The Glycoprotein of Measles Virus

EXTERNAL RADIOACTIVE LABELLING OF ITS CARBOHYDRATE AND PARTIAL CHARACTERIZATION OF THE GLYCOPEPTIDE

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Measles virus was propagated in VERO cells and purified from the culture supernatants by two successive tartrate-density-gradient centrifugations. Surface carbohydrates were labelled both *in vitro* and *in vivo* with ^3H after treatment with galactose oxidase/ NaB^3H_4 or with ^3H glucosamine. The major labelled glycoprotein in measles virions had a mol.wt. of 79 000. After labelling with periodate/ NaB^3H_4 , which would result in specific labelling of sialic acid residues, the 79 000-mol.wt. glycoprotein was very weakly labelled. This suggests that there is no or a very low amount of sialic acid in the virions. Further analysis of the glycoprotein showed that galactose is the terminal carbohydrate unit in the oligosaccharide, and the molecular weight of the glycopeptide obtained after Pronase digestion is about 3000. The oligosaccharide is attached to the polypeptide through an alkali-stable bond, indicating a *N*-glycosidic asparagine linkage.

Measles virus is an enveloped virus which belongs to the Paramyxovirus group. The glycoprotein structure of measles virus differs, however, from the structure of typical paramyxoviruses. Conflicting reports exist on the molecular weights of the measles-virus glycoproteins. Hall & Martin (1973, 1974) reported that the viral envelope contains two glycoproteins, with mol.wts. of 53 000 and 69 000. Hardwick & Bussell (1978) also found two glycoproteins with apparent mol.wts. of 20 000 and 76 000. Others have found only one glycoprotein, with an apparent mol.wt. of 79 000 (Mountcastle & Choppin, 1977; Tyrrell & Norrby, 1978; Vainionpää *et al.*, 1978). The 20 000-mol.wt. glycoprotein has been suggested to correspond to the F2 protein of other paramyxoviruses (Hardwick & Bussell, 1978). A further difference between measles virus and typical paramyxoviruses is the neuraminidase. The enzyme has been found in paramyxoviruses, but has not been demonstrated in measles virus (Howe & Schluederberg, 1970). The virus-coded neuraminidase hydrolyses neuraminic acid from myxo- and paramyxo-viruses during virus maturation.

In the present study we have analysed the virus after radioactive labelling of its carbohydrate portions *in vitro* and *in vivo*. We here show that it con-

tains only one major glycoprotein, which is exposed on the surface. It has an apparent mol.wt. of 79 000. The glycoprotein contains a complex type of oligosaccharide with terminal galactose and no or very little sialic acid. The oligosaccharide is evidently linked through a *N*-acetylglucosaminyl residue to an asparagine residue in the polypeptide.

Materials and Methods

Cells and virus

Measles virus was grown in monolayer cultures of VERO cells, which were maintained in Eagle's basal medium supplemented with 10% foetal calf serum. The wild-type measles-virus strain was cloned five times as described earlier (Vainionpää *et al.*, 1978) and stored at -70°C until used. VERO-cell monolayers were infected with a multiplicity of 0.01. After adsorption for 1 h, the maintenance medium, consisting of Eagle's basal medium supplemented with 0.2% bovine serum albumin and 5% tryptose/phosphate broth, was added. The purification of measles virus has been described earlier in detail (Vainionpää *et al.*, 1978). In brief, extracellular virus was harvested when the cytopathic effect was 75–100%. After 10-fold concentration by vacuum dialysis, the

supernatant was layered on a step gradient consisting of 10 ml of 18% and 5 ml of 36% (w/w) potassium tartrate and centrifuged at 25 000 rev./min for 90 min in a Spinco SW 27 rotor. The 18%-36%-tartrate interface material was diluted and layered on a 24 ml linear 18–36% potassium tartrate gradient and centrifuged at 25 000 rev./min for 3 h in a Spinco SW 27 rotor. The virus was collected, pelleted at 80 000 g for 30 min, and resuspended in Dulbecco's phosphate-buffered saline, pH 7.4 (Flow Laboratories, cat. no. 18-610-54).

