Identification and ultrastructural characterization of a novel virus from fish

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During routine investigations on fish, a virus (isolate DF 24/00) with novel morphological features and hitherto undescribed morphogenesis was isolated from a white bream (Blicca bjoerkna L.; Teleostei, order Cypriniformes). Cell-free virions consist of a rod-shaped nucleocapsid (120–150 × 19–22 nm) similar to that seen in baculoviruses. The virion has a bacilliform shape (170–200 × 75–88 nm) reminiscent of rhabdoviruses with an envelope containing coronavirus-like spikes (20–25 nm). DF 24/00 replicated well in various fish cell lines. Inhibitor studies with 5-iodo-2'-deoxyuridine indicated that the viral genome consists of RNA and chloroform sensitivity correlated with ultrastructural demonstration of enveloped virions. The buoyant density of the virus determined in sucrose was 1.17–1.19 g/ml. Preliminary biochemical characterization revealed the presence of six antigenic glycoproteins, three of which contain sugars with concanavalin-A specificity. Ultrastructurally, morphogenesis of virus progeny was detected only in the cytoplasm. Nucleocapsids were observed to bud through membranes of the endoplasmic reticulum and/or Golgi apparatus into dilated vesicles. Egress of mature virions occurs primarily by exocytosis and, only very rarely, by budding directly at the plasma membrane. Morphologically similar viruses had previously been isolated from grass carp (Ctenopharyngodon idella), blue crab (Callinectis sapidus), European shore crab (Carcinus maenas) and shrimp (Penaeus monodon). To date, none of them has been classified. In summary, the first characterization of a new virus that might represent a member of a novel virus family that has morphological features resembling those found in rhabdo-, corona- and baculoviruses is presented.

Introduction

Viruses infecting and causing disease in fish are being increasingly recognized. In 1981, only 16 fish viruses had been isolated in cell culture and an additional 11 had been observed by electron microscopy. By 1988, the number of detected viruses that had been isolated increased to 34 and an additional 25 had been visualized but not yet isolated (Wolf, 1981, 1988). By 1993, another 35 new viruses had been identified (Hetrick & Hedrick, 1993). The fish viruses described were classified as members or tentative members of 10 virus families (Ahne & Kurstak, 1989; van Regenmortel et al., 2000): the DNA-containing Iridoviridae and Herpesviridae families and the RNA-containing Picornaviridae, Birnaviridae, Reoviridae, Rhabdoviridae, Orthomyxoviridae, Paramyxoviridae, Retroviridae and Coronaviridae families. Generally, by their morphological and morphogenetical features, all newly isolated viruses could be classified as members of virus families established already. A hitherto unclassified new fish virus, the causative agent of infectious salmon anaemia of the Atlantic salmon (Salmo salar L.) (Hovland et al., 1994; Nylund et al., 1995), has now been proposed to be a member of the Orthomyxoviridae family based on molecular biological, virological and electron microscopical data (Falk et al., 1997; Koren & Nylund, 1997).

Electron microscopy is being used regularly in diagnostic investigations of tissues from diseased fish or cell cultures.
infected with isolates from different fish species. Diagnostic studies of cyprinid cell cultures incubated with homogenized fish tissues during a monitoring program for pathogens in wild fish populations in the Federal State of Saxonia-Anhalt (Germany) led to the isolation of a cytopathic virus from a white bream (Blicta bjoerkna L.; Teleostei, order Cypriniformes). This virus, detected in cell culture supernatants and infected cells by electron microscopy, showed an ultrastructure and morphogenesis that differed significantly from all known members or tentative members of the virus families established by the International Committee on Taxonomy of Viruses (ICTV) (van Regenmortel et al., 2000). Here, we present first results on characterization, primarily by electron microscopy, of the newly isolated virus.

