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Effect of Alkaline Protease on the Antigenic Nature of *Wiseana* Nuclear Polyhedrosis Virus Polyhedron Protein

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Polyhedron protein from *Wiseana* spp. nuclear polyhedrosis virus was found to be degraded by an alkali protease when polyhedra are dissolved in alkali. The protease activity did not occur at high pH (0.1 M NaOH) and was inactivated by heating polyhedra to 70°C for 3 h. The products from the protease degradation of *Wiseana* spp. nuclear polyhedrosis virus polyhedron protein retain the antigenicity of undegraded polyhedron protein as measured by the direct solid-phase radioimmunoassay and immunoadsorption. Degradation products below 27,000 daltons could not be detected by the sandwich radioimmunoassay, indicating that they are probably monovalent.

Serology provides a quick and sensitive method for the detection and identification of many plant and vertebrate viruses. Its use for baculovirus identification has been hampered by the cross-reaction between baculoviruses from different insect hosts (7, 8). Baculovirus serology is further complicated by the presence in the inclusion bodies of an alkaline protease (3, 6, 7, 11). The dilute alkali dissolution procedure used in the disruption of baculovirus inclusion bodies (1) provides suitable conditions for protease activity, and any inclusion body protein dissolved by this method is rapidly degraded (10). Many of the early serological investigations of baculovirus inclusion body protein (7, 8) used the dilute alkali disruption procedure, and consequently protease-degraded inclusion body proteins were used to determine serological relationships.

This study was initiated to confirm the presence of an alkaline protease in the inclusion bodies of *Wiseana* spp. nuclear polyhedrosis virus (NPV) and study the antigenic nature of the polyhedron protein prepared by alkali dissolution.

A radioassay similar to that developed by Kalmakoff et al. (J. Kalmakoff, C. C. Payne, and J. S. Mahood, submitted for publication) for *Spodoptera littoralis* NPV was used to study the degeneration of alkali-dissolved polyhedron protein by protease. The assay uses [^{3}H]iodoacetate-labeled polyhedron protein as the substrate for the protease. The proteolytic activity is determined by measuring the amount of labeled protein that is not precipitated by 0.2 M acetate buffer, pH 5.0. Using this method, it can be shown that proteolytic activity is inactivated when polyhedra are dissolved in 0.1 M NaOH (Fig. 1). Heat treatment of the polyhedra (3 h, 70°C) prior to dissolution destroys proteolytic activity, indicating that the "activity" is probably due to the putative protease enzyme rather than chemical decomposition.

Polyhedra from *Wiseana* spp. NPV and *Epiphyas postvittana* NPV were dissolved by two different procedures: 0.1 M NaOH dissolution for 5 min at room temperature (little or no protease degradation) and 0.1 M Na_2CO_3 dissolution for 2 h at 37°C (protease-degraded polyhedron protein). The protein solutions were then labeled with [^{3}H]iodoacetate, using the method of Parkinson and Kalmakoff (9).

Immunoglobulins were purified either by ammonium sulfate fractionation (4) or by elution from a Sepharose column conjugated with polyhedron protein. Proteins were conjugated to cyanogen bromide-activated Sepharose by incubating at 4°C for 16 h in 0.1 M Na_2CO_3/$NaHCO_3$ buffer (pH 8.3) containing 0.5 M NaCl and 5 mg of protein per ml of the Sepharose. Remaining sites were blocked using 0.1 M Tris buffer, pH 8.0, and conjugates were washed alternatively with acetate buffer (0.1 M, pH 4.0, containing 1 M NaCl) and borate buffer (0.1 M, pH 8.0, containing 1 M NaCl).

^{3}H-labeled polyhedron protein antigens were eluted from immunoadsorption columns using 0.1 M acetic acid containing 10% dioxan. These were brought to neutrality with NaOH and characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

