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Influenza Virus Haemagglutinin. Structural Predictions Suggest that the Fibrillar Appearance is Due to the Presence of a Coiled-coil

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Abstract

Predictions of secondary structure for the two chains HA1 and HA2 of the haemagglutinin from the Hong Kong influenza virus A/Memphis/102/72 reveal a striking contrast between the potential conformations of the two chains. HA1 is predicted to be rich in β-structure while HA2 is highly helical. The predictions further suggest that coiled-coil type interactions between the central helical segments of the HA2 chains may hold the haemagglutinin monomers together in the virus.

Introduction

The haemagglutinin is the major coat protein of influenza virus and is the major antigen against which neutralizing antibodies are directed (Laver and Kilbourne 1966). Changes in antigenicity accompany the appearance of new influenza variants and are associated with changes in the amino acid sequence of the haemagglutinin (Laver *et al.* 1979, 1980). The haemagglutinin occurs in the intact virus, as 400–1000 (Tiffany and Blough 1970) densely packed spikes projecting out from the viral surface. Each spike is a trimer (Wiley *et al.* 1977) and when viewed under the electron microscope has the appearance of a triangular-shaped rod 120–160 Å (12–16 nm) long and 40–60 Å (4–6 nm) wide (Laver and Valentine 1969; Griffith 1975). The haemagglutinin monomer consists of a single heavy (HA1) and light (HA2) chain and each three monomers are held together in the spike in unstaggered parallel register with the hydrophobic tail region of their light chains embedded in the viral membrane (Skehel and Waterfield 1975).

Nothing is known about the nature of the interactions that hold the haemagglutinin monomers together in the spike or of the structural features which give the spike its fibril-like appearance. In this paper we have applied Chou and Fasman's empirical procedures of secondary structure prediction to the recently completed amino acid sequence data for the haemagglutinin chains from the Hong Kong influenza variant A/Memphis/102/72 (Dopheide and Ward 1978, 1979, 1980*a*; Ward and Dopheide 1979, 1980; Sleigh *et al.* 1980; Ward *et al.* 1980). We report a striking contrast between the predicted structures for the heavy (HA1) and light (HA2) chains and discuss these features in relation to the overall morphology of the haemagglutinin spike.

Predictions of Secondary Structure

The complete amino acid sequence of A/Memphis/102/72 haemagglutinin is shown in Fig. 1. It is coded for by a single monocistronic segment of RNA (Scholtissek

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HA1  Glx Asp Phe Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly His His Ala Val Pro Asn Gly Thr Leu Val Lys Thr Ile Thr
                                 CHO                                                                                     CHO                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
HA2  Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ser Glu
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Fig. 1. Amino acid sequence of heavy (HA1) and light (HA2) chains of A/Memphis/102/72 haemagglutinin. Half-cystine residues occur in HA1 at positions 14, 52, 64, 76, 97, 139, 277, 281 and 305 and in HA2 at positions 137, 144, 148, 195, 210, 217 and 220. Oligosaccharide units are attached at residues 8, 22, 38, 81, 165 and 285 in HA1 and 154 in HA2. Data is from Dopheide and Ward (1978, 1979, 1980*a*), Ward and Dopheide (1979, 1980), Ward *et al.* (1980) and Sleigh *et al.* (1980).

1978), and is synthesized as a single polypeptide chain (mol. wt 77000). It is proteolytically processed (Air 1979; Waterfield *et al.* 1979) to give the two chains HA1 and HA2 held together by disulfide bonds (Laver 1971; Dopheide and Ward 1980*b*). The Hong Kong HA1 contains 328 amino acid residues including six glycosylated asparagines. HA2 contains 221 amino acid residues including one glycosylated asparagine.