Protein determination

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

External radioactive labelling of measles-virus carbohydrate

Purified measles virus (120 µg of protein) was labelled with NaB³H₄ (8.6 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) after treatment in Dulbecco's phosphate-buffered saline with 5 units of galactose oxidase (Kabi AB, Stockholm, Sweden) with or without 12 units of *Vibrio cholerae* neuraminidase (Behringwerke, Marburg/Lahn, Germany) as described in detail previously (Gahmberg & Hakomori, 1973; Gahmberg, 1976). The neuraminidase and galactose oxidase preparations did not contain proteolytic activity when assayed as described (Gahmberg & Hakomori, 1973), and the galactose oxidase preparation was free from neuraminidase activity (Gahmberg & Hakomori, 1973).

Alternatively, virus was labelled with NaB³H₄ after treatment with 1 mM-sodium metaperiodate (Merck A.G., Darmstadt, Germany) at 0°C for 10 min. This would result in relatively specific labelling of sialic acid residues (Gahmberg & Andersson, 1977). Labelled virus was precipitated with 5% trichloroacetic acid at 0°C, washed in acid and residual acid removed by ether extraction (Luukkonen *et al.*, 1977). The preparation was dissolved in electrophoresis sample buffer (Laemmli, 1970) and boiled for 2 min.

Radioactive labelling of measles virus in vivo

[³H]Glucosamine (20 Ci/mmol) was obtained from The Radiochemical Centre. Labelling with [³H]glucosamine (50 µCi/ml) was done for 48 h in Eagle's basal medium lacking D-glucose but supplemented with 10 mM-fructose and 2% foetal calf serum. The supernatants were harvested when the cytopathogenic effect was spread over the monolayer, and the virions were purified as described above.

Polyacrylamide-gel electrophoresis

Electrophoresis was performed as described by Laemmli (1970) in the presence of sodium dodecyl

sulphate on cylindrical gels with an acrylamide concentration of 8% in the separating gels. After electrophoresis the gels were cut into 2 mm slices with a gel slicer, solubilized in NCS solubilizer (Amersham/Searle, Arlington Heights, IL, U.S.A.) and counted for radioactivity after incubation for 2 h at 50°C in a toluene-based scintillation fluid (Gahmberg & Hakomori, 1973) in a Wallac-LKB 81000 liquid-scintillation counter. The ¹⁴C-labelled (Rice & Means, 1971) standard proteins have been described previously (Gahmberg & Andersson, 1977).

Isolation and analysis of the glycopeptide from the major glycoprotein

The major glycoprotein labelled with ³H after galactose oxidase/NaB³H₄ treatment or with [³H]-glucosamine was isolated by preparative polyacrylamide-gel electrophoresis. Gel slices were eluted with water and portions counted for radioactivity (Bray, 1960). Fractions containing the glycoprotein were pooled and freeze-dried. The preparations were then dissolved in Dulbecco's phosphate-buffered saline containing 0.1% sodium dodecyl sulphate and 0.5% Pronase (Sigma, St. Louis, MO, U.S.A.). After incubation at 60°C for 48 h the samples were applied to a column (1.0 cm × 80.0 cm) of Bio-Gel P-6 (100–200 mesh; Bio-Rad, Richmond, CA, U.S.A.) in 0.15 M-Tris/HCl, pH 7.8, containing 0.1% sodium dodecyl sulphate. The void volume of the column was determined by using Blue Dextran 2000 (Pharmacia, Uppsala, Sweden). The molecular-weight markers were thyroglobulin glycopeptide (mol.wt. 4100), an octasaccharide (mol.wt. 1434) and a triose (mol.wt. 504) (Gahmberg *et al.*, 1979), which were provided by Professor O. Renkonen, Department of Biochemistry, University of Helsinki.

Determination of labelled carbohydrate

For hydrolysis of neutral sugars a portion of the isolated glycoprotein labelled after galactose oxidase/NaB³H₄ treatment and containing about 10 000 c.p.m. was hydrolysed in 1 M-H₂SO₄ for 5 h at 100°C. After hydrolysis the sample was passed through Dowex anionic and cationic resins and the neutral sugars were eluted with water. Labelled sugars were analysed by descending paper chromatography on Whatman 3 MM paper with ethyl acetate/pyridine/acetic acid/water (5:5:1:3, by vol.). On both sides of the chromatograph standard monosaccharides were applied. The markers were detected by staining in a solution containing diphenylamine (1 g), aniline (1 ml), phosphoric acid (85%, w/v; 5 ml) and acetone (50 ml). The paper was sliced in 3 cm × 1 cm pieces and the radioactivities were determined by counting in Bray's (1960) solution.

