The Use of the Enzyme Linked Immunosorbent Assay to Detect a Nuclear Polyhedrosis Virus in *Heliothis armigera* Larvae

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**SUMMARY**

The enzyme linked immunosorbent assay (ELISA) has been shown to be a specific and sensitive method to detect a nuclear polyhedrosis virus in *Heliothis armigera* larvae. 1 ng/ml of purified virus particle antigen could be detected. The presence of larval extracts did not interfere in the assay. The growth of the virus in *Heliothis armigera* larvae was analysed by ELISA and the conventional counting of virus polyhedra and this showed that ELISA was a superior detection method.

Baculoviruses, particularly nuclear polyhedrosis viruses, are currently being used as agents to control insect pests of crops over large areas of agricultural and forest lands, in some instances on a commercial basis (Summers *et al.* 1975). Consequently there is a requirement for rapid and specific diagnostic methods to detect and monitor baculoviruses in ecological and epidemiological studies. Currently it is usual to screen larvae for baculovirus infections using Giemsa stained larval smears to detect polyhedra, a method which is laborious, has low-detection efficiency and is of unknown specificity (Wigley, 1976). Serological methods which have previously been shown to be sensitive for discrimination between selected baculoviruses are complement fixation and immunodiffusion (Harrap *et al.* 1977). Neither of these methods is readily applicable to large scale screening of infected larvae. Radioimmune assay for the polyhedron protein of nuclear polyhedrosis viruses has been studied by Kalmakoff and co-workers (Crawford & Kalmakoff, 1977; Crawford *et al.* 1977; Kalmakoff *et al.* 1977), but the sensitivity of the method for detecting virus or polyhedra in infected larvae has not yet been demonstrated.

The enzyme linked immunosorbent assay (ELISA) technique is known to be a rapid, specific and sensitive method for detecting and quantifying animal and plant viruses (Voller *et al.* 1976a, b) and recently the technique has been applied successfully to detect and discriminate between small iridescent viruses in insect larvae (Kelly *et al.* 1978). In this communication we report the use of the ELISA technique to detect a nuclear polyhedrosis from *Heliothis armigera* (McKinley, 1971), and an evaluation of the specificity and specificity of this technique.

In these experiments the double antibody ELISA method (Voller *et al.* 1976b) was used. Briefly, this method involves sensitizing polystyrene microtitre plates by absorbing specific immunoglobulin. A solution containing antigen is then incubated with the sensitized plate and the excess washed away. A conjugate of specific immunoglobulin chemically linked to alkaline phosphatase is incubated with the bound antigen and the amount of enzyme linked immunoglobulin bound is estimated by determining the rate at which a substrate (*p*-nitrophenyl phosphate) is degraded.

In the experiments reported we have chosen to monitor the presence of virus particle antigen rather than polyhedron protein antigen in test larvae for the following reasons.
Firstly, virus particle antigens are more specific than polyhedron protein antigens (Harrap et al. 1977). Secondly, it is not known whether the polyhedron surface ‘membrane’ interferes in the antigen–antibody reaction when purified polyhedra are used to produce the antibody. Finally, polyhedron protein production is a very late step in the production of nuclear polyhedrosis virus (D. C. Kelly and T. Lescott, unpublished observations) and so is an inappropriate biological marker to use if it is required to detect low levels of virus early in infection.

The nuclear polyhedrosis virus of *Heliothis armigera*, a singly enveloped virus, was grown in *H. armigera* larvae and virus particles were extracted from purified polyhedra as previously described for a granulosis virus (Brown et al. 1977). Multiply enveloped nuclear polyhedrosis viruses derived from *Spodoptera frugiperda*, *Spodoptera exempta*, *Spodoptera exigua*, *Spodoptera littoralis* and *Mamestra brassicae*; a singly enveloped nuclear polyhedrosis virus from *Neodiprion sertifer*; and a granulosis virus from *Pieris brassicae* were purified by methods previously described (Harrap et al. 1977; Brown et al. 1977). An antiserum to *Heliothis armigera* nuclear polyhedrosis virus particles was prepared in rabbits by injecting intramuscularly a suspension of 500 μg of antigen in 1 ml of water emulsified with an equal volume of Freund’s complete adjuvant, followed at weekly intervals by two similar inocula containing incomplete adjuvant. The serum used in the assays was taken (by bleeding from the marginal ear vein) 3 weeks after the final injection.

The enzyme linked immunosorbent assay was performed in polystyrene microtitre plates (M129A; Dynatech Ltd., Billingshurst, Sussex) essentially as described by Clark & Adams (1977). Purified specific immunoglobulin was conjugated with alkaline phosphatase and the amount of conjugated enzyme present in an assay was determined by measuring the absorbance at 405 nm (in a 1 cm cell) of the hydrolysed product using a Pye-Unicam 1800 spectrophotometer (Kelly et al. 1978). All assays were performed in duplicate.

