Structural Polypeptides of Measles Virus

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SUMMARY

The structural polypeptides of two strains of measles virus grown in Vero cells were analysed in SDS–PAGE slab gels. Six major polypeptides were identified with mol. wt. of 79,000, 72,000, 60,000, 43,000, 40,000 and 36,000. The largest polypeptide was sensitive to trypsin digestion and was the dominant glycosylated polypeptide identified when the virus was grown in medium containing $^3$H-fucose or $^3$H-glucosamine or when the virus was treated with galactose oxidase and labelled with $^3$H-sodium borohydride. It is concluded that the 79,000 mol. wt. polypeptide represents the haemagglutinin. Treatment with non-ionic detergent removed this polypeptide and also the 40,000 mol. wt. polypeptide from the virus envelope. The 40,000 mol. wt. polypeptide is probably associated with haemolysin and cell fusion activities and is analogous to the F1 of paramyxoviruses. A polypeptide of mol. wt. approx. 20,000 detected after glycoprotein labelling may represent the F2 of measles virus. The 43,000 mol. wt. polypeptide co-migrates with cellular actin and is the only major measles polypeptide that is heavily labelled when the virus is grown on Vero cells prelabelled with $^{35}$S-methionine. Thus it may represent cellular actin incorporated into the virus during maturation. The quantity of the 72,000 mol. wt. polypeptide relative to the other major polypeptides varied considerably in different virus preparations. The role of the polypeptide could not be defined. By analogy with previously published data the 60,000 and 36,000 mol. wt. polypeptides are inferred to represent nucleocapsid and membrane proteins, respectively.

INTRODUCTION

The introduction of successful vaccination has significantly reduced the incidence of acute measles and its complications (Krugman, 1977). However, the identification of measles virus as the causative agent in patients with sub-acute sclerosing panencephalitis (SSPE) and the accumulation of serological data suggesting a possible role for measles virus in multiple sclerosis, lupus erythematosus and connective tissue diseases have provided the continued stimulus for further research into the biology of measles virus and its interactions with the host. A prerequisite for studying this interaction is better knowledge of the measles virion polypeptides.

The first sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) pattern of measles virion polypeptides was published by Hall & Martin (1973). Six major

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polypeptides with mol. wt. of 75,000, 69,000, 60,000, 53,000, 51,000 and 45,700 were described. The 69,000 and 53,000 mol. wt. polypeptides were glycosylated. These results were confirmed by Schluederberg et al. (1974) and were similar to the pattern of virion polypeptides of other paramyxoviruses (Mountcastle et al. 1971). Concurrent with the above study, Waters & Bussell (1973) described six major polypeptides in SDS-PAGE of measles virions with mol. wt. of 78,000, 71,250, 61,000, 53,000, 45,000 and 37,500. Further studies indicated that only the 78,000 mol. wt. polypeptide was glycosylated (Bussell et al. 1974). This pattern differed from the pattern described by Hall & Martin (1973) in some mol. wt. determinations, but most striking was the finding of only one glycosylated polypeptide. Recently Mountcastle & Choppin (1977) have analysed four strains of measles virus in an attempt to clarify these discrepancies. They found six major polypeptides with mol. wt. of 80,000, 70,000, 61,000, 55,000, 42,000 and 37,000. Only the 80,000 mol. wt. polypeptide was glycosylated and confirmed the earlier results of Bussell et al. (1974).

In parallel with this recent work, we have analysed the polypeptides of two strains of measles virus. Both whole and partially degraded viruses were studied under various labelling conditions in an attempt to define the structure-function relationship of measles virion polypeptides.