Methods

- **Viruses and cells.** DF 24/00 was isolated during a monitoring program for microbial pathogens in wild fish populations in the Federal State of Saxonia-Anhalt (Germany) by incubation of homogenized tissues (heart, spleen, kidney and swim-bladder) from a white bream (B. bjoerkna L.) with Epithelioptamus papulosum cyprini (EPC) cell cultures, in accordance with the requirements of the EU (Anon., 1992). The aquatic rhabdoviruses swim viraemia of carp virus (SCV) isolate VF78 (Dresenkamp, 1992), viral haemorrhagic septicemia virus (VHSV) strain F1 (Deuter & Enzmann, 1986) and infectious haemotopoietic necrosis virus (iHNV) isolated from rainbow trout in Oregon 1969 (generously provided by P. de Kinkel, France), all obtained from the National Reference Centre for Viral Fish Diseases of the Federal Research Centre for Virus Diseases of Animals (Germany) were used for cell culture infections. Transmissible gastroenteritis virus strain TO (Asagi et al., 1986) was used as the representative member of the Coronaviridae family. The wild-type baculovirus Autographa californica multiple nucleopolyhedrovirus was obtained from ICC Biotechnology.

- Cell lines used in this study were the fish cell lines EPC, blue gill fry (BF-2), chinook salmon embryo (CHSE) and fathead minnow (FHM) (Fryer & Medicine of the Federal Research Centre for Virus Diseases of the Federal State of Saxonia-Anhalt (Germany) used in this study were the fish cell lines EPC, blue gill fry (BF-2), chinook salmon embryo (CHSE) and fathead minnow (FHM) (Fryer & Medicine of the Federal Research Centre for Virus Diseases of the Federal State of Saxonia-Anhalt (Germany) led to the isolation of a cytopathic virus from a white bream (Blicta bjoerkna L.; Teleostei, order Cypriniformes). This virus, detected in cell culture supernatants and infected cells by electron microscopy, showed an ultrastructure and morphogenesis that differed significantly from all known members or tentative members of the virus families established by the International Committee on Taxonomy of Viruses (ICTV) (van Regenmortel et al., 2000). Here, we present first results on characterization, primarily by electron microscopy, of the newly isolated virus.

- **Antibodies.** For the generation of a polyclonal antiserum against isolate DF 24/00, a rabbit was immunized three times by intramuscular injection of 0.5 ml (approx. 5 µg) of purified virus suspended in 0.5 ml Freund’s complete (for the first immunization) or incomplete (for booster immunizations) adjuvant. Serum obtained after the third immunization was used for labelling experiments.

- **Determination of the viral nucleic acid and lipid solvent sensitivity.** Cell cultures were infected with isolate DF 24/00, catfish iridovirus or infectious pancreatic necrosis virus (IPNV) at an m.o.i. of 0.1 and grown after the addition of 1000 or 100 µg/ml of 5-ido-2’-deoxyuridine (IDU) (Serva). Virus infectivity was determined 4 days post-infection by titration. Lipid solvent sensitivity was determined by the addition of 10% chloroform (Roth) to pools of isolate DF 24/00, IPNV or VHSV for 4 h at 4 °C. After centrifugation at 2000 g, the supernatants were titrated.

- **Electron microscopy.** For negative staining, infected cell cultures were scraped off the plate and pelleted by low-speed centrifugation. The pellet was resuspended in PBS. Formvar-coated grids were placed for 7 min onto drops of cell culture supernatant or resuspended pellet. Negative staining was performed with 2% phosphotungstic acid (PTA, pH 7.4) for 7 min, PTA (pH 6.0) for 1–7 min, 2% ammonium molybdate (AMo, pH 6.5) for 1 min, 2% methylamine tungstate (MAT, pH 5.8) for 1 min and 1% aqueous uranyl acetate (UAc) for 4–45 s.

For immunoelectron microscopy studies on isolated DF 24/00, purified virions adsorbed to grids were incubated with the polyclonal serum in appropriate dilutions, followed by gold-tagged protein A (10 nm) (British Bio Cell). Finally, grids were stained with 2% PTA, pH 7.4.

