NOTES

Further Morphological Characterization and Structural Proteins of Infectious Bursal Disease Virus

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An outer layer surrounding the capsid of infectious bursal disease virus was evident from electron micrographs of intact virus particles having diameters of 62 to 63 nm. The capsid was found to be composed of large morphological units or capsomeres, measuring about 12 nm in diameter. The architecture of the capsid appears to be that of T = 3 symmetry, with a probable 32 morphological units by rotational enhancement of image detail. Structural proteins of infectious bursal disease virus consist of seven species, two major and five minor polypeptides. These are P1 to P7, with molecular weights of $133 \times 10^3$, $124 \times 10^3$, $98 \times 10^3$, $51 \times 10^3$, $33 \times 10^3$, $26 \times 10^3$, and $23 \times 10^3$, respectively.

Infectious bursal disease (IBD) is a naturally occurring virus-induced immune deficiency of chickens characterized by necrosis and depletion of lymphoid elements. A recent study has suggested that surface immunoglobulin M-bearing B lymphocytes are target cells for infection by the IBD virus (IBDV) (3). Electron microscopy has shown that IBDV is a nonenveloped icosahedron with diameters ranging from 55 to 65 nm (1, 2, 5, 13). In a previous study (5), we suggested that the capsid of the virion consisted of 32 capsomeres, each composed of five or six structural units, and that IBDV resembled bluetongue virus in morphology. However, it is known that there are biological and physicochemical differences between IBDV and bluetongue virus. Apart from the initial study by Nick et al. (10), little information is available on the structural proteins of IBDV, using polyacrylamide gel electrophoresis. The exact number of polypeptides and their locations within the virion are not yet clearly understood.

This report is concerned with a reexamination of two criteria: the fine structure of virions viewed with high-resolution electron microscopy and rotational enhancement of image detail and the polypeptide structure of IBDV, using polyacrylamide gel electrophoresis.

Samples of the GBF-1 strain of IBDV were obtained as previously described (4, 6). Briefly, susceptible chickens were inoculated with virus from passage 15 to 20, and affected bursae of Fabricius were harvested for virus extraction. The purification procedure was essentially the same as that described previously (5). Electron microscopy was performed as previously described (5). Rotational enhancement of image detail was accomplished with a double-wheel device modified from the pinwheel apparatus described by Markham et al. (8). These modifications eliminated the hole produced at the axis of rotation. Virus samples were solubilized by incubation for 2 h at 37°C with equal amounts of the 0.01 M phosphate buffer (pH 7.2) containing 2% sodium dodecyl sulfate, 6 to 8% 2-mercaptoethanol, and 50% glycerol, followed by heating for 2 min at 100°C before electrophoresis. The polypeptide composition of the virus was determined in 7.5% polyacrylamide gels as described by Spandidos and Graham (16). Electrophoresis was carried out at a constant current of 5 mA/gel for 8 h. The molecular weights of polypeptides determined on 7.5% gels by the procedure of Shapiro et al. (15) were used for the final results.