METHODS

Virus growth and purification. Mycoplasma free Vero cells were grown on 200 cm² Roux bottles in 150 ml Eagle’s minimal essential medium (MEM) with 5% calf serum. When the monolayer was 80 to 90% confluent, the cells were infected with either the SSPE strain LEC (Barbanti-Brodano et al. 1970) or the Edmonston strain of measles virus at an m.o.i. of 0.01 TCID₅₀/cell in MEM with 2% calf serum. At 72 h the medium was changed and the extracellular virus was harvested on the 6th day post infection. The virus was purified either by the method described by Hall & Martin (1973) or by the following procedure. The medium was clarified by centrifuging at 10,000g for 10 min and filtering through no. 0 Munktell’s filter paper. The virus was pelleted from the clarified medium at 28,000g for 1 h in a Sorvall RC-5 centrifuge with a GSA rotor. The virus pellet was suspended in 0.005 M-tris-HCl, 0.001 M-EDTA, pH 7.4 (TE buffer) using 1% of the original volume and was sonicated for 15 s at 20 kHz and 50 μm in a SP 180 Rapids Ultrasonic apparatus with a 3 mm probe. Aggregated material was removed by centrifuging at 5000g for 5 min. The suspension of virus was banded at an interface between 40 and 60% (w/w) sucrose at 45,000g for 45 min. The virus was collected, diluted in TE buffer and centrifuged to equilibrium in a 15 to 40% (w/w) potassium tartrate gradient at 90,000g for 3 h. The virus band was collected and dialysed against phosphate-buffered saline (PBS) for 12 h. The protein content was determined (Lowry et al. 1951) and the virus suspension was used directly or stored at −70°C.

Preparation of labelled virus. Vero cells were grown as monolayers in roller bottles (1500 cm²) in MEM with 5% foetal calf serum. Monolayers were infected with measles virus at an m.o.i. of 0.01 TCID₅₀/cell in maintenance medium. After 72 h, when the cells began to show cytopathic changes, the medium was exchanged for radiolabel-containing medium. ³H-fucose was added at 2 μCi/ml in MEM with 2% calf serum; ³H-glucosamine was added at 2 μCi/ml in MEM with 2% calf serum and fructose substituted for glucose. Protein labelling with ³⁵S-methionine was done at 5 μCi/ml in MEM with 2% calf serum and methionine reduced to 4 mg/l. After 72 h labelling, virions were purified as previously described.

Pre-labelling of Vero cells. Vero cells were grown in roller bottles (1500 cm²) with MEM
containing 5% foetal calf serum, methionine at 4 mg/l and 5 μCi/l ³⁵S-methionine. At 80 to 90% confluency the medium was removed and the cells were washed twice with regular maintenance medium. After 6 h the medium was changed and the monolayers were infected with measles virus at an m.o.i. of 0.01 TCID₅₀/cell. The L-methionine was increased to 60 mg/l and on the 6th day post infection the virus was harvested.

**Tween 80-ether, Cutscum and trypsin treatment of virus.** Purified virion samples containing 80 to 100 μg/ml of protein suspended in PBS were treated with 0.125% Tween 80 in an ice bath for 5 min and then extracted for 15 min with 0.5 vol. ether at 4°C. After centrifuging at 3000g for 15 min the aqueous phase was removed and the ether evaporated (Norrby, 1962). Similar virus samples were treated with 0.1% or 1.0% Cutscum for 15 min at 22°C and with trypsin at 0.1% or 0.002% for 2 h at 37°C. After each treatment, solubilized proteins were separated from sedimentable virus particles by centrifugation on a discontinuous caesium chloride gradient of 1.15 g/ml (9 ml) on 1.40 g/ml (2 ml) at 100000g for 1 h.

**External labelling of glycoprotein.** Surface glycoproteins were labelled using the method of Luukkonen et al. (1977). Each virus sample containing 80 μg of protein in 200 μl of Dulbecco’s phosphate-buffered saline, pH 7.4, was incubated with 25 units of galactose oxidase for 1 h at 37°C. Then 200 μCi of NaB³H₄ in 30 μl of 0.01 M-NaOH was added at 22°C for 30 min. The virus was then precipitated with 10% trichloroacetic acid (TCA) and prepared for slab gel electrophoresis.

