The Use of a Protein A Conjugate in an Indirect Enzyme-linked Immunosorbent Assay (ELISA) of Four Closely Related Baculoviruses from *Spodoptera* Species

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**SUMMARY**

The virus particles from four closely related insect nuclear polyhedrosis virus (NPVs) isolated from their homologous *Spodoptera* spp. hosts were examined using an indirect enzyme-linked immunosorbent assay (ELISA), employing both swine anti-rabbit and protein A conjugate. Results from both systems indicated that *S. littoralis* NPV is serologically distinct from the other three viruses, *S. frugiperda* NPV, *S. exempta* NPV and *S. exigua* NPV, which are closely related. The relatedness of these viruses is discussed with reference to existing knowledge and the results obtained from both conjugate systems.

The family Baculoviridae contains many nuclear polyhedrosis viruses (NPVs) which are potential control agents for insect pests; the need to develop or improve criteria for identification and diagnosis is very important. The enzyme-linked immunosorbent assay (ELISA) has already been employed for detection of baculoviruses by a number of workers (Kelly *et al.*, 1978; Longworth & Carey, 1980) and all of the more common methods of assay have been compared in a comprehensive study by Crook & Payne (1980). This latter work indicated the versatility of the indirect assay method especially when employing purified gamma-globulins. Since then, other workers have used ELISA for the detection of baculovirus structural proteins (Langridge *et al.*, 1981a) and as an indicator of virus replication (Langridge *et al.*, 1981b).

Until now, all conjugates of baculoviruses prepared for ELISA have used either rabbit antiviral gamma-globulins (IgG) bound to an enzyme, usually alkaline phosphatase, or enzyme-labelled anti-rabbit IgG. *Staphylococcus aureus* protein A has an affinity for the Fc portion of IgG molecules of several mammalian species including the rabbit (Goudswaard *et al.*, 1978), and can be coupled to alkaline phosphatase for use in immunoassays (Engvall, 1978). In the present work, a protein A conjugate was used under the same assay conditions as a swine anti-rabbit conjugate to differentiate between four closely related NPVs isolated from four insect species. These were in the genus *Spodoptera*, which contains many pest insects of economic importance.

Three of these viruses, namely *S. littoralis* NPV, *S. frugiperda* NPV and *S. exempta* NPV, have previously been extensively characterized and their relatedness assessed using other serological methods (Harrap *et al.*, 1977). All four viruses, including *S. exigua* NPV, have been examined for nucleotide homology using reassociation kinetics (Kelly, 1977). The four isolates (*S. littoralis* NPV from K. M. Smith, Egypt origin; *S. frugiperda* NPV from J. Vaughn, U.S. origin; *S. exempta* NPV from K. M. Smith, Kenya origin; *S. exigua* NPV from L. A. Falcon, U.S. origin) were grown in their homologous insect hosts and virus particles were extracted and purified from polyhedra by the method recommended by Harrap & Longworth (1974). Suspensions of purified virus particles were diluted to working concentrations using 0.05 M-carbonate–bicarbonate buffer pH 9.6.

Antisera to purified virus particles were raised for all four viruses in rabbits and gamma-globulins precipitated from 10-fold dilutions of antiviral and preimmune sera by addition of...
an equal volume of saturated ammonium sulphate solution. The gamma-globulins were then extensively dialysed against phosphate-buffered saline (PBS) before dilution with PBS–Tween (PBS containing 0.05% Tween 20).

Alkaline phosphatase (Boehringer; grade I) was coupled to swine anti-rabbit gamma-globulins (Dako Laboratories, Denmark) using the glutaraldehyde method of Avrameas (1969). Protein A (Sigma) was bound to the same enzyme and subsequently purified using gel filtration as described by Engvall (1978). Both conjugates were prepared by the two-step procedure and used in indirect assays carried out in polystyrene plates as described by Crook & Payne (1980).

A classic chequerboard system was used to establish optimum gamma-globulin concentrations (1 µg/ml) and also conjugate dilutions (swine anti-rabbit, 1/1600; protein A, 1/3200). Simultaneously, it was noted that polystyrene plates (Flow/Titertek) showed no significant well-to-well variation and were preferable to PVC plates (Falcon Microtest III). In both cases the choice of coating buffers for these assays was carbonate–bicarbonate, which gave slightly higher readings than PBS although differences were slight.