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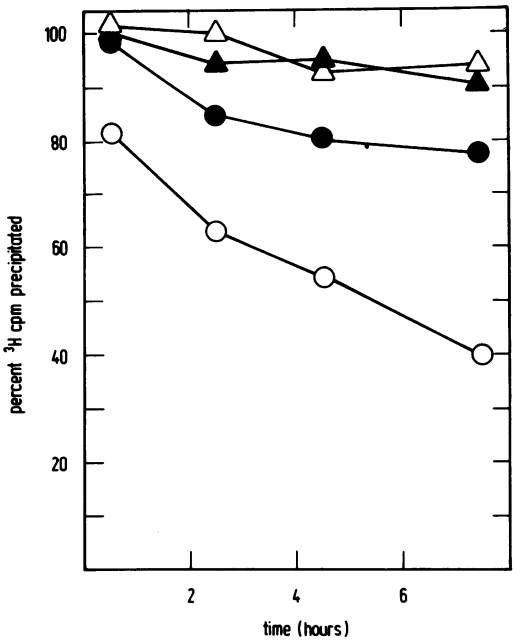

FIG. 1. *Radioassay for proteolytic activity in alkali-dissolved Wiseana spp. NPV. Polyhedra (1 mg in 0.5 ml of distilled water) were either heated (70°C, 3 h) or not heated prior to alkali dissolution in either 0.1 M NaOH or 0.1 M Na_2CO_3. After leaving the dissolved polyhedra solutions to incubate overnight at room temperature, ^{3}H-labeled polyhedron protein (20 μg in 100 μl of 0.05 M Na_2CO_3/$NaHCO_3$ buffer, pH 9.6) was added. Duplicate samples (50 μl) of each mixture were removed at various times, and the proteolytic activity was determined by measuring the amount of labeled protein that could be precipitated by 0.2 M acetate buffer, pH 5.0. Symbols: (△) unheated, NaOH dissolved; (▲) heated, NaOH dissolved; (○) unheated, Na_2CO_3 dissolved; (●) heated, Na_2CO_3 dissolved.*

(7% gels run at 4 mA/gel for 8.5 h in the phosphate buffer system).

E. postvittana NPV was used as the virus control; neither NaOH- nor Na_2CO_3-dissolved *E. postvittana* NPV polyhedron proteins bound to the Sepharose-*Wiseana* spp. NPV-immunoglobulin conjugate. Immunoglobulin from normal rabbit serum conjugated to Sepharose did not bind either the *E. postvittana* or *Wiseana* spp. NPV polyhedron protein preparations. These controls indicate that the immunoadsorption of *Wiseana* spp. NPV polyhedron protein antigens to the Sepharose-immunoglobulin conjugate was specific.

The results presented in Fig. 2 and 3 are radioactive profiles of SDS-polyacrylamide gels sliced into 1-mm sections. The upper profiles (A) are of the NaOH- or Na_2CO_3-dissolved, ^{3}H-labeled polyhedra of *Wiseana* spp. NPV before immunoadsorption, and the lower profiles (B) are of the components eluted from the immunoadsorption columns. Both NaOH- and Na_2CO_3-dissolved *Wiseana* spp. NPV polyhedron proteins appear to bind to the Sepharose-immunoglobulin conjugate. The ratios of degraded to undegraded polyhedron protein in both the NaOH- and Na_2CO_3-dissolved *Wiseana* spp. NPV polyhedra are similar after immunoadsorption. This would indicate that both the degraded and undegraded polyhedra protein can be bound by the same antibody.

