

A New Genetic Marker for Influenza Virus Subunits

Brief Report

By

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With 3 Figures

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Several recombinant strains of influenza virus are produced in which the two virus-coded subunits, haemagglutinin (HA) and neuraminidase (Ndase) are derived from the respective parent strains. These studies in addition to suggesting the basis for the natural emergence of new strains of influenza virus (6) also have practical application in vaccine production (3).

Various markers have been used to characterize these recombinants and their parents (4); these include thermal stability of the HA, differential susceptibility to non-specific inhibitors, rate of elution from erythrocytes, virulence in reference hosts, plaque type and/or plaque morphology, virion morphology (spherical or filamentous), and surface antigens including Ndase. Most of these tests are cumbersome to perform, and are liable to variation. On the other hand, a number of these tests are applicable to only limited number of strains of influenza virus. In the course of a detailed study of the effect of guanidine hydrochloride on several strains of influenza virus (DAVID-WEST and BELYAVIN, in preparation) a striking differential effect of the compound on the viral haemagglutinin and neuraminidase activities was consistently observed. The genetic significance of this was later tested, using different sets of parent-recombinant combinations. The results of these experiments are reported in this communication.

Three recombinants were used: X-7 (F1), a hybrid of A₀/NWS and A₂/R15, having the Ndase antigen of the A₂ parent and the HA antigen of the A₀ parent (5); X-31, having both Ndase and HA derived from A₂/Hong Kong/68, but with the soluble (S) antigen from A₀/PR8 (3). The third is a recombinant between A₀/BEL/42 and A₂/Singapore/57; the HA derived from the former parent and the Ndase from the A₂ parent (7). All the strains were kindly provided by Dr. G. C. Schild, World Influenza Centre, National Institute for Medical Research, Mill Hill, London.

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The viruses were grown in the allantoic cavity of 10 or 11-day-old embryonated hens eggs, and were purified by differential centrifugation, followed by rate zonal centrifugation through a linear (10–50%) sodium glutamate gradient in Tris-saline, pH 7.5.

Guanidine hydrochloride (Sigma Grade 1) was prepared in Tris-saline and mixed with virus to give final molar concentrations of 0.5, 1.0 and 1.5. The virus-guanidine mixtures were maintained at 37° C in a water bath for 30 minutes. After stopping the reaction by chilling in crushed ice, followed by dilution in buffer, the residual haemagglutination and neuraminidase activities were determined.

Haemagglutinin was assayed by the microtitre method using Takatsy loops. Equal volumes of freshly prepared 0.5% fowl red blood cells suspension were added to the virus dilutions, and the test read after 30–45 minutes at room temperature (20°–22° C).

Neuraminidase assays were performed by mixing virus with colloidal mucoid substrate in 0.02 M sodium acetate buffer, pH 6.0, in 0.15 M sodium chloride. The mixtures were incubated overnight at 37° C, and the free sialic acid released assayed by the Aminoff method (1). All tests were set up in duplicate, and repeated at least three times.

The results obtained with the three hybrids and corresponding parent strains are summarized in the diagrams (Figs. 1, 2 and 3) as per cent residual HA or

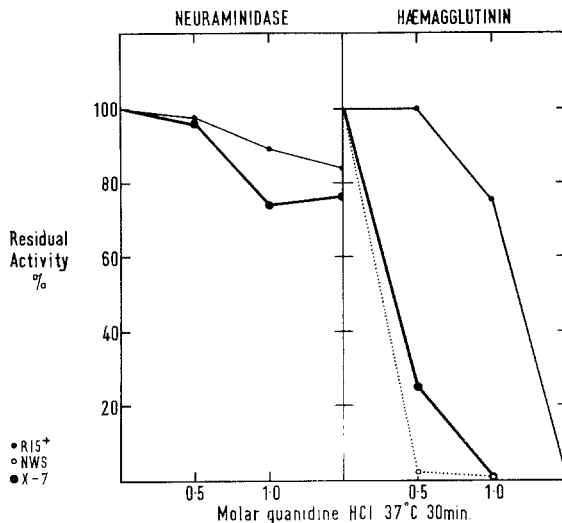


Fig. 1. Guanidine hydrochloride inactivation kinetic curves of influenza virus X-7 (F1) and its corresponding parent influenza virus strains. Note: Under the conditions of the experiment the neuraminidase of strain NWS was inactive. This is in accord with the observation of BURNET (1951)

