Biosynthesis of Virus-Specific Proteins in Cells Infected with Infectious Bursal Disease Virus and Their Significance as Structural Elements for Infectious Virus and Incomplete Particles

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Received 12 April 1982/Accepted 24 June 1982

It has previously been shown that infectious bursal disease virus is a naked icosahedral particle with a diameter of about 60 nm and a genome consisting of two segments of double-stranded RNA (Müller et al., J. Virol. 31:584–589, 1979). One of the two major structural polypeptides (molecular weight, 40,000) of this virus could not be found in lysates of infected cells; it is derived from a precursor polypeptide demonstrable inside the cells in relatively large quantities and seems to be processed during virus assembly or later. The precursor molecule is regularly present in the infectious virus particle (buoyant density, 1.33 g/ml in minor proportions, but it represents an outstanding structural element of incomplete noninfectious particles (“top components”; buoyant density, 1.29 g/ml) which contain viral RNA. This type of incomplete particles is mainly produced by chicken embryo fibroblasts in contrast to lymphoid cells from the bursa of Fabricius. Precursor-product relationships also seem to exist in the biosynthesis of the other viral polypeptides. In contrast to some other viruses with a segmented double-stranded RNA genome, none of the structural proteins of infectious bursal disease virus is appreciably glycosylated.

Infectious bursal disease virus (IBDV), the causative agent of a highly contagious disease of young chickens (Gumboro disease) resulting in severe necrotic lesions in the bursa of Fabricius (BF), is a nonenveloped icosahedral particle with a diameter of about 60 nm. The genome consists of two segments of double-stranded RNA with molecular weights of $2.5 \times 10^6$ and $2.2 \times 10^6$ (19, 24), which places IBDV within a new group of viruses that includes strains isolated from fish, molluscs, and Drosophila (8).

In several reports from different laboratories, four structural proteins (designated as VP1, VP2, VP3, and VP4) have been described for IBDV; among these polypeptides, two of about 40 kilodaltons (kd) and 35 kd represent the major structural constituents (7, 8, 20, 25). In all our virus preparations, even after purification by repeated runs through sucrose and cesium chloride gradients, another minor polypeptide (termed VP-X; 7) could regularly be detected (see reference 1). A peptide analysis (7) showed that its fingerprints were very similar to those of one of the two major structural polypeptides, indicating that a precursor-product relationship exists between these two components. Major objectives of this study were to trace the synthetic steps for these two and perhaps other viral polypeptides and to define the significance of these products for the formation of infectious virus particles and immature forms.

Since it has been reported that reoviruses and rotaviruses, which have essential structural features in common with IBDV, contain glycoproteins in their capsids (4, 15, 23), it had to be clarified whether synthetic events in IBDV-infected cells include glycoproteins.

MATERIALS AND METHODS

Propagation of virus and cell cultures. Plaque-purified strain Cu-1 of IBDV (20) was propagated in various cell types (see below) or in 3- to 6-week-old, specific-pathogen-free chickens (Lohmann, Cuxhaven, West Germany) as described (13, 20).

For the preparation of lymphoid cells from the BF, thymus, or spleen, these organs were aseptically removed from uninfected chickens, minced very carefully with scissors, and suspended in RPMI 1640 (with 25 mM HEPES [N-2-hydroxyethyl]piperazine-N'-2-ethanesulfonic acid] and 2 mM L-glutamine; GibCO Europe, Karlsruhe, West Germany). After coarse material was allowed to settle for 5 min, 3 parts of the cell suspension were layered over 1 part of Ficoll-Paque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and centrifuged at $600 \times g$ for 30 min at 12°C. Cells were removed from the interphase, washed twice with...
RPIM 1640, and incubated for 30 min at 39°C in the presence of an iron-containing lymphocyte separator reagent (Technicon; Technicon Instruments Corp., Tarrytown, N.Y.); phagocytizing cells were then removed with a magnetic bar (17). Nonadhering, mostly lymphoid cells were washed and counted. Chicken embryo fibroblasts (CEF) were prepared by standard procedures.

Cells were infected at a multiplicity of infection of 10 PFU/cell. After 60 min (this was taken as zero time) at 39°C, cells were washed twice, covered with RPMI 1640, and incubated at 39°C.