**Polyacrylamide gel electrophoresis.** The procedure used essentially followed the method of Laemmli (1970). The purified virions were precipitated with 10% TCA. Solubilized proteins were precipitated with 200 μg/ml insulin as carrier since the insulin migrates at the front of the gel and does not interfere with the virus protein pattern (L. Payne, personal communication). The precipitated samples were centrifuged at 3000g for 20 min at 4°C. The protein film was washed successively with cold ethanol, cold 50% ether in ethanol (v/v) and cold ether. When insulin was used as a protein carrier, ethanol was omitted from the washing procedure. TCA in these samples was removed by re-centrifugation for 5 min and aspiration before washing the TCA pellet with cold ether. The protein film was dissolved in a dissociating buffer of 0.0625 M-tris-H₂PO₄ (pH 6.8), 2% sodium dodecyl sulphate (SDS), 0.2% dithiothreitol, 0.1% EDTA-Na₂ and 10% glycerol. The samples were dissociated by boiling in a waterbath for 2 min.

The slab gel apparatus was similar to that described by Studier (1973). The gels measured 8.0 x 12.0 x 0.15 cm. The spacer gel contained 4.5% acrylamide and 0.12% N,N'-methylene-bisacrylamide (bis). The separation gel contained 10% or 15% acrylamide and 0.18% bis. The acrylamide and bis were treated with Amberlite MB-1 for 5 h and stored under nitrogen prior to use. The buffer system was modified from Laemmli’s system by substituting tris-H₂PO₄ for tris-HCl in the spacer gel. The gels were polymerized with N,N,N',N'-tetramethylethylenediamine (TEMED) and ammonium persulphate at 0.06% each in the spacer gel and 0.03% each in the separation gel. The electrophoresis was performed at room temperature for 6 to 8 h at 1 to 2 W. The migration front was followed by adding bromophenol blue to the electrode buffer. Gels were stained for 12 h using 0.25% Coomassie brilliant blue in 35% ethanol, 10% acetic acid in water and were destained in the same solution lacking the stain.

**Scintillation autofluorography.** The gels were prepared for scintillation autofluorography after protein staining and were destained as described above or directly after the electrophoresis run was completed. The gels were equilibrated in dimethylsulphoxide (DMSO), impregnated with 2,5-diphenyloxazole (PPO) in 20% (w/w) DMSO and soaked in water.
(Bonner & Laskey, 1974). The gels were dried (Maizel, 1971) and exposed to Wicorx-RP film at −70 °C for 72 h. Exposed films were developed in Kodak liquid X-ray developer for 3 min, put in 1% acetic acid for 20 s, fixed for 3 min in Kodak rapid fixer, washed for 30 min and dried.

**Chemicals and isotopes.** H-fucose, H-glucosamine, 35S-methionine and H-sodium borohydride were obtained from The Radiochemical Centre, Amersham, England. Trypsin and galactose oxidase were purchased from Sigma Chemicals Company. Cutscum detergent was obtained from Fischer Scientific and Tween 80 was obtained from Light and Co. Ltd, Colnbrook, England. Actin prepared from rabbit skeletal muscle was a generous gift of Rigmor Thorstensson, Department of Immunology, National Bacteriological Laboratory, Stockholm. The LEC strain of measles virus was kindly provided by Hilary Koprowski, The Wistar Institute, Philadelphia.

**RESULTS**

**The polypeptide pattern of measles virus**

Fig. 1 shows the polypeptide banding pattern of the LEC strain of measles virus in 10% polyacrylamide gel electrophoresis. Six major polypeptides were consistently seen in the gel preparations of this strain of measles virus grown in Vero cells. A similar pattern was seen when the LEC strain was grown in green monkey kidney cells or when the Edmonston strain was grown in either Vero or green monkey kidney cells. In most experiments we used the LEC strain grown in Vero cells as this combination yielded the largest quantities of virus.

We have compared two methods for the purification of measles virus. Originally, we purified measles virus on two successive potassium tartrate equilibrium gradients (Hall & Martin, 1973). However, there were a number of minor polypeptide bands that occurred in varying concentrations in different preparations. Most of these minor polypeptide bands co-migrated with polypeptides of uninfected Vero cells and appeared in the autofluorograms of measles virus isolated from Vero cells pre-labelled with 35S-methionine. On this basis most of the minor polypeptide bands seen in the gels using measles virus purified on two successive potassium tartrate gradients were considered to be cellular contaminants.