Extracts of infected and healthy larvae were prepared by triturating 0.5 to 2.5 larvae/ml in phosphate buffered saline containing 0.05% (v/v) Tween 20 (PBST) for 3 min in a Colworth stomacher (A. C. Steward Ltd, Bury St Edmunds, U.K.). The extracts were filtered through muslin and centrifuged at 1000g for 15 min. The supernatant was removed and assayed immediately.

Using a chequerboard system to determine an efficient method of detecting purified virus particles it was found that a combination of 1 μg of coating immunoglobulin/ml and a 1/800 dilution of enzyme conjugated immunoglobulin was appropriate. The chequerboards incorporated all combinations of 10, 5, 1 and 0.1 μg coating immunoglobulin/ml and 1/200, 1/800 and 1/3200 dilutions of conjugated immunoglobulin to react with tenfold dilutions of virus (100 μg/ml to 1 ng/ml). Fig. 1(a) shows a typical dilution curve for the titration of virus particles by ELISA. Ten ng virus particle antigen/ml could be detected with ease and 1 ng/ml gave *A*₄₀₅ readings significantly above background.

The efficiency of detection of virus particle antigen in extracts of *H. armigera* larvae was tested by adding 100 μg of pure virus particles to 10 ml samples of PBST; adding 1, 5, 10 and 25 healthy, frozen and thawed larvae and triturating the mixture. The extracts were titrated by ELISA and Fig. 1(b) shows the effect of the added extracts on the *A*₄₀₅ obtained. When the *A*₄₀₅ is adjusted to compensate for volume changes on addition of larvae, Fig. 1(b) shows that the equivalent of 2-5 larvae (about 1 g) per ml has little effect. The compensation is approximate because of difficulties in determining the exact change in volume (Kelly et al. 1978). The experiment showed that it is technically possible to detect virus particle antigen efficiently in the presence of large amounts of larval extract.

The growth of *Heliothis armigera* nuclear polyhedrosis virus in *H. armigera* larvae was
Fig. 1. ELISA absorbance values for purified *Heliothis armigera* nuclear polyhedrosis virus particles: (a) diluted in PBST; (b) 10 μg virus particles/ml in the presence of various concentrations of *H. armigera* last instar larvae uncorrected (● – ●) and corrected (○ – ○) for volume changes. ---, represents background absorbance values.

Fig. 2. The production of virus particle antigen determined by ELISA, and numbers of polyhedra synthesized, during the reproduction of *Heliothis armigera* nuclear polyhedrosis virus in *H. armigera* larvae. Initiation of infection occurred at the third instar. ● – ●, Virus protein; ○ – ○, polyhedra.

determined in order to evaluate the detection of virus particle antigen in diseased larvae. In this experiment third instar larvae were individually infected *per os* with purified polyhedra (100 μl; 2.45 × 10⁸ polyhedra/larva). At various times after infection 5 larvae were sacrificed by freezing. These larvae were added to 3 ml of PBST, triturated in a stomacher and processed as outlined earlier. Polyhedra were recovered from the 1000 g pellet,
resuspended in 1 ml PBST and counted by an improved dry counting method (which involved polyhedra being counted in known areas across the radius of a standard circle, each sample being mathematically weighted for its position in the circle; Wigley, 1976). The results, expressed as ng of virus antigen or numbers of polyhedra/larva, are shown in Fig. 2. A significant increase in $A_{405}$ was observed 12 h after infection, equivalent to 3 ng virus particle antigen per larva rising to 106 ng virus particle antigen 72 h after infection. Polyhedra were first detected 24 h after infection although significant counts of polyhedra ($\geq 10^7$/ml) were not achieved until 36 h after infection. The ELISA technique thus appeared to be superior to conventional counting of polyhedra, detecting infection 12 h earlier qualitatively and 24 h earlier quantitatively. *Heliothis armigera* nuclear polyhedrosis virus particles do not possess antigens in common with polyhedron protein (J. S. Robertson and D. A. Brown, unpublished observations) and so the antigen detected at 12 h after infection must represent progeny virus (input virus particles released from polyhedra would be considerably less than 3 ng/larva). Uninfected control larvae at zero time (third instar) and at 144 h (last instar) gave $A_{405}$ values of 0.11, the background level.

Late in infection (from 72 h) the amount of virus particle antigen dropped while the numbers of polyhedra increased. An explanation is that virus particles are becoming occluded in the polyhedra being assembled so withdrawing them from the antigen pool. Earlier in infection the increase in virus particle antigen considerably precedes the assembly of polyhedra agreeing with the known biology of nuclear polyhedrosis viruses.

The specificity of the ELISA double antibody detection method was checked by substituting purified virus particles of seven baculoviruses (listed earlier) for the *Heliothis armigera* virus. Dilutions of 100 µg to 1 ng per ml failed to evoke a significant increase in absorbance above background. This demonstrates that the ELISA technique is a highly specific method for the detection of baculoviruses.

During the coming year we plan to evaluate the use of the ELISA method in a field situation. We also plan to look at polyhedron protein antigen production and the inter-relationship of this with virus particle antigen using viruses derived from *Mamestra brassicae* and *Neodiprion sertifer* as model systems.

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REFERENCES


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