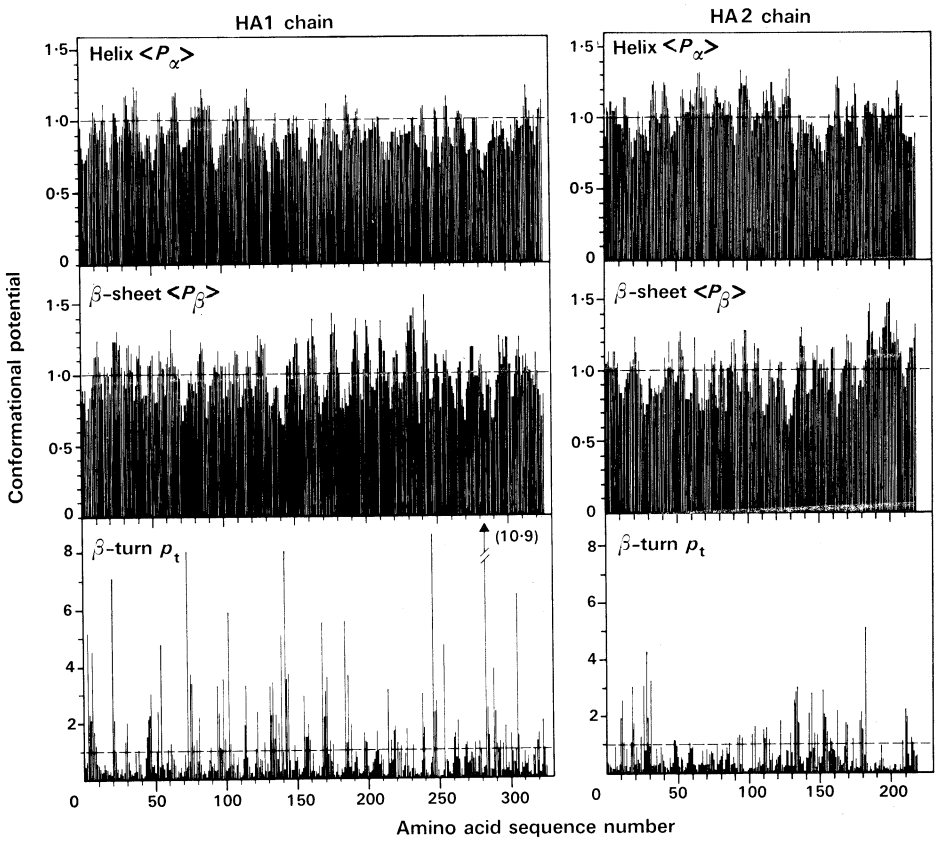

Fig. 2. Conformational profiles of heavy (HA1) and light (HA2) chains. The helical potential $\langle P_\alpha \rangle$ and β-sheet potential $\langle P_\beta \rangle$ at residue i are the sum of the appropriate P_α or P_β values at residue i to $i+3$ divided by 4 (Chou and Fasman 1978). Tetrapeptide values above 1·0 have helical or β-sheet potential. The β-turn potential, p_t, at residue i is obtained by the expression: $p_t = f_i \times f_{i+1} \times f_{i+2} \times f_{i+3} \times 10^4$ (Chou and Fasman 1978). Values above 1·0 have β-turn potential.

Secondary structure predictions were made using the algorithms of Chou and Fasman (1974). For helix and β-sheet predictions $\langle P_\alpha \rangle$ and $\langle P_\beta \rangle$ values were computed for runs of 4, 5 and 6 consecutive residues along the entire sequence using the parameters listed in Chou and Fasman (1978). For β-turns both $\langle P_t \rangle_4$ and p_t values were computed using the parameters given in Chou and Fasman (1977). All calculations were carried out on an Olivetti 101 desk-top computer. The distribution of polar and non-polar residues in the predicted helical regions of HA2 were examined by the helical net approach of McLachlan and Stewart (1975).

Results and Discussion

The predicted conformational profiles for the haemagglutinin chains are shown in Fig. 2 and the final assignments summarized in Table 1. These predictions place the haemagglutinin in the $\alpha+\beta$ class of protein structures (Levitt and Chothia 1976) typified by many extracellular proteins (Sternberg and Thornton 1978). The heavy chain HA1 is very rich in potential β-structure. It contains 19 β-strands including several found in sheets of three or more antiparallel strands connected by hairpin β-bends (residues 10–38, 108–133, 175–214 and 222–246). HA1 contains only four short sections of α-helix and 44 β-turns. The distribution of β-turns is fairly regular throughout HA1 and the number predicted in good agreement with that expected (43) from the linear relationship of Rose and Wetlaufer (1977).

Table 1. Predicted secondary structure for A/Memphis/102/72 heavy (HA1) and light (HA2) haemagglutinin chains

HA1 chain			HA2 chain		
α-helix	β-sheet	β-turn	α-helix	β-sheet	β-turn
	10–16	3–6, 6–9	2–10		11–14
	24–30	20–23, 30–33			18–21
39–45	33–38	46–50, 52–55			24–27
65–71		61–64, 73–76			26–29
	83–88	76–79, 81–84	34–40		28–31
89–94		94–98, 98–101	41–48		31–34
	108–113	102–105, 114–117	57–74	52–56	48–51
	117–122	122–125	77–92		93–96
	125–133	131–135, 135–138	96–103	104–111	104–107
		139–142, 142–145	113–123		110–113
	150–155	155–158	125–133		133–137
	163–168	158–162, 168–171		137–144	142–145
	175–184	171–174, 184–187			144–147
	191–198	198–201			148–151
	201–207	206–209			152–155
	209–214	214–217, 216–219			157–160
	222–226	219–222, 227–230		170–173	162–165
	230–238	238–241	174–180		168–171
	242–246	246–249, 253–257			179–182
		260–263, 263–166		185–211	182–185
	273–279	268–272, 278–281		214–221	210–213
		283–286, 288–291			
	294–298	292–295, 296–300			
	300–305	305–308			
313–318	308–312	320–324, 323–326			

The predicted conformations for the light chain are very different. The distribution of β-bends in HA2 is uneven and confined largely to the two ends of the polypeptide chain. There are very few potential β-turns in the region between residues 34 and 133 (see Fig. 2) and only four tetrapeptide sequences that fulfill β-turn requirements. As shown in Table 1 this central region of HA2 is predicted to be highly helical with 78 of the 100 residues occurring in seven sections of α-helix. Of the three short sections of assigned β-sheet in this region two contain sequences (residues 54–56 and 138–143) that favour α-helix almost as well as β-structure, and one contains a potential β-bend (residues 104–107).

