The structure and role of the carbohydrate moieties of influenza virus haemagglutinin

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The haemagglutinin glycoprotein is the major surface antigen of influenza virus and is responsible for receptor binding, membrane fusion and antigenic variation. The 1968 Hong Kong haemagglutinin contains seven Asn-Xaa-Ser/Thr sequences, all of which are glycosylated (13000 daltons of carbohydrate per monomer, 20% carbohydrate by wt.). The three-dimensional structure of the haemagglutinin (Wilson et al., 1981) has shown the carbohydrate to be located on the surface of the trimer, distributed along the length of the 13.5 nm glycoprotein spike. The high-mannose moieties on HA1 (residues 165 and 285) are more clearly visible in the electron density map, whereas the complex oligosaccharides are less well defined (residues, 22, 38, 81 of HA1) or have little or no density in the native unrefined electron density map (residues HA1 8, HA2 154). The weak density is presumably due to positional disorder or heterogeneity of the carbohydrate.

The biological role of the carbohydrate is not yet clearly understood. Some of the carbohydrate chains may be structurally important and stabilize the tertiary or quaternary structure of the trimer. The carbohydrate also covers potential proteolytic cleavage sites. The oligosaccharides in different viral strains and subtypes may play a role in masking or altering the nature of the protein's antigenic sites and hence play a role in the process by which the virus escapes recognition by the immune system.

Fig. 1. Stereo view and scheme of the location of the glycosylation sequences on the haemagglutinin of influenza virus

The stereo view shows the carbon-a co-ordinates of HA1, 1-328, and HA2, 1-175, with the Asn-Xaa-Ser/Thr sequences likely to be glycosylated from different influenza strains 1933-1979 shows in larger circles. The scheme is a ribbon diagram with helices and ß-structure indicated and was produced by a computer program (Lesk & Hardman, 1982). The potential carbohydrate attachment sites are indicated by large filled circles on HA1, 1-328, and HA2, 401-575.

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Location and variation in carbohydrate

Analysis of the haemagglutinin sequences of 14 human A-viruses (two H1-WSn/33 (Hitzi et al., 1981); PR/8/34 (Winter et al., 1981); one H2-Jap/305/57 (Gething et al., 1988); 11 H3- (for a review 1967-1977 see Laver et al., 1980), two related viruses (B/Lee/1940; Krystal et al., 1982) show 32 potential carbohydrate attachment sites with Asn-Xaa-Thr/Thr sequences. One Asn-Pro-Ser (HA 1 144) sequence (JAP/305/57; Waterfield et al., 1980) is not glycosylated and two consecutive potential glycosylation sequences have the Asn glycosylated on only the first Asn (Asn*-Asn-Thr-Ser, HA1, 169-172, Jap/305/57; Brown et al., 1982) or the second (Asn-Asn*-Ser-Thr, HA1, 20-24; Waterfield et al., 1980). Two additional Asn-Xaa-Thr/Thr sequences are located in the membrane anchoring peptide. Of the 26 probable glycosylation sites, (Fig. 1) 21 have Asn-Xaa-Thr and five have Asn-Xaa-Ser sequences. The only regions where carbohydrate is present on all strains and subtypes examined are either around residues 20-22 or residues 33-38 of HA1 where the polypeptide part of the haemagglutinin has the smallest radius in the trimer. All the potential carbohydrate sites labelled in Fig. 1 are accessible to the surface of the haemagglutinin trimer and hence no buried carbohydrate is expected.

Conformation of the glycosylated peptides

The conformation of the polypeptide chain around six of the seven glycosylated asparagines for X:31 Hong Kong is shown in Table 1. The asparagines are in various secondary structures; two are in β-sheet, two in β-bends (one left-handed) and two in α-helical-type structures. Three of the sequences have Gly residues at position Xaa and these Gly residues have positive α-helical-type structures. Three of the sequences have Gly after two are in β-sheet, two in β-bends (one left-handed) and two in β-sheet, two in β-bends (one left-handed) and two in β-sheet, two in β-bends (one left-handed) and two in β-sheet, two in β-bends (one left-handed) and two in β-sheet, two in β-bends (one left-handed) and two in β-sheet, two in β-bends (one left-handed) and two in β-sheet.
The complex carbohydrate at Asn-81 follows closely the polypeptide chain of an adjacent strand, residues 120–122, whereas the two simple carbohydrates extended directly out from the surface of the molecule.

Role of the carbohydrate

The total number of glycosylation sites varies from five to nine oligosaccharides in those sequences analysed. In the Hong Kong X:31 haemagglutinin less than 20% of the total accessible surface area appears to be buried by the hexoses. However, seven potential chymotryptic and tryptic cleavage sites are partially covered by the carbohydrate, which may protect against such degradative enzymes. The carbohydrate at Asn-165 is at the trimer interface.

The location of regions on the haemagglubinin molecule to which antibodies bind and which are involved in antigenic variation have been proposed (Wiley et al., 1981) based on the structure of 1968 Hong Kong haemagglutinin. It was noted that some residues in these regions were sites of oligosaccharide attachment in other viral strains (e.g. fowl plague virus residue 133 (site A), 158 (site B); A/JAP/305/57 residue 240 (site D); A/PR/8/34 residue 169 (D); WSN/33 residues 129 and 131 (A/B)). This raises the possibility that variations in oligosaccharide attachment may play a role in antigenic variation by effectively masking certain regions of the molecular surface from interactions with the immune system. The loss of a carbohydrate site at Asn-81 and creation of a new site at Asn-63 which occurred between the 1968 and 1975 strains may have altered immunological recognition of that site (E?). Similarly, the presence of carbohydrate at residue 131 (site A/B) and its absence from residue 165 in the A/PR/8/34 strain may modify the size and extent of the two adjacent antigenic regions.

References


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