The Polypeptide Composition of Isolated Surface Projections of Avian Infectious Bronchitis Virus

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SUMMARY

Disruption of avian infectious bronchitis virus (IBV) particles with 4% Triton X-100 and 1.0 M-KCl and centrifugation through a sucrose gradient containing 0.1% Triton X-100 and 1.0 M-KCl enabled separation of the petal-shaped surface projections. By negative-contrast electron microscopy the separated projections appeared mainly as rosettes containing 3 to 12 projections radiating from a central core, although single projections and rosettes containing up to 16 projections were seen. SDS-PAGE of these preparations revealed two polypeptides of 86,000 and 66,000 mol. wt. The larger polypeptide was glycosylated.

Studies aimed at identifying the structural polypeptides associated with the surface projections (spikes or peplomers) of coronaviruses have generally relied on specific digestion of these structures from the intact virus particles by proteases and the association of polypeptides by their absence after such treatment (Bingham, 1975; Garwes & Pocock, 1975; Hierholzer, 1976; Hierholzer et al. 1972; Macnaughton et al. 1977; Sturman, 1977). However, in studies with other enveloped viruses such as avian myeloblastosis virus (Bolognesi et al. 1972) Semliki Forest virus (Simons et al. 1973) and Newcastle disease virus (Scheid & Choppin, 1973), the use of non-ionic detergent or non-ionic detergent and KCl at high ionic strength enabled the isolation of functional undigested surface projections from virus particles. Using the latter method, we have isolated the surface projections of avian infectious bronchitis virus (IBV) and determined the associated polypeptides by polyacrylamide gel electrophoresis (PAGE).

IBV strain Massachusetts 41 was grown in embryonated fowls' eggs and purified by sucrose density gradient centrifugation as described by Alexander & Collins (1977). Concentrated purified virus (0.8 ml) containing 1 mg protein, estimated by the method of Lowry et al. (1951), was dissociated by adding 0.2 ml 20% (v/v) Triton X-100 in 0.01 M-tris-HCl buffer, pH 7.0 and KCl to a concentration of 1.0 M. The mixture was left at room temperature for 2 h before applying to a 4 ml 20 to 65% (w/w) sucrose gradient in 0.01 M-tris-HCl buffer, pH 7.0 containing 0.1% Triton X-100 and 1.0 M-KCl and centrifuged at 104,000 g for 18 h. After centrifugation, 30 five-drop fractions were taken from the gradient. Absorbance at 260 nm indicated three peaks, one at the top of the gradient with density 1.14 g/ml, a smaller peak with density 1.18 g/ml and spread of 1.17 to 1.19 g/ml and a very broad peak with density greater than 1.19 g/ml and including some pelleted material. The fractions containing the three peaks were pooled, dialysed against 0.01 M-tris-HCl buffer, pH 7.0, to remove sucrose and KCl and then concentrated by dialysis against polyethylene glycol. Negative-contrast electron microscopy of the three peaks revealed amorphous material in the lightest and densest peaks but the small peak of 1.17 to 1.19 g/ml consisted of distinct rosette-like structures formed from typical petal-shaped surface projections of the virus. Although single projections or rosettes having as many as 16 projections were seen, more usually rosettes were made up of 3 to 12 projections (Fig. 1 a). The diameters of the
Fig. 1. Negative-contrast electron micrographs of preparations of IBV surface projections. Staining was with 2% phosphotungstate at pH 6.6. The projections were dissociated with 4%, Triton X-100 and 1 M-KCl and separated by sucrose density gradient centrifugation. (a) Typical rosette structures formed from the petal-shaped projections; (b) higher magnification of a single rosette; (c) aggregate of rosettes seen in concentrated preparations.
Fig. 2. Analysis of IBV structural polypeptides on 10% acrylamide gels by discontinuous SDS-PAGE. (a to c) Whole virus; (d to f) isolated petal-shaped surface projections. (a), (c), (d) and (f) stained with Coomassie brilliant blue; (b) and (e) stained with Schiff’s reagent for glycopolypeptides. Figures represent apparent mol. wt. × 10^-3 estimated by comparison with standards of known mol. wt.

Rosettes were in the range of 40 to 47 nm, i.e. slightly longer than twice the length of the projections on intact virus particles. The petal-shaped projections, with wide end outermost, radiated from a dense central core to form the rosettes (Fig. 1 a, b). This arrangement gave the impression that each projection ended in a small knob which made up the central core in the rosettes but would be concealed in the envelope of intact viruses. In the concentrated preparations large aggregates or clumps of rosettes were frequently seen (Fig. 1 c).

Preparations of separated IBV projections were subjected to SDS-PAGE as described (Alexander et al. 1979) and staining with Coomassie brilliant blue revealed two polypeptides of mol. wt. 86,000 and 66,000. Both corresponded to polypeptides present in whole virus preparations (Fig. 2) although both the polypeptides associated with the projections repre-
represented relatively minor bands of the whole virus polypeptide profile. SDS–PAGE and staining with Schiff's reagent (Alexander & Collins, 1977) indicated that the 86,000 mol. wt. polypeptide was strongly glycosylated and, on some gels, a band of faint Schiff's staining corresponded to the 66,000 mol. wt. polypeptide (Fig. 2).

In other studies with IBV virus particles have been treated with bromelain to remove surface projections and polypeptides associated with the surface projections by their absence from the polypeptide profiles after treatment. This method led to the association with the projections of five polypeptides of mol. wt. 180,000, 130,000, 106,000, 83,000 and 70,000 of which 180,000 and 83,000 were glycosylated (Bingham, 1975) and three glycopolypeptides of mol. wt. 130,000, 105,000 and 74,000 (Macnaughton et al. 1977). Although the polypeptide profiles of the whole virus reported by Bingham (1975), Macnaughton et al. (1977) and ourselves are not absolutely comparable, our present results would suggest that the protease treatment used in their studies removed more than just the petal-shaped surface projections. Whether or not the additional material is present as another surface projection, morphologically distinct from the petal-shaped species, as suggested by Macnaughton et al. (1977) has not been determined.

It would be expected that the surface projections would play an important role in the immunogenicity of IBV. It is therefore of interest that polypeptide analysis of the structural polypeptides of different serotypes of IBV has indicated differences in the migration of the glycopolypeptide corresponding to the 86,000 mol. wt. polypeptide of Massachusetts 41. However, variation in the migration of the glycopolypeptide corresponding to the 31,000 mol. wt. polypeptide of Massachusetts 41 was also seen in the different serotypes (Collins & Alexander, 1980).

Work is in progress on the separation and comparison of the surface projections of serologically and/or morphologically distinct IBV strains.

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REFERENCES


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