Internal Proteins of Influenza Virus: $^{35}$S-Methionine Peptide Maps as Genetic Markers


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(Accepted 25 April 1977)

SUMMARY

Methods are described for the preparation in vivo of $^{35}$S-methionine-labelled influenza viruses, the purification of the nucleoprotein (NP) and matrix (M) proteins and the separation of peptides obtained by protease digestion by two-dimensional thin-layer chromatography. The maps of the M proteins of A/Okuda/57 (H2N2) and A/Finland/4/74 (H3N2) were very similar overall but differed in three peptides. Hence they could be clearly distinguished. Maps of the NP proteins of the same strains showed a greater number of differences.

A recombinant strain having the haemagglutinin and neuraminidase of the A/Finland/4/74 parent and the virulence of the A/Okuda/57 parent was shown to have the M and P proteins of A/Okuda/57.

INTRODUCTION

Influenza viruses are distinguished by having nucleoprotein (NP) antigens which have type A, B or C specificity. Except by refined serological analysis (Lief, Fabiyi & Henle, 1958), type A specific NP antigens from strains carrying different haemagglutinin or neuraminidase antigens cannot be distinguished from each other. More recently Schild (1972) has shown that the other major internal protein of influenza type A viruses, the matrix (M) protein, is also type specific.

Influenza type A viruses are well known to undergo genetic interactions. However, the extent of these interactions cannot be fully appreciated unless each gene product can be distinguished and assigned to a particular parent. Up to the present time the majority of such studies have been limited to the haemagglutinin and neuraminidase. We report in this paper methods which have enabled us to analyse the NP and M proteins by mapping their $^{35}$S-methionine peptides. These methods have been used to identify the parents of the NP and M proteins of a potential live vaccine strain.

METHODS

Viruses. The influenza strains used for peptide mapping were A/Okuda/57 (H2N2), A/Finland/4/74 (H3N2) and a recombinant strain designated WRL 105 which was cloned from the progeny of a mixed infection between these two viruses (McCahon, Beare & Stealey, 1976). WRL 105 has the haemagglutinin and neuraminidase of A/Finland and the avirulence for man of A/Okuda. All viruses were received from Dr N. B. Finter as allantoic fluid and were not repassaged during this study. A/FPV/Rostock/34 (Hav1N1) and A/Japan/
305/57 (H₂N₂) were also used. They were grown and purified as previously described (Kelly & Dimmock, 1974).

**Preparation of ³⁵S-methionine-labelled virus.** Viruses were grown in 12-day-old de-embryonated chicken's eggs by a modification of the method described by Bernkopf (1949). Routinely we used 10 eggs per strain. A circular section of the shell plus membranes was cut with scissors from the pointed end of the egg and the embryo tipped out so that the chorioallantoic membrane remained attached to the shell. The membrane was rinsed twice with standard medium (Fazekas de St Groth & White, 1958) and drained. The hole was then covered with an aluminium foil cap which was sealed with melted paraffin wax. Each egg was injected through the cap with 5 ml standard medium, 0·1 ml virus and 200 μCi ³⁵S-methionine (sp. act. 280 Ci/mmol; Radiochemical Centre, Amersham, U.K.). Eggs were held horizontally between two egg trays which were rotated at 1 rev/7 min on a modified tissue culture tube roller machine at 33 °C for about 17 h. The medium was then removed, chilled and centrifuged at 15000 rev/min for 5 min at 4 °C to remove debris. Virus multiplication was checked by assaying virus haemagglutinin with doubling dilutions in plastic trays and 0·5% chicken erythrocytes.

**Purification of ³⁵S-methionine virus.** About 50 ml virus was precipitated by adding solid (NH₄)₂SO₄ to 60% saturation at 4 °C with constant stirring. After 20 min the precipitate was collected by centrifugation at 10000 rev/min in the MSE 6 × 100 angle rotor for 30 min at 4 °C. The pellets could be completely solubilized in 7 ml phosphate buffered saline (PBS), pH 7. Virus was then layered on to a single 50 ml gradient of 15 to 45% (w/v) sucrose in 0·15 M-NaCl, 0·01 M-tris, pH 7·4 and centrifuged in an MSE 3 × 70 swing-out rotor at 23500 rev/min for 75 min at 4 °C. Fractions of 2 to 3 ml were taken from the gradient and assayed for haemagglutinin and radioactivity. Where the peaks of activity coincided, at the middle of the gradient, fractions were pooled. To these was added 1 mg unlabelled purified influenza type A virus and the sucrose diluted about four-fold. Virus was pelleted in the MSE 8 × 50 angle rotor at 30000 rev/min for 3 h at 4 °C.