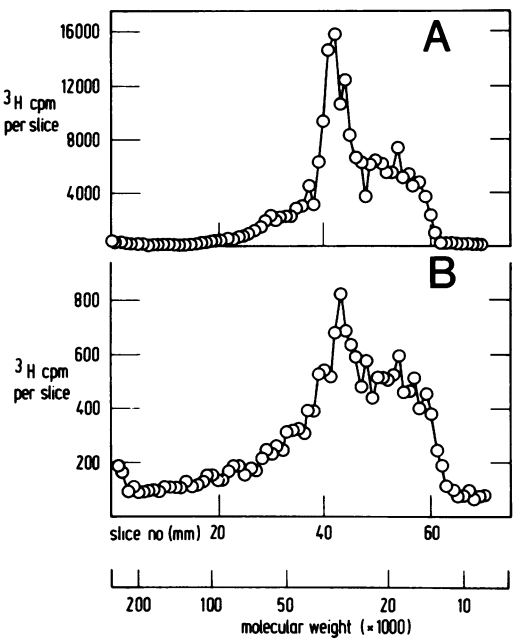

FIG. 2. *SDS-PAGE analysis of ^{3}H-labeled, 0.1 M NaOH-dissolved polyhedra before and after immunoadsorption to Sepharose conjugated with immunoglobulin purified from antiserum prepared against Wiseana spp. NPV. Three milligrams of Wiseana spp. NPV polyhedra was dissolved in 0.1 M NaOH, for 5 min at room temperature, and labeled with [^{3}H]iodoacetate, and then the labeled polyhedron protein solution was run on SDS-polyacrylamide gels. (A) Radioactive profile obtained by slicing the gels (1-mm slices) and determining the radioactivity of each slice. (B) A 0.5-mg amount of the labeled polyhedron protein was added to Sepharose conjugate prepared with immunoglobulin prepared against Wiseana spp. NPV. After washing the immunoadsorption column with 0.2 M Tris buffer (pH 8.0) and 0.5 M NaCl, the immunoadsorbed components were eluted from the conjugate with 0.1 M acetic acid and 10% dioxan. These components were then run on SDS-polyacrylamide gels, and the radioactivity of each slice was determined.*

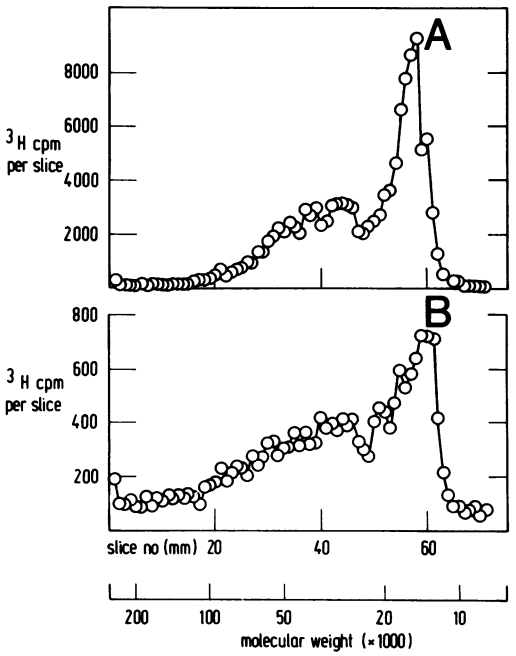

FIG. 3. *SDS-PAGE analysis of ^{3}H-labeled, 0.1 M Na_2CO_3-dissolved polyhedra. Polyhedra were dissolved in 0.1 M Na_2CO_3 for 2 h at 37°C before labeling. (A) Before immunoadsorption; (B) after immunoadsorption. For further details, see Fig. 2.*

To test for antigenicity by radioimmunoassay (RIA), the NaOH- or Na_2CO_3-dissolved polyhedra from *Wiseana* spp. or *E. postvittana* NPV were run in SDS-polyacrylamide gels. The gels were sliced, and the protein eluted from each slice. Half of the eluted protein was assayed by the direct solid-phase RIA, and half was assayed by the sandwich solid-phase RIA (2, 5). The results from NaOH- and Na_2CO_3-dissolved *Wiseana* spp. NPV polyhedra are presented in Fig. 4 and 5, respectively. In each of the figures the upper profile (A) is the result of the sandwich RIA and the middle profile (B) is the result of the direct RIA. An indication of the amount of protein eluted from each gel slice is given in the lower profile (C), which shows the ^{3}H counts per minute (labeled polyhedron protein) fixed to the wells in the direct RIA. Proteins from *E. postvittana* NPV, treated in a similar manner, did not show any binding to *Wiseana* spp. NPV antibody.

From Fig. 4 and 5 it can be seen that the sandwich assay does not detect those proteins below 27,000 molecular weight, whereas the direct assay detects all the proteins eluted from the gel. In the direct assay the antigen (polyhedron protein) is fixed to the microtiter well (Limbro S-MRC-96) and detected by adding labeled antibody. The sandwich assay uses a primary antibody coat fixed to the microtiter well surface (first layer): the antigen is added and is bound by this antibody coat; the antigen is then detected by adding ^{125}I-labeled antibody (second layer). The difference in antigen requirements between the two assays is that the sandwich assay requires a multivalent antigen, whereas the direct assay will detect monovalent antigens as well. Failure of the direct sandwich RIA to detect polyhedron protein degradation products below 27,000 molecular weight and their detection by the direct RIA suggest that the degradation products are monovalent antigens.