After three equilibrium centrifugations in cesium chloride, the major band of IBDV occurred at a buoyant density of 1.34 g/ml, and three faint minor bands occurred at buoyant densities of 1.35, 1.36, and 1.37 g/ml, respectively. No cellular debris was observed in any band when examined by electron microscopy. Bands at den-
sities of 1.34 and 1.35 g/ml both contained nu-
terous particles with hexagonal or roughly 
spherical profiles. Hexagonal virus particles pre-
dominated at the density of 1.34 g/ml, whereas 
the band at the density of 1.35 g/ml contained 
mostly spherical particles. Intact virions were 
generally hexagonal in profile, and surface caps-
osome arrangements of the morphological unit 
was not clearly resolved (Fig. 1a and b). These 
particles averaged from 62 to 63 nm in diameter 
and appeared to be covered by an outer layer 
composed of T-shaped structures (Fig. 1b). The 
outer layer was continuous. Spherical particles 
without an apparent outer layer predominated 
at the density of 1.35 g/ml (Fig. 1c). Their di-
ameters were 54 to 55 nm, and morphological 
units were clearly observed. At a higher magni-
fication, each morphological unit seemed to be 
composed of smaller subunits in hexagonal-pen-
tagonal arrays around a central hole (Fig. 2a and 
d). Subunits appeared to be shared by neighbor-
ing structural units. In previous ultrastructural 
studies on the morphology of IBDV (5), we 
suggested that the capsid of the virion consisted 
of 32 capsomeres arranged in 5:3:2 symmetry. In 
an attempt to further elucidate the capsomere 
arrangement, rotational enhancement of image 
detail has been accomplished with a few IBDV 
particles. When viewed on a threefold vertex 
(Fig. 2a), six capsomeres were clearly seen on an 
n = 6 rotation (Fig. 2b) but not on an n = 5 
rotation (Fig. 2c). Peripheral capsomeres may 
not have been penetrated by stain and, thus, 
were not clearly seen. On a fivefold axis of sym-
metry (Fig. 2e), capsomeres were enhanced by 
an n = 5 rotation but not by an n = 6 rotation 
(Fig. 2f). Peripheral capsomeres were clearly 
enhanced by an n = 5 rotation. The diameters 
of the morphological unit and central hole were 
about 12 and 4 nm, respectively. Bands at den-
sities of 1.36 and 1.37 g/ml both contained de-
graded virus particles and other smaller parti-
cles. However, a higher percentage of smaller 
particles was observed in the band at the density 
of 1.36 g/ml than in the band at the density of 
1.37 g/ml. The latter band contained mostly 
stringlike structures. The smaller particles had 
a mean diameter of 15 nm and a range of 10 to 
20 nm. These particles occurred singly, in large 
groups, and occasionally on the surface of de-
grading IBDV particles (Fig. 1d). The stringlike 
structures observed in the 1.37g/ml CsCl frac-
tion (Fig. 1e) were 10 to 23 nm in width and 
appeared to be composed of smaller linked 
doughnut-shaped structures. Degraded virions 
were observed occasionally at a stage in which 
structural components were being released like 
an unwinding ball of string.

The 5 and 10% gels used for electrophoresis 
did not alter the IBDV polypeptide profiles ob-
tained in 7.5% gels. Further heat dissociation 
of viral proteins before electrophoresis also did 
not alter the electrophoretic profiles. Electrophore-
sis of the major CsCl fraction having a buoyant 
density of 1.34 g/ml was done in more than 30 
gels. A representative stained gel is shown in 
Fig. 3a. From these results, seven polypeptides 
representing viral structural proteins were evi-
dent and designated as P1, P2, P3, P4, P5, P6, 
and P7 (descending molecular weight). Two ma-
jor bands, P3 and P4, were obvious, as indicated 
by their dense staining and high profiles. Five 
minor bands were also evident, of which two, 
P1 and P2, were seen as sharp but faint bands. 
The other minor bands, P5, P6, and P7, stained more 
diffusely. The molecular weights of viral polyp-
epptides were determined by comparison with 
the relative mobilities of proteins of known mo-
olecular weights. An almost linear relationship 
was obtained between ratios of migration and 
molecular weight in the range of 12 x 10^3 to 160 
X 10^3 for 7.5% gels (Fig. 4). The molecular 
weights of the seven structural components, rep-
resenting averages of 15 separate determina-
tions, were 133 x 10^3 (P1), 124 x 10^3 (P2), 98 
X 10^3 (P3), 51 x 10^3 (P4), 33 x 10^3 (P5), 26.5 x 10^3 
(P6), and 23 x 10^3 (P7). In Table 1, the molecular 
weights of the IBDV polypeptides are compared 
with those of rotavirus, bluetongue virus, and 
avian reovirus. The stained gel of the CsCl frac-
tion at a density of 1.35 g/ml (Fig. 3b) did not 
differ from the gel of the major fraction shown 
in Fig. 3a. Electrophoresis of fractions at den-
sities of 1.36 and 1.37 g/ml revealed that the 
polypeptide concentration of P4 was apparently 
less than that of P3, contrary to the standard 
profile of the virion. Other minor bands were not 
detected in these fractions (Fig. 3c).

