF complex (27), the tritrophic interactions among the PIF complex, midgut proteases, and SDS within the insect midgut are not currently known. Interestingly, a lepidopteran midgut serine protease, trypsin, has been shown to activate the per os infectivity of ODVs of *Autographa californica* multiple nucleopolyhedrovirus by cleaving P74, a PIF protein in the PIF complex (21). This finding, together with the inhibition of the activity of similar insect-derived serine proteases by SDS (17,24), suggests that higher concentrations of SDS may inhibit the activity of the particular serine proteases that are involved in the activation of PIF proteins. Thus, such blockage of PIF activation may delay the initiation of the infection process, resulting in losses in the biological activity of baculoviruses.

In conclusion, SDS concentrations of 0.1%–1.0% could be used based on the biological activity of the virus; however, 1% provided the best biological activity in our study when both LD_{50} and LT_{50} values were taken into consideration. Concentrations higher than 1% caused significant loss of biological activity. Therefore, the concentration of SDS should be considered when analyzing the biological data. Proteases associated with the OBs and insect midgut, and PIF proteins involved in the baculovirus infection process and their interaction with SDS, would be an interesting area of research in order to understand the mode of action of SDS on baculovirus activity.

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