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GENE HOMOLOGY AND ANTIGENIC SPECIFICITY OF ENVELOPE PROTEINS OF AVIAN INFLUENZA VIRUS STRAINS POSSESSING HAEMAGGLUTININ Hav1

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Summary. — Comparison of some avian influenza virus strains possessing haemagglutinin Hav1 revealed the greatest differences in strains A/FPV/Weybridge and A/FPV/Rostock/34. These strains differed in the degree of homology of eight genome fragments, electrophoretic mobility of the majority of proteins, size of plaques and rct_{42} marker and displayed significant differences in antigenic specificity of haemagglutinin. Strains A/FPV/ /Weybridge and A/FPV/Dobson proved to be more close in the degree of genome homology but differed in three genes, electrophoretic mobility of some proteins, size of plaques, rct_{42} marker and antigenic specificity of haemagglutinin. The data obtained indicate that avian influenza virus strains of the Hav1 subtype may differ from each other in the degree of gene homogoly and some other properties including antigenic specificity of haemagglutinin like influenza viruses with other haemagglutinin subtypes.

Key words: orthomyxoviruses; avian influenza viruses; gene homology; electrophoretic mobility of proteins; antigenic specificity of haemagglutinin

Introduction

Avian influenza viruses possessing haemagglutinin Hav1 had been isolated much earlier than human influenza viruses. The first strain A/hen/Breschia/ 1902 was isolated in 1902 and strain A/FPV/Dutch/27 was so in 1927. All these strains were originally designated as fowl plague viruses (FPV) and were maintained in veterinary laboratories by passages in chicks (Gorbunova and Pisina, 1973). Later, a number of additional similar strains were described, namely Rostock, Ruzetce, Dobson, Weybridge (Gorbunova and Pisina, 1973). Subsequently these strains were found to belong to orthomyxoviruses and they have become widely used as models of influenza A virus in

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basic research mainly to solve problems connected with replication and genetics of orthomyxoviruses.

This viral group was considered to be antigenically homogeneous without any significant differences. The haemagglutinin typical of this group was classified as a particular subtype Hav1 (WHO, 1971) although there were indications that FPV haemagglutinins were related to subtype Heq1 possessed by strains isolated from horses.

The present paper deals with a comparative study on the homology of RNA fragments (genes), electrophoretic mobility of viral proteins, antigenic specificity of haemagglutinin and neuraminidase and some biological properties of several FPV strains with haemagglutinin Hav1.

Materials and Methods

Viruses. The following virus strains were used: A/FPV/Rostock/34, A/FPV/Dutch/27, A/FPV/Weybridge, A/FPV/Dobson, A/turkey/England/64, A/turkey/Oregon/71 and A/equi/Prague/56. They were grown in chick embryos.

Immune sera were prepared by immunizing rats and rabbits. Before used in serological reactions, the sera were freed from inhibitors by treatment with receptor-destroying enzyme.

The haemagglutination inhibition (HI) test was performed by the method recommended by WHO (1959) and *the neuraminidase inhibition (NI) test* was carried out according to Aymard-Henry *et al.* (1973).

Electrophoretic mobility of viral proteins. Chick embryo cell (CEC) cultures were infected with purified and concentrated viruses at a multiplicity of 100 EID_{50}/cell and incubated at 36 °C for 4 hr in Eagle's medium without methionine. ^{35}S-Methionine was then added (20 μCi per culture; ^{35}S-mehtionine, specific activity 1300 Ci/mmol; Radiochemical Centre, Amersham) and incubation continued for 30 min. The cells were solubilized and subjected to electrophoresis in 25 or 15% polyacrylamide gel as described by Ghendon *et al.* (1979).

Analysis of the degree of gene homology. Electrophoretic analysis of double-stranded (ds) hybrids of cRNA and vRNA treated with nuclease was carried out as described by Hay *et al.* (1977) and Ghendon *et al.* (1979). Briefly, CEC cultures were infected with the viruses (100—300 EID_{50}/cell) and incubated in the presence of cycloheximide (100 μg/ml) at 36 °C for 60 min. ^{3}H-Uridine was then added (100 μCi/ml) and incubation continued at 36 °C for 4 hr. From the infected cells viral cRNA labelled with ^{3}H-uridine was isolated and then hybridized with an excess of unlabelled vRNA isolated from purified virions. The ds complex was treated with S-1 nuclease and subjected to electrophoresis in 4% polyacrylamide gel as described by Hay *et al.* (1977).

