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Carbohydrate composition of the oligosaccharide units of the haemagglutinin from the Hong Kong influenza virus A/Memphis/102/72

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The haemagglutinin from the Hong Kong influenza virus A/Memphis/102/72 contains seven oligosaccharide units attached to asparagine residues 8, 22, 38, 81, 165 and 285 in the heavy chain (HA_1) and to residue 154 in the light chain (HA_2). The single oligosaccharide unit in HA_2 and four of the oligosaccharide units of HA_1 (at residues 8, 22, 38 and 81) contain the four monosaccharides *N*-acetylglucosamine, mannose, galactose and fucose and are of the *N*-acetyllactosamine (or 'complex') type. The two other oligosaccharide units on HA_1 are of the oligomannoside (or 'simple') type and contain only two residues of *N*-acetylglucosamine and five or six residues of mannose. The data are discussed in relation to the differences in the carbohydrate compositions of other influenza haemagglutinins.

Influenza virus contains 5–7% carbohydrate, bound to two glycosylated coat proteins and to membrane glycolipid (Frommhagen *et al.*, 1959). Most of the protein-bound carbohydrate is attached to the haemagglutinin (see White, 1974) and is synthesised via dolichyl phosphate intermediates (Waechter & Lennarz, 1976; Schwarz *et al.*, 1977; Elder *et al.*, 1979) by host-cell, not viral-coded, enzymes. It resembles the carbohydrate on host glycoproteins in its antigenic character (Knight, 1944; Harboe, 1963; Laver & Webster, 1966; Jackson *et al.*, 1979).

In previous communications we have reported the carbohydrate content, composition (Ward & Dopheide, 1976) and amino acid sequences (Dopheide & Ward, 1978, 1979, 1980; Ward & Dopheide, 1979; 1980*a*; Ward *et al.*, 1980*a*) for the heavy (HA_1; 328 residues) and light (HA_2; 221 residues) chains of the haemagglutinin from the Hong Kong influenza virus A/Memphis/102/72. Oligosaccharide units were found attached to six asparagine residues (positions 8, 22, 38, 81, 165 and 285) in HA_1 and to one asparagine residue (position 154) in HA_2. No carbohydrate was found *O*-glycosidically linked to serine or threonine. In the present communication we present the carbohydrate compositions and amino acid sequences for these seven glycopeptides.

Abbreviations used: HA_1, haemagglutinin heavy chain; HA_2, haemagglutinin light chain.

Materials and methods

Peptide preparation and characterization

The procedures for haemagglutinin preparation, peptide fragmentation, amino acid analysis and amino acid sequencing have been fully described (Dopheide & Ward, 1978; Ward & Dopheide, 1979, 1980*a*).

Analytical methods

Glucosamine was determined on an amino acid analyser after hydrolysis in 3.0M-*p*-toluenesulphonic acid at 100°C for 24h (Allen & Neuberger, 1975). Neutral sugars were determined as alditol acetates by g.l.c. (Albersheim *et al.*, 1967). All samples (21–90nmol), except HA_2, were hydrolysed in 2ml of 2.5M-trifluoroacetic acid at 100°C for 2h in a sealed tube under N_2. The extreme insolubility of HA_2 necessitated the use of more severe hydrolysis conditions. A sample of HA_2 (70nmol) was swollen in 2ml of 90% (v/v) formic acid at 100°C for 2h, dried under N_2 and then hydrolysed in 2ml of 2.5M-trifluoroacetic acid at 100°C for 7h. After hydrolysis, the acid was removed by rotary evaporation, and the hydrolysates were reduced with $NaBH_4$ and acetylated (Albersheim *et al.*, 1967). The resulting alditol acetate derivatives were separated by g.l.c. on a column (1.85m × 4mm) of 0.2% ethyleneglycol succinate, 0.2% ethyleneglycol adipate and 1.4% silicone XE-60 on Gas-Chrom Q 100/120.

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Results

The monosaccharide compositions of whole A/Mem/72 (A/Memphis/102/72) HA_1 and the six glycopeptides derived from it are shown in Table 1 together with the monosaccharide compositions of HA_2. The amino acid sequences of these glycopeptides and the location of the glycosylated asparagine residues in the sequence of the Hong Kong influenza virus haemagglutinin are shown in Fig. 1. The single oligosaccharide unit in HA_2 and four of the oligosaccharide units on HA_1 (at residues 8, 22, 38 and 81) contain the four sugars *N*-acetylglucosamine, mannose, galactose and fucose and are of the *N*-acetyllactosamine (or 'complex') type (see Clamp & Johnson, 1972; Montreuil & Vliegenthart, 1979). The two other oligosaccharide units on HA_1 are of the oligomannoside (or 'simple') type and contain only two residues of *N*-acetylglucosamine and five or six residues of mannose.

