Host Antigen as the Sulphated Moiety of Influenza Virus Haemagglutinin

By JEAN C. DOWNIE*

Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T. Australia

(Accepted 24 May 1978)

SUMMARY

Inorganic sulphate (35S) was incorporated into the haemagglutinin molecule of A/Memphis/1/71 (H3N2) influenza virus when a keratosulphate-like host antigen was also incorporated into the glycoproteins of virus grown in the chorioallantoic membrane of the embryonated hen’s egg. Little or no 35S-sulphate was incorporated when this host antigen was not present in the glycoproteins of virus grown in chick embryo kidney cells or in the chorioallantoic membrane of embryonated duck eggs.

The presence of the keratosulphate-like host antigen was required for the stability of the haemagglutinin molecule in sodium dodecyl sulphate (SDS). The haemagglutinin molecules from virus grown in hens’ eggs were stable in SDS, whereas those from virus grown in duck eggs or in chick embryo kidney cells were not and could not be isolated on cellulose acetate.

Chemical analysis showed that there were 87 glucosamine residues and three molecules of sulphate per haemagglutinin subunit as calculated for a trimer molecule having a mol. wt. of 200000. There was one sulphate molecule per HA1 polypeptide chain and this was associated with the slowest migrating carbohydrate-protein complex of an HA1 tryptic digest separated by polyacrylamide gel electrophoresis.

INTRODUCTION

There are at least three distinct antigenic determinants on the haemagglutinin molecule of influenza virus grown in the embryonated hen’s egg. Two of these, the common and specific determinants, are virus-coded (Laver et al. 1974), while the third is a host cell-coded antigen composed mainly of carbohydrate and covalently linked to the polypeptide chain of the virus protein (Laver & Webster, 1966). Laver and Webster also found this host antigen in uninfected cells of the chorioallantoic membrane of the chick embryo. Earlier, Harboe and co-workers (Harboe, 1963; Strandli et al. 1964; Haukenes et al. 1965) had described the isolation of a host factor from the allantoic fluid of embryonated hens’ eggs which was mainly carbohydrate in nature.

Meyer et al. (1967) later chemically analysed a mucopolysaccharide (CAFS) from the allantoic fluid of embryonated hens’ eggs infected with influenza virus which was similar to the host-factor of Harboe or the host antigen of Laver and Webster. It was found to be

* Present address: National Biological Standards Laboratory, Viral Products Section, Private Bag No. 7, Parkville, Victoria 3052 Australia.
chemically similar to a group of sulphated, uronic acid-free, proteoglycans called keratosulphates. Meyer suggested that the keratosulphate-like CAFS may represent a class of compounds widely distributed in epithelial mucins and cell membranes.

Therefore, since sulphate may be incorporated into influenza glycoproteins as part of the carbohydrate moiety coded for by the host cell, a comparison was made of incorporation of inorganic sulphate into the haemagglutinin of influenza virus grown in cells differing in their ability to synthesize the keratosulphate-like host antigen found in the allantoic fluid of the embryonated hen’s egg. The present study also estimates the number of glucosamine and sulphate residues per molecule of haemagglutinin and confirms the findings of Compans & Pinter (1975) on the incorporation of inorganic sulphate into the virus glycoproteins.

METHODS

Virus. An H3N2 strain of influenza virus, A/Memphis/1/71, which is similar to the prototype Hong Kong strain, was used. An antigenic hybrid possessing the haemagglutinin (HA) subunits of A/Memphis/1/71 influenza virus and the neuraminidase (NA) subunits of A/Bel/42 (HoN1) strain of influenza virus was prepared as previously described (Webster, 1970) and will be referred to as Mem/71-Bel. The virus was grown in the allantoic sac of 11-day-old chick embryos, in the surviving cells of the chorioallantoic membrane (CAM)-on-shell (Fazekas de St Groth et al. 1958), in monolayers of primary chick embryo kidney (CEK) cells, and in the allantoic sac of 14-day-old duck embryos.

The virus particles were purified by adsorption-elution on fowl erythrocytes followed by differential centrifugation and sedimentation through a 10 to 40% sucrose density gradient as previously described (Laver, 1969).

Radioactive labelling. Carrier-free inorganic 35S-sulphate (100 to 200 μCi) with specific activity of 93.9 μCi/mmol (Radiochemical Centre, Amersham, England) were inoculated into each egg at the time of virus inoculation. The eggs were incubated for 48 h at 36 °C before the allantoic fluids were harvested.

