THE DISULPHIDE BONDS OF A HONG KONG INFLUENZA VIRUS HEMAGGLUTININ

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1. Introduction

The major antigen of influenza virus is a hemagglutinin [1,2] which attaches the virus to the cell surface receptors on infection [3]. This hemagglutinin consists of a heavy chain (HA1, mol. wt 47 000 [4]) and a light chain (HA2, mol. wt 30 000 [4]), which are linked by disulphide bonds [5]. Three hemagglutinin molecules join by hydrophobic bonding to form a trimeric spike [6,7], protruding from the viral membrane [8].

These features and functions imply a high level of structural organisation, in which the disulphide bonds would be expected to play an important role, especially since we have found 9 $Cys_{1/2}$ in HA1 [9] and 7 $Cys_{1/2}$ in HA2 [10].

This letter reports the isolation and pairing of disulphide peptides in influenza hemagglutinin. We show by referring to published sequence data [9,11] that 3 S–S bonds link the 2 chains, and that HA1 contains 3 intrachain S–S bonds.

The other 4 $Cys_{1/2}$ residues in the light chain occur in the hydrophobic tail [10] and can only be paired amongst themselves.

2. Materials and methods

Viral hemagglutinin (HA) was prepared as in [9]. HA was digested with thermolysin for 24 h at 37°C, in 0.1 M *N*-ethylmorpholine acetate (pH 8.0) at a 100:1 substrate to enzyme level.

Amino acids were analysed on a phthalaldehyde-based analyser; 100–200 pmol samples were hydrolysed at 110°C in 20 μl constant boiling HCl.

Diagonal peptide maps were prepared at pH 6.5 essentially as in [12]. The neutral spots were further resolved by diagonal mapping at pH 1.9.

The paper used was Whatman 3 MM, washed with 5% acetic acid to reduce possible background contamination, significant at these low levels of detection. Peptides were visualised on the maps with fluorescamine [13]. They were sequenced essentially by the micro-method in [14] with the modification, that after each cyclisation the thiazolinone was extracted with butylacetate and converted to the corresponding amino acid [15] which was characterised by amino acid analysis.

3. Results and discussion

Details of the peptide pairs isolated from the diagonal maps are shown in table 1. We have isolated peptides relating to 3 intrachain pairs on HA1 (52–277, 64–76 and 281–305) and 2 interchain pairs (HA1 14–HA2 137 and HA1 139–HA2 144). A third interchain pair is assumed to connect HA1 97 and HA2 148, although we have not isolated the HA1 partner of this pair. However, there are no other possibilities for pairing, apart from the 4 $Cys_{1/2}$ occurring in the C-terminal area of HA2 [10]. These can be eliminated as interchain candidates or from interaction with the other light chain $Cys_{1/2}$ by the fact that a truncated hemagglutinin, which only lacks the tail end of HA2, can be prepared by proteolytic digestion of whole virus [16].

We have no evidence concerning these terminal $Cys_{1/2}$ since procedures to disrupt this very aggregated and hydrophobic area are too drastic to allow the isolation of disulphide bonded pairs.

In fig.1 we show a diagrammatic arrangement of the disulphide pairs within the general framework of the sequence. It is obvious that the HA1 chain is

Table 1
Cysteic acid-containing peptides, obtained from A_2/Mem/72 influenza hemagglutinin through a diagonal mapping procedure; their mobilities before, Mob 1, and after, Mob 2, performic acid oxidation, and their sequence

Pair	Mob 1	Mob 2	Sequence	Source
1a	−0.35	−0.65	Leu−Cys^{14}	HA1
1b		−0.55	Leu−Arg−Glu−Asn−Ala−Glu−Asp−(Met O_2−Gly−Asn−Gly−Cys^{137})	HA2
2a	0.40	Neutral	Ile−Cys^{52}−Asn−Asn−(Pro−His−Arg)	HA1
2b		−0.45	Ile−Gly−Thr−Cys^{277}	HA1
3a	−0.60	−0.40	Ile−Asp−Cys^{64}−Thr	HA1
3b		−0.70	Leu−Gly−Asp−Pro−His−(Cys^{76}−Asp−Gly)	HA1
4a	Neutral[a]		Ile−Ser−Glu−Cys^{281}	HA1
4b			Gly−Ala−Cys^{305}−(Pro−Lys−Tyr)	HA1
5a	0.20	−0.55	(Lys−Cys^{144}−Asp)	HA2
5b		−0.05	(Ala−Cys^{139}−Lys−Arg−Gly−Pro−Asp)	HA1
6	Neutral	−0.60	(Asn−Ala−Cys^{148})	HA2
			Partner not resolved	

[a] Neutral peptides were further resolved through a pH 1.9 diagonal separation. Brackets indicate identifications on the basis of composition only. Mobilities are given with respect to the mobility of aspartic acid (−1.0 at pH 6.5)

The numbers over the $Cys_{½}$ residues correspond with the numbers of the $Cys_{½}$ in the chains [9,11]

structurally highly constrained through this disulphide bonding system. Comparison between the HA1 structures of A/Jap/57 [17] and A2/Mem/72 [18] shows a 55% difference in sequence, but also shows an absolute conservation of the $Cys_{½}$ relative positions. This observation can be extended to the first 3 $Cys_{½}$ in the HA2 chain [11]. This may be seen as evidence of the importance of the disulphide bonds in determining the spatial structure necessary for the function of the hemagglutinin.

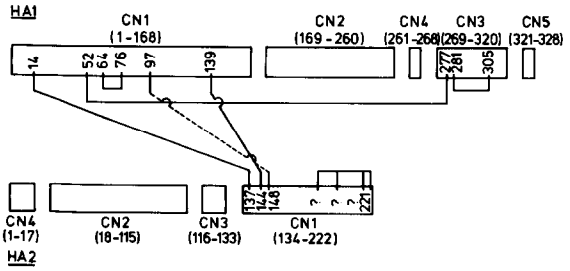

Fig.1. Diagrammatic structure of influenza hemagglutinin. The cyanogen bromide fragments are indicated as boxes, with the numbers within the boxes indicating the position of the $Cys_{½}$ residues in the sequence. The bracketed numbers refer to the position of each cyanogen bromide fragment within the protein sequence [9,11]. The dotted bridge is assumed, and question marks indicate $Cys_{½}$ which are present, but not placed.

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