Heterogeneity in the genome RNAs and polypeptides of five members of a novel group of rotavirus-like viruses isolated from aquatic animals

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Biochemical characteristics of five rotavirus-like viruses isolated from striped bass (Morone saxatilis), turbot (Scophthalmus maximus), smelt (Osmerus mordax) and Atlantic salmon (Salmo salar) in North America and Europe were compared. The genome of each isolate was composed of 11 segments of dsRNA and each isolate had a unique electropherotype in polyacrylamide gels. Agarose gel electrophoresis showed similar RNA profiles for all four isolates from North America, whereas the RNA profile of the isolate from Europe was different. Analysis of virion proteins revealed that each virus had five structural proteins ranging in Mr from 130 000 to 34 000. Each isolate had a unique polypeptide profile but their overall polypeptide patterns were similar. Reciprocal RNA-RNA blot hybridization demonstrated that all these rotavirus-like viruses cross-hybridized with each other except for the isolate from Europe which did not hybridize with the RNA from any of the other isolates. No genetic relationship was found between these rotavirus-like viruses of fish and a true group A rotavirus (SA11).

Viruses belonging to the family Reoviridae have been isolated from different species of fish and shellfish (Plumb et al., 1979; Meyers, 1979; Winton et al., 1981; Hedrick et al., 1984; Amend et al., 1984; Ahne & Kolbl, 1987; Hsu et al. 1989; Marshall et al. 1990). Some of these viruses were isolated from diseased animals whereas others were isolated during the routine examination of apparently healthy fish. Although the pathogenic potential of these isolates is not well studied, some of them caused disease leading to mortality in experimental infection (Plumb et al. 1979; Meyers, 1979; Winton et al., 1987; Amend et al., 1984).

The physical and biochemical characteristics of this group of viruses have been described (Hedrick et al., 1984; Winton et al., 1987); they are ether-resistant, stable at pH 3, approximately 75 nm in diameter with a double layer capsid and have a genome composed of 11 segments of dsRNA. Presently, the status of the genus of this group of viruses has not been decided, but these characteristics are similar to members of the genus Rotavirus in the family Reoviridae. However, unlike rotaviruses, these viruses replicate in cells of fish origin at low temperature (16 °C), forming plaque-like syncytia, and their infectivity is not enhanced by treatment with trypsin.

Morphological and biochemical characteristics of four aquatic rotavirus-like viruses isolated in North America also have been compared (Winton et al., 1987). All isolates were approximately 75 nm in diameter, all produced syncytia in cell monolayers and each virus could be distinguished from the other by its cell culture spectrum. The genome of each virus contained 11 segments of dsRNA and had a unique electropherotype. Each virus contained five structural proteins ranging in Mr from 34 000 to 135 000, with the 34 000 and 70 000 polypeptides being present in the highest concentration. However, the genetic relationships amongst isolates of aquatic rotavirus-like viruses from different geographical regions have not been examined. In this study, genome RNAs and polypeptides of five additional viruses isolated from fish during the past 2 years in North America and Europe were compared. The genomic RNAs were examined by polyacrylamide and agarose gel electrophoresis and by reciprocal RNA–RNA blot hybridization to determine the extent of genetic relatedness among these isolates by genomic segment. Furthermore, the genetic relationship between rotavirus-like viruses of fish and a true group A rotavirus (SA11) was also examined.

The five rotavirus-like viruses used in this study were isolated during the past 2 years from several species of fish. The SBR strain was isolated from a striped bass (Morone saxatilis) in Maryland, U.S.A (Baya et al., 1990). The HBR and ASR strains were isolated from Atlantic

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Fig. 1. RNA profiles of five isolates of aquatic rotavirus-like viruses (SBR, lane 1; ASR, lane 2; SRV, lane 3; HBR, lane 4; TRV, lane 5), simian rotavirus SA11 (lane 6) and reovirus type 1 (lane 7). Genomic dsRNAs were end-labelled with [5'-32P]pCp and subjected to electrophoresis in a 5% polyacrylamide gel, 1.2 mm thick and 22 cm long. Electrophoresis was for 18 h at 200 V. The RNA segments were detected by autoradiography.

Fig. 2. RNA profiles of five isolates of aquatic rotavirus-like viruses (TRV, lane 2; HBR, lane 3; ASR, lane 4; SRV, lane 5; SBR, lane 6) and reovirus type 1 (lane 1) in a 1% agarose gel. Genomic dsRNAs were extracted from purified viruses and subjected to electrophoresis in 1% agarose Tris–borate-buffered gels. The RNA segments were detected by staining with ethidium bromide.

The virus genomic dsRNA was extracted from purified virus by the method of McCrae & Joklik (1978). Samples of rotavirus-like virus genomic RNA were analysed using a 5% polyacrylamide gel system as described by Laemmli (1970) or by 1% agarose gel electrophoresis using a Tris/borate/EDTA buffer system as described by Maniatis et al. (1982). Results of this analysis are shown in Fig. 1. Each of the five viruses exhibited a distinctive profile of 11 dsRNA segments in polyacrylamide gels. The three large dsRNA segments of ASR and SRV strains were difficult to resolve in most gels but were resolved in longer gels (results not shown). The dsRNA profile of the two Maryland isolates (SBR and HBR) were similar and although similarity in dsRNA mobility between two isolates does not necessarily correlate with genetic relatedness (Clarke & McCrae, 1982), the possibility that a single virus strain could be circulating in Maryland exists. Therefore, more definitive RNA analysis is necessary to distinguish the two isolates. The dsRNA profiles of the five aquatic rotavirus-like viruses were unlike those of simian rotavirus (SA11) and reovirus type 1 (Fig. 1). These differences in the profiles of the genomic dsRNA of different aquatic rotavirus-like viruses seen in polyacrylamide gels can be used for molecular epidemiological studies to monitor virus outbreaks and transmission.