Six × 10⁶ cells of either CEK tissue culture cells or CAM-on-shell of 10-day-old chick embryos were grown on 60 mm Petri dishes. The CAM of 10-day-old chick embryos has been estimated to contain 30 × 10⁶ cells (Cairns & Fazekas de St Groth, 1957). Thus the CAM-on-shell of 10-day-old embryos was cut into five equal pieces and each piece (6 × 10⁶ cells) was placed in a 60 mm Petri dish and treated as for CEK tissue culture cells. The cells were inoculated with 10⁷.2 EID₅₀ of Mem/71-Bel influenza virus and 50 to 90 μCi of ³⁵S-sulphate per plate. Both the CEK cells and CAM-on-shell cells were incubated in an humidified incubator for 48 h at 36 °C in the presence of 5% CO₂.

The incorporation of ³⁵S-sulphate into acid precipitable material was measured in a Packard Tri-Carb liquid scintillation spectrometer.

Titration of virus. Haemagglutination (HA) titrations and haemagglutination inhibition (HI) tests were done as previously described (Fazekas de St Groth & Webster, 1966). HI tests for the detection of host antigen in the virus particle were done as described by Laver & Webster (1966).

Antiserum. Hyperimmune antiserum to the isolated HA subunits and to host antigen was raised in rabbits as previously described (Laver & Webster, 1966; Laver et al. 1974).

Isolation of the haemagglutinin subunits and separation of the HA1 and HA2 polypeptides. The haemagglutinin subunits were isolated by electrophoresis on cellulose acetate strips as previously described (Laver, 1964), and the HA1 and HA2 polypeptide chains were
Sulphated influenza virus host antigen

separated by centrifugation on a guanidine hydrochloride-dithiothreitol (DTT) density gradient as described by Laver (1971).

Haemagglutinin subunits were also released from the virus particle by bromelain digestion and purified by sucrose density gradient centrifugation (Brand & Skehel, 1972).

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was done as previously described (Laver, 1971) using 7% acrylamide. Gels were stained for protein with Coomassie brilliant blue dye (Imperial Chemical Industries; Laver, 1971) and for carbohydrate by the periodic acid–Schiff reagent method as modified by Zacharius et al. (1969). After staining, the gels were sliced vertically, dried and exposed to RP Royal X-Omat film (Kodak) for 1 to 3 weeks.

Protein assay. Protein was assayed by the method of Lowry et al. (1951) using five times crystallized ovalbumin (Swartz-Mann) as standard.

Hexosamine assay. Samples of protein (50 to 300 μg) were hydrolysed with 4 N-HCl in sealed evacuated tubes for 4 h at 100 °C. Hexosamine was assayed by the method of Rondle & Morgan (1955) as modified by Kraan & Muir (1957). D-Glucosamine-HCl (BDH) was used as standard. The hexosamine detected by this assay method was assumed to be glucosamine and will be referred to as such in the text.

Inorganic sulphate determination. Mem/71-Bel influenza virus was grown in embryonated hens' eggs in the presence of 35S-sulphate which had been allowed to equilibrate for 4 h before the virus was inoculated. After incubation of the eggs for 48 h at 36 °C, the virus was recovered from the allantoic fluid by adsorption on to fowl erythrocytes and the resulting supernatant was dialysed overnight at 4 °C against five times its own volume of distilled water. The dialysate was made acid with 4 N-HCl and inorganic sulphate was precipitated with BaCl₂ according to the method of Lloyd (1961). The resulting Ba₃₅SO₄ precipitate was washed four times with 0.1 N-HCl and dried overnight in a hot-air oven (60 °C). Triplicate samples (300 to 500 μg) of Ba₃₅SO₄ precipitate were weighed on a Sartorius micro-balance and resuspended in distilled water. The suspensions were then sonicated for 1 min before the number of disintegrations per min for 35S contained in 1 μg and 10 μg of precipitate were counted in a Packard Tri-Carb liquid scintillation spectrometer.

