Thermal Inactivation of Infectious Hematopoietic Necrosis and Infectious Pancreatic Necrosis Viruses

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A plaque assay was used to follow the inactivation kinetics of infectious hematopoietic necrosis virus and infectious pancreatic necrosis virus in cell culture media at various temperatures. Inactivation of infectious hematopoietic necrosis virus in a visceral organ slurry was compared with that in culture media.

Information concerning the heat inactivation kinetics of infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) is important both to preserve infectivity for laboratory or diagnostic studies and to prevent the spread of disease through process wastes and movement of fish products. In studies by other investigators, the inactivation rate of IPNV at 60°C increased when the pH was adjusted to 3 or 10 (3, 5); IHNV grown in cell culture was completely inactivated within 8 h at 32°C, but infective virions were detected after 1 week at 27°C (4).

We used a plaque assay to follow the inactivation kinetics of IPNV and IHNV in culture media at various temperatures and compared inactivation of IHNV in a visceral organ slurry with that in culture media.

Isolates of IHNV from sockeye salmon, Oncorhynchus nerka (KarluK Lake, Alaska, and Cedar River, Wash.), and an isolate of IPNV from rainbow trout, Salmo gairdneri (Wizard Falls State Hatchery, Ore.), were used. The KarluK Lake IHNV isolate was passed three times and the Cedar River IHNV isolate was passed twice in epithelioma papillosum carpio (EPC) cells before this study. Stock IHNV was harvested from EPC cell cultures that were incubated at 15°C. Stock IPNV (exact number of passages uncertain, but at least 15) was produced in bluegill (Lepomis macrochirus) cell cultures (BF-2) kept at 15°C.

EPC and BF-2 cells were used in the assays for IHNV and IPNV, respectively. Cells were grown in Eagle minimum essential medium (MEM) buffered to pH 7.2 with 7.5% NaHCO3 and supplemented with 10% fetal bovine serum (MEM-10). All media contained penicillin (100 U/ml), streptomycin (0.1 mg/ml), and gentamicin (0.1 mg/ml).

Sockeye salmon viscera, including gut, pyloric ceca, kidney, spleen, and liver, were homogenized in MEM-10 (1:10, wt/vol) supplemented with amphotericin B (0.25 mg/ml; Fungizone, GIBCO Laboratories) in a Waring blender. A Cedar River IHNV suspension was diluted 1:13 with the visceral slurry.

A temperature gradient incubator (model TN-3, Scientific Industries, Inc.) was used for the inactivation experiments. Stock virus was diluted 10-fold with MEM at pH 7.2 (final suspension contained 1% fetal bovine serum [MEM-1]) for the studies. Visceral slurry was also used as a diluent. Samples were taken at various times, and the infectivity titer was determined with a plaque assay.

In the plating procedure for IHNV, as previously described (1), 2 ml of an EPC cell suspension of about 106.5 cells per ml was pipetted into a tissue culture dish (35 by 10 mm, Lux Scientific Corp.), and the cells were incubated overnight at 27°C. The medium was removed by aspiration, and 0.1 ml of inoculum was dripped onto the center of the dish. One hour was allowed for adsorption, and then 2 ml of overlay (0.75% methyl cellulose in MEM-10, plus 0.25 mg of amphotericin B per ml) was added. After plaque formation had occurred (usually within 7 days), the cells were fixed and stained.

The plating procedure for IPNV was similar to that for IHNV, although different cells (BF-2), larger dishes (60 by 15 mm, Falcon Plastics), and gum tragacanth (0.8%, Sigma Chemical Co.) overlay were used (2). Plaque formation occurred in 6 to 8 days. Inactivation curves were compared with one-way analysis of covariance. Significance was determined at the α = 0.05 level.

The IHNV was sensitive to thermal inactivation, and the inactivation rate increased with temperature (KarluK Lake isolate, 2.53 × 107 plaque-forming units per ml, Fig. 1). The Cedar River IHNV isolate (1.52 × 107 plaque-forming units per ml) was completely inactivated (infec-
tious particles, 0/ml) within 140 min at 38°C and within 24 h at 32°C (data not shown). A survival ratio of about 10⁻⁴ was detected after 7.3 h at 32°C, although complete inactivation of a suspension containing 10⁴ to 10⁶ mean tissue culture infective doses per ml within 8 h at this temperature has been reported (4). The survival curves of the two IHNV isolates (Karluk Lake and Cedar River) were not significantly different at 32°C. The viral suspension (Karluk Lake IHNV) was incubated at 27°C for 1 h before infectivity decreased detectably. We detected no significant inactivation after 5 h at 22°C; consequently, routine laboratory manipulations can apparently be performed near room temperature without significant decrease in titer.

The survival curves of Karluk Lake IHNV at 32 and 38°C had a two-component character (Fig. 1). The initial rapid inactivation rate persisted until 99.9% of the infectious particles were inactivated, and then it decreased. All survivor curves were significantly different except those at 22 and 8°C.

When the virus was suspended in a viseraI homogenate, the only significant variation from MEM-1 inactivation rates occurred at 28°C. The inactivation rate was increased in the slurry. We suggest that this increase could be due to the activity of intracellular enzymes released during homogenization of the organs and infer from its occurrence only at 28°C that the activity of the enzymes was temperature dependent.

The results clearly showed the thermal stability of IPNV (Fig. 2). Complete inactivation occurred within 16 h at 60°C. Low levels of IPNV infectivity (10⁻⁶ survival ratio) after 5 h at 60°C have been reported (3). Over 95% of the infectivity was rapidly lost at 50°C, but the inactivation rate decreased abruptly and infectious virions were detected after 22 h. The difference in inactivation rates between 50 and 37.5°C occurred in the first rapid phase; the slopes of the second components were similar. All three curves were significantly different. The effects of pH on the thermal inactivation of IPNV at 60°C also occur during the initial rapid phase of the two-component process (3).

Although we examined the inactivation of IHNV and IPNV in defined media, some of the information has practical application. Potentially, fish products may be contaminated with IPNV and IHNV and their use in fish culture may result in the spread of disease. The thermal stability of IPNV is particularly important in this regard.