Optical Brighteners Provide Baculovirus Activity Enhancement and UV Radiation Protection

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Received June 26, 1995; accepted January 3, 1996

Fluorescent brighteners increase insect viral activity and provide protection against UV inactivation. The relative amount of viral UV protection has not previously been determined due to the dual nature of these compounds. In this study, two distinct viral–host systems, a nuclear polyhedrosis virus of the gypsy moth (Lymantria dispar) infecting its homologous host and a nuclear polyhedrosis virus of the alfalfa looper (Autographa californica) infecting the cabbage looper (Trichoplusia ni), were treated with various regimes of UV light from 0.5 to 120 min and fluorescent brightener 28 and were subsequently assayed with and without fluorescent brightener 28. A comparison of LC50s from the various treatments produced similar viral bioassay profiles in both systems. The fluorescent brighteners protected insect viruses from UV inactivation and enhanced residual activity of active virus. For the gypsy moth virus, the enhancement was 214-fold for its homologous host and for the A. californica virus, the enhancement was 41-fold for T. ni.

KEY WORDS: Nuclear polyhedrosis virus; Lymantria dispar; Trichoplusia ni; cabbage looper; fluorescent brightener; gypsy moth.

INTRODUCTION

Optical (fluorescent) brighteners, initially described by Paine et al. (1937) and later by Eggert and Wendt (1939), are a group of substituted stilbenes that are routinely utilized in many industrial processes for lightening or brightening paints, fibers, coatings, etc. Numerous adjunct uses have been described for these compounds including fluorochromes for microorganisms (Darken, 1961). More recently, Shapiro (1992) has described the use of fluorescent brighteners as radiation protectants for the gypsy moth, Lymantria dispar L., multiple-embedded nuclear polyhedrosis virus (LdMNPV). Shapiro’s initial description of this effect and more comprehensive studies conducted and described by a patent (Shapiro et al., 1992) revealed the UV radiation protective effect of several of these compounds on a variety of microorganisms suitable for biological control of agricultural pests.

The UV protection efficiency of some fluorescent brighteners was such that 100% of original activity of LdMNPV preparations remained compared with total inactivation of untreated virus. These data are important because inactivation by solar radiation is one of the largest problems of utilizing microbial control agents under field conditions (Ignoffo et al., 1977).

In addition to the described UV radiation protection afforded by fluorescent brighteners, these compounds are described as viral enhancers capable of increasing viral activity up to 2000-fold as with LdMNPV, when assayed against its homologous host. Early UV inactivation protection studies (Shapiro, 1992; Shapiro et al., 1992), however, have not adequately distinguished between UV protection and activity enhancement of residual virus.

In this study our objective was to separately determine the degree of UV protection and viral enhancement provided by fluorescent brighteners by UV inactivating virus in either water (no protection) or 1% fluorescent brightener (protection) and diluting the virus in either water (no enhancement) or 1% fluorescent brightener (enhancement). Two viruses Autographa californica multiple-embedded nuclear polyhedrosis virus (AcMNPV) and LdMNPV, were utilized against their respective hosts. Trichoplusia ni (Hübner) and L. dispar.

METHODS AND MATERIALS

Insect Colonies and Viruses

The colonized L. dispar (New Jersey strain) was obtained from USDA Forest Service, Hamden, Connecticut, and T. ni which are susceptible to AcMNPV were obtained from the USDA Agricultural Research Service, Biological Control of Insects Research Laboratory, Columbia, Missouri. The insects were received as eggs and reared on a wheat germ diet (Bell et al., 1981). Both diet volumes and containers were used as described by
Shapiro (1992). Insects were maintained at 28°C under nonregulated photoperiod conditions.

The LDP-67 strain of LdMNPV was produced and purified as described previously (Guzo et al., 1991). The 6R strain of AcMNPV was produced in T. ni larvae and similarly purified. Briefly, virus-infected cadavers were ground in a Dounce homogenizer in distilled water. The slurry was poured through cheesecloth and layered on a 40% sucrose pad. Virus occlusion bodies (OBs) were pelleted after 1 h centrifugation at 25,000 g. OBs were washed three times in distilled water and enumerated with an improved Neubauer hemocytometer.