**Purification of nucleoprotein (NP) and matrix protein (M)**

**Chromatography through concanavalin A-Sepharose.** Virus glycoproteins, haemagglutinin (HA) and neuraminidase (NA) were separated from non-glycosylated proteins by their affinity for the plant lectins, a principle described by Hayman, Skehel & Crumpton (1973). This separation is necessary since the glycoproteins have mol. wt. which are similar to NP or M and therefore could not be separated by the polyacrylamide gel electrophoretic procedure described below. We used concanavalin A (Con A) linked to Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) and dissociated the virus by heating at ~ 100 °C in 2·5% (w/v) SDS in PBS for 1·5 min. This was then dialysed overnight at room temperature against a 50-fold excess of 0·25% SDS, 0·14 M-NaCl and 0·1 M-tris, pH 7·5. Con A-Sepharose (5 ml) was packed under gravity into a 10 mm internal diam. column and equilibrated with 20 ml 0·1 M-tris, 0·05% (w/v) SDS, pH 7·5. The virus proteins were heated to ~ 100 °C, cooled and applied to the column. Non-glycosylated proteins were eluted with washing buffer and collected in 1 ml fractions. These were assayed for radioactivity and peak fractions were pooled.

**Polyacrylamide gel electrophoresis.** Proteins eluting from the Con A column were separated by electrophoresis in a slab of polyacrylamide gel. The apparatus was based on that of Studier (1973) and the buffer system on that of Laemmli (1970). The gel was cast between two glass plates (20 × 20 cm) separated by strips of silicone rubber 2 mm thick. The gel consisted of 10% acrylamide, 0·26% bisacrylamide, 0·1% (w/v) SDS, 0·5 M-urea,
Peptide maps of NP and M proteins

0.375 \text{M-tris-HCl, pH 8.6, 0.0003\% (v/v) TEMED and 0.07\% (w/v) ammonium persulphate. The gel was polymerized under water-saturated butanol. This was removed and 10 ml stacker gel was added [4.5\% acrylamide, 0.12\% bisacrylamide, 0.4\% (w/v) SDS, 0.5 \text{M-tris-HCl, pH 6.8, 0.003\% (v/v) TEMED and 0.07\% (w/v) ammonium persulphate]. Electrophoresis buffer solution contained 0.1\% (w/v) SDS, 0.02\% (w/v) glutathione, 0.05 \text{M-tris, 0.38 M-glycine, pH 8.6.}}

Virus proteins were denatured under reducing conditions with 1.4\% (w/v) SDS, 1 \text{M-urea and 1\% \text{\textbeta-mercaptoethanol by heating to \sim 100 °C for 2 min. Up to 10 ml of this was loaded on to the gel and electrophoresed for 13 h at 60 V. The gel was then supported on filter paper and dried under vacuum between sheets of silicone rubber in a water bath at about 95 °C. Before autoradiography, radioactive markers were spotted on to the gel so that the film could be re-located accurately after it had been developed.}

Protease digestion. A tracing of the autoradiograph was aligned with the gel so that NP and M proteins could be excised. Accuracy was checked by re-exposing the gel. The gel slices were then processed by a modification of the method used by Clegg, Brzeski & Kennedy (1976). They were washed by shaking continuously with 500 ml 25\% ethanol and 10\% acetic acid in water overnight at room temperature, with fresh solution for a further 2 h and with 10 \text{mM-ammonium bicarbonate for 2 cycles of 90 min. The filter paper backing was removed and the gel placed in 8 ml 10 \text{mM-ammonium hydroxide (giving approx. pH 8). Proteins were digested with 50 \mu g/ml trypsin (treated with 1.1-tosylamido-2-phenylethyl chloromethyl ketone from Worthington Biochemical Corp., Freehold, N.J., U.S.A.) or with 25 \mu g/ml trypsin+25 \mu g/ml chymotrypsin in ammonium hydroxide on a rolling machine at 37 °C for 6 h. The gel was re-extracted with two successive vol. of 8 ml 10 \text{mM-ammonium hydroxide at 37 °C for about 2 h each. The digest and the eluates were pooled and incubated for a further 4 h at 37 °C, centrifuged to remove debris and then lyophilized. Peptides were oxidized at \sim 8 °C with performic acid (Bray & Brownlee, 1973). The sample was then dried, re-dissolved in water and taken through three successive lyophilizations before dissolution in 30 \mu l water. This was stored at \sim 70 °C.}}