Tryptic peptides. Fractions from the guanidine hydrochloride gradients containing the ³⁵S-labelled HA1 polypeptide chain of A/Memphis/1/71 HA subunits were pooled, dialysed against saline and precipitated with ethanol. The protein was then digested with 0.1% (w/v) trypsin (Trypsin-TPCK, Worthington Biochemicals) and the tryptic peptides soluble at pH 6.5 were separated by electrophoresis on 14% polyacrylamide gels. After staining for protein (Coomassie blue) or carbohydrate (periodic acid–Schiff reagent) the gels were sliced vertically, dried and exposed to RP Royal X-Omat film (Kodak) for 3 weeks.

RESULTS

Incorporation of ³⁵S-sulphate into the virus particle and HA subunits

Purified Mem/71-Bel virus grown in embryonated hens' eggs was extracted twice with chloroform/methanol (2:1) and the residue was tested for radioactivity. The results showed that 99% of the ³⁵S-label remained in the defatted, acid-precipitable fraction.

20000 HA units of purified virus (5500 ct/min) were disrupted with SDS and subjected to electrophoresis on cellulose acetate strips. The results showed that 64 to 77% of the ³⁵S-label incorporated into the virus particle was located in the main HA subunit fraction. ³⁵S-labelled HA subunits were also isolated by digestion of the virus particle with bromelain followed by centrifugation on a sucrose density gradient. The results shown in Fig. 1...
Fig. 1. Incorporation of $^{35}\text{S}$-sulphate into the HA subunits of Mem/71-Bel influenza virus released by bromelain digestion and purified on a 5 to 20% (w/v) sucrose density gradient. Nineteen fractions were collected from a hole in the bottom of the tube and each was tested for protein (2 μl) on cellulose acetate by staining with Coomassie blue and for the incorporation of $^{35}\text{S}$-sulphate (25 μl) into acid precipitable material. Fractions containing protein (9 to 13) were pooled, dialysed against 0.15 M-NaCl and identified as A/Mem/1/71 HA by double immunodiffusion using hyperimmune rabbit serum.

indicate that the $^{35}\text{S}$-label was incorporated into that portion of the virus haemagglutinin which was released by the enzyme. This portion has lost the hydrophobic property of the HA molecule but contains the region where the antigenic determinants are located.

Fig. 2 shows the separation of $^{35}\text{S}$-labelled HA subunits on a guanidine hydrochloride density gradient into the HA1 and HA2 polypeptide chains. Seventy per cent of the $^{35}\text{S}$-label was incorporated into the HA1 polypeptide chain (fractions 14 to 19), while only 13% was incorporated into the HA2 polypeptide chain (fractions 6 to 9). The ratio of HA1 to HA2 for protein and glucosamine was 2:1, while for incorporation of $^{35}\text{S}$-sulphate it was 5:1.

Incorporation of $^{35}\text{S}$-sulphate into virus grown in tissue culture and embryonated eggs

The host antigen which is covalently linked to the HA of influenza virus grown in the embryonated hen’s egg has not been demonstrated in influenza virus grown in other
Fig. 2. Separation of the HA1 and HA2 polypeptide chains of Mem/71-Bel influenza virus haemagglutinin on a guanidine hydrochloride density gradient. Twenty fractions were collected from a hole in the bottom of the tube and each was tested for protein by spotting 2 μl on to cellulose acetate and staining with Coomassie blue and for incorporation of 35S-sulphate (10 μl) into acid precipitable material. Counts per min (ct/min) and % of total 35S-sulphate incorporated (INC) were determined for each fraction. The cellulose acetate strip was also developed by autoradiography.

epithelial tissue or in the embryonated duck’s egg (Laver & Webster, 1966). Therefore, to determine whether or not 35S incorporation varied with the host cell, virus was grown in the presence of 35S-sulphate in either CEK tissue culture cells or in embryonated ducks’ eggs and was compared with virus similarly grown in the surviving cells of CAM-on-shell or in embryonated hens’ eggs.

HA units (2000 to 5000) of purified virus from each host cell type were tested for the incorporation of 35S-sulphate into acid-precipitable material. The results, shown in Table 1, indicate that incorporation of 35S into virus grown in CAM-on-shell or embryonated hen’s egg was 70 to 95% greater than that of virus grown in CEK cells or embryonated ducks’ eggs.