Table 1. *Carbohydrate composition of the seven oligosaccharide units of A/Memphis/102/72 haemagglutinin*
The peptides used were isolated as described in the original papers on the amino acid sequences (Ward & Dopheide, 1980*a*; Dopheide & Ward, 1978, 1980). Neutral sugars were determined by g.l.c. as their alditol acetates. Glucosamine was determined on an amino acid analyser and is assumed to be *N*-acetylated. Peptides C1, C2·Th4, C2·Th5, C6 and CN3 also contained 7, 3.7, 0.7, 1.9 and 2.0 mol of glucose/mol of peptide respectively, presumably derived from the paper or Sephadex supports used during their isolation.

Peptide	Asparagine residue no.	Content (mol/mol of peptide) of: *N*-Acetylglucosamine	Mannose	Galactose	Fucose	Carbohydrate type
HA_1		19.1	29.4	9.5	4.9	
HA_1·CN1·C1	8	4.1	4.1	4.8	1.8	'Complex'
HA_1·CN1·C2·Th4	22	3.7	2.1	2.2	1.3	'Complex'
HA_1·CN1·C2·Th5	38	4.0	5.4	2.5	0.6	'Complex'
HA_1·CN1·C6	81	2.9	2.5	2.1	0.2	'Complex'
HA_1·CN1·C16	165	1.9	5.8			'Simple'
HA_1·CN3	285	2.0	5.1			'Simple'
	Total HA_1 glycopeptides	18.6	25.0	11.6	3.9	
HA_2	154	4.0	2.6	2.2	1.1	'Complex'

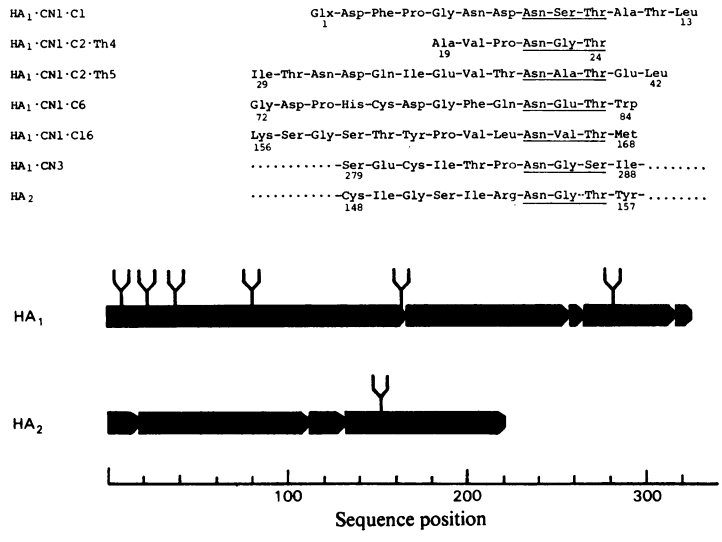

Fig. 1. *Distribution of oligosaccharide units on A/Mem/102/72 haemagglutinin*
The position in the sequence of the glycosylated asparagine residues and the amino acid sequences of the glycopeptides analysed are shown. The nodes on the segments represent positions of cleavage by CNBr. The tripeptide units containing the glycosylated asparagine residues are underlined.

The *N*-acetylglucosamine, galactose and fucose compositions for the six HA_1 glycopeptides account well for that found in whole HA_1. Some four residues of mannose were not recovered in the isolated glycopeptides and may have been lost during the production and isolation of the peptides. Large quantities of glucose were also found in most of the glycopeptides isolated by high-voltage paper electrophoresis or gel filtration on Sephadex. This glucose was presumably derived from these supports since the original HA_1 did not contain any of this sugar (Table 1).

When the hydrolysis conditions used for HA_1 and its glycopeptides were applied to HA_2, only small amounts (less than molar ratios) of neutral sugars were recovered (Ward & Dopheide, 1976). The extreme insolubility of HA_2 necessitated the use of longer, more drastic, hydrolysis conditions for the release of neutral sugars. Under these conditions the monosaccharides galactose, mannose and fucose were detected (Table 1) but degradative losses, especially of fucose, would be expected.

N-Acetylneuraminic acid was not determined since it is known to be absent from mature influenza virions (Klenk & Choppin, 1970; Palese *et al.*, 1974), due to the presence of an active neuraminidase as one of its two coat proteins.