Peptide mapping. Peptides were separated in two dimensions by chromatography on silica gel-coated glass plates (20 × 20 cm, 0.25 mm gel thickness from Schleicher and Schüll, Dassel, West Germany). The solvent in the first dimension was methyl acetate/isopropanol/25\% ammonium hydroxide (3:2:2 by vol.) and in the second was butan-1-ol/acetic acid/water (3:1:1 by vol.). Plates were dried in an oven after chromatography in the first dimensions. A dye marker (xylene cyanol FF) was run to a predetermined location. The silica gel was autoradiographed with Kodirex film (Kodak Ltd) to locate radioactive peptides. Usually 20,000 ct/min were applied to each plate which then required an exposure of about 20 days.

Neuraminidase assay. Activity was measured using fetuin as substrate by the method described by Webster & Laver (1967) in which the development of a coloured product is measured at 549 nm.

RESULTS

Multiplication and labelling of virus in de-embryonated eggs

Far more \textsuperscript{35}S-methionine was incorporated into virus grown in de-embryonated eggs than in embryonated eggs resulting in an improved specific activity (ct/min/\mu g virus protein) in excess of 1000-fold. However, the yield of virus was always less in de-embryonated eggs by 5- to 10-fold. Virus (A/FPV) grown in de-embryonated eggs without radiolabel was purified and shown to have the same major proteins by polyacrylamide gel electrophoresis.
Fig. 1. Polyacrylamide gel electrophoresis of A/FPV grown in (a) embryonated eggs and (b) de-embryonated eggs. Gels were cast in tubes of 7 mm i.d. and proteins stained with Coomassie brilliant blue. The trace was made with a Gilford recording spectrophotometer (Gilford Instruments Ltd, Teddington, Middlesex, U.K.). Nomenclature of the proteins follows the convention of Kilbourne et al. (1972). P3 (Inglis et al. 1976; Lamb & Choppin, 1976) did not separate from P2. Electrophoresis is from left to right.

Fig. 2. Distribution of 35S-methionine in the proteins of A/Okuda after electrophoresis on a slab of 10% polyacrylamide gel. The slab was autoradiographed and the film analysed with a densitometer (a) before and (b) after chromatography on concanavalin A-Sepharose. Electrophoresis is from left to right.

as virus grown in embryonated eggs (Fig. 1). To date we have used 23 strains (22 type A and 1 type B) isolated from man, birds and horses in de-embryonated eggs, all of which incorporate sufficient 35S-methionine for peptide mapping of NP and M proteins.

Incorporation of 35S-methionine into virion proteins

Viruses were purified as described under Methods. The specific activities of virus purified as far as the sucrose gradient stage (ct/min/μg protein) were A/Okuda: 3.9 × 10⁵, A/Finland: 6.8 × 10⁵ and WRL 105: 2.7 × 10⁵. The distribution of radioactivity between Okuda virion proteins can be seen after polyacrylamide gel electrophoresis (Fig. 2a). The majority of radioactivity is present in M and NP. These proteins were shown (see below) to be non-glycosylated, to have mol. wt. of approx. 58000 and 24000 and on electrophoresis to move the same distance as similar proteins of other strains (whereas the distance moved by
Peptide maps of NP and M proteins

Fig. 3. Removal of glycoproteins from A/FPV by chromatography on Con A-Sepharose. Virus was dissolved in SDS and the protein in Peak I (see Fig. 4) was precipitated with ethanol and then electrophoresed on polycrylamide gel. (a) Untreated virus (250 µg); arrows show areas containing carbohydrates as shown by Schiff staining. (b) Virus proteins (250 µg) passing through the Con A column. None stained with the Schiff reagent. (a) and (b) were cylindrical gels and proteins were stained with Coomassie brilliant blue. Electrophoresis is from top to bottom.

Fig. 4. Affinity chromatography of A/Okuda on Con A-Sepharose. Virus was disrupted with SDS before it was applied to the column. Peak I was not retained by the column. Bound material (Peak II) was eluted with methyl-α-D-glucopyranoside (arrow).

Influenza virus HA and NA glycoproteins is known to vary according to the strain. The identities of M and NP were confirmed by immune precipitation with specific antiserum raised to type A ribonucleoprotein (Kelly & Dimmock, 1974) or against type A M protein (Oxford & Schild, 1975) followed by electrophoresis in a slab gel adjacent to non-fractionated virus (unpublished data). The distribution of 35S-methionine in A/Okuda was Pt 0.5%, P2 and 3 0.8%, HA9 2.3%, NP 22.2%, HA1 and NA 7.9%, M 57.1% and HA2 9.4%. The ratio of 35S-methionine/mol of M and NP was calculated as 1.7:1 using the numbers of mol/virion quoted by Choppin & Compans (1975).