To test whether or not the low level of 35S-label (132 ct/min) detected in the acid-precipitable material of CEK grown virus had been incorporated into the HA subunit,
Table 1. Incorporation of $^{35}$S-sulphate into Mem/71-Bel influenza virus grown in various host cells

<table>
<thead>
<tr>
<th>Host cell</th>
<th>Presence* of host antigen</th>
<th>Concentration† of $^{35}$S-sulphate</th>
<th>HA units tested</th>
<th>$^{35}$S$^\ddagger$ incorporated§ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonated hen's egg</td>
<td>1/240</td>
<td>100 $\mu$Ci/egg</td>
<td>3000</td>
<td>662</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000</td>
<td>1089</td>
<td></td>
</tr>
<tr>
<td>Embryonated duck's egg</td>
<td>&lt; 1/10</td>
<td>100 $\mu$Ci/egg</td>
<td>3000</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>CAM-on-shell</td>
<td>1/240</td>
<td>50 $\mu$Ci/plate</td>
<td>2000</td>
<td>484</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86 $\mu$Ci/plate</td>
<td>2000</td>
<td>868</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000</td>
<td>1286</td>
<td></td>
</tr>
<tr>
<td>CEK tissue culture cells</td>
<td>&lt; 1/10</td>
<td>50 $\mu$Ci/plate</td>
<td>2000</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86 $\mu$Ci/plate</td>
<td>2000</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5000</td>
<td>257</td>
<td>20</td>
</tr>
</tbody>
</table>

* The virus grown in each cell type was titrated against an anti-chick CAM serum as described by Laver & Webster (1966).
† Concentration of $^{35}$S-sulphate used to label the virus as described in Methods.
‡ Counts per min of $^{35}$S-incorporated into acid-precipitable material as measured in a Packard Tri-Carb liquid scintillation spectrometer.
§ % of $^{35}$S-sulphate incorporated into non-host antigen containing virus as compared to virus containing host antigen.

2000 HA units of CEK and CAM-on-shell grown virus were applied to cellulose acetate strips and electrophoresed in the presence of SDS. These results are shown in Fig. 3(a, b). Virus grown in CAM-on-shell cells (Fig. 3a) showed a protein and $^{35}$S-sulphate incorporation profile similar to virus grown in the embryonated hen's egg. Seventy-one per cent of the total $^{35}$S-sulphate incorporation into the virus was recovered in fractions 3 and 4 which correspond to the HA subunits. On the other hand, it was found that the HA of CEK grown virus was not stable in SDS (Fig. 3b) and was not recovered in active form on cellulose acetate. The HA of virus grown in embryonated ducks' eggs was also not stable in SDS and gave a similar electrophoretic pattern as CEK grown virus on cellulose acetate.

These results indicate that $^{35}$S-sulphate was probably incorporated into the influenza virus HA as part of the host antigen component and that the stability of the HA molecule was dependent on the nature of this carbohydrate moiety.

Estimation of glucosamine and sulphate residues in the HA molecule

Mem/71-Bel influenza virus was grown in embryonated hens' eggs in the presence of $^{35}$S-sulphate. Purified HA subunits were isolated on cellulose acetate and assayed for protein, glucosamine and the incorporation of $^{35}$S-sulphate in acid-precipitable material. Inorganic sulphate was precipitated from the allantoic fluid of the harvested virus as described in Methods. It was assumed that the ratio of $^{35}$S to $^{32}$S was the same in the virus HA molecule as it was in the allantoic fluid.

The results for two experiments showed that there were 87 molecules of glucosamine and 3 molecules of sulphate per HA subunit as calculated for a trimer molecule having an average mol. wt. of 200,000 (Table 2). The glucosamine assay of this (9.4% of protein) and a previous study (9.1% of protein, Laver, 1971), indicated that the theoretical number of glucosamine residues per HA molecule with a mol. wt. of 200,000 would be 85 to 87.

The HA1 and HA2 polypeptide chains of HA subunits isolated on cellulose acetate were separated on a guanidine hydrochloride density gradient and it was estimated that there was one molecule of sulphate per HA1 polypeptide chain (Table 3).
Fig. 3. Incorporation of $^{35}$S-sulphate into CAM-on-shell and CEK grown Mem/71-Bel influenza virus. Purified virus was disrupted with 1% SDS and electrophoresed on a cellulose acetate strip for 4 h at 125 V. One half of the strip was stained for protein with Coomassie blue and the other was cut into 13 horizontal strips 8 cm wide which were dried and counted for incorporation of $^{35}$S-sulphate. Counts per min (ct/min) and % of total $^{35}$S-sulphate incorporated (INC) were determined for each strip. (a) 2000 HAU (868 ct/min) of CAM-on-shell grown MEM/71-Bel virus. (b) 2000 HAU (131 ct/min) of CEK grown Mem/71-Bel virus.

