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Evolution of the Hong Kong influenza A sub-type

Structural relationships between the haemagglutinin from A/duck/Ukraine/1/63 (Hav 7) and the Hong Kong (H3) haemagglutinins

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The relationship between the haemagglutinin from the influenza virus A/duck/Ukraine/1/63 (Hav 7) and the human Hong Kong variants (H3) has been investigated. Amino-acid-sequence analysis shows that the Hav 7 haemagglutinin closely resembles the 1968 human H3 haemagglutinin in structure. However, the number of amino-acid-sequence differences (23) suggest that the Hong Kong haemagglutinin gene did not come directly from A/duck/Ukraine/1/63 but from a virus derived from it by antigenic drift during the period 1963–1968.

Considerable evidence supports the suggestion (Laver & Webster, 1973) that genetic reassortment is one of the mechanisms by which major antigenic change (shifts) can occur in influenza virus (Webster & Laver, 1975; Laver & Webster, 1979; Young & Palese, 1979; Bean *et al.*, 1980; Hinshaw *et al.*, 1980). In particular, at least one human sub-type, the Hong Kong virus, may have been formed by genetic reassortment between the existing Asian-influenza variant and either an animal virus related to A/equine/Miami/1/63 (Heq 2, Neq 2) or a bird virus related to A/duck/Ukraine/1/63 (Hav 7, Neq 2). Both of these viruses had been isolated some 5 years earlier and showed antigenic and structural similarities to the human Hong Kong strains (Laver & Webster, 1973; Scholtissek *et al.*, 1978).

Since the base-sequence homology between Hav 7 and H3 is only 80% (Scholtissek *et al.*, 1977) compared with the 92% homology between Hav 7 and H3 (Scholtissek *et al.*, 1978), the duck/Ukraine/1/63 virus would appear to be the more likely donor of the Hong Kong haemagglutinin gene.

In a previous paper (Ward *et al.*, 1981) the A/duck/Ukraine/1/63 haemagglutinin was shown to closely resemble the human Hong Kong haemagglutinin in amino-acid composition and *N*-terminal (30 residues) sequences of its heavy (HA1) and light (HA2) chains. Here we present the complete amino-acid sequence and oligosaccharide composition for the Hav 7 haemagglutinin except for the highly aggregated portion (residues 180–207) near the *C*-terminal end of HA2.

Abbreviations used: HA1, haemagglutinin heavy chain; HA2, haemagglutinin light chain.

Materials and methods

The virus used was a recombinant possessing the haemagglutinin of A/duck/Ukraine/1/63 (Hav 7) and the neuraminidase from A/Bel/42 (N1). The inoculum was obtained from Dr. W. G. Laver, Department of Microbiology, Australian National University, Canberra, Australia. The procedures employed were: virus cultivation and purification (Laver, 1969); haemagglutinin isolation and separation into heavy (HA1) and light (HA2) chains (Ward & Dopheide, 1980); *S*-carboxymethylation with iodo[2-^{14}C]acetate, cleavage with CNBr and separation of CNBr peptides (Dopheide & Ward, 1978), enzymic digestion, peptide fractionation, amino-acid analysis, carbohydrate analysis and amino-acid-sequence determination (Ward & Dopheide, 1979, 1980, 1981; Ward *et al.*, 1980).

Results

The amino-acid composition and *N*-terminal-sequence analysis of A/duck/Ukraine/1/63 HA1 and HA2 (Ward *et al.*, 1981) indicated that this Hav 7 haemagglutinin was very closely related to that of the human Hong Kong strains and indicated that its complete structure could be rapidly determined by the comparative peptide approach used previously (Ward & Dopheide, 1981) to determine the structure of the haemagglutinin from the early Hong Kong variant A/Aichi/2/68. This involved CNBr cleavage of HA1 and HA2, followed by tryptic digestion of the isolated CNBr peptides. Large tryptic peptides were further digested with thermolysin, chymotrypsin, pepsin or *Staphylococcus aureus* proteinase. The

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Fig. 1. *Amino-acid sequence of A/duck/Ukraine/1/63 haemagglutinin*

Methionine residues occur at positions 168, 260, 268 and 320 in HA1, and 17, 115, 133 and the insoluble-hydrophobic-tail region of HA2. A partial replacement of isoleucine for methionine at position 133 in HA2 was observed.

Table 1. *Carbohydrate composition of the five oligosaccharide units on A/duck/Ukraine haemagglutinin*
The values are expressed as nmol of sugar/30nmol of peptide. The values in parentheses are the number of residues per molecule of peptide. Glucosamine was determined in an amino-acid analyser and is assumed to be *N*-acetylated. Neutral sugars were determined by g.l.c. as their alditol acetates. There was no carbohydrate on HA2.

Peptide	Asparagine residue no.	Sugar ...	Sugar composition (nmol/30nmol of peptide) GlcNAc	Man	Gal	Fuc
CN1 T1 Th2	8		86.7 (2.9)	56.7 (1.9)	20.7 (0.7)	12.0 (0.4)
CN1 T1 Th6	22		59.3 (2.0)	49.5 (1.7)	62.7 (2.1)	51.9 (1.7)
CN1 T2 Sa2	38		54.6 (1.8)	111 (3.7)	9.3 (0.3)	4.2 (0.1)
CN1 T10	165		52.8 (1.8)	129.6 (4.3)	12.3 (0.4)	6.0 (0.2)
CN3 T1 Th4	285		53.7 (1.8)	177 (5.9)		

amino-acid sequence of each resulting peptide was deduced by comparing its chromatographic behaviour, electrophoretic mobility, amino-acid composition and *N*-terminus with that of the corresponding peptides from the haemagglutinin of Aichi/2/68 and Mem/102/72, whose structures are known (Ward & Dopheide, 1980, 1981; Dopheide & Ward, 1980).