UV Inactivation Assay

OBs were diluted to a final concentration of $1 \times 10^6$ OBs/ml for AcMNPV and $1 \times 10^8$ OBs/ml for LdMNPV in either sterile distilled water or 1% w/v fluorescent brightener 28 solution (Sigma Chemicals, St. Louis, MO). Approximately 6 ml of aqueous viral suspension ($10^6$ OBs/ml) was placed in a 60 × 15 mm (28.25 cm²) plastic dish (Fisher Scientific, Pittsburgh, PA) and irradiated on a Fotodyne, UV 440, Analytical Transilluminator, Model 3-4400 (Fotodyne, New Berlin, WI) containing six 15-W, 302-nm bulbs. A fan was directed toward the apparatus to diminish heat build up from the bulbs. The temperature of the viral suspension was maintained at 28°C under these conditions.

To determine the relative role of activity enhancement and UV protection associated with fluorescent brightener, both LdMNPV and AcMNPV ($10^6$ OBs/ml) were subjected to UV inactivation conditions while suspended in water (no protection) or 1% fluorescent brightener (UV protection). Each virus sample was then diluted by serial 10-fold dilutions in either water (no enhancement) or fluorescent brightener (enhancement). Thus, the UV treatment/dilution protocol consists of water/water (maximum inactivation/no enhancement), water/fluorescent brightener (maximum inactivation/maximum enhancement of remaining virus), fluorescent brightener/water (maximum protection/no enhancement), and fluorescent brightener/fluorescent brightener (maximum protection/maximum enhancement of remaining virus). One milliliter of viral suspension was pipetted on the diet surface and spread in a fine film to uniformly cover the surface. All dilutions were performed in triplicate and the experiments replicated three times.

Ten second instar larvae were placed in each diet cup containing various viral treatments (30 larvae/dilution). Six 10-fold dilutions ($10^0$–$10^3$ OBs/ml) were assayed for each treatment. Insects were monitored until pupation or larval death. Larval mortality was calculated using the statistical method for probit analysis written by Dr. R. Peaden, USDA, Agricultural Research Service, Prosser, Washington.

RESULTS AND DISCUSSION

In this study, fluorescent brightener enhancement of LdMNPV activity decreases the LC$_{50}$ 214-fold ($3.71 \times 10^3$ vs $1.73 \times 10^1$ OBs/ml). The 41-fold decrease in LC$_{50}$ for AcMNPV ($8.31 \times 10^1$ vs $2.04 \times 10^0$ OBs/ml) is a smaller, yet real, enhancement of a virulent baculovirus. To date, fluorescent brighteners provide the most enhancement of viruses having a high LC$_{50}$ (LdMNPV) and are less active in increasing activity of viruses having a low LC$_{50}$ (e.g., AcMNPV). The virulence enhancement noticed with LdMNPV in laboratory studies has been confirmed under field conditions (Webb et al., 1994). Whether the small increase in activity found with AcMNPV will likewise be validated under field conditions remains to be investigated. The dual nature of fluorescent brighteners (reported activity enhancement and UV protection), however, justifies use of these compounds as UV protectants whether activity is enhanced or not.

The focus of this study uncouples the dual nature of fluorescent brighteners. Do they give both UV protection and activity enhancement or is the surviving virus from the UV inactivation simply enhanced to the original activity? LdMNPV is very susceptible to UV inactivation. Under the conditions described for these assays, viral activity of UV-treated aqueous LdMNPV suspensions quickly declined until no viral activity could be detected after 30 min (Fig. 1). UV-treated aqueous LdMNPV suspensions diluted in 1% fluores-
cent brightener had lower LC_{50} values after equivalent periods of UV treatment due to viral enhancement by the brightener. However, no viral activity could be rescued (enhanced) by 30 min, suggesting either that all LdMNPV was inactivated or that residual active virus was insufficient to produce a LC_{50} even in the presence of 1% fluorescent brightener.