Until recently all the serology of baculoviruses was carried out using dilute alkali-dis-

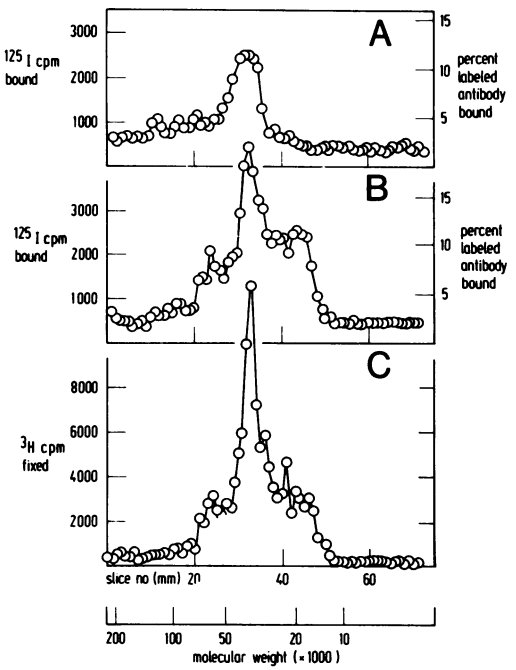

FIG. 4. *RIA of SDS-PAGE-separated components of 0.1 M NaOH-dissolved Wiseana spp. NPV polyhedra. Wiseana spp. NPV polyhedra were dissolved in 0.1 M NaOH for 5 min at room temperature, labeled with [^{3}H]iodoacetate, and then run in a 7% SDS-polyacrylamide gel. The gels were sliced in 1-mm sections, and each slice was placed in 0.2 ml of phosphate-buffered saline and incubated at 4°C overnight to allow elution of the protein from the slices. A 0.1-ml amount was assayed using the sandwich RIA (A), and the remaining 0.1 ml was assayed by the direct RIA (B). The amount of protein in each slide (C) was obtained by measuring the ratio of ^{3}H to ^{125}I in the wells used for the direct RIA.*

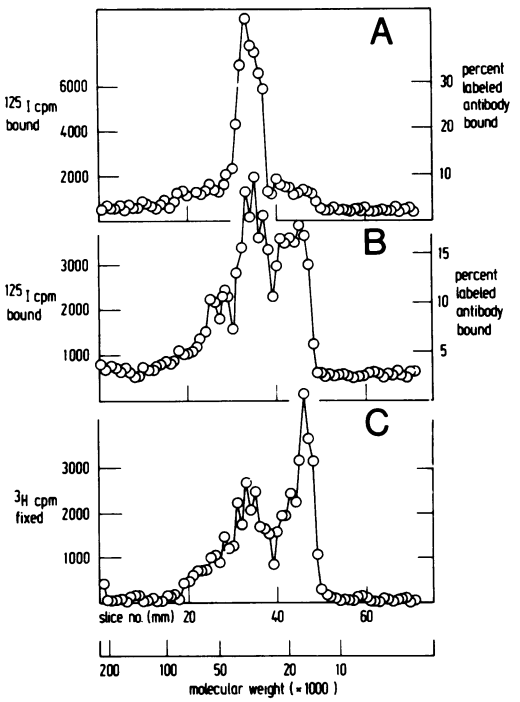

FIG. 5. *RIA of SDS-PAGE-separated components of 0.1 M Na_2CO_3-dissolved Wiseana spp. NPV polyhedra. Wiseana spp. NPV polyhedra were dissolved in 0.1 M Na_2CO_3 for 2 h at 37°C and assayed as described for Fig. 4: sandwich RIA (A), direct RIA (B), and protein in each slice (C).*

solved inclusion bodies (i.e., protease-degraded proteins). In this laboratory we have routinely used 0.1 M NaOH for dissolution of polyhedra, a condition that inhibits protease activity in *Wiseana* spp. NPV (2, 4, 5). It is concluded that since alkaline protease degradation products of *Wiseana* spp. NPV were found to retain the same antigenic specificity as undegraded polyhedron protein, previous baculovirus serology using dilute alkali to dissolve baculoviruses remains valid, providing the method of assay had no requirement for a multivalent antigen. There appears to be no attenuation of the virion proteins by protease activity (unpublished data); those serological results would likewise be valid.

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