The periodate/ NaB^3H_4 -labelled glycoprotein was hydrolysed with $0.05\text{ M-H}_2\text{SO}_4$ for 1 h at 80°C . At the same time an erythrocyte membrane preparation labelled in a similar way (Gahmberg & Andersson, 1977) was hydrolysed. After hydrolysis both samples were neutralized with NaOH and filtered through a Sephadex G-25 column in water. The radioactive peaks eluted in the low-molecular-weight regions were recovered. After freeze-drying the samples were dissolved in water/methanol (1:1, v/v) and descending paper chromatography was performed. On both sides of the virus sample, the labelled erythrocyte preparation was applied, and after chromatography in butyl acetate/acetic acid/water (3:2:1, by vol.) the paper was dried and both the markers and the virus-sample strips were cut into pieces and the radioactivities determined as described above for the galactose oxidase/ NaB^3H_4 -labelled samples.

Results

Polyacrylamide-gel electrophoresis of measles virus labelled in vivo and in vitro

Labelled measles-virus proteins were analysed by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. One major labelled glycoprotein with an apparent mol.wt. of 79 000 was seen after treatment of intact virus with neuraminidase and galactose oxidase (Fig. 1a). No change in

the labelling profile was seen when the virus had been treated with galactose oxidase only (Fig. 1b). This suggested that the 79 000-mol.wt. glycoprotein contains no or very little sialic acid. A weakly labelled 79 000-mol.wt. peak was observed after periodate/ NaB^3H_4 labelling (Fig. 1c). A similar profile was obtained by using NaB^3H_4 alone without any pretreatment (Fig. 1d).

Because of the possibility of the presence of glycoproteins that had not been oxidized by galactose oxidase or periodate and therefore would not have been labelled by external labelling, measles-virus glycoproteins were also analysed after labelling *in vivo* with $[^3\text{H}]$ glucosamine. Fig. 2 shows that only one major glycoprotein peak, corresponding in molecular weight to that obtained by external labelling, was seen after labelling with this mono-saccharide. The labelled material running in the front (Figs. 1a, 1b, 2) most probably represents labelled glycolipids, because the label was strongly increased after treatment with galactose oxidase (cf. Figs. 1a and 1d).

Analysis of the glycopeptide obtained from the measles-virus glycoprotein

Gel filtration on a Bio-Gel P-6 column of the Pronase digest of the glycoprotein labelled with ^3H after galactose oxidase treatment gave one rather broad glycopeptide peak with a mean apparent molecular weight of about 3000 (Fig. 3a). A similar

Fig. 1. Polyacrylamide-gel electrophoresis of surface-labelled measles virus

(a) Labelling pattern after treatment with galactose oxidase and neuraminidase. (b) Labelling pattern after treatment with galactose oxidase only. (c) Labelling pattern after treatment with 1 mM-periodate. (d) Labelling pattern after treatment with NaB^3H_4 only. Abbreviations: BPB, position of Bromophenol Blue marker dye; GP79, major labelled glycoprotein with an apparent mol.wt. of 79 000. For details see the Materials and Methods section.

pattern was obtained for the [^3H]glucosamine-labelled glycoprotein (Fig. 3b). No radioactivity was released from the glycoprotein by 0.05 M-NaOH/1 M-NaBH₄ (Finne, 1975) (results not shown), which indicates that the oligosaccharide is bound to an asparagine residue through an alkali-stable bond.

Fig. 2. Polyacrylamide-gel-electrophoresis pattern of [^3H]glucosamine-labelled measles virus. For abbreviations and details see legend to Fig. 1.

Fig. 3. Gel filtration of Pronase-treated glycoprotein (GP79) on Bio-Gel P-6

(a) Glycopeptide from galactose oxidase + neuraminidase/ NaB^3H_4 -labelled glycoprotein (GP79). (b) Glycopeptide from [^3H]glucosamine-labelled glycoprotein (GP79). For details see the text. V_0 , void volume.

