pH Dependence of Influenza A Virus-induced Haemolysis is Determined by the Haemagglutinin Gene

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SUMMARY

The pH-dependent profiles of haemolysis by influenza viruses AHK/8/68 (HK virus) and APR/8/34 (PR8 virus) were found to possess characteristic differences. Both viruses were highly haemolytic at pH 5.0, but while HK virus-induced haemolysis was undetectable above pH 5.5 to 5.6, PR8 virus-induced haemolysis persisted at higher pH values, becoming undetectable above pH 5.7 to 5.8. In order to determine whether these haemolysis profiles were genetically determined, we studied the pH dependence of haemolysis by eight recombinants of HK and PR8 viruses. The pH profile of each recombinant clearly resembled that of one parent or the other, showing that it is an inherited trait. The pH profile was found to be conferred exclusively by the haemagglutinin (HA) gene.

Viral haemolysis is generally accepted to be a measure of virus–erythrocyte fusion. Several strains of influenza virus have recently been shown to be haemolytic at pH values between 5.0 and 6.0 although the pH-dependent profile of haemolysis differs for each strain (Huang et al., 1981; Lenard & Miller, 1981; Maeda & Ohnishi, 1980).

The low-pH requirement for influenza haemolysis has been considered as evidence for the similarity of influenza uncoating to that of other enveloped viruses, in which uncoating presumably occurs following endocytosis by fusion of the virus membrane with host cell lysosomal or endocytotic membranes in the acid milieu of those organelles (Lenard & Miller,
Fig. 2. pH dependence of haemolysis by influenza PR8, HK and their recombinants in 344 mOsm buffer. Values for highest haemolysis measured are normalized to 1.0. Profile was independent of amount of haemolysis. (a) and (b) represent separate experiments. (a) O—-O, PR8; O---O, HK; ■, R11; □, R13; ▲, R15; △, R19; ●, R22. (b) O—-O, PR8; O---O, HK; □, R1; △, R3; ●, R2.

Table 1. Protein composition of influenza PR8, HK and their recombinants*

<table>
<thead>
<tr>
<th>Protein derivation</th>
<th>P1 to 3, NP, NS</th>
<th>M</th>
<th>NA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR8</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>R1</td>
<td>H</td>
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<td>H</td>
<td>P</td>
</tr>
<tr>
<td>R3</td>
<td>P</td>
<td>P</td>
<td>H</td>
<td>P</td>
</tr>
<tr>
<td>R13</td>
<td>P</td>
<td>H</td>
<td>P</td>
<td>P</td>
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<tr>
<td>R19</td>
<td>P</td>
<td>H</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>R22</td>
<td>P</td>
<td>H</td>
<td>P</td>
<td>P</td>
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<tr>
<td>HK</td>
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<td>R2</td>
<td>P</td>
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<td>R11</td>
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<td>R15</td>
<td>P</td>
<td>H</td>
<td>H</td>
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</tr>
</tbody>
</table>

* Data are from Lubeck et al. (1978). P indicates protein derived from PR8; H indicates protein derived from HK.
† Protein derived from PR8 except for P1.
It is therefore of interest to determine which protein(s) of the influenza virion is responsible for low-pH-mediated fusion.

Influenza strains and recombinants were kindly provided by Drs P. Palese and J. L. Schulman, Mt. Sinai School of Medicine, New York, U.S.A. They were grown in 10- to 11-day-old embryonated eggs and purified on 5 to 40% potassium tartrate gradients. The recombinant strains are identified by the numbers used by Lubeck et al. (1978). Haemolysis was carried out as described previously, using a citrate-phosphate buffer system (Gomori, 1955). In preliminary experiments, very similar pH profiles to those reported here were obtained using the MES--BES buffer system employed previously (Lenard & Miller, 1981), except that buffers below a pH of about 5·3 gave rise to appreciable spontaneous haemolysis. The pH profile of influenza strain WSN was similar in citrate--phosphate buffer to that reported in MES--BES buffer (Lenard & Miller, 1981; data not shown). pH was measured with a Radiometer model 26 pH meter at room temperature (20 ± 2 °C) with an accuracy of ±0·02 pH units. pH did not vary throughout the incubation. Since variations in osmolarity were found to alter both the extent of haemolysis and the pH profile (Fig. 1), osmolarity was measured (Advanced Instruments, Model 3L osmometer) and adjusted with NaCl to keep it constant throughout the pH range. The characteristic difference in the pH profiles of haemolysis by influenza viruses AHK/8/68 (HK virus) and APR/8/34 (PR8 virus) was similar at all osmolarities tested (Fig. 1).

The pH dependence of haemolysis of PR8, HK and eight of their recombinants is shown in Fig. 2. The parent strains differ in their pH profiles in that the HK curve is about 0·2 pH units more acidic than that reported for PR8. These results are very similar to those reported by Huang et al. (1981) using an acetate buffer system. All of the recombinants tested displayed pH profiles which clearly resembled one parent or the other. As shown in Fig. 2, all eight recombinants displayed the pH profile characteristic of the parent that had contributed the HA protein. Most dramatically, the recombinant R1 contains only the HA protein from the PR8 parent, all other proteins being derived from the HK parent, while R2 is the exact reverse (see Table 1). We conclude that the gene for HA alone determines the pH dependence of haemolysis, with no significant effect of the M gene, the NA gene, or the genes of any of the other virus proteins.

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REFERENCES


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