Determination of plaque size and plaque formation at different temperatures (rct marker). CEC cultures were infected with various virus dilutions and after 30 min adsorption at room temperature overlaid with agar overlay. The infected cultures were incubated at 36, 40 and 42 °C for three days and then the plaques were counted. When determinig the size of plaques, at least 200 plaques formed at 36 °C were measured.

Results

Analysis of the degree of gene homology

The comparative study on the homology of genome fragments of three FPV strains, Rostock, Dobson and Weybridge, was done by cross-hybridization of virus-specific complementary RNAs (cRNA) and virion RNAs (vRNA), subsequent treatment of the ds complexes with S-1 nuclease and electrophoresis. When the hybridized cRNA and vRNA fragments of different strains are completely homologous, the electrophoretic mobility

of heterologous (cRNA of one and vRNA of another strain) or homologous (both cRNA and vRNA of the same strain) complexes remains identical after treatment with nuclease; in incomplete homology, the nuclease-treated complex changes its electrophoretic mobility or disappears at all.

Fig. 1 (Plate VIII) shows that on homologous hybridization of cRNA and vRNA of FPV strains Dobson and Weybridge (gels 2 and 4), bands corresponding to eight complexes of the respective genes were revealed. On cross hybridization of FPV strains Dobson and Weybridge we found (gels 1 and 3) that genes 1, 2, 3, 5 and 8 were completely homologous and that bands of ds RNAs migrated at the same rate as homologous ds RNAs (gels 2 and 4). At the same time bands corresponding to genes 4, 6 and 7 located in a slightly different position at cross-hybridization as compared with the same bands formed by ds homologous complexes of cRNA/vRNA. The study of the degree of gene homology of FPV strains Rostock and Weybridge showed (gels 5 and 6) that heterologous complexes of all genes treated with nuclease differed significantly in electrophoretic mobility from the complexes formed on homologous hybridization of cRNA/vRNA of FPV Weybridge (gel 4). Differences in all the genome fragments were observed when comparing FPV strains Dobson and Rostock, both in our tests (not shown) and in those of Almond *et al.* (1979).

Our results thus showed that many genes of FPV strains Dobson and Weybridge are very similar if not identical, and that only genes 4, 6 and 7 which code for haemagglutinin, neuraminidase and M protein are slightly different. At the same time these FPV strains differ significantly from the FPV strain Rostock in all the eight genes.

Electrophoretic mobility of virus-specific proteins

The results concerning electrophoretic mobility of virus-specific proteins formed in CEC cultures are illustrated in Fig. 2 (plate IX). Assay in 15% gel yielding better separation of polypeptides showed (Fig. 2 - I) that P1, P2, and P3 proteins of FPV strains Weybridge and Dobson have identical electrophoretic mobilities whereas the mobility of these proteins in the Rostock strain is slightly different. Haemagglutinin of Rostock and Dobson strains did not differ in electrophoretic mobility and that of Weybridge strain had a different electrophoretic mobility. Assay in 25% gel yielding better separation of small polypeptides showed (Fig. 2 - II) that electrophoretic mobility of M protein of the three strains differed, while that of NS protein was similar.

Size of plaques and rct marker

All three FPV strains examined differed in the size of plaques formed in CEC cultures (Table 1): strain Weybridge formed the largest plaques, strain Rostock medium plaques and strain Dobson the smallest plaques. Based on the plaque-forming ability at high temperature (rct marker), all the FPV strains tested also differed from each other: the Weybridge

Table 1. Plaque formation by FPV strains

Virus strain	Plaque size at 36 °C* (mm)	Titre (PFU/ml) at 36 °C	40 °C	42 °C
Weybridge	3 ± 0.4	1.7×10^9	2.4×10^9	1.1×10^9
Dobson	1.5 ± 0.3	2.3×10^9	1.1×10^9	3×10^5
Rostock	2.0 ± 0.2	3.0×10^8	1.3×10^7	$< 10^1$

*At least 200 plaques were measured 72 hr post inoculation.

strain formed plaques equally at 36, 40 and 42 °C; the titre of the Dobson strain at 42 °C was markedly lower and Rostock strain formed no plaques at 42 °C.