- **Detection of viral glycoproteins.** Glycoproteins in purified virus preparations were detected after electrophoretic separation and blotting by specific insertion of Biotin-LC-Hydrazide (Pierce Chemicals) into the carbohydrate moieties followed by reaction with a streptavidin–

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Results

Ultrastructure of purified virions

Numerous enveloped rod-shaped virus particles were observed in pelleted material from supernatants of DF 24/00-infected cell cultures (Fig. 1A), even without further purification or concentration steps, indicating a high number of particles. The virion was of a bacilliform shape with parallel sides and rounded ends (Fig. 1B). After staining with 2% PTA (pH 7.4), the observed particles varied from 130 to 160 nm in length and from 37 to 45 nm in diameter (measured without the spikes). A cilindro-rod-like core structure of about 120–150 × 19–21 nm, discernible only within some virions, appeared rather rigid (Fig. 1C). Virus particles with two enveloped nucleocapsids were occasionally observed (Fig. 1F). The envelope enclosed the nucleocapsid tightly and was covered by prominent surface projections of about 20–25 nm in length. These spikes were of a fine and fuzzy appearance and formed a dense coronavirus-like rim around the particle (Fig. 1B). Spikeless virions were never found. However, virions that still connected to membranous cell remnants were occasionally detected. Many of the virions showed characteristic blebbing at one end, interpreted as extrusion of parts of the envelope (Fig. 1A).

After staining with 2% PTA (pH 6.0) for 1 min, the beginning of virion disintegration was observed (data not shown). After rupture of the envelope, only single intact nucleocapsids were visible, while most of them were completely disrupted (Fig. 1C). Staining for 7 min with PTA (pH 6.0) resulted in the disruption of nearly all of the virus particles, leaving mostly remnants of the envelope with surface projections (Fig. 1D).

After staining with 1% UAc for 45 s, envelope spikes disappeared and the bacilliform shape changed to a more ovoid appearance. Staining with 1% UAc for shorter periods of time (5–15 s) reduced, but did not completely prevent, the structural alterations of the virus particles (data not shown). Table 1 summarizes the estimated sizes of the virions and their components using the different negative staining methods and after ultrathin sectioning.

Fig. 2 shows a comparison between virions of isolate DF 24/00 (Fig. 2A, E), rhabdovirus (Fig. 2B, F), coronavirus (Fig. 2C, G) and baculovirus (Fig. 2D, H). Similarities in morphology of the virus particle to the rhabdo- and baculoviruses, and to coronavirus spikes are apparent. However, no electron-dense layer of viral (M) protein between the rod-shaped nucleocapsid and envelope, which is present in rhabdoviruses and coronaviruses, was observed in DF 24/00- and baculovirions (Fig. 2E–H). Mature rhabdovirus particles carried only short surface projections of only up to 10 nm in length (Fig. 2B, F). In contrast to rhabdoviruses and DF 24/00, the envelope of extracellular mature baculovirions was regularly loose fitting and enclosed the rod-shaped nucleocapsid (Fig. 2D, H). Only at both ends of extracellular baculovirions were spikes of 12–15 nm in length visible (Fig. 2H).

Determination of the viral nucleic acid, lipid solvent sensitivity and buoyant density

In Table 2, the results of an IDU inhibition test are shown. In contrast to the DNA-containing catfish iridovirus, the replication of DF 24/00 and the RNA-containing IPNV was not influenced by the drug. Thus, isolate DF 24/00 contains an RNA genome. Moreover, preliminary data suggest the presence of a nonsegmented ssRNA genome of more than 20 kb in size (data not shown). Correlating with the ultrastructure, DF 24/00 proved to be sensitive to chloroform (Table 3), which indicates the presence of a lipid envelope. As expected, VHSV was also sensitive to the lipid solvent treatment, whereas IPNV was resistant.

The buoyant density of DF 24/00 in sucrose was determined to be between 1.17 and 1.19 g/cm³ based on the results of titration as well as electron microscopy of aliquots after centrifugation.