IDBV apparently has an outer layer, as indi-
cated by the large size of intact particles with 
hexagonal outlines. Moreover, the capsomeric 
detail on the main capsid surface of IBDV often 
appeared partially obscured by such a layer. 
This outer layer was very thin (7 to 8 nm) and 
continuous, with T-shaped structures suggestive 
of the configuration reported for rotavirus but 
not as clearly defined (9, 12, 17). Although the 
outer layers of reovirus and orbivirus are also 
indistinct, they are featureless (7, 17). The outer 
layer of IBDV may exert some stability to the 
structure of the underlying major capsid layer. 
By rotational enhancement of image detail, the 
results of this study confirmed our previous re-
port (5) that IBDV has an icosahedral symmetry of 
T = 3, with a probable 32 large capsomeres. 
This type of symmetry and the unique feature of 
subunit sharing are morphological character-
istics reported for the Reoviridae family (11, 12).
FIG. 1. Electron micrographs of negatively stained IBDV particles. (a) Intact virions of IBDV recovered from the 1.34-g/ml CsCl. Bar = 100 nm. X130,000. (b) Higher magnification of an intact virion, showing the outer layer. The surface of the particles appear to have T-shaped morphology which is continuous. Bar = 50 nm. X450,000. (c) IBDV particles recovered from the 1.35-g/ml CsCl fraction. Intact virions of IBDV (arrow) and particles with clear capsomers showing the loss of the outer layer (double arrow). Bar = 100 nm. X190,000. (d) Smaller particles recovered from the 1.36-g/ml CsCl fraction. Smaller particles on the surface of a degraded particle. Each particle appears to be a structural unit of the capsid. Bar = 50 nm. X380,000. (e) Stringlike structures recovered from the 1.37-g/ml CsCl fraction. Bar = 100 nm. X33,000.
Fig. 2. (a-c) Single IBDV particle viewed on a threefold axis of symmetry, representing $n = 0$, $n = 6$, and $n = 5$ rotations, respectively. Enhancement of capsomeres is evident in the $n = 6$ rotation. Bar = 50 nm. ×390,000. (d-f) Single IBDV particle viewed on a fivefold axis of symmetry, representing $n = 0$, $n = 5$, and $n = 6$ rotations, respectively. Enhancement of capsomeres is evident in the $n = 5$ rotation. Bar = 50 nm. ×390,000.
The results of this study demonstrated that IBDV is composed of seven polypeptides, two major and five minor proteins. The major polypeptides were associated with the smaller subunit particles and stringlike structures, both having the same electrophoretic profile. Therefore, these polypeptides may be the capsomeric proteins of IBDV. These results differed from those reported by Nick et al. (10) with regard to the number of polypeptides and their molecular weights. Only four polypeptides were found in the previous study, three of which did compare closely in molecular weight with our P4, P5, and P6 proteins. However, a polypeptide having a molecular weight of $11 \times 10^3$ was not detected in our preparations. Their failure to detect no more than four structural polypeptides may have resulted from partial disruption of virus particles before sodium dodecyl sulfate-polyacrylamide gel analysis. Thus, the limited amount of purified IBDV obtained may not have provided sufficient material or detection of all structural polypeptides. As shown in Table 1, the molecular weights of the IBDV proteins compared closely with those reported for the human rotavirus (14). However, the major polypeptide species (P3 and P4) of IBDV did not correspond with those major proteins of the human rotavi-

### Table 1. Comparison of molecular weights of IBDV proteins with those of rotavirus, bluetongue virus, and avian reovirus

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight (x10^3)</th>
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<tbody>
<tr>
<td>IBDV</td>
<td>133</td>
</tr>
<tr>
<td>Human rotavirus*a</td>
<td>127</td>
</tr>
<tr>
<td>Bluetongue virus*b</td>
<td>140</td>
</tr>
<tr>
<td>Avian reovirus*c</td>
<td>140</td>
</tr>
<tr>
<td>124</td>
<td>103</td>
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<tr>
<td>98</td>
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<td>26</td>
</tr>
<tr>
<td>23</td>
<td>21</td>
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*a Rodger et al. (14).
*b Verwoerd et al. (18).
*c Spandidos and Graham (16).
rus, nor did IBDV have an eighth polypeptide of molecular weight 88 x 10^6. Our results concerning the morphology of IBDV and the similarity of its protein composition to the rotavirus group suggest its tentative inclusion into the Reoviridae family.

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LITERATURE CITED