The genomic dsRNAs of these virus isolates were analysed in 1% agarose gels. The characteristic electrophoretic patterns of these five isolates seen in polyacrylamide gels were not present in agarose gels (Fig. 2). The dsRNA profiles of all four North American isolates were
similar, whereas the dsRNA profile of the European isolate was different. It has been shown that, in agarose gels, the mobilities of the dsRNA segments of bluetongue virus isolates are proportional to their $M_r$s and the RNA profile is common amongst all bluetongue virus isolates but is different from that seen in members of other orbivirus serogroups (Bodkin & Knudson, 1985; Kowlik & Li, 1987; Pedley et al., 1988). Therefore, our results indicate that all four isolates of aquatic rotavirus-like virus from North America have dsRNA segments of similar $M_r$s whereas the isolate from Europe has dsRNA segments of different $M_r$s.

Virion proteins of the five isolates were analysed in 12.5% polyacrylamide gels (Laemmli, 1970). The isolates had two large, one medium and two small virion proteins ranging in $M_r$ from 137000 to 34000 (Fig. 3). Each isolate had a unique polypeptide profile but their overall polypeptide patterns were similar. Polypeptide profiles of aquatic rotavirus-like virus isolates were different from those of simian rotavirus (SA11) and reovirus type 1. Further work using radiolabelled viral proteins is needed to identify the differences more clearly.

Fig. 3. Structural polypeptide profiles of five isolates of aquatic rotavirus-like viruses (SBR, lane 3; SRV, lane 4; ASR, lane 5; HBR, lane 6; TRV, lane 7), simian rotavirus SA11 (lane 2) and reovirus type 1 (lane 8). Purified virions were lysed into sample buffer and subjected to electrophoresis in 12.5% Tris-glycine-buffered gels, 1.2 mm thick and 22 cm long, that were run for 18 h at 8 mA. Virion proteins were detected by staining with Coomassie blue. Lane 1 contains $M_r$ markers.

The isolates were further examined by reciprocal RNA–RNA blot hybridization of genomic RNA. Total genomic dsRNA probes were prepared following standard protocols. Briefly, the viral genomic dsRNA was extracted from purified virus by the method of McCrae & Joklik (1978) and the 3' end was labelled with 3',5'-cytidine [5'32P]bisphosphate using RNA ligase as described by England & Uhlenbeck (1978) and England et al. (1980). The method of Bodkin & Knudson (1985) was used to transfer viral dsRNA from polyacrylamide gels electrophoretically to GeneScreen Plus membranes. The conditions used for the prehybridization and hybridization of membranes were similar to those of Bodkin & Knudson (1985). Blots were prehybridized for 2 to 4 h at 42°C in 50% (v/v) formamide, 5 × SSC, 50 mM sodium phosphate pH 6.5, sonicated salmon testes DNA (100 µg/ml), 0.2% SDS and 0.1% each of bovine serum albumin, Ficoll and polyvinylpyrrolidone and hybridized overnight at 52°C. Blots were washed twice for 30 min at room temperature in 2 × SSC and 0.1% SDS and once for 1.5 h in 0.1 × SSC and 0.1% SDS at 50°C. The results suggested that all rotavirus-like viruses cross-hybridized with each other, except for the isolate from Europe which did not hybridize with the RNA from any other

Fig. 4. Reciprocal RNA–RNA blot hybridization analysis of the five isolates of aquatic rotavirus-like viruses. Genomic dsRNAs were extracted from purified viruses and subjected to electrophoresis in 10% Tris–glycine-buffered gels and transferred to GeneScreen Plus membranes using a Trans-Blot cell. Total genomic dsRNAs from each virus were end-labelled with [5'32P]pCp and hybridized separately to a membrane containing genome profiles of the five viruses. Hybridization took place at 52°C in the presence of 50% formamide. Lanes 1, TRV; lanes 2, ASR; lanes 3, HBR; lanes 4, SBR; lanes 5, SRV.
isolates (Fig. 4). Furthermore, the genetic homology between rotavirus-like viruses of fish and a true group A rotavirus (SA11) was examined by reciprocal dot–blot hybridization (Fig. 5). Our results demonstrate that rotavirus-like viruses of fish do not share any sequence homology with this group A rotavirus.

Our results confirm the observation of Winton et al. (1987) that isolates of rotavirus-like virus of fish exhibit variability in the pattern of migration of the dsRNA and polypeptides on polyacrylamide gels. Data provided by Winton et al. (1987) are extended by the demonstration that genetically distinct groups exist amongst isolates of rotavirus-like viruses of fish in nature and none of these genetic groups shares close sequence homology with a group A rotavirus (SA11). Further investigation of the relationship of these isolates with isolates from other geographical areas is necessary for taxonomic classification of these viruses. This may provide insight as to their phylogeny.

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References


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