Duplicate samples of each virus dilution were assayed by adding 200 µl per well of 1 µg/ml, 5 µg/ml and 10 µg/ml of each virus suspension in 0.05 M-carbonate-bicarbonate buffer pH 9.6 which was allowed to adsorb overnight at 4 °C. One plate represented one antiviral gamma-globulin and the corresponding preimmune serum against each of the four viruses. After washing the plates three times in PBS–Tween, 200 µl gamma-globulin was added where appropriate and left overnight at 4 °C. After washing extensively, 200 µl of appropriate conjugate was added to each well and the plates were refrigerated overnight. Bound conjugate was assayed using 200 µl of substrate [0.1% disodium p-nitrophenyl phosphate (Sigma) in 10% ethanolamine–HCl pH 9.8] which was added to each well and left for 1 h at room temperature. The reaction was stopped by the addition of 50 µl 3 M-NaOH.

On each plate the background was measured for each conjugate, using PBS–Tween only, and sufficient controls were included to enable detection of both non-specific binding of gamma-globulins and of the reaction of conjugate directly with each virus. Background was automatically subtracted for each conjugate by the ELISA plate reader (Dynatech MR580) and the results minus plate control values are represented graphically in Fig. 1 (swine anti-rabbit) and Fig. 2 (protein A).

All reactions with S. littoralis NPV, other than with the homologous gamma-globulin (Fig. 1 a, 2 a), gave the lowest readings with both conjugates. The swine anti-rabbit conjugates (Fig. 1) show that S. frugiperda (Fig. 1 b) was exceptional in that higher values were obtained with both S. exempta NPV and S. exigua NPV than with the homologous reaction. This observation has been made before by other workers using the same viruses in complement fixation tests but unfortunately S. exigua was not included in those experiments. A similar situation was apparent when using protein A conjugate in the S. exigua assay (Fig. 2 d). All other reactions using protein A produced highest absorbance values for the homologous reactions.

The results suggest that S. littoralis NPV, although related to the other three, remains serologically distinct. With the other viruses there is a greater antigenic relationship, a situation observed previously for S. frugiperda and S. exempta NPVs. The inclusion of S. exigua NPV in the present serological study has confirmed its relatedness and position in the grouping, by DNA homology, of NPVs by Kelly (1977).

In general, there was a high degree of cross-reactivity between S. exigua NPV and S. exempta NPV with the exception of results obtained using S. exempta NPV gamma-globulin and swine anti-rabbit conjugates (Fig. 1 c). The S. frugiperda NPV results suggest that, although it is related closely to the other two NPVs, anomalies of cross-reaction occurred which did not clarify its position in the general scheme of relatedness.
Fig. 1. Results of indirect ELISA using swine anti-rabbit-alkaline phosphatase conjugates. NPVs shown are *S. littoralis* (○), *S. frugiperda* (■), *S. exempta* (●) and *S. exigua* (▲). (a) to (d) show the coating gamma-globulins from *S. littoralis*, *S. frugiperda*, *S. exempta* and *S. exigua* sera respectively.

Fig. 2. Results of indirect ELISA using protein A–alkaline phosphatase conjugates. NPVs shown are *S. littoralis* (○), *S. frugiperda* (■), *S. exempta* (●) and *S. exigua* (▲). (a) to (d) show the coating gamma-globulins from *S. littoralis*, *S. frugiperda*, *S. exempta* and *S. exigua* sera respectively.

It is difficult to compare directly the relative merits of the two conjugate systems because different working dilutions were used for each. The protein A could possibly be used at even lower dilutions, since the 1-in-3200 dilutions caused curve flattening in Fig. 2(b, d), and may in some instances prove to be preferable in this type of assay for certain viruses.
Although not definitely determining a preferential conjugate for the indirect ELISA of baculoviruses, this work does provide the basis for an alternative which could prove useful for certain modifications of the classic assay system. This is also the first report of the usefulness of protein A for baculovirus serology. Further work is under way to improve and adapt the ELISA for use with other members of the baculovirus group, with a view to using it as a more definitive tool for characterization and comparison.

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REFERENCES


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