Stabilizing Effect of Magnesium Sulfate on Avian Infectious Bronchitis Virus Propagated in Chicken Embryo Kidney Cells

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The Beaudette strain of avian infectious bronchitis virus propagated in chicken embryo kidney cells is stabilized by exposure to 1 m MgSO₄ at 50 C for 80 min, at pH values ranging from 4 to 10.

Avian infectious bronchitis virus (IBV) strains propagated in embryonating chicken eggs (CE) vary in their thermostability at 56 C (1, 7, 8, 13). Low egg-passaged virus is inactivated over a range of 30 to 150 min (3), whereas the high egg-passaged Beaudette strain is inactivated within 10 min (4). Virus diluted in 20% horse serum retained its infectivity after exposure at 56 C for 45 min, but the same dilution without horse serum was inactivated by 20 min (8). A recent report indicates that the CE-propagated Beaudette strain is stabilized by MgSO₄ during exposure at 50 C for 80 min (9).

Investigations of pH lability indicate the Beaudette strain is stable in allantoic fluid at pH 3 for 14 days, labile at pH 10.6 for 2 days, and stable at pH 7.8 for 170 days (5). This same strain is stable at room temperature at pH 2 for 1 hr (11). In cell culture fluid, the Beaudette strain is stable for 30 min at pH 3, but labile at pH 11 if tests are conducted at 4 C (14).

This study reports on the effect of various pH values on thermostability of the cell culture-adapted Beaudette strain of IBV suspended in 1 m MgSO₄.

MATERIALS AND METHODS

Virus. The chicken embryo-lethal Beaudette strain (IBV-42) of IBV was used at the 33rd passage in chick embryo kidney (CEK) cells (2). The titer of IBV-42-33 in CEK cells was 10⁶.⁴ plaque-forming units (PFU)/ml.

Cell cultures. Monolayers of primary CEK cells were prepared from the kidneys of 18-day-old chicken embryos. The cells were dispersed with 0.25% trypsin, washed three times in phosphate-buffered saline (PBS), and resuspended in medium 199 (M-199) containing 5% fetal calf serum. A 5-ml amount of the cell suspension was seeded into plastic petri dishes (60 by 15 mm; Falcon Plastics, Los Angeles, Calif.). Cell cultures were incubated at 40 C in an atmosphere containing 2.5 to 3.0% CO₂, and were generally ready for use within 24 hr.

Virus diluent. A sufficient quantity of reagent grade MgSO₄ was dissolved in double glass-distilled water to make a 1 m solution. The volume of water used as solvent was reduced 10% to allow for addition of IBV-infected cell culture fluid, thus giving a final salt concentration of 1 m. This resulted in a 10⁻¹ dilution of the virus. The pH range of 1.0 to 10.0 of stock salt solutions and distilled water was adjusted by addition of 1 N HCl or NaOH. Values above pH 10.0 could not be obtained with MgSO₄, because of crystallization of the salts. All solutions were sterilized by autoclaving.

Experimental procedure. Cell culture fluid containing IBV-42 was clarified by centrifugation at 580 x g for 10 min at 4 C. The supernatant fluid was diluted 1:10 with salt solution at each pH value. Dilutions were made in culture tubes (15 by 100 mm) equipped with Morton closures (Bellco Glass Co., Vineland, N.J.). Culture tubes were kept in a water bath at 4 C while dilutions were made. Time-temperature tests were done by placing the tubes in a 50 C circulating water bath, allowing a 2-min temperature equilibration interval before timing the tests. Samples were removed from the 50 C water bath at 0, 20, 40, 60, and 80 min and were placed immediately in a 4 C water bath. Virus control samples were diluted 1:10 in distilled water at pH values of 1.0 to 10.0 and were handled similarly to the virus-salt mixtures.