The amino-acid sequence of the A/duck/Ukraine/1/63 haemagglutinin is shown in Fig. 1, along with the peptides used to determine the structure. The sequence is complete except for the highly aggregated hydrophobic region (residues 180–207) near the *C*-terminal end of HA2, which could not be resolved by peptide sequencing. There are 23 differences in amino-acid sequence between this Hav 7 haemagglutinin and that of the Aichi/2/68 isolate studied by Verhoeyen *et al.* (1980). Of these, fifteen occur in HA1 (positions 4, 25, 62, 81, 92, 135, 137, 144, 145, 182, 186, 193, 226, 228 and 309) and eight in HA2 (positions 2, 67, 71, 106, 132, 133, 154 and 161). The isoleucine-for-methionine replacement at position 133 in HA2 was only partial.

A/duck/Ukraine/1/63 haemagglutinin contains only five oligosaccharide units and these are attached at asparagine residues 8, 22, 38, 165 and 285 in HA1. It lacks the glycosylation sites at positions 81 in HA1 and 154 in HA2 (see Ward & Dopheide, 1981). The late Hong Kong variant A/Vic/3/75 also lacks the glycosylation site at position 81 in HA1 (Min-Jou *et al.*, 1980). The sugar compositions for the five oligosaccharide units are shown in Table 1. As found for A/Mem/72 (Ward *et al.*, 1980) and Aichi/2/68 (Ward & Dopheide, 1981) the carbohydrate on residues 8 and 22 are of the *N*-acetyl-lactosamine ('complex') type, whereas that on 285 is of the oligomannoside (or 'simple') type. In A/duck/Ukraine HA1 the sugar units on residues 38 and 165 also resemble the oligomannoside type but contains small, but significant, amounts of galactose and fucose. In both Aichi/2/68 and Mem/72 the carbohydrate at residue 165 was 'simple', whereas that at residue 38 was 'complex' in Mem/72 but very similar to that found here for A/duck/Ukraine in Aichi/2/68 (Ward & Dopheide, 1981).

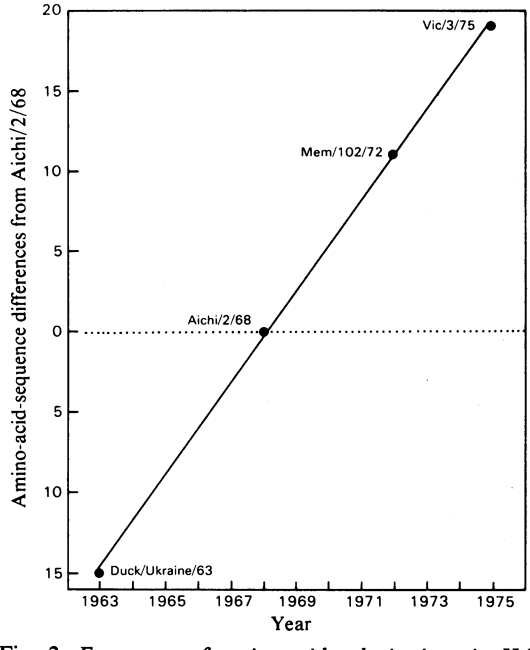

Fig. 2. *Frequency of amino-acid substitutions in HA1*
The number of differences in the amino-acid sequences of the HA1 polypeptides of the various strains are shown. Duck/Ukraine falls on the same line that shows the rate of change in the human Hong Kong sub-type, when extrapolated back to 1963. The data sources are: Aichi/2/68 (Verhoeyen *et al.*, 1980; Ward & Dopheide, 1981); Memphis/102/72 (Ward & Dopheide, 1980); Vic/3/75 (Min-Jou *et al.*, 1980).

Discussion

Although the results clearly show that the Hav 7 haemagglutinin belongs to the H3 sub-type (see also

Schild *et al.*, 1980), it appears that the haemagglutinin gene of the human Hong Kong strains was not directly donated by the duck/Ukraine virus, since their amino-acid sequences differ at fifteen positions in HA1 and eight positions in HA2. The data suggest, however, that the 1968 Hong Kong haemagglutinin may have come from a virus derived from A/duck/Ukraine/1/63 by antigenic drift during the period 1963–1968. Firstly, the rate of drift required, in the period 1963–1968, to accommodate the changes between A/duck/Ukraine/1/63 and A/Aichi/2/68 is similar to the rate of drift found to occur from 1968 to 1975 in the Hong Kong sub-type (Fig. 2). Secondly, an examination of the sequence data available for other Hong Kong variants (see Ward & Dopheide, 1981 for summary) shows that the duck/Ukraine/1/63 is identical with Aichi/2/68 at most of the positions that subsequently changed during antigenic drift in the Hong Kong sub-type. This would be expected, since antigenic drift involves the gradual accumulation of changes at different loci rather than sequential changes at a single or limited number of positions (see Laver *et al.*, 1980; Ward & Dopheide, 1981).

Although nothing is known about the rate of antigenic drift in bird influenza infections, it has been shown that the mutation rate in the antigenically stable Sendai and vesticular-stomatitis viruses (Portner *et al.*, 1980) and in the 'non-selected' internal matrix and non-structural proteins of influenza virus (Air *et al.*, 1981) are similar to that found in influenza-virus haemagglutinin. These observations are consistent with the hypothesis, but do not prove, that the haemagglutinin gene for the Hong Kong influenza A pandemic came from a virus derived from A/duck/Ukraine/1/63.

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