Immunolabelling of isolate DF 24/00

Labelling experiments with polyclonal rabbit antiserum showed the high reactivity of antibodies with the virion (Fig. 1E, F). Only the surface of the virions, presumably the spikes, were labelled, whereas freely accessible nucleocapsids within partly disrupted virus particles and areas of the envelope without spikes were not labelled (Fig. 1F). This indicated that the antiserum was directed primarily against viral envelope proteins.
Identification of viral glycoproteins

Preparations of purified virus were analysed for the presence of glycoproteins (Fig. 3) after SDS–PAGE. Fig. 3(A) shows a Coomassie-stained polyacrylamide gel with six distinguishable protein bands, designated p1, p2 and p4–p7. Western blotting with the rabbit antiserum (Fig. 3B, WB) demonstrated the presence of another protein designated p3. Carbohydrate-specific labelling with Biotin-LC-Hydrazide (Fig. 3B, Bio) detected six viral proteins, p1–p5 and p6 or p7. Here, the smallest protein p7 migrates with a different apparent molecular mass in lanes WB and Bio due to the integration of biotin into the protein or p6 was Biotin-LC-Hydrazide labelled. As a control, the critical oxidizing step has been omitted (Fig. 3B, Co). This confirms that the major antigenic proteins (p1–p4 and p7) are indeed glycoproteins. Three of them,
### Table 1. Size determination (nm) of isolate DF 24/00

<table>
<thead>
<tr>
<th>Structure</th>
<th>2% PTA, pH 7.4</th>
<th>2% PTA, pH 6.0</th>
<th>1% UAc</th>
<th>2% AMo</th>
<th>2% MAT</th>
<th>Ultrathin section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core length (virion)</td>
<td>120–150</td>
<td>140–150</td>
<td>140–148</td>
<td>138–147</td>
<td>136–148</td>
<td>170–180</td>
</tr>
<tr>
<td>Core length (cytoplasm)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>170</td>
</tr>
<tr>
<td>Virion diameter (including spikes)</td>
<td>75–88</td>
<td>70–85</td>
<td>60–75</td>
<td>84–89</td>
<td>78–81</td>
<td>70–75</td>
</tr>
<tr>
<td>Virion diameter (excluding spikes)</td>
<td>37–45</td>
<td>37–43</td>
<td>35–43</td>
<td>33–42</td>
<td>43–45</td>
<td>40–45</td>
</tr>
</tbody>
</table>

ND, Not determined.

### Ultrastructure of virus morphogenesis

Fig. 4 shows intracellular morphogenesis of DF 24/00 in EPC cells (Fig. 4 A–H). Similar results were obtained using other fish cell lines, as described in Methods (data not shown).

namely p1, p2 and p7, show affinity to con–A (Fig. 3C), indicating that they contain α-mannose. These three proteins were also detectable using the lectins MAA, DSA, GNA and SNA from the DIG glycan differentiation kit (Roche) (data not shown).


Table 2. IDU inhibition test

Virus titre is tabulated as TCID₅₀ per 10 μl of supernatant.

<table>
<thead>
<tr>
<th>Virus</th>
<th>No IDU</th>
<th>100μg/ml IDU</th>
<th>1000μg/ml IDU</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF 24/00</td>
<td>10⁵⁻³</td>
<td>10⁵⁻³</td>
<td>10⁵⁻³</td>
</tr>
<tr>
<td>Catfish iridovirus</td>
<td>10⁶⁻³</td>
<td>10⁶⁻³</td>
<td>–</td>
</tr>
<tr>
<td>IPNV</td>
<td>10⁵⁻³</td>
<td>10⁵⁻³</td>
<td>10⁵⁻³</td>
</tr>
</tbody>
</table>

Table 3. Results of chloroform sensitivity test

Virus titre is tabulated as TCID₅₀ per 10 μl of supernatant.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Without chloroform</th>
<th>With chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF 24/00</td>
<td>10⁵⁻³</td>
<td>–</td>
</tr>
<tr>
<td>IPNV</td>
<td>10⁵⁻³</td>
<td>10⁵⁻³</td>
</tr>
<tr>
<td>VHSV</td>
<td>10⁴⁻³</td>
<td>–</td>
</tr>
</tbody>
</table>