**Labeling of infected cells and IBDV particles with [35S]methionine.** For labeling of proteins in infected cells with [35S]methionine. CEF were grown in plastic petri dishes (about 10^6 cells each), and lymphoid cells were kept in plastic centrifuge tubes (about 5 x 10^6 cells each). RPMI 1640 was removed, and the cells were washed twice and incubated for 90 min with Eagle minimum essential medium without methionine (Flow, Meckenheim, West Germany) before they were pulsed with [35S]methionine (SJ 204; Amersham Buchler, Braunschweig, West Germany; 50 μCi in 0.5 ml of minimum essential medium without methionine) for various time periods. Thereafter the cells were either washed twice with ice-cold phosphate-buffered saline and then lysed, or washed twice with medium containing an excess of unlabeled methionine and then incubated further at 39°C for various time periods in pulse-chase experiments.

For the preparation of protein-labeled virus particles, CEF grown in 15-cm plastic petri dishes were infected as described above, and each dish was covered with 15 ml of minimum medium deficient in methionine at zero time after infection. This medium did not reduce the yield of progeny virus significantly. At 2 h after infection, 200 μCi of [35S]methionine, as described above, was added to each plate. Equal amounts of radioactivity were added a second time 10 h later, and the medium was collected 24 h after infection.

**Labeling of infected cells and IBDV particles with 3H-sugars.** Labeled or unlabeled lymphoid cells or CEF were covered with Earle balanced salt solution containing 10 mM sodium pyruvate instead of glucose as an energy source and supplemented with 20 μCi of one of the following radioactive sugars, all obtained from Amer sham, per ml: [3H]galactose (TRK 233; 29 Ci/mmol), [3H]glucosamine (TRK 398; 29 Ci/mmol), [3H]glucose (TRK 366; 54 Ci/mmol), or [3H]mannose (TRK 364; 10.4 Ci/mmol). After 3 or 6 h at 39°C, the cells were washed twice with ice-cold phosphate-buffered saline and processed for immunoprecipitation. For labeling virus particles, CEF grown in 15-cm plastic petri dishes were covered with 15 ml of Earle balanced salt solution (with pyruvate instead of glucose), containing a 20-μCi/ml concentration of one of the tritiated sugars listed above, for 24 h.

**Extraction and purification of virus particles.** IBDV was extracted from BF or CEF with Frigen 113 (Hoechst AG, Frankfurt, West Germany) and purified as described previously (19), with one additional step of centrifugation in a sucrose gradient and one additional step of centrifugation to equilibrium in CsCl gradients (mean density, 1.32 g/ml; 18 h; 40,000 rpm; SW60 Ti rotor) before the virus particles were finally pelleted.

**Antisera.** Antisera against IBDV structural polyepitides were produced in rabbits (rabbit anti-IBDV serum) by three intramuscular injections of purified virus emulsified in Freund incomplete adjuvant at 3-week intervals. For the removal of antibodies elicited against BF cellular components, sera were passed through a column of Sepharose 6B/CL (Pharmacia) to which an extract from BF of uninfected chickens had been conjugated after CNBr activation (21, 22). Chickens that had survived a peroral infection with IBDV-containing BF homogenate were injected intramuscularly three weeks later with UV-inactivated purified virus in adjuvant. Chickens were bled after 3 weeks (chickens anti-IBDV serum). Neutralization titers in both types of serum were greater than 1:10,000 as determined by plaque reduction.