Since the measles virus preparation was heavily aggregated in the potassium tartrate, some cellular contaminants may have become trapped in or adsorbed on to the aggregates. To prevent aggregation, the virus was banded at an interface between 40 and 60% (w/w) sucrose for 45 min at 45000g before banding in a potassium tartrate equilibrium gradient. This eliminated considerable cellular contamination. Some experiments described below were performed using virus prepared on two successive potassium tartrate gradients before we recognized the benefits of banding the virus at the 40/60% (w/w) discontinuous sucrose interface.

Six major polypeptides with mol. wt. of 79000, 72000, 60000, 43000, 40000 and 36000 were seen (Fig. 1). Two high molecular weight proteins were also frequently identified in the slab gels. The amount of the heavier protein (mol. wt. ~ 200000) varied considerably in different virus preparations and was considered to be a cellular contaminant. The smaller protein (mol. wt. ~ 185000) was a consistent finding and may be analogous to the 'L' protein seen in other paramyxoviruses.

**Comparison of 35S-methionine labelled virus with virus grown on 35S-methionine pre-labelled cells**

The polypeptide banding patterns of 35S-methionine labelled virus and virus grown on 35S-methionine pre-labelled cells are compared in Fig. 2(a, b). With the labelled virus poly-
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Fig. 1. Coomassie brilliant blue stained 10% slab gel of (a) standard proteins: phosphorylase (90), bovine serum albumin (67), human IgG heavy chains (55), actin (43) and human IgG light chains (23), (b) actin and (c) measles virus purified on a discontinuous sucrose gradient and an equilibrium potassium tartrate gradient. Details of the procedures are given in Methods. Mol. wt. × 10^{-3} is shown.

Fig. 2. Autofluorogram of a 10% slab gel of (a) 35S-methionine labelled measles virus, (b) measles virus grown on 35S-methionine pre-labelled Vero cells, (c) 3H-glucosamine labelled virus, (d) 3H-fucose labelled virus and (e) virus externally labelled with galactose oxidase and 3H-sodium borohydride. Details of the procedures are given in Methods. Mol. wt. × 10^{-3} is shown.
peptides, all six major polypeptides are heavily labelled. However, the 43 000 mol. wt. polypeptide was the only major measles polypeptide that was heavily labelled in virus grown on 35S-methionine pre-labelled cells. This band co-migrates with cellular actin. The relatively heavy labelling of the minor polypeptide bands in virus grown on 35S-methionine pre-labelled Vero cells suggests these polypeptides are of cellular rather than virus gene origin.

The glycoproteins of measles virus

The autofluorograms of the LEC strain of measles virus grown in the presence of 3H-fucose or 3H-glucosamine are shown in Fig. 2(c, d). Of the major polypeptides in measles, only the 79 000 mol. wt. protein was glycosylated. A large glycoprotein of mol. wt. 185 000 was visualized. In addition, a rather diffuse glycoprotein band migrated near the electrode buffer front. This glycoprotein has a mol. wt. of approx. 20 000.

Measles virus was incubated with galactose oxidase for 1 h at 37 °C followed by reduction with 3H-sodium borohydride at 20 °C for 30 min. This specifically labels the glycoproteins of purified Semliki Forest virus (Luukkonen et al. 1977). The 79 000 mol. wt. polypeptide was labelled by this procedure but the other major polypeptides of measles virus were not (Fig. 2e). Several other minor proteins were labelled by this procedure, but none co-migrated with the major polypeptides of measles. Labelling of a diffuse band in the region of 20 000 daltons was seen.

Treatment of virus with trypsin

Treatment of virions with trypsin prevents the induction of haemagglutinin inhibiting (HI) antibodies (Norrby & Gollmar, 1975). However, this treatment does not prevent the induction of haemolysin-inhibiting (HLI) antibodies by measles virus. Trypsin treated measles virus was compared with untreated measles virus on SDS-PAGE to try to explain the differential sensitivity of HA and HL antigens to this treatment. In Fig. 3, the pattern of untreated measles virus is compared to the pattern of measles virus treated with 0.002 % or 0.1 % trypsin for 2 h at 37 °C. The 79 000 mol. wt. polypeptide band was removed and cleaved into smaller fragments under both conditions of treatment whereas the other major polypeptides were unchanged even after treatment with 0.1 % trypsin.