Especially striking is the strong proliferation of intracytoplasmic vesicles in DF 24/00-infected cells (Fig. 4A). Virus components in different stages of morphogenesis of DF 24/00 were found only within the cytoplasm. The most prominent viral components were rigid cylindrical core structures—presumably nucleocapsids—measuring from 22 to 25 nm in diameter which were found scattered in the cytoplasm (Fig. 4B). At higher magnifications, cross sections of nucleocapsids showed a central electron-lucent hole of 2–5 nm in diameter (Fig. 4C). The surrounding capsid appeared to be organized by subunits, presumably in helical symmetry similar to rod-shaped plant viruses, e.g. tobacco mosaic virus particles. The same nucleocapsid ultrastructure was visible in cross-sectioned mature virions (Fig. 4D). The rod-shaped nucleocapsid structures were often observed arranged side by side in association with smooth cytoplasmic membranes, presumably degranulated rough endoplasmic reticulum and/or Golgi vesicles (Fig. 4E). After budding at cytoplasmic membranes (Fig. 4F), enveloped mature virus particles were located within slightly dilated smooth membranous vesicles or cisternae (Fig. 4B, F), often near the cell surface where virus egress may occur by exocytosis. Occasionally, mature virions were found within the perinuclear space (Fig. 4G). Only in very rare cases could nucleocapsids be detected budding at the plasma membrane (Fig. 4H).

In comparison, maturation and release of rhabdoviruses occurs primarily by budding at the plasma membrane and only occasionally at intracytoplasmic membranes into vesicles or cisternae (Fig. 5A, B and D). No preformed rod-shaped core structures are detectable in the cytoplasm, in the virion or at the site of budding (Fig. 5A–D). The only recognizable intracellular virus elements were granular accumulations (Fig. 5E) and ribonucleoprotein strands of twisted filamentous appearance that were either accumulated in inclusion bodies or seen dispersed in the cytoplasm (Fig. 5F).

In baculoviruses, virions occur in two different phenotypes, as occlusion-derived virus (ODV) or budded virus (BV). Both contain one (BV) or more (ODV) rod-shaped nucleocapsids of 30–60 nm in diameter and 250–300 nm in length with cap-like structures at their ends (Fig. 5J). For our comparative analysis, only formation of BV is of interest. Baculovirus nucleocapsids assemble at membranous structures in the nucleus (Fig. 5G, H) and acquire and lose a first envelope during traversal of the nuclear membrane. They obtain their final envelope by budding at the plasma membrane (Fig. 5K, L). Cross sections of empty and DNA-filled capsids (Fig. 5G–I) demonstrated neither a recognizable capsomer subunit nor an electron-lucent centre, as was observed in DF 24/00 nucleocapsids.

Discussion

The electron microscopy results of our investigation of the virus isolate DF 24/00 from a white bream (B. bjoerkna L.) demonstrate the presence of a new bacilliform virus in a bony
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Fish of the order Cypriniformes. This new virus exhibits morphological features resembling those found in members of the Coronaviridae (length and morphology of spikes), Rhabdoviridae (bacilliform shape of the virion) and Baculoviridae (rigid rod-shaped nucleocapsid) families. However, comparative investigations on ultrastructure and morphogenesis demon-

Fig. 4. Steps in morphogenesis of DF 24/00. EPC cells infected with DF 24/00 were analysed by electron microscopy after ultrathin sectioning. (A) An overall view of a DF 24/00-infected EPC cell. Nucleocapsids (arrowheads) and enveloped virions (arrows) in cisternae were found in the cytoplasm (B). Cross sections of intracytoplasmic nucleocapsids (C) and virions (D) are also shown. Nucleocapsids were detected side by side at smooth membranes (E). After maturation of nucleocapsids by budding at cytoplasmic membranes (F, arrowheads), virions accumulated in vesicles or were detected in the perinuclear cisternae (G). A rare example of virus egress by budding at the cell membrane is also shown (H). Bars: 2.5 µm (A), 250 nm (B), 50 nm (C, D), 100 nm (E, G and H) and 200 nm (F).
strate that DF 24/00 exhibits only partly the dimensions and morphogenetic characteristics of the known rhabdoviruses (Darlington et al., 1972; Granzow et al., 1997), the BV of baculoviruses (Blissard & Rohrmann, 1990; Fraser, 1986; McKinnon et al., 1974; van Regenmortel et al., 2000) and coronaviruses (Dubois-Dalcq et al., 1984).