**Immunoprecipitation.** When comparative assays were carried out, all cells were treated alike and lysed simultaneously. Immunoprecipitation was performed as described by Gilead et al. (12) and Gielkens et al. (11) by lysing radiolabeled cells on ice with a 10 mM sodium phosphate buffer (pH 7.2) containing 10 mM EDTA, 1% (vol/vol) Triton X-100, 1% (vol/vol) sodium deoxycholate, 0.1% (wt/vol) sodium dodecyl sulfate (SDS), and 2% (vol/vol) Trasylol (Bayer, Leverkusen, West Germany). Lysates were clarified by centrifugation (50 min, 35,000 rpm, 4°C, SW60 Ti rotor), and 200 μl was mixed with 5 μl of anti-IBDV serum raised in chickens or in rabbits. After incubation at room temperature, 50 μl of a fully precipitating dilution of a goat anti-chicken immunoglobulin G serum (Miles Laboratories, Elkhart, Ind.) was added, when chicken serum was used, or 100 μl of a freshly prepared 10% (vol/vol) suspension of heat-inactivated, Formalin-fixed Staphylococcus aureus (14) diluted with lysing buffer was added in the case of rabbit anti-IBDV serum. After incubation on ice for 60 min, the antigen-antibody complexes bound to anti-globulin or protein A were collected by centrifugation (1 min, 12,000 rpm) and washed twice with a buffer containing 10 mM sodium phosphate (pH 7.0), 1 M NaCl, 10 mM EDTA, 40 mM NaF, and 0.2% (vol/vol) Triton X-100 and once with double-distilled water. Final wash in electrophoresis buffer was added, the complexes were dissociated by heating and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

**SDS-PAGE.** Purified virus particles, lysates of radiolabeled cells, or immune complexes were dissociated with electrophoresis buffer (50 mM Tris-hydrochloride, pH 6.8, containing 2% [wt/vol] SDS, 4 M urea, 2% [vol/vol] 2-mercaptoethanol, 10% [vol/vol] glycerol, and a trace of bromphenol blue) by heating to 100°C for 2 min and immediately subjected to SDS-PAGE according to Laemmli (16) as previously described (19). The acrylamide-bisacrylamide ratio was 50:1.5 in the stacking gel or 30:0.8 in the resolving gels (15% acrylamide). All gels contained 2.5 M urea and were run at 100 V for 14 h. Unlabeled protein bands were stained with Coomassie brilliant blue R-250 and monitored with a Gilford spectrophotometer at a wavelength of 578 nm. Gels with radiolabeled proteins were fixed with 7.5% (vol/vol) acetic acid and processed for fluorography as described by Bonner and Laskey (3). Gels were dried and exposed to Kodak X-omat AR films. The individual viral proteins were designated with a "p" in combination with their molecular sizes in kd.
Iodination of IBDV structural proteins and two-dimensional tryptic peptide mapping. IBDV structural polypeptides were identified on SDS-polyacrylamide gels by Coomassie brilliant blue staining, excised, and radioiodinated with $^{125}$I (NEZ-033L, ca. 17 mCi/mmol; New England Nuclear Corp., Dreieich, West Germany) in the gel slice by a modification of the chloramine-T method as described by Elder et al. (10). Thereafter the samples were suspended in 0.05 ammonium bicarbonate buffer (pH 8.0) supplemented with 50 $\mu$m of tolysalloyl phenylalanyl chloromethyl ketone containing trypsin (Worthington Biochemicals Corp., Freehold, N.J.) per ml and digested for 14 h at 37°C. Samples were lyophilized, washed with ammonium bicarbonate buffer twice, and subjected to electrophoresis on cellulose-coated glass plates (20 by 20 cm; E. Merck, Darmstadt, West Germany) with acetic acid-pyridine-water (5:0.5:94.5, vol/vol) at 500 V for 1.5 h. The plates were chromatographed in the second dimension with n-butanol-acetic acid-pyridine-water (90:18:78:78, vol/vol) as the solvent for 4 to 5 h at 20°C. The plates were dried and covered with X-ray films as above.

Staining of glycoproteins with lectins labeled with FITC. Fluorescein isothiocyanate (FITC)-labeled concanavalin A and wheat germ agglutinin were purchased from Sigma (Munich, West Germany). After SDS-PAGE, parallel gels were stained with Coomassie brilliant blue or with the FITC-labeled lectins as described by Maher and Molday (18). Fowl plague virus with well-defined lectin-binding glycoproteins (2) was included as a control. Fluorescent bands were visualized with a short-wave transilluminator (Ultra-violet Products, San Gabriel, Calif.).