Treatment of virus with Tween 80-ether

Treatment of measles virus with Tween 80 and ether has been shown to increase markedly the haemagglutinating activity of the virus (Norrby, 1962). After this treatment the polypeptide banding pattern remains unchanged if the solubilized proteins are not separated from the sedimentable components. After separation on caesium chloride discontinuous gradients, the solubilized protein contained only small amounts of the 79 000 mol. wt. polypeptide and the 40 000 mol. wt. polypeptide. Some of the 43 000 dalton band is removed by this treatment (Fig. 4c).

Treatment of virus with Cutscum

After treatment of measles virus with Cutscum at concentrations of 0.1 % and 1 %, the sedimentable particles were separated from solubilized proteins on caesium chloride gradients. As seen in Fig. 4(e, g) the 79 000 and 40 000 mol. wt. polypeptides were removed from the virus particle and were recovered among solubilized polypeptides. The 72 000, 60 000, 43 000 and 36 000 mol. wt. polypeptides remained as a sedimentable unit after Cutscum treatment (Fig. 4d, f).
DISCUSSION

The polypeptide banding pattern of LEC strain measles virus in SDS-PAGE is quite similar to the previously reported patterns of measles virus (Bussell et al. 1974; Mountcastle & Choppin, 1977). As in these studies, the largest polypeptide had a mol. wt. of 79,000 and was the main glycosylated polypeptide of measles virus. This protein was easily removed from the measles virion by trypsin digestion while the other polypeptides remained unaltered even after treatment with relatively large amounts of trypsin. Norrby & Gollmar...
(1975) have shown that treatment of measles virions with trypsin removes the surface projections and that the projectionless virions fail to induce HI antibodies in rabbits. The results in SDS-PAGE are in agreement with the morphological and serological data and confirm that the large glycosylated polypeptide of measles is responsible for the haemaggluti-
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nating (HA) activity. It is analogous to the large glycoprotein found in other paramyxoviruses (Choppin & Compans, 1975).

While trypsin treatment reduces the ability of measles virus to induce HI antibodies, it enhances the ability of the virus to induce HLI antibodies (Norrby & Gollmar, 1975). The 40,000 mol. wt. polypeptide remains with the virus particle after trypsin treatment, but is removed by Cutscum treatment, indicating that it is an envelope protein. Surprisingly, this protein is not glycosylated since it is not labelled with $^3$H-fucose, $^3$H-glucosamine or after treatment with galactose oxidase and $^3$H-sodium borohydride. However, we believe it is a likely candidate for the protein component of the structure responsible for haemolysin and cell fusion activities. Graves et al. (1977) have studied the synthesis of measles polypeptides in Vero cells in which cell fusion was inhibited. Under these conditions they found that two polypeptides with mol. wt. of 80,000 and 62,000 were glycosylated. A 40,000 mol. wt. polypeptide appeared in pulse-chase experiments. Possibly the polypeptide responsible for haemolysin and cell fusion is glycosylated in the cell and transported to the external surface of the cell membrane as the 62,000 mol. wt. glycosylated protein. On maturation of the virus this polypeptide may be cleaved to a non-glycosylated protein of mol. wt. 40,000. This would explain the pulse-chase experiments and the fact that there is only one glycosylated polypeptide in the purified measles virions. This sequence of events would be analogous to the cleavage of the F protein to $F_1$ and $F_2$ proteins in other paramyxoviruses (Scheid & Choppin, 1977). In these viruses the $F_2$ protein is relatively carbohydrate rich in comparison with $F_1$. In the measles system the uneven distribution of carbohydrate between $F_1$ and $F_2$ could be exaggerated, resulting in an absence of carbohydrate in $F_1$. Hardwick & Bussell (1976) reported the presence of two glycoproteins in measles polypeptides analysed under non-reducing conditions. Mountcastle & Choppin (1977) have confirmed this original report. The glycoprotein of approx. mol. wt. 20,000 may represent the $F_3$ of measles. This polypeptide band is not seen on Coomassie brilliant blue stained gels due to its diffuse nature which can be attributed to its carbohydrate content.