The slight differences observed in the size of the isolated virions and their subunits, estimated by negative staining, seemed to be a result of the effect of different staining conditions. More strikingly, different staining conditions had a profound effect on the morphology of the virion. The acid pH of 6.0 of 2% PTA and a staining time exceeding 1 min resulted in the disruption of nearly all of the virion envelopes and also, although only partly, of the nucleocapsids of the virus particles.

Fig. 5. Morphogenesis of rhabdo- and baculoviruses. EPC cells infected with rhabdovirus (A–F) or baculovirus-infected EAA cells (G–L) were analysed by electron microscopy after ultrathin sectioning. Bars: 75 nm (C), 150 nm (A, B, D, F and I–K), 300 nm (H) and 500 nm (E, G and L).
Staining with other acidic stains, such as 2% AMo and 2% MAT, for 1 min led to less dramatic alterations, whereas staining of virions with 1% UAc for about 45 s caused striking damage to the virions. This is obviously due to the sensitivity of the envelope to acid pH.

By comparison of our data on the size of DF 24/00 virions (19–21 × 130–160 nm) to baculo- (30–60 × 250–300 nm) and rhabdoviruses (45–100 × 100–430 nm) (van Regenmortel et al., 2000), it appears that DF 24/00 virions are distinctly smaller. In overall shape, DF 24/00 virions resemble most closely the bacilliform rhabdoviruses of plants (Jackson et al., 1987). In contrast, the rigid rod-shaped nucleocapsid structure is similar to the BV nucleocapsids of baculoviruses, whereas rhabdovirus nucleocapsids appear as flexible structures of varying length. The long surface projections observed on DF 24/00 were never found on rhabdo- and baculoviruses but are characteristic for coronaviruses, which carry spikes of more than 15 nm in length. However, members of the Coronavirus family are either spherical (genus Coronavirus) or toroid (genus Torovirus) in shape (van Regenmortel et al., 2000) and do not contain a rod-shaped core as seen in DF 24/00.

Comparing the intracytoplasmic virus components, two typical inclusions that clearly differed from the intracellular nucleocapsid structures of DF 24/00 are present in rhabdovirus-infected cells. One of the rhabdovirus inclusion bodies evidently contains typical viral nucleocapsids (Dubois-Dalcq et al., 1984), whereas the second form, visible as a granular electron-dense matrix, possibly contains only the viral nucleoprotein (N), which is synthesized in excess early in infection and results in small cytoplasmic granula. Rhabdoviruses primarily bud at the plasma membrane where virus particles are immediately released from the cell. In contrast to rhabdo- and baculoviruses, DF 24/00 virions were only rarely observed during budding at the cell surface but acquired their envelope by budding through smooth intracytoplasmic membranes. This correlated with the consistent finding of enveloped virions inside cytoplasmic vesicles beneath the cell surface, probably on the way to release by exocytosis.

Although a similar rigid rod-shaped nucleocapsid was detected in DF 24/00 and BV of baculoviruses (Blissard & Rohrmann, 1990; Fraser, 1986; McKinnon et al., 1974; van Regenmortel et al., 2000), pronounced differences exist regarding its size (30–60 nm diameter for BV in comparison to 19–25 nm for DF 24/00), morphology of the ends of the nucleocapsids, the intracellular location of the nucleocapsids and the site of capsid assembly. Morphogenesis of baculovirus nucleocapsids occurs in the nucleus and at their blunt end capsid-like structures are present. In contrast, DF 24/00 nucleocapsids were only detected in the cytoplasm and lacked a cap-like structure. Similar to baculovirus nucleocapsids, DF 24/00 nucleocapsids were found arranged side by side at intracellular membranes, but these are located in the nucleus in baculoviruses and in the cytoplasm in DF 24/00. In contrast to baculoviruses, in the cytoplasm of DF 24/00-infected cells, smooth membranes seemed to proliferate and vesicles were often surrounded by more than one membrane. Envelopment of both viruses takes place by budding, but BV gain their final envelope by budding at the cell surface, whereas DF 24/00 primarily buds at intracytoplasmic membranes. Mature BV of baculoviruses are characterized by spikes only at the rounded ends of the enveloped particle, whereas DF 24/00 virions were completely surrounded by long and fuzzy spikes (van Regenmortel et al., 2000; this report).