RESULTS

Polypeptide composition of infective IBDV particles. IBDV was purified from the BF of chickens within 40 h after infection, when the animals exhibited serious clinical symptoms or were moribund (5, 13). Infectious IBDV particles (Fig. 1a; buoyant density, 1.33 g/ml in cesium chloride gradients), when subjected to PAGE, show an overall distribution of viral polypeptides (Fig. 2A) which is in accordance with previously published results (7, 8). However, it is apparent from these tracings that one of the two major structural proteins (VP 3, 35 kd) exists in two distinct bands (33 and 32 kd). Further structural details became evident when IBDV particles grown in CEF and labeled with $^{35}$S methionine were analyzed by PAGE. VP-X, present in Fig. 2A as one relatively broad peak, shows up in Fig. 3 as two closely adjacent but clearly discernible bands. In the high-molecular-weight region another protein (p95) can be seen besides VP 1 (90 kd) in many gels stained with Coomassie brilliant blue, and here again the existence of these two large polypeptides could be verified by prolonged exposure of gels with $^{35}$S methionine-labeled proteins to the films (see Fig. 5, lane f, and Fig. 8, lane e). Finally, it should be mentioned that another band (34 kd) was regularly observed when the gels were heavily overloaded or when they stayed in contact with the films for a long time (not shown). Since VP-X and possibly the other minor polypeptides described above and listed in Table 1 might represent precursor molecules which are regularly incorporated into the virion, all virus-specific proteins described in this paper were designated by "p," followed by their molecular size in kd. The proportions of the polypeptides found by these methods did not vary significantly when different batches of virus were examined or when the purification schedule was slightly modified. According to their molecular sizes they can be placed into three groups: large (about 90 kd), medium (40 to 50 kd), and small (about 30 kd).

The polypeptide analysis in the preceding paragraph refers to infectious virus particles with a density of 1.33 g/ml in CsCl gradients.
Besides this prominent band containing particles with the typical appearance of IBDV (Fig. 1a), another band regularly formed at a density of 1.32 g/ml. Particles forming this band contained a relatively small amount of double-stranded RNA, and a third virus population banding at a density of 1.31 g/ml did not contain any RNA at all. The morphology of the particles in these two bands did not differ from the electron micrographs of "standard" IBDV with a buoyant density of 1.33 g/ml; the polypeptide patterns of virus particles banding at either density were also the same (not shown). This is generally true for virus purified from the BF or grown in CEF.

**Polypeptide composition of IBDV "top component."** After centrifugation to equilibrium in CsCl gradients a fourth virus band formed at a density of 1.29 g/ml. This was regularly the case when IBDV had been grown in CEF; IBDV purified from the BF only formed very faint or no clearly discernible bands at this density. The morphology of particles in this top component of tissue culture-grown virus differed considerably from that of standard infectious particles. As shown in Fig. 1b, stain penetrates into these particles, which have an irregular shape and do not appear to be solidly assembled. Although these particles contain the usual amount of double-stranded RNA (data not shown), they were not infectious. Their polypeptide pattern turned out to be substantially different from that of standard infectious virus.

The tracing of a stained gel in Fig. 2b and the autoradiograph in Fig. 3 show that in top component particles there is a relative abundance of proteins of between 44 and 50 kd, at the expense of both major structural polypeptides p40 and p32. Furthermore, the large polypeptides p95 and p90 as well as the small p33 and p28 are missing (Table 1).

**Tryptic peptide analysis.** The combined molecular size of infectious IBDV structural polypeptides listed in Table 1 amounts to 449 kd, which considerably exceeds the coding capacity of the IBDV genome for approximately 230 kd. This discrepancy indicated that some of the proteins are not primary gene products. To define a potential precursor-product relationship, the polypeptides described above were analyzed by tryptic peptide mapping. Structural polypeptides

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**FIG. 2.** (A) Densitometric tracing of the polypeptides of infectious IBDV particles (Fig. 1a; buoyant density, 1.33 g/ml) purified from the BF of infected chickens. (B) Densitometric tracing of the polypeptides of particles banding at a buoyant density of 1.29 g/ml (top component, Fig. 1b), grown in CEF. Both samples were electrophoresed on the same 15% polyacrylamide gel, stained with Coomassie brilliant blue, and scanned as described in the text. The arrows represent molecular size markers of 90, 53, and 25 kd. The structural polypeptides are characterized by "p," followed by their molecular sizes in kd. The polypeptides described by Dobos (7) and Dobos et al. (8) are indicated in parentheses.
separated by SDS-PAGE were labeled with $^{125}$I in vitro. Since the amounts of p95, p34, and p33 available were too small, these polypeptides could not be considered. p49 and p48 were treated as one protein. As shown in Fig. 4, all of the major peptides present in p48/49 were also present in the pattern of the p40 molecule, except for one and possibly two other oligopeptides. These results indicate that p40 is generated by specific cleavage of the 48-49-kd protein. The peptide maps of the other structural proteins which could be included in this analysis (p90, p32, p28) were totally different from each other.