Treatment of purified measles virus with Tween 80-ether greatly increases the HA activity (Norrby, 1962). After this treatment the HA activity is associated with particles displaying varying sedimentation characteristics distributed in sucrose gradients (Norrby, 1966). The soluble HA particles exhibit high HA activity but very low HA-specific complement fixing (CF) antigenic activity. The high density sedimentable HA particles exhibit relatively low HA activity, but high HA-specific CF antigenic activity. In our results the major portion of the 79,000 mol. wt. polypeptide remained with the sedimentable particles and represents the HA-specific CF antigen. A small amount of the 79,000 mol. wt. polypeptide is solubilized and represents the soluble HA antigen.

The 60,000 mol. wt. polypeptide was the most abundant polypeptide in the slab gels of LEC strain measles. By analogy to the previous studies on measles virus (Hall & Martin, 1973; Bussell et al. 1974; Mountcastle & Choppin, 1977) and other paramyxoviruses (Mountcastle et al. 1970; Waters & Bussell, 1973), this is the nucleocapsid protein (NP).

A polypeptide band at 43,000 daltons was a consistent finding in our preparations. This polypeptide was heavily labelled in virus grown on pre-labelled Vero cells while the other major polypeptides were not, indicating that this polypeptide is of cellular origin and not coded for by the virion genome. This protein co-migrates with cellular actin. Actin has been shown to be present by polypeptide analysis of other enveloped viruses (Fleissner & Tress, 1973; Scheid & Choppin 1974; Wang et al. 1976), other paramyxoviruses (Lamb et al. 1976) and in measles virus (Mountcastle & Choppin, 1977). Actin is sensitive to cleavage by trypsin (Lazarides & Lindberg, 1974). Since the actin band remains unaltered when whole
virions are treated with high trypsin concentrations, most of the actin found in the measles virus is presumably protected inside the virion.

The smallest measles polypeptide had a mol. wt. of 36000 and corresponds to the membrane (M) protein of paramyxoviruses (Scheid & Choppin, 1974). The ability of the M protein to bind to nucleocapsid has been shown in other paramyxoviruses (McSharry et al. 1975; Schimizu & Ishida, 1975). Treatment of measles virus with Cutsen removed the major surface proteins but left the complex of NP protein, M protein and actin. Actin may have a role in binding the M protein to NP protein or binding the M−NP protein complex to the envelope. However, as a note of caution, actin is a very sticky protein and the decision as to whether actin is a structural component of measles virus or not has to await a purification system that eliminates all cellular contamination or until a functional role for actin in the virus can be defined.

Previous studies (Hall & Martin, 1973; Bussell et al. 1974; Mountcastle & Choppin, 1977) have indicated that measles virus polypeptides are also found at approx. 70000 and 53000 daltons. In our preparations a polypeptide band was seen at 72000 daltons. Mountcastle & Choppin (1977) have seen this polypeptide in association with nucleocapsids isolated under conditions inhibiting proteolysis. We have not used specific proteolysis inhibitors during the purification of measles virus which might explain why this polypeptide band varied in amount in different preparations.

In the studies of Hall & Martin (1973) the 53000 dalton polypeptide is clearly defined and glycosylated. Bussell et al. (1974) and Mountcastle & Choppin (1977) also found a small amount of protein banding at 53000 daltons, but it was not glycosylated. We have detected a measles virus polypeptide at 56000 daltons in some virus preparations. However, this was an inconsistent finding and when present, it was heavily pre-labelled and not glycosylated. For this reason we believe this polypeptide represents a cellular contaminant.

A number of minor polypeptide bands are seen in the gels of measles virus. Most of these polypeptides co-migrate with clearly defined polypeptides in Vero cells. It has been suggested that the budding process of measles virus maturation entraps host cytoplasmic components, making purification of measles virus free of cellular contaminants very difficult in the present cell culture systems (Mountcastle & Choppin, 1977). At present only the major structural proteins can be identified with the SDS–PAGE methods. The identification of less abundant virus proteins and clarification of the presence and function of actin in measles virus await improved methods of virus growth and purification.

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