Removal of glycoproteins by chromatography on concanavalin A-Sepharose

This procedure was employed to remove glycoproteins which could contaminate M and NP. Virus was dissolved in SDS and passed through a column of Con A-Sepharose. Fig. 2 shows 35S-methionine labelled protein of A/Okuda separated by gel electrophoresis (a) before and (b) after Con A chromatography. HA1 and HA2 were removed by this procedure. Other conditions of electrophoresis which clearly separated HA2 from M confirmed that HA2 proteins were retained by Con A. The efficiency of the Con A column is further shown in Fig. 3 where A/FPV proteins and glycoproteins are visualized by staining with Coomassie blue and Schiff reagent respectively. After passage through Con A (Fig. 3b) there were no
Table 1. Removal of neuraminidase by concanavalin A-Sepharose chromatography

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total neuraminidase activity $E_{549}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Japan before chromatography*</td>
<td>672.0</td>
</tr>
<tr>
<td>After chromatography—sum of 8 x 1 ml fractions</td>
<td>$\leq 0.2$</td>
</tr>
<tr>
<td>Elution with 0.1 M-methyl-α-D-glucopyranoside†</td>
<td>7.0</td>
</tr>
<tr>
<td>Elution with 0.1 M-methyl-α-D-mannopyranoside†</td>
<td>26.0</td>
</tr>
<tr>
<td>Assay of enzyme bound to the concanavalin A-Sepharose after the above elution procedures</td>
<td>206.0</td>
</tr>
</tbody>
</table>

* 1 mg A/Japan (H2N2) was dissociated in SDS at room temperature as described in Methods.
† In 0.1 M-tris, pH 7.5, and 0.05 % SDS.

Fig. 5. Polyacrylamide gel electrophoresis on a slab of 10 % gel of A/Okuda non-glycosylated proteins. Fractions containing the peak of radioactivity from the eluate of concanavalin A chromatography were applied to a stacker consisting of 4.5 % gel. Electrophoresis is descending and was continued for 18 h at 60 V. The gel was autoradiographed and the NP and M proteins excised as shown by the arrows.

stained bands in the region of HA1 and no stained carbohydrate in the M/HA2 region indicating that HA2 glycoprotein had been removed (data not shown). Fig. 3(b) also shows that Con A leached from the Sepharose and ran in a series of bands adjacent to the M protein. This was not a problem when using radiolabelled virus.

A quantitative measure of the efficiency of the removal of glycoproteins was made by the measuring of the loss of neuraminidase activity. This could be done since the N2 neuraminidase is active in the presence of SDS. In this experiment 1 mg purified A/Japan (H2N2) was dissolved in SDS and passed through the affinity column. The original virus in SDS and the material passing through the column were assayed by the standard procedure. The neuraminidase activity of the eluate was reduced by over 103-fold (Table 1).

The majority of the enzyme bound so firmly to the Con A that it could not be freed by either 0.1 M-methyl-α-D-glucopyranoside or methyl-α-D-mannopyranoside, a standard procedure for recovering glycoproteins from lectins.

A typical preparative chromatography run is shown in Fig. 4. The non-glycosylated proteins passing through the column (Peak I) were then separated as described below. About 20 % of the total radioactivity applied was retained and the majority of this (Peak II), presumably HA1 + HA2, could be eluted with 0.1 M-methyl-α-D-glucopyranoside.
Peptide maps of NP and M proteins

Table 2. Recovery of radioactivity during the preparation of NP and M peptides from A/Okuda*

<table>
<thead>
<tr>
<th>Procedure</th>
<th>NP (ct/min × 10^-3)</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH4)2SO4 precipitation, sucrose gradient centrifugation and pelleting. Dissolved in SDS</td>
<td>94</td>
<td>66</td>
</tr>
<tr>
<td>Concanavalin A affinity chromatography</td>
<td>90</td>
<td>240</td>
</tr>
<tr>
<td>Preparative polyacrylamide electrophoresis: protein band excised and solubilized</td>
<td>4.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Protease digestion and elution</td>
<td>2.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Lyophilizations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Virus was grown in de-embryonated eggs in the presence of 35S-methionine.