Immunoprecipitation of IBDV specific polypeptides synthesized in infected cells. To define a precursor-product relationship in more detail and to gain insight into the mechanism of virus replication, lymphoid cells isolated from the BF of susceptible chickens were infected in vitro, labeled with $^{[35]}$Smethionine, and lysed, and the polypeptides were analyzed by SDS-PAGE after immunoprecipitation. This approach was valid since the relative amounts of $^{[35]}$Smethionine incorporated into individual structural polypeptides corresponded well with the amounts of dye

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**TABLE 1. Catalog of IBDV structural proteins and IBDV-specific polypeptides in infected cells identified by one-dimensional SDS-PAGE**

<table>
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<tr>
<th>IBDV structural components identified in:</th>
<th>IBDV-specific polypeptides identified after:</th>
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<tr>
<td>Infective particles $^a$</td>
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<td>Top component $^c$</td>
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$^a$ Infected BF lymphoid cells were labeled with $^{[35]}$Smethionine and analyzed after immunoprecipitation. Polypeptides are identified by "p", followed by molecular size in kd.

$^b$ Buoyant density in CsCl, 1.33 g/ml.

$^c$ Buoyant density in CsCl, 1.29 g/ml.

$^d$ Only trace amounts of p40 can be demonstrated.

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FIG. 3. Fluorographs of IBDV polypeptides labeled with $^{[35]}$S methionine. (a) Infectious virus particles (buoyant density, 1.33 g/ml) and (b) top component particles (buoyant density, 1.29 g/ml) were grown in CEF. Conditions for SDS-PAGE and fluorography were as described in the text. Viral polypeptides are designated by "p," followed by the molecular size in kd. The film was slightly underexposed to improve the resolution of individual protein bands in the gel.

FIG. 4. Two-dimensional analysis of the tryptic peptides of p48/49 (A) and p40 (B). IBDV structural polypeptides were separated by SDS-PAGE, labeled with $^{125}$I, and digested as described in the text. Electrophoresis of the polypeptides was from left to right (I), and ascending chromatography in the second dimension was from bottom to top (II). The digests were spotted on the plates as indicated by arrows. Oligopeptides not present in p40 are marked by circles.
bound to the respective virion proteins after staining with Coomassie blue in polyaclrylamide gels. Immunoprecipitation of virus-specific proteins synthesized in lymphoid cells from the BF, which is the main target organ of IBDV (13), was chosen since virus-specific polypeptides could be detected among cellular proteins in infected CEF only with difficulty, and IBDV replication proved to be extremely sensitive to actinomycin D (data not shown).

In a first series of immunoprecipitation experiments, chicken convalescent-phase serum and rabbit hyperimmune serum against purified virus were used in parallel for comparative purposes. These experiments showed that identical IBDV-specific polypeptides were precipitated by both types of antiserum and that the amounts of radioactivity in the individual protein bands did not differ. However, the amount of host cell-specific polypeptides trapped by rabbit anti-IBDV serum plus S. aureus protein A was significantly less than in precipitates formed by chicken serum plus anti-chicken immunoglobin G serum. Rabbit anti-IBDV was therefore used in all other immunoprecipitation experiments. Although variable amounts of host cell-specific polypeptides were precipitated when this technique was employed, the resolution of individual IBDV-specific polypeptides was clearly improved. Either they had no counterparts in uninfected cells, or the intensity of these bands increased after infection and they could thus be considered to be virus specific.