The composition of the viral envelope of rhabdoviruses, coronaviruses, baculoviruses and DF 24/00 virions also differs. Rhabdovirus envelopes contain one viral glycoprotein and coronaviruses contain two or three viral glycosylated polypeptides (Dubois-Dalcq et al., 1984; van Regenmortel et al., 2000). BV of baculoviruses contain one major envelope glycoprotein (gp64) (Rohrmann, 1992; van Regenmortel et al., 2000). Our preliminary biochemical analysis indicated the presence of at least six glycoproteins in purified virions. Five of them also reacted with an antisera that had been produced against purified virions and which, in immunoelectronic microscopy, only labels virion surface components but not nucleocapsids. Thus, in complexity of the viral envelope, DF 24/00 differs from baculo-, rhabdo- and coronaviruses.

In 1987, Ahne et al. (1987) isolated a syncytia-inducing virus from cultured grass carp (Ctenopharyngodon idella), which, in dimension and bacilliform shape, is similar to DF 24/00. However, contrary to our findings, in one electron micrograph after negative staining, the grass carp virus seemed to be without recognizable surface projections. Further detailed studies about the ultrastructure and morphogenesis in cells were not performed. Based on our experiences on the influence of different staining conditions on virion morphology, the absence of spikes might have been an artefact. Thus, both viruses could, in fact, have identical morphologies. More than two decades ago, Yudin & Clark (1978, 1979) and Chassard-Bouchaud et al. (1976) each described a new virus (ecdyssial gland virus and Y-organ virus) infecting the blue crab (Callinectes sapidus) and European shore crab (Carcinus maenas). Although not well characterized, these viruses also appear to be similar in virion morphology to DF 24/00. The highest similarity in ultrastructure and morphogenesis was found in newly discovered RNA-containing viruses isolated in Thailand from black tiger shrimp (Penaeus monodon), which suffered from yellow-head disease (YHD) (Chantanachookin et al., 1993; Wongteerasupaya et al., 1995). Similar viruses were also isolated from P. vannamei and P. stylirostris in Hawaii (Nadala et al., 1997) and in Australia from shrimp with pathological signs that differed from YHD (Spann et al., 1995, 1997). Preliminary data indicate that DF 24/00 contains a non-segmented ssRNA genome of more than 20 kb and the classification of these isolates has been a subject of controversial discussion. Based on the ultrastructure and the shape of the detected nucleocapsids as well as its animal host (crustacean), Boonyaratpalin et al. (1993) and Chantanachookin
et al. (1993) reported it as a granulosis-like baculovirus (Anderson & Prior, 1992; Edgerton et al., 1996; Groff et al., 1993; Hedrick et al., 1995; Johnson & Lightner, 1988; Mari et al., 1993; van Regenmortel et al., 2000). After Wongteerasupaya et al. (1995) identified this virus as an RNA-containing pathogen, Nadala et al. (1997) suggested that it was rhabdovirus-like. Until now, no exact classification of these virus isolates by the ICTV was performed. Therefore, these viruses are missing in the virus index of the seventh report of the ICTV (van Regenmortel et al., 2000). In summary, we demonstrate that the novel virus isolate DF 24/00 from a white bream (B. bjerkna L., order Cypriniformes) shares ultrastructural and morphogenetic features with three virus families, Coronaviridae, Rhabdoviridae and Baculoviridae, without meeting all of the criteria for inclusion into any of these families.

Therefore, additional comparative investigations are needed for the comprehensive characterization of the vertebrate and invertebrate viruses with similar morphology that may constitute members of a new virus family. Further studies on the biology and molecular biology of these viruses are underway. These studies will help to find the correct taxonomic status for these viruses.

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References


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