Assay. All samples were serially diluted in 10-fold steps at 4 C in Tryptose phosphate broth containing 100 µg of penicillin and 0.1 mg of dihydrostreptomycin per ml. Duplicate PBS-washed CEK cell cultures were inoculated with 0.5 ml of each virus dilution. The virus was allowed to adsorb to the cells for 90 min at 40 C. The inoculum was removed, and the cell sheet was washed twice with PBS and then overlaid.
with 5 ml of agar overlay medium (2). Plaques were counted 48 hr postadsorption after staining the cultures with 3 ml of neutral red solution (2).

RESULTS

The Beaudette strain of IBV propagated in CEK cells was stabilized by 1 mM MgSO₄ at certain pH values during exposure at 50 C for 80 min (Fig. 1). This stability was enhanced as pH values were changed from acid toward neutrality. At pH 1 and 2, the virus was inactivated at 20 and 60 min, respectively. IBV-42 at pH 1 to 3 was protected at zero time by 1 mM MgSO₄ (Fig. 1), whereas virus at these pH values in distilled water had a loss of 10⁴ (pH 1) and 10² (pH 2 and 3) PFU at the same time (Fig. 2). At pH 3, virus in 1 mM MgSO₄ was inactivated at a slower rate than at pH 2. IBV-42 at pH 4, 5, and 6 was stabilized in a solution of 1 mM MgSO₄. Virus controls in distilled water at pH values of 4 to 10 have a similar rate of inactivation (Fig. 2 and 3).

In Fig. 1, PFU titers below 10⁴ for pH 1 and 2 were not used, as they would not lend any additional information. However, PFU titers below 10⁴ were used in Fig. 2 and 3 to illustrate the lower titers obtained with control samples, especially at pH 1, at zero time.

![Graph showing pH values and time](image)

**Fig. 1. Stabilizing effect of 1 mM MgSO₄ on cell culture propagated IBV-42 at 50 C in pH range 1 to 6.**

Thermostability of IBV-42 in the presence of 1 mM MgSO₄ in pH range 7 to 10 is given in Fig. 4. Virus at pH 7 and 8 was stabilized similarly to virus at pH 4, 5, and 6, with some decrease in stabilizing activity at pH 9 and 10.

**DISCUSSION**

Results of these experiments indicate that the Beaudette strain of IBV propagated in CEK cells is stable in a 1 mM MgSO₄ solution at 50 C for 80 min. These results are similar to those reported for this virus strain propagated in chicken embryos, subjected to similar conditions (9). CEK-propagated virus at pH 3 was as stable at 50 C as it was at 4 C (14). Thermostability at pH 2 or below is still open for investigation, as only one report is known where the sample tested was considered stable after exposure at room temperature for 1 hr (11).

Control of IBV by vaccination with modified live virus vaccines has its disadvantages because of transmission from chicken to chicken. Even though these vaccines contain virus with a reduced pathogenicity for the chicken, they do retain their immunogenicity. In contrast,
inactivated IBV vaccines have the advantage of being noninfectious, but they have not proved to be entirely satisfactory in being immunogenic. Formalin-inactivated IBV has been reported to be nonimmunogenic (6) and partially immunogenic (16). Formalin inactivation of poliovirus at 50 C results in a loss of antigenicity (12); however, if inactivated in the presence of divalent cations at 50 C, its antigenicity is retained (10). Further work with Formalin-inactivated IBV in the presence of MgSO4 may produce similar results.

Thermostabilization of IBV by 1 m MgSO4 at 50 C may be helpful in eliminating certain contaminating avian viruses that often appear in embryonating chicken eggs and chicken embryo origin cell cultures. Salt solutions containing divalent cations have been reported to enhance the inactivation of poxviruses, herpesviruses, adenoviruses, influenza virus, mumps virus, and parainfluenza virus at 50 C (15). Thus, treatment of IBV at 50 C in the presence of 1 m MgSO4 could eliminate such contaminating avian viruses as chick embryo lethal orphan, infectious laryngo-tracheitis, Marek’s, avian influenza, fowlpox, and Newcastle disease without destroying IBV, if such treatment were conducted at pH values of 6 or above.

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