Table 2. Estimation of the number of glucosamine and sulphate residues per molecule of influenza virus haemagglutinin

<table>
<thead>
<tr>
<th></th>
<th>Protein†</th>
<th>Glucosamine†</th>
<th>$^{35}$S‡</th>
<th>µg per 2458 ct/min of $^{35}$S</th>
<th>No. of molecules* x 10$^{14}$</th>
<th>No. of molecules per HA molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol. wt.</td>
<td>200000</td>
<td>215.65</td>
<td>35</td>
<td>25.8</td>
<td>0.774</td>
<td>86.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.42</td>
<td>67.30</td>
<td>3.02</td>
</tr>
</tbody>
</table>

* As calculated from the formula: no. of molecules = Avogadro’s no. mol. wt. x $10^8$ x µg per 2458 ct/min of $^{35}$S.
† Virus haemagglutinin.
‡ Ba$^{35}$SO$_4$ precipitate.

Tryptic peptides

An attempt was made to locate the sulphate moiety on the HA molecule. The HA1 $^{35}$S-labelled polypeptide chain isolated on a guanidine hydrochloride density gradient was digested with trypsin and the tryptic peptides soluble at pH 6.5 were separated by polyacrylamide gel electrophoresis. These results are shown in Fig. 4. There were three bands which stained for both carbohydrate and protein (C-P1, C-P2, C-P3) to a greater or lesser extent and a fourth band which stained only for protein (P4). $^{35}$S-sulphate appeared to be
Table 3. *Estimation of the number of molecules of sulphate per molecule of HA1 and HA2 polypeptide chain*

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Mol. wt.</th>
<th>µg of protein per 1828 ct/ min of 35S</th>
<th>No. of molecules per I828 ct/min</th>
<th>No. of molecules 35S* per molecule poly peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA1</td>
<td>47 000†</td>
<td>15.80</td>
<td>2.02</td>
<td>1.16</td>
</tr>
<tr>
<td>HA2</td>
<td>28 000†</td>
<td>36.56</td>
<td>7.82</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* As calculated for 2.34 × 10^{14} molecules of 35S giving 1828 ct/min.
† Ward & Dopheide, 1976.

Fig. 4. Polyacrylamide gel electrophoresis of HA1 tryptic peptides. The 35S-labelled HA1 polypeptide chain of Mem/71-Bel influenza virus was isolated on a guanidine hydrochloride density gradient and digested with trypsin. Tryptic peptides soluble at pH 6.5 were separated by electrophoresis on 14% polyacrylamide gels. After staining for protein (P) with Coomassie blue or carbohydrate (C) with periodic acid-Schiff reagent, the gels were sliced vertically and developed by autoradiography (A).
mainly associated with the slowest of the carbohydrate–protein complexes (C-P1). There were two other fainter bands on the autoradiograph, A2 and A3, but the detection of these could not be repeated.

The staining procedures considerably altered the size of the gels (the protein stain shrinking and the carbohydrate stain swelling the gels) making direct comparison difficult. Therefore it was attempted to photograph the gels to the same overall length. The autoradiograph was taken from the carbohydrate stained gel.

**DISCUSSION**

Inorganic sulphate (\(^{35}\)S) was incorporated into influenza virus haemagglutinin when the virus was grown in the chorioallantoic membrane of the embryonated hen’s egg, a membrane known to contain a carbohydrate host component similar to that which is incorporated into the virus glycoproteins (Laver & Webster, 1966). When influenza virus was grown in cells that do not contain this host antigen (CEK tissue culture cells or the CAM of the embryonated duck’s egg), 70 to 95 % less \(^{35}\)S-sulphate was incorporated into the virus particle. The difference in incorporation of the \(^{35}\)S-label between virus grown in duck and chick embryos was not due to a difference in the amount of inorganic sulphate present in these cells for both cell types contained an equal amount of unbound sulphate. The difference was probably due to the nature of the glycosyl transferases present in the two types of chorioallantoic membranes.