Antigenic specificity of haemagglutinin and neuraminidase

Six strains of avian and one strain of equine influenza virus, isolated in different years, were studied. Four of them (A/FPV/Dutch/27, A/FPV/ /Rostock/34, A/turkey/England/63 and A/equi/Prague/56) represent international reference strains. As shown in Table 2, the antigenic structure of

Table 2. Antigenic relationship of envelope proteins of FPV strains

Antigens	HI test with antisera: Dutch/27	Rostock/34	Weybridge	turk/Engl./63	turk/Oreg/71	equi/Prague/56	NI test with antisera: Dutch/27	Rostock/34	Weybridge	equi/Prague/56
A/FPV/Dutch/27 (Hav1Neq1)	2560	640	640	640	320	80	270	0	—	60
A/FPV/Rostock/34 (Hav1N1)	320	640	80	320	80	20	0	270	0	0
A/FPV/Weybridge (Hav1N ?)	320	320	2560	320	40	0	270	0	270	180
A/FPV/Dobson (Hav1Neq1)	320	80	320	320	80	0	180	30	90	60
A/turkey/England/63 (Hav1Nav3)	160	160	160	2560	320	0	—	—	—	—
A/turkey/Oregon/71 (Hav1Nav2)	640	640	160	2560	2560	320	—	—	—	—
A/equi/Prague/56 (HeqlNeql)	640	320	40	40	80	2560	270	0	270	180

— means not done.

haemagglutinin of the strains studied is heterogeneous. Antiserum to strain Dutch/27 reacted with haemagglutinins of the other FPV strains only up to 1/8—1/4 of the homologous titre and serum to strain Weybridge reacted with strains Dutch, Dobson and Rostock up to 1/4, 1/8 and 1/32 of homologous titre, respectively. Haemagglutinin of Dobson strain also differed from all the other strains tested not less than by 1/8 of the homologous titre.

Strains A/FPV/Weybridge and A/turkey/Oregon/71 proved to differ greatly form international reference strains: antisera against them poorly neutralized haemagglutinins of the remaining representatives.

Differences were also observed in investigating the antigenic relationship of strains of the Hav1 subtype with equine influenza virus of the Heq1 subtype. No relationship was found between strain A/turkey/England/63 and equine influenza virus. A certain unilateral relationship was found between strain Weybridge and A/equi/Prague/56. Interaction between haemagglutinins of Rostock and Dutch strains and antiserum to influenza virus A/equi/Prague/56 was very weak.

The NI test showed (Table 2) that neuraminidase of FPV strain Weybridge is antigenically identical with that of A/equi/Prague/56 and FPV strain Dutch, but that it differs greatly from neuraminidase of FPV strain Rostock. Neuraminidase of FPV strain Dobson is closer to that of A/equi/Prague/56 and FPV strain Dutch as far as antigenic specificity is concerned, though it has certain common groups with neuraminidase of FPV strain Rostock. Consequently, FPV strain Weybridge has neuraminidase of Neq1 subtype. Neuraminidase in FPV strain Dobson might also be referred to Neq1, although it slightly differs from that of the reference equine influenza virus strain.

Discussion

The present data showed that the FPV strains studied, containing haemagglutinin Hav1, differ from each other in antigenic specificity of haemagglutinin, rct marker, plaque size, electrophoretic mobility of proteins and degree of gene homology. The most distinct proved to be FPV strains Weybridge and Rostock which significantly differed in the degree of homology of all the eight genes, electrophoretic mobility of most proteins, rct_{42} marker, plaque size and antigenic specificity of haemagglutinin.

Our comparative study on FPV haemagglutinin showed that the Hav1 subtype has antigenic strain variants. The apathogenic strain A/turkey/ /Oregon/71, one of the rare representatives of this group isolated in recent years, differed significantly from all the reference strains belonging to the Hav1 subtype. Strain A/FPV/Weybridge, although its haemagglutinin is antigenically similar to that of strains Rostock and Dutch, cannot be considered identical with any reference strain and should be probably classified as an antigenic variant.

The FPV strains studied fall into 2 groups as concerns their relationship to haemagglutinin of equine influenza virus Heq1. Strains Dutch and Rostock exhibited a certain relationship to equine virus, whereas in strains Weybridge and Dobson these relationships proved to be unilateral and insignificant.

The prevailing opinion in the literature was that FPV strains of the Hav1 subtype possess rather similar properties and that they do not change greatly in nature. Our results obtained on comparing two wild FPV strains — — Rostock and Weybridge — clearly showed that fowl plague viruses might differ from each other in the degree of homology of many genes and in a number of biological properties including antigenic specificity of haemagglutinin like influenza viruses possessing other subtypes of haemagglutinin.

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