One-percent fluorescent brightener suspensions of LdMNPV provided protection against viral inactivation by UV light. Basically, fluorescent brightener 28-protected virus has significantly lower LC_{50} than unprotected virus irrespective of dilution in water or fluorescent brightener. Using data from the protected virus, we can distinguish between protection and enhancement. UV-treated LdMNPV 1% fluorescent brightener suspensions diluted in water revealed significantly higher LC_{50} levels at 60 min (4.66 \times 10^3 OBs/cup) than irradiated LdMNPV 1% fluorescent brightener suspensions diluted in 1% fluorescent brightener (3.09 \times 10^2 OBs/cup). The former were UV protected and not enhanced, while the latter were both UV protected and enhanced approximately 15-fold.

A similar profile emerges for AcMNPV (Fig. 2). Basically, samples of UV-treated viral suspensions in aqueous suspensions (no protection) and diluted in either water (no enhancement) or 1% fluorescent brightener solutions (viral enhancement) showed quick inactivation profiles. The viral samples diluted in 1% fluorescent brightener consistently had lower LC_{50}S; therefore, the fluorescent brightener was enhancing residual viral activity after UV treatment. By 60 min there was no detectable activity in either control or 1% fluorescent brightener-enhanced viral samples. Again, either the viral sample was totally inactivated by the prolonged UV treatment or there were nondetectable amounts of residual virus which could not be enhanced by fluorescent brightener to be detected in this assay.

In those samples that were UV-treated in the presence of 1% fluorescent brightener, viral enhancement could be distinguished from UV protection until the last time period tested (2 h). AcMNPV samples that were UV-treated in the presence of fluorescent brightener and then diluted in water (protection/no enhancement) had an initial LC_{50} decline of approximately 6.6-fold from 10 to 20 min (3.71 \times 10^2 to 2.45 \times 10^3 OBs/cup); however, LC_{50} values remained relatively stable over the next 40 min until 2 h when the LC_{50} was in excess of 1 \times 10^6 OBs/cup. AcMNPV samples that were treated in the presence of 1% fluorescent brightener (protection) and diluted in 1% fluorescent brightener (enhancement) consistently had lower LC_{50} values until 2 h post-UV treatment than samples diluted in water. Data from 60-min UV irradiation revealed that both AcMNPV samples were protected by 1% fluorescent brightener. However, those samples diluted in fluorescent brightener showed approximately a 17-fold enhancement over those samples diluted in distilled water (1.86 \times 10^2 vs 3.09 \times 10^2 OBs/cup).

The two virus–host systems utilized in these studies, LdMNPV–L. dispar–fluorescent brightener and AcMNPV–T. ni–fluorescent brightener, gave similar profiles when viral samples were UV-treated in either water or 1% fluorescent brightener followed by dilution in either water or 1% fluorescent brightener. These studies, which uncouple UV protection from viral activity enhancement, demonstrated that 1% fluorescent brightener not only protected baculoviruses from UV inactivation but also enhanced the viral activity of the remaining viable OBs. Interestingly, these studies produced the same general profile when two very different virus–host systems were used. AcMNPV is associated with lepidopteran pests of row crops, such as T. ni, whereas LdMNPV is associated with L. dispar, a hardwood defoliator. AcMNPV is considered a virulent virus for T. ni and other similar hosts and is moderately enhanced by 1% fluorescent brightener solutions (2 vs 83 OBs/cup LC_{50} = 41.5-fold enhancement). LdMNPV has a high LC_{50} associated with its homologous host and also has a much greater viral activity enhancement (1.73 \times 10^5 vs. 3.71 \times 10^3 OBs/cup LC_{50} = 214-fold enhancement).

Finally, these viruses differ greatly in their susceptibility to UV inactivation by 302-nm light sources. AcMNPV is much more resistant to 302-nm light, retaining viral activity under all conditions at 40 min posttreatment, whereas LdMNPV activity is totally inactivated by 30-min posttreatment. Thus, these
data indicate that fluorescent brightener can provide both UV protection and viral enhancement to at least two viral bioinsecticides.

REFERENCES


Shapiro, M. 1992. Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera:Lymantriidae) nuclear polyhedrosis virus. J. Econ. Entomol. 85, 1682–1686.
