Stability of Infectious Influenza A Viruses to Treatment at Low pH and Heating

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With 3 Figures

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Summary

We have measured the infectivity of influenza A virus strains grown either in embryonated eggs or in chick embryo cells in culture after treatment at low pH. At pH values at which hemolysis occurs there was an irreversible loss of infectivity. The threshold pH, at which the infectivity was lost, depended on the hemagglutinin subtype of the virus strain. All H5 and H7 strains tested were extremely labile at low pH. In contrast, all H3 strains were relatively stable, independent of the species from which the viruses were isolated. With several H1 viruses the hemagglutination (HA) activity was irreversibly lost at intermediate pH values causing inactivation of infectivity. Strains with noncleaved hemagglutinins were much more stable. These observations might explain why duck influenza viruses can easily survive in lake water and wet faeces, and multiply in the intestinal tract, where trypsin is present. There are also significant differences in heat stability exhibited by influenza A strains. In contrast to pH stability this is not a specific trait of the hemagglutinin, since it can be influenced by reassortment. There is no correlation between the stability of infectivity at low pH and heat.

Introduction

We were interested in the question why some influenza A virus strains such as fowl plague virus which are highly pathogenic, do not spread well, whereas nonpathogenic strains such as duck influenza viruses, spread very well. These differences in infectivity may be related to contrasting stabilities of
the strains to low pH and heat. Recently it has been shown that influenza viruses cause hemolysis and cell fusion (5, 6, 8, 10, 11, 12, 14, 17, 18) at about pH 5 and that at this pH an irreversible conformational change of the hemagglutinin occurs (15). These properties are thought to correlate with the entry of the nucleocapsid into the cell. The threshold pH at which hemolysis, cell fusion or the conformational change occur, is strain-specific and depends on the cleavage of the hemagglutinin. Therefore we have tested the infectivity of many strains belonging to different subtypes with respect to pH and heat stability and we have compared strains with cleaved hemagglutinin with those of noncleaved hemagglutinin.

Materials and Methods

Virus Strains and Cell Cultures

The influenza A virus strains as listed in Table 1 were investigated. We are grateful to Drs. P. A. Bachmann, C. Hannoun, V. S. Hinshaw, M. Lipkind, K. F. Shortridge, J. J. Skehel, and R. G. Webster for providing strains that were not in the collection of our institute. Primary chick embryo cells in culture were prepared from 11-day-old chick embryos and were used 48 hours after seeding. Plaque and hemagglutination (HA) tests were performed according to established procedures (7).

Treatment of Virus Strains at Low pH

Allantoic fluids containing infectious virus were diluted by phosphate buffer, pH 6.4, to give a HA titer of about 1/64. The desired pH was adjusted exactly by using 1 M acetic acid, and aliquots were removed and incubated for different lengths of time at 20°C. After adding a drop of phenol red the samples were neutralized rapidly with 1 M NaOH. Thereafter a plaque test was performed, or tissue cultures (6 x 10^6 cells) were infected with about 64 HA units/ml. If a strain with a cleavable hemagglutinin was investigated, the cultures were processed 7 hours after infection, in order to avoid a second cycle of infection. The majority of strains contained a hemagglutinin that was not cleaved in chick embryo cells. In these cases the cells were processed 8 to 9 hours after infection by three cycles of freezing and thawing. The HA units were determined after removal of the cell debris by centrifugation.

If virus with noncleaved hemagglutinin was used for the pH treatment, large tissue cultures of about 10^9 cells were infected. After incubation overnight at 37°C the supernatant medium was used without further dilution for pH adjustment with 1 M acetic acid. Multiplication of these viruses in chick embryo cells was followed by adding 10 μg/ml of trypsin to the medium.

Heat Treatment of Virus Strains

Allantoic fluids containing infectious virus diluted 1:5 with phosphate buffered saline were incubated for either 10 or 60 minutes at 50°C or for 10, 20 or 60 minutes at 54°C, respectively. After cooling, the samples were used to infect primary chick embryo cells as described above. For viruses with a noncleaved hemagglutinin, the supernatant medium from infected primary chick embryo cells after incubation overnight was used without further dilution.