**Polypeptide synthesis and pulse-length variation.** The polypeptides precipitated by rabbit anti-IBDV serum from lysates from infected lymphoid BF cells pulse-labeled 2 h after infection with [35S]methionine for various periods of time are shown in Fig. 5. After 5- or 10-min pulses (Fig. 5a and b) five polypeptides with molecular sizes of 90, 50, 33, 32, and 28 kd were seen. When the cells were pulsed for 30 or 60 min (Fig. 5c and d), two additional polypeptides with molecular sizes of 95 and 34 kd could be detected, and after a 120-min labeling period (Fig. 5e) two smaller polypeptides with sizes of 26 and approximately 10 kd (already present as faint bands after a 60-min pulse) became apparent. It has to be emphasized that a polypeptide comigrating with p40, one of the main structural proteins, could only be seen as a faint band when the gels were overexposed. Variable amounts of host cell-specific polypeptides were trapped in the precipitates (e.g., the protein band of about 80 kd in Fig. 5), but the polypeptides described above and listed in Table 1 were regularly found in a number of experiments and could therefore be considered to be IBDV specific. When the polypeptide pattern obtained after a 60-min pulse at 6 h after infection (Fig. 6b) was compared with the pattern obtained at 3 h after infection (Fig. 6a) it virtually was the same as described above, but the relative
amounts of radioactivity in two of the polypeptides had changed. At 6 h after infection the amounts of radioactivity in p95 had decreased whereas the amounts of $^{35}$S incorporated into the low-molecular-weight proteins increased as compared to 3 h after infection. Again, p40 was present only as a very faint protein band. These polypeptides could be detected as soon as 90 min after infection.

**Pulse-chase experiments.** To trace this transposition of radioactivity among the polypeptides more precisely, chase periods of 2, 4, or 6 h were allowed to follow a 30-min labeling period at 2 h postinfection. Figure 7 shows that after a 4-h chase, i.e., at 6 h after infection when virus replication proceeds at an exponential rate, the radioactivity in most proteins of all size groups had decreased.

In the high-molecular-weight region the radioactivity in p95 was significantly reduced, whereas p90 did not appear to be altered; a new protein band of 85 kd became visible now. In the medium-size polypeptides, the amount of radioactivity in p50 was considerably reduced. Even after the long chase periods p40 did not become visible. In the small size group the radioactivity of all polypeptides was drastically reduced; p34 had a tendency to disappear in the course of the chase. Finally, one or two faint protein bands of approximately 8 kd became visible after a 4-h chase period (Table 1).

**Analysis of the culture medium.** The virion-containing supernatant fluid of the cells used in the pulse-chase experiments described above was centrifuged for 60 min at 54,000 rpm in an SW60 Ti rotor. The resulting pellets were dissolved and immediately used for immunoprecipitation without prior pelleting of unsolubilized proteins. The polypeptides precipitated in this experiment are shown in Fig. 8. IBDV-specific proteins were noticeable immediately after the pulse, but they became clearly visible after a 4-h chase. All polypeptides mentioned in Table 1, and in particular a heavily labeled polypeptide in the position of p40, could be seen. Another prominent feature of this figure is the strong labeling of p28 in the supernatants. The amount of radioactivity in this band exceeds the radioactivity in the analogous bands obtained with labeled standard IBDV (Fig. 8b) and in the autoradiographs of intracellular proteins (Fig. 7).

**Attempts to demonstrate carbohydrates in IBDV-specific polypeptides.** IBDV was propagated in the presence of tritiated galactose, glucosamine, fucose, or mannose as described above. None of these radioactive sugars was incorporated into virus particles in substantial amounts, and in PAGE they were not found to comigrate with any one of the virus-specific polypeptides. Furthermore, these bands could not be stained with FITC-labeled concanavalin A or wheat germ agglutinin, whereas both lectins specifically stained the well-defined glycoproteins of fowl plague virus. These results demonstrate that appreciable amounts of common carbohydrates are not present in IBDV.

**DISCUSSION**

There is general agreement that IBDV is essentially made up of four structural proteins (1, 7, 8, 20, 25), which has been pointed out as a
common characteristic of a new group of viruses possessing bisegmented genomes of double-stranded RNA (8). When PAGE techniques which offer a higher resolution were used, one of the major structural polypeptides, p32, always appeared as two closely adjacent bands or at least as a split peak. This could be confirmed when 35S-labeled virus was employed and the autoradiographs were exposed for relatively short periods; two bands could be resolved by this technique which normally overlapped to form a single broad area. When gels were heavily overloaded or the films were overexposed to the gels, a large size class protein of 95 kd and a polypeptide of 34 kd were discernible.