Ndase activity remaining after 30 minutes following exposure to the corresponding guanidine molarity at 37° C. The residual activity is proportional to the rate of inactivation, assuming approximately equivalent kinetics over the relevant period of measurement. It is evident from the data that: (i) The haemagglutinin and neuraminidase of an influenza virus strain each has a demonstrable inactivation “profile” following exposure to varying concentrations of guanidine hydrochloride.

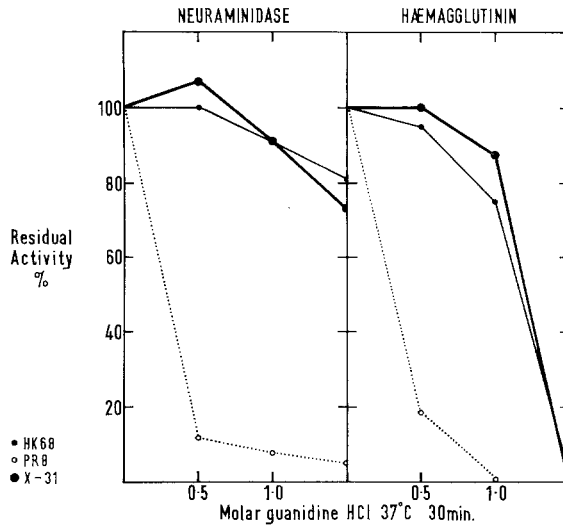


Fig. 2. Similar inactivation kinetic curves for influenza virus X-31, and its corresponding parent strains

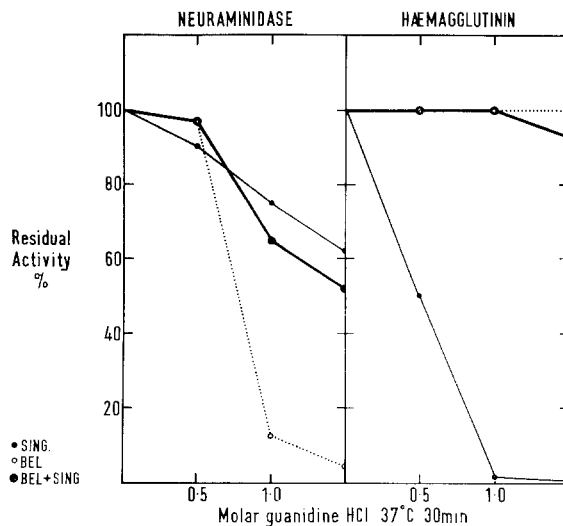


Fig. 3. Similar inactivation kinetic curves for influenza virus hybrid BEL + SINGAPORE, and its corresponding parent strains

(ii) The “profile” concerned is determined by the viral genome, as the characteristic response of a parent virus strain in the case of either haemagglutinin or neuraminidase is likely to be retained in a hybrid strain.

In so far as observation (ii) above were to be generally true among influenza virus strains, the implication would be that biological activity in the case of both haemagglutination and neuraminidase activity was dependent upon specific intra-molecular configurations, which are sensitive to inactivation by guanidine. On this basis, some broad relationship between guanidine inactivation kinetics

and antigenic specificities may be expected. Detailed work along these lines are in progress.

The tests for the guanidine hydrochloride (GH) marker described here could thereby complement the conventional serological tests at present commonly used in influenza virus hybridization studies. Because the tests for the GH marker are easy to perform, and need fewer reagents, they can be used readily to monitor the efficiency of recombination at various stages of the hybridization process. Furthermore, there are indications that the tests could have broader application in influenza virus typing.

Acknowledgment

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