Table 2. Heptad distribution of non-polar residues in the helix-favouring central region (residues 34–133) of A/Memphis/102/72 haemagglutinin light chain HA2
Positions *a* to *g* are the seven positions that make up every two turns of the helix (McLachlan and Stewart 1975). The hydrophobic residues in positions *a* and *d* are indicated. The corresponding arrangement for the first 154 residues of α-tropomyosin (McLachlan and Stewart 1975) is shown for comparison. The residues in positions *a* and *d* form a spiral hydrophobic spine down the length of the α-helices which can interact and hold the helices together in coiled-coils

Tropomyosin

a	b	c	d	e	f	g
Met	Asp	Ala	Ile	Lys	Lys	Lys^{7}
Met	Gln	Met	Leu	Lys	Leu	Asp
Lys	Glu	Asn	Ala	Leu	Asp	Arg
Ala	Glu	Glu	Ala	Glu	Ala	Asp
Lys	Lys	Ala	Ala	Glu	Asp	Arg
Ser	Lys	Gln	Leu	Glu	Asp	Glu
Leu	Val	Ser	Leu	Gln	Lys	Lys
Leu	Lys	Gly	Thr	Glu	Asp	Glu
Leu	Asp	Lys	Tyr	Ser	Glu	Ala
Leu	Lys	Asp	Ala	Gln	Glu	Lys
Leu	Glu	Leu	Ala	Glu	Lys	Lys
Ala	Thr	Asp	Ala	Glu	Ala	Asp
Val	Ala	Ser	Leu	Asn	Arg	Arg
Ile	Gln	Leu	Val	Glu	Glu	Glu
Leu	Asp	Arg	Ala	Gln	Glu	Arg
Leu	Ala	Thr	Ala	Leu	Gln	Lys
Leu	Glu	Glu	Ala	Glu	Lys	Ala
Ala	Asp	Glu	Ser	Glu	Arg	Gly
Met	Lys	Val	Ile	Glu	Ser	Arg
Ala	Gln	Lys	Asp	Glu	Glu	Lys
Met	Glu	Ile	Gln	Glu	Ile	Gln
Leu	Lys	Glu	Ala	Lys	His	Ile_{154}

A/Memphis/102/72 HA2

a	b	c	d	e	f	g				
			^{34}Gln	Ala	Ala	Asp^{37}				
Leu	Lys	Ser	Thr	Gln	Ala	Ala				
Ile	Asp	Gln	Ile	Asn	Gly	Lys				
Leu	Asn	Arg	Val	Ile	Glu	Lys	Thr	Asn	Glu	Lys_{62}
Phe	His	Gln	Ile	Glu	Lys	Glu				
Phe	Ser	Glu	Val	Glu	Gly	Arg				
Ile	Gln	Asp	Leu	Glu	Lys	Tyr				
Val	Glu	Asp	Thr	Lys	Ile	Asp				
Leu	Trp	Ser	Tyr	Asn	Ala	Glu				
Leu	Leu	Val	Ala	Leu	Glu	Asn	Gln	His	Thr_{107}	
Ile	Asp	Leu	Thr	Asp	Ser	Glu				
Met	Asn	Lys	Leu	Phe	Glu	Lys	Thr			
Arg	Arg	Gln	Leu	Arg	Glu	Asn				
Ala	Glu	Asp	Met_{133}							

Even more striking is the observation that there is a heptad distribution of non-polar residues in this region, similar (Table 2) to that found in α-tropomyosin (McLachlan and Stewart 1975) and other fibrous proteins (Parry *et al.* 1977; Doolittle *et al.* 1978). This raises the possibility that the three haemagglutinin monomers are held together in the spike by hydrophobic coiled-coil type interactions between these helical regions of their light chains. The marked absence of potential β-turns, which are generally situated at the solvent-accessible surface of proteins (Kuntz 1972), is consistent with this region forming a central core in the haemagglutinin spike. Similar interactions have been implicated in the structures of tropomyosin, α-keratin and fibrinogen which, like the influenza haemagglutinin (Wrigley *et al.* 1977), occur in parallel, unstaggered, symmetrical form.

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