In all of our virus preparations a polypeptide of medium size could be demonstrated which proved to be a double band of about 48 and 49 kd (p48/49). A comparative analysis of the tryptic peptides of most of the prominent structural proteins revealed that the tryptic maps of p48/49 and p40 are virtually identical, which is in agreement with previous results (7). It is therefore safe to assume that our p48/49 corresponds to the polypeptide which Dobos (7) termed VP-X and found only inconsistently in his virus preparations. In view of the almost identical fingerprints, he designated this component as a precursor of p40, one of the two major structural proteins.

When we followed the biosynthesis of IBDV-specific proteins, only a polypeptide of 50 kd could be demonstrated in cell extracts in substantial amounts, whereas p40 could never be found in infected cells. In pulse-chase experiments p50 sometimes was chased to form p49 (data not shown). In the supernatant medium of the same cell cultures, however, p40 was readily demonstrable besides the other structural proteins. This could mean that posttranslational modification of p50 does not take place right after translation, because the final product, p40, does not accumulate intracellularly as the other structural proteins do. Cleavage seems to go on during virus assembly, and one could imagine, therefore, that cleaving of the precursor p50 to the structural protein p40 via p48/49 proceeds while virus particles are transported to the cell surface or later.

The most obvious inadequacy among the structural elements in incomplete virus particles of low density and irregular morphology (top components, Fig. 1b) is not the RNA but an overwhelming amount of insufficiently or incorrectly modified precursor molecules of approximately 44 to 47 kd. The viral double-stranded RNA can obviously be bound to these precursors. This is in contrast to the results described by Todd and McNulty (25), who could not demonstrate viral RNA in their low-density "empty capsids." The general polypeptide pattern of these incomplete particles, however, is comparable to our results. One reason for this inadequate virus assembly may reside in the proteolytic enzymes which can be furnished by the host system for an efficient cleavage of the precursor polypeptides. The observation that this type of incomplete particle is mainly produced by CEF and can rarely be detected in virus populations replicated in lymphoid cells of the BF suggests such an interpretation. Incomplete particles with densities of 1.32 or 1.31 g/ml, either grown in CEF or purified from the BF, usually have all structural proteins in regular proportions, but they incorporate an insufficient amount of RNA or do not contain nucleic acid at all.

The formation of incomplete virus particles, especially of top components in CEF, might also explain the loss of pathogenicity for chickens when IBDV is passaged repeatedly in this type of cells. It should be pointed out in this context that the accumulation of an inefficiently processed capsid protein precursor inside cells infected with infectious pancreatic necrosis virus, which is a member of the same virus group (8), may have been related to low virus yields (17a).

Besides the obvious precursor-product relationship among the medium-size proteins, there is good evidence that precursor proteins are also formed in the large size group, where p95 virtually disappears completely after a 2-h chase. p33 may represent an intermediate stage of a p34-to-p32 transition, and it obviously is able to serve as a structural component besides p32. Since it was not possible until now to trace the final cleavage products, definitive proof for a precursor-product relationship, possibly with intermediate stages, cannot be established yet. This question can be definitively settled when the individual polypeptides are analyzed by tryptic peptide mapping. Another point which remains to be elucidated for the small proteins is the fact that p28 is present in relatively large quantities in immunoprecipitates from the supernatants of infected cells, whereas this protein represents only a minor constituent in purified IBDV particles.

The general picture of precursor-product relationship emerging from this study is in accordance with replicative events in cells infected with infectious pancreatic necrosis virus; in particular, a two-step cleavage process of medium-size polypeptides has been reported for the maturation of that virus (6, 9, 17a). The rate of synthesis of the various virus-specific proteins does not seem to be identical throughout the replication cycle (Fig. 6). Whereas the large-
class proteins are predominantly formed early after infection, the smaller ones are synthesized at a higher rate during the later phase. The regulation which underlies this phenomenon and the transcription of the mRNAs for the individual polypeptides from the two RNA segments of the genome has not been clarified yet.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich Gielkens, 11.

We are obliged to Kerstin Braun for technical assistance and to Ruth Back for the preparation of the electron micrographs.

LITERATURE CITED