Discussion

There is considerable interest in the factors that influence protein glycosylation and the number and type of oligosaccharide units attached (Rosner *et al.*, 1980). Studies on influenza-virus haemagglutinins may provide some insight into these factors, since the virus can be propagated in a variety of cell types and is known to exhibit both host-cell- and virus-strain-dependent differences in carbohydrate type and composition (Schwarz *et al.*, 1977; Nakamura & Compans, 1978, 1979). Furthermore, the primary structures of several different haemagglutinins are now available (Porter *et al.*, 1979; Ward & Dopheide, 1980*a*; Dopheide & Ward, 1980; Gething *et al.*, 1980; Min-Jou *et al.*, 1980; Both *et al.*, 1980) and the three-dimensional crystal structure of one Hong Kong variant haemagglutinin will soon be completed (Wilson *et al.*, 1980).

As found for other glycoproteins (Marshall, 1972) the glycosylated asparagine residues in A/Mem/72 haemagglutinin occur in tripeptide sequences of the type Asn-X-Ser/Thr. However, unlike most other glycoproteins, all such potential glycosylation sites in this protein do carry carbohydrate. Generally only 20% of such sequences on average are glycosylated in other proteins (Hunt & Dayhoff, 1970). Examination of these tripeptide sequences further invalidates the suggestion (Jackson & Hirs, 1970) that the nature of the amino acid in position X dictates the type of oligosaccharide unit attached. As shown in Fig. 1, only two of the 'complex' type glycopeptides contain polar residues at position X, whereas glycopeptides with the same residue (glycine) in the X position carry 'simple' (HA_1·CN3) or 'complex' (HA_1·CN1·C2·Th4 and HA_2) sugar units. It is interesting to note, however, that the two tripeptides of identical sequence (HA_1·CN1·C2·Th4 and HA_2) have carbohydrate units of almost identical compositions attached.

As Clamp (1975) points out, other factors such as the relationship of the tripeptide sequence to the surface of the protein and the extended specificity requirements of the initial glycosyltransferase must also be involved in the final recognition of glycosylation sites. In ovomucoid, the four major glycosylation sites are close to regions predicted to have β-turn conformation (Beeley, 1976) and the same applies to A/Mem/72 haemagglutinin. The glycosylated asparagine residue in HA_2, and four of those in HA_1 (residues 8, 22, 81 and 285), occur in sequences with high β-turn potential (Ward & Dopheide, 1980*b*).

When the amino acid sequence for A/Mem/72 haemagglutinin is compared with that now available for the haemagglutinin from fowl plague virus (Porter *et al.*, 1979), the Asian influenza virus strain A/Jap/57 (Waterfield *et al.*, 1979; Gething *et al.*, 1980) and another Hong Kong influenza virus A/Vic/75 (Min-Jou *et al.*, 1980), it can be seen that the previously observed differences in type (Schwarz *et al.*, 1977; Nakamura & Compans, 1978, 1979) and amount (Waterfield *et al.*, 1979) of carbohydrate on haemagglutinins from different virus strains grown in the same cell type are probably due to differences in their amino acid sequences. A/Jap/57 HA_1 contains only four oligosaccharide units at positions 21, 33, 170 and 289 (numbers refer to the A/Mem/72 sequence) whereas fowl plague virus HA_1 contains five (at residues 22, 38, 158, 165 and 240). In addition, the carbohydrate groups attached in similar regions of the various proteins are of different types. That on Asn-285 in A/Mem/72 HA_1 is 'simple' (Table 1) whereas that on Asn-289 in A/Jap/57 HA_1 is 'complex' (Waterfield *et al.*, 1979). All oligosaccharide units on fowl plague virus HA_1 are believed to be of the 'complex' type (Schwarz *et al.*, 1977) but it is not known if all five potential glycosylation sites, predicted from the RNA sequence data, are utilized. With regard to their light chains, both A/Jap/57 and fowl plague virus haemagglutinins contain the glycosylation site at Asn-154, but unlike that of A/Mem/72 also contain an additional potential carbohydrate attachment site. In fowl plague virus HA_2 this second position (residue 82) probably carries carbohydrate, since both 'simple' and 'complex' oligosaccharides have been reported for this polypeptide (Schwarz *et al.*,

1977). In A/Jap/57 HA_2 it seems unlikely that there are two oligosaccharide units, since the second position (residue 213) occurs near the membrane-embedded tail region of HA_2.

Differences also exist between strains within the same subtype. Comparison of the A/Mem/72 haemagglutinin sequence with that of A/Vic/75 (Min-Jou *et al.*, 1980) shows that the latter has lost the threonine residue at position 83 in HA_1 and presumably has lost the carbohydrate unit attached to Asn-81. The data presented in this report represent the first stage in the characterization of the carbohydrate units of the Hong Kong influenza virus haemagglutinin. Studies are needed to establish which of these oligosaccharides constitute the so-called viral 'host antigen' (Ward *et al.*, 1980*b*) and which elicit the production of anti-carbohydrate antibodies (Jackson *et al.*, 1979).

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