Separation of NP and M by preparative polyacrylamide gel electrophoresis

Fractions from the Con A eluate containing the peak of radioactivity were pooled, reduced and re-denatured. The solution was applied to a slab of polyacrylamide gel with a stacker without any reduction in volume (Fig. 5). After electrophoresis and autoradiography protein could be resolved with a 3 h exposure or less. The radioactive bands were excised as indicated by the arrows in Fig. 5.

Efficiency of recovery of radiolabelled virus proteins and peptides

The gel slices containing NP and M were processed as described under Methods and then digested with proteases. A summary of the recovery of radioactivity from a preparation of purified A/Okuda is presented in Table 2. These figures are characteristic of a large number of influenza A strains. The data are not corrected for differences in quenching although these are likely to be small since sample volumes for radioactive counting were less than 10 µl. The approximate recovery after Con A chromatography was 70%. After electrophoresis of this material samples of the NP and M bands were solubilized and counted: 50% of the applied radioactivity was in the combined NP and M proteins. When the bands were digested with proteases and eluted in the usual way, 48% NP and 30% M present were released from the gel. Recovery after oxidation and the reduction in volume by lyophilization from 25 ml to 30 µl was greater than 56%.

Peptide mapping by two-dimensional chromatography

M protein

Preliminary experiments showed that trypsin released few methionine-containing peptides from this polypeptide. Consequently, we used a mixture of trypsin and chymotrypsin to obtain greater digestion. This released considerably more peptides, thus allowing more stringent comparisons to be made between the M proteins from different virus strains.

Maps of the M proteins of A/Finland (H3N2), A/Okuda (H2N2), and their recombinant WRL 105 (H3N2) are shown in Fig. 6 (a to c). The overall distribution of peptides from A/Okuda and A/Finland is similar and they appear to have the majority of peptides in common. However, there are peptides (arrowed in Fig. 6) which enable the viruses to be distinguished. A/Finland has a peptide (no. 1) which moves fastest in the second dimension which is absent in A/Okuda. In addition, A/Okuda has a peptide (no. 2) which is absent in A/Finland and peptide no. 3 which is over twice as heavily labelled relative to the adjacent peptide above it in A/Okuda than in A/Finland as shown by scanning with a Joyce-Loebl densitometer.
Fig. 6. Maps of $\textsuperscript{35}$S-methionine peptides of M proteins of (a) A/Finland (b) A/Okuda and (c) WRL 105. Proteins were digested with a mixture of trypsin and chymotrypsin and mapped by 2-dimensional chromatography on a thin layer of silica gel. Peptides were applied to the lower left corner of the plate. The solvent in the first dimension (arrow 1) was 3:2:2 methyl acetate/isopropanol/25% ammonium hydroxide and in the second dimension (arrow 2) was 3:1:1 butan-1-ol/acetic acid/water. Maps were developed simultaneously in the same chromatography tank. About 20000 ct/min were loaded on to each plate together with a xylene cyanol FF dye marker which migrated 6 cm and 2.5 cm in the first and second dimensions respectively. The silica gel plate was autoradiographed with Kodirex film with an exposure of 20 days.

Fig. 7. Maps of $\textsuperscript{35}$S-methionine-containing tryptic peptides of NP proteins (a) A/Finland, (b) A/Okuda and (c) WRL 105. The procedure is described in Fig. 6 and in Methods. The dye marker migrated 6 cm in the first dimension (arrow 1) and 3 cm in the second dimension (arrow 2). About 20000 ct/min were loaded on to each plate. The exposure was 20 days.
Having successfully distinguished between the M proteins of A/Okuda and A/Finland we mapped the M protein of their recombinant WRL 105 (Fig. 6c) to determine which of the parental genes is expressed. The map shows that WRL 105 M protein lacks the peptide (no. 1) and contains peptide no. 2 and the densely labelled peptide no. 3. Thus we conclude that it is expressing the A/Okuda M protein. Radioactivity at the origin of each map may represent protease-resistant core material.

**NP protein**

Maps of the tryptic peptides of the NPs of A/Finland, A/Okuda and WRL 105 are shown in Fig. 7 (a to e). There are about 14 and 18 35S-peptides in the maps of A/Finland and A/Okuda respectively. Many differences can be seen between their maps, one of the most characteristic being a group of 4 peptides of A/Okuda (arrowed in Fig. 7b) which is absent from A/Finland. A second difference is peptide no. 1 (A/Okuda) which migrates with the leading edge of the elongated peptide in the first dimension, this being absent from A/Finland. Radioactivity at the origin may be trypsin-resistant core material.

