Enhancement of Aquareovirus Infectivity by Treatment with Proteases: Mechanism of Action

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The effects of protease digestion on the polypeptide composition and on the infectivity of striped bass virus, an aquareovirus, were examined. Both trypsin and chymotrypsin enhanced the infectivity of the virus. Enhancement of infectivity was correlated with the digestion of the outer capsid protein, VP7. These studies support the assertion that VP7 is the outermost capsid protein and suggest that VP4 and VP5 are exposed on the outer surface of infectious particles. The possible role of VP7 in the variation in virulence observed among aquareovirus isolates is discussed.

Aquareoviruses have been isolated from a wide variety of aquatic organisms. While the roles of these viruses as pathogens in these organisms appear to be varied, it is clear that many of these viruses play a significant role in the morbidity and mortality of aquatic populations (reviewed in reference 9).

Aquareoviruses are members of the family Reoviridae and as such possess a multilayered capsid. The virion has a diameter of about 70 nm and consists of seven structural proteins (VP1 to -7) and a genome composed of 11 segments of double-stranded RNA (5, 14).

Work with other members of the family Reoviridae has shown that specific protease treatments can influence infectivity. Treatment of orthoreoviruses with trypsin or chymotrypsin removes or3 from the outer capsid, but most of the other outer capsid proteins remain (although some are modified by the protease treatment [1, 7]). Protease treatment converts virions into morphologically distinct subviral particles (ISVPs). These ISVPs are significantly more infectious than intact virions (13). Treatment of rotaviruses with trypsin cleaves VP4, an outer capsid protein. The “activated” rotavirus particles retain the cleavage products and exhibit enhanced infectivity in comparison with nonactivated particles. Chymotrypsin does not enhance rotavirus infectivity (2-4, 6).

In this study we report the effect of protease treatment on the structure and infectivity of striped bass aquareovirus (SBR virus). Treatment of SBR virus with trypsin resulted in the complete digestion of VP4 and VP7 and the digestion of all or most of VP5 (Fig. 1). Treatment with chymotrypsin also yielded the complete digestion of VP4 and VP7, but all or most of VP5 was left intact. In these studies we did not determine whether the cleavage products of digestion were solubilized or whether they remained associated with the virus. Neither of the proteases examined affected the VP1 to -3 or VP6 present in virions. These data are consistent with the earlier assertion that VP7 is a major outer capsid protein (11).

Examination of purified viruses treated with trypsin showed core-like particles with a diameter of about 60 nm. These particles exhibited projections that displayed a sixfold symmetry by electron-microscopic examination (Fig. 2). Chymotrypsin treatment yielded particles 60 nm in diameter but without the projections seen in the trypsin-treated viruses (data not shown).

As happens with other members of the Reoviridae, treatment of SBR virus with protease enhanced infectivity. Treatment

FIG. 1. Effect of protease treatment on the polypeptides of purified SBR virus. Virus labeled with [35S]methionine was purified as previously described (14). Purified viruses were treated with 200 μg of chymotrypsin (CHYT) or trypsin (TRYF) per ml at 37°C for 60 min and analyzed by electrophoresis in a 6% Tris-glycine-buffered polyacrylamide gel with 4% bisacrylamide cross-linkage. The resulting autoradiograph is shown. MW, molecular weight markers.
with 10 μg of trypsin per ml for 5 min at 37°C increased infectivity more than 100-fold. Longer treatments with trypsin consistently decreased virus titers. The virus was optimally activated when VP7 was digested but before the digestion of VP4 and/or VP5 progressed (Fig. 3). This indicates that while VP7 is the most exposed surface protein of the virion (11, 12, 14), the intact protein might not play a direct role in attachment or in the replication process per se. It is possible that VP7’s major role is that of providing stability for the virion. If this is the case, the VP7 of aquareovirus would perform a function similar to that of the σ3 protein of orthoreovirus (10).

It is possible that the increase in infectivity seen with trypsin treatment is more closely associated with the appearance of a 52,000-molecular weight (52K) polypeptide (Fig. 3) than it is with the digestion of VP7. Since the appearance of the 52K polypeptide was correlated with the digestion of VP4 and/or VP5, it is likely that the 52K polypeptide is a cleavage product of one of these polypeptides. Noting that the digestion of VP5 preceded VP4 (11; also, data not shown), we suggest VP5 as the leading candidate for the source of this cleavage product.

The fact that there was a correlation between decreased titer and digestion of VP4 and/or VP5 suggests that intact VP4 and/or VP5 play an essential role in the viral replication process. The indication that either or both of these proteins are exposed on the outer surface of the virion (11, 12) hints that one of them might be the viral attachment protein.

Chymotrypsin also increased the infectivity of SBR virus (data not shown); however, since the increase was not as great as that shown with trypsin, these studies were not pursued.

By examining SBR virus particles under different gel conditions, we were able to better resolve VP1-VP2-VP3 and VP4-VP5 (Fig. 4). Such analysis supported the earlier conclusion that VP1, VP2, and VP3 are not digested by trypsin treatment. A time-course study indicated that the order in which virus structural proteins are digested by trypsin is VP7, VP5, VP4 (data not shown).

In an effort to determine whether the enhancement of infectivity by protease treatment was limited to the SBR virus (genogroup A), we examined viruses from five other genogroups of aquareoviruses (genogroups B through E) (8). Preliminary studies suggested that trypsin treatment enhanced the infectivity of representative members of each of the aquareovirus groups tested (data not shown).
Our finding that the digestion of the outermost capsid protein (VP7) of an aquareovirus was associated with increased infectivity suggests an explanation for the variation in virulence associated with different isolates of aquareoviruses. It is possible that in some low-virulence infections most virus particles have an intact VP7. In this case most of the viruses would be noninfectious. In cases where VP7 is digested by pancreatic enzymes, virulence might increase. It will be interesting to determine if enzymatic treatment can alter the virulence of aquareoviruses.

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REFERENCES