It was also found that the haemagglutinin of CEK and duck embryo grown influenza virus was not stable in SDS and HA subunits could not be isolated on cellulose acetate. Both Haslam *et al.* (1970) and Compans *et al.* (1970) noted that the stability and electrophoretic mobility of the haemagglutinin depended on the host cell in which the virus was grown and suggested that this might be due to the carbohydrate moiety varying with the host cell.

There is considerable evidence that this carbohydrate moiety of influenza virus and purified haemagglutinin grown in the embryonated hen’s egg is composed of only four sugars; N-acetyl glucosamine, galactose, mannose and fucose, and that neither galactosamine nor uronic acid is incorporated into the influenza virus glycoproteins (Ada & Gottschalk, 1956; Frommhagen *et al.* 1959; Laver & Webster 1966; Ward & Dopheide, 1976; Allan *et al.* 1977). It was therefore assumed that the hexosamine detected by the Rondle & Morgan (1955) assay method was entirely glucosamine and that the host antigen component of the virus glycoprotein was uronic acid free.

Chemical analysis of Mem/71-Bel influenza virus haemagglutinin showed that there were 87 glucosamine residues per HA subunit having an average mol. wt. of 200000. This would allow 29 residues of glucosamine per HA monomer (HA1 plus HA2) and is in good agreement with the findings of Ward & Dopheide (1976) who found 26 residues by amino acid analysis. It was also found that there were three molecules of sulphate per haemagglutinin subunit and that this sulphate was predominately associated with the HA1 polypeptide chain. Tryptic digests of the HA1 polypeptide chain separated by polyacrylamide gel electrophoresis suggest that the sulphated moiety was mainly associated with the slowest of the three protein–carbohydrate complexes detected.

Keratosulphate is a term used to describe a family of closely related proteoglycans rather than to describe a single compound. Their basic structure is that of a linear polysaccharide made up of repeating units of monosulphated N-acetyl glucosamine polymerized to galactose through \(\beta 1,3\) linkages (Gottschalk, 1972). They are esterified by sulphate
predominately in the 6 position of the N-acetyl glucosamine residue (Bhavanandan & Meyer, 1967). Other monosaccharide components are mannose, fucose and sialic acid but their position on the polysaccharide chain is unknown. The main difference between the two major classes of keratosulphates is that skeletal keratosulphates also contain galactosamine whereas corneal keratosulphates do not (Lindahl & Roden, 1972).

Therefore, it seems probable for the following reasons, that the haemagglutinin of influenza virus grown in the embryonated hen’s egg contains a carbohydrate moiety which most resembles that of the corneal keratosulphates. Firstly, the sugar composition of influenza virus haemagglutinin is very similar to that of the corneal keratosulphates. Although sialic acid has not been found in influenza virus, it has been suggested that one of the functions of the enzyme neuraminidase is to remove sialic acid from the envelope of the virus (Klenk et al. 1970). Secondly, since sulphate has been found to be incorporated into the keratosulphate-like CAFS almost entirely as N-acetyl-glucosamine-6-sulphate and that its molar ratio to glucosamine was close to one (Meyer et al. 1967), the present findings would suggest that there could be one sulphated glucosamine residue per HA1 polypeptide chain. Thirdly, it also seems likely that there would be at least one molecule of galactose available to form one keratosulphate disaccharide unit per HA1 polypeptide chain since Ward & Dopheide (1976) found ten molecules of galactose in the HA1 polypeptide chain of A/Memphis/102/72 influenza virus; a strain which is antigenically related to A/Memphis/1/71 influenza virus.

This paper confirms and extends the findings of Compans & Pinter (1975) that inorganic sulphate is incorporated into the haemagglutinin of influenza virus, but suggests that it is incorporated probably as the N-acetyl-glucosamine-6-sulphate ester of the keratosulphate-like host antigen found in the CAM of chick embryos. This sulphated host antigen component is probably incorporated into the virus glycoproteins by the host cell’s own glycosyl transferase complexes. Cells which lack the particular glycosyl transferase involved in forming the sulphate ester of N-acetyl glucosamine will not contain this keratosulphate moiety in their cell membranes and influenza viruses grown in such cells will not incorporate sulphate into their glycoproteins.

The author would like to thank Dr Joan Fildes for analysing the purity of the BaSO₄ precipitate.

REFERENCES


Sulphated influenza virus host antigen


(Received 28 February 1978)