The peptide map of the NP protein of WRL 105 (Fig. 7c) is seen to have the central group of 4 peptides and peptide (no. 1) which are characteristic of A/Okuda. These two strains have all other peptides in common. Thus WRL 105 is expressing both the NP and M of the A/Okuda parent.

**DISCUSSION**

We have devised and demonstrated methods for the preparation and mapping of the 35S-methionine containing peptides of the NP and M proteins of three influenza A viruses. The methods seem generally applicable to type A or B strains (N. J. Dimmock, unpublished data). Our report complements and extends the accompanying paper by Brand, Stealey & Rowe (1977) describing the mapping of iodinated peptides of the same viruses.

We chose to label virus in vivo. The advantage is that only 10 de-embryonated eggs per strain are required. The virus was dissolved and NP and M proteins were freed of virus glycoproteins (Ha and NA) by allowing the glycoproteins to bind to the plant lectin concanavalin A. The final purification and separation of NP and M was readily achieved by their greatly differing mobilities on polyacrylamide gel. Proteins were digested from slices of washed gel with an excess by weight of trypsin (NP) or trypsin and chymotrypsin (M).

We have now mapped a large number of M proteins from a variety of human and animal influenza A strains (data not shown). Despite them being prepared, digested and mapped on different occasions the similarity between the peptide maps is remarkable. The maps shown in this paper were prepared from viruses which were grown, processed and mapped simultaneously to allow the best comparison between them. The three maps of the M proteins shown above are fundamentally very similar but there are small but reproducible differences between the M proteins of A/Finland and A/Okuda which allow them to be distinguished. That the methodology can produce internally consistent maps of proteins obtained from a variety of sources is shown by studies on alphavirus proteins (Clegg et al. 1976) and by our own unpublished experiments in which we have obtained identical peptide maps of NP proteins from A/FPV/Rostock/34 virus and from infected cells.

The object of this report is to describe methods which can be used for a comparative study of the M and NP proteins of influenza viruses. We have used a representative of the H1N1 and of the H2N2 subtypes. Our peptide maps of the M proteins of these subtypes are very similar showing that their M proteins have substantially the same primary structure. However, there are differences in certain methionine peptides by which these strains can be
clearly distinguished. We have not yet looked at sufficient strains to show if the differences are representative of the subtypes. Differences in M proteins of H\textsubscript{2}N\textsubscript{2} and H\textsubscript{3}N\textsubscript{2} strains have been shown by mapping of unlabelled peptides (Laver & Downie, 1976) and of iodinated peptides (Brand et al. 1977). M proteins cannot be distinguished by immunological methods (Schild, 1972).

Maps of tryptic peptides of the NPs of the H\textsubscript{2}N\textsubscript{2} and H\textsubscript{3}N\textsubscript{2} strains were clearly different, indicating that there was a greater degree of difference than between the M proteins from the same strains. However, the two NPs were sufficiently related for them to be recognizably more similar to each other than to the M proteins. The structural difference in NPs is presumably the basis of the immunological differences which have been reported between type A nucleoprotein antigens (Lief, Fabiyi & Henle, 1958).

Our mapping procedures which have enabled us to distinguish between the NP and M proteins of A/Okuda (H\textsubscript{2}N\textsubscript{2}) and A/Finland (H\textsubscript{3}N\textsubscript{2}) have thereby allowed us to investigate a recombinant (WRL 105) produced between them. The recombinant expresses the HA and NA of A/Finland and we have shown it to express the NP and M of A/Okuda. The conclusion regarding the M proteins was confirmed by Brand et al. (1977) who used different methods of preparation and examined the tyrosine-containing tryptic peptides. In our recombinant we can account for four proteins, two being derived from each parent.

This report complements the recent work of others (reviewed by Palese, 1977) who have analysed the parental contribution to the genome of recombinant strains. This was possible because the equivalent parental RNA segments migrated at different rates on polyacrylamide gel electrophoresis. However, a detailed examination of the proteins such as we have made will be useful in analysing recombinants made from parents with RNA segments of the same electrophoretic mobility.

We are grateful to Professor D. C. Burke and Dr R. G. Webster for helpful discussions and to the Medical Research Council for financial support. Dr N. B. Finter (Wellcome Research Laboratories, Beckenham, Kent) provided the virus strains and Dr J. S. Oxford (National Institute for Biological Standards and Control, London) the antiserum to M protein.

REFERENCES


Peptide maps of NP and M proteins


(Received 10 January 1977)