Identification of labelled sugars from externally labelled measles-virus glycoprotein

When the hydrolysed sugars from the galactose oxidase/ NaB^3H_4 -labelled glycoprotein were eluted from the Dowex columns with water, about 90% of the applied radioactivity was recovered. Fig. 4(a) shows the paper-chromatography profile. Only one radioactive peak co-migrating with the galactose marker was obtained. Mild acid hydrolysis of periodate/ NaB^3H_4 -labelled virus followed by paper chromatography showed no radioactivity in the positions of 5-acetamido-3,5-dideoxy-L-arabino-2-octulosonic acid or 5-acetamido-3,5-dideoxy-L-

Fig. 4. Descending paper chromatography of hydrolysed sugars obtained from labelled glycoprotein (GP79) and labelled erythrocyte membranes

(a) Neutral sugars hydrolysed from galactose oxidase + neuraminidase/ NaB^3H_4 -labelled glycoprotein (GP79). The position of the galactose marker is indicated by the shaded box. (b) Pattern obtained after hydrolysis in 0.05 M- H_2SO_4 of periodate/ NaB^3H_4 -labelled glycoprotein (GP79). (c) Pattern obtained after hydrolysis in 0.05 M- H_2SO_4 of periodate/ NaB^3H_4 -labelled erythrocyte membranes. For details see the text.

arabino-heptulosonic acid (Fig. 4b) (Gahmberg & Andersson, 1977), whereas the similarly treated erythrocyte membrane gave the two expected peaks (Fig. 4c).

Discussion

Measles is an important viral disease, and characterization of the virus is a prerequisite for successful understanding and management of the disease. Compared with many other enveloped viruses, measles virus is difficult to grow and purify, and this largely explains the meagre and conflicting literature on the virus. We have therefore initiated a more detailed biochemical characterization of its membrane, and here we report the partial elucidation of the structure of its major glycoprotein.

Our studies confirm those reports claiming that measles virus contains only one major glycoprotein, with a mol.wt. of 79 000. This glycoprotein was radioactively labelled by NaB^3H_4 after treatment with galactose oxidase. This shows that the glycoprotein is exposed on the surface of the viral particle. It contains terminal D-galactose. Incubation with neuraminidase did not change the labelling from NaB^3H_4 after galactose oxidase treatment. This strongly indicates that the major glycoprotein contains no or very little sialic acid. This notion was further strengthened by using the periodate/ NaB^3H_4 -labelling method, which is specific for sialic acids (Gahmberg & Andersson, 1977). It should, however, be pointed out that O-acetylated sialic acids may not be oxidized by periodate under these conditions, and thus not be labelled.

Dore-Duffy & Howe (1978) claimed that purified measles virus contains sialic acid. This was based on the liberation of sialic acid-like material after neuraminidase treatment. It is, however, difficult to exclude contamination by host components.

The classical paramyxoviruses do not contain sialic acid, evidently because they contain a virus-coded neuraminidase (Klenk *et al.*, 1970; Klenk & Choppin, 1971). Whether measles virions also contain a neuraminidase is unclear. Howe & Schluederberg (1970) did not detect neuraminidase activity in purified measles virions, but our finding of a virtual absence of sialic acid from the major glycoprotein indicates an exposure to neuraminidase somewhere during the maturation.

Previously, there was no information available on the structure of the carbohydrate component(s) of measles-virus glycoproteins. We found that the oligosaccharide of the major glycoprotein contains glucosamine and galactose and is attached to the polypeptide through an alkali-stable linkage. From the presence of both galactose and glucosamine in the glycopeptide and from its mol.wt. of about 3000

we can expect a structure similar to the desialylated 'lactosamine type' of oligosaccharides found in other enveloped viruses (Mattila *et al.*, 1976; Nakamura & Compans, 1978). No evidence was obtained for the presence of mannose-rich 'simple' oligosaccharides, which are found in influenza virus and Semliki Forest virus (Nakamura & Compans, 1978; Mattila *et al.*, 1976). Like other enveloped viruses, measles virus does not contain O-glycosidic alkali-labile oligosaccharides.

The major glycoprotein is expected to have an amphiphilic structure containing a hydrophobic amino acid sequence (Gahmberg *et al.*, 1972). This should make it possible to get it incorporated into liposomes for vaccine production (Morein *et al.*, 1978), thereby circumventing side-effects commonly seen during measles vaccination.

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