Lymphocytic Choriomeningitis Virus. III. Structural Proteins of the Virion

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SUMMARY

Analysis of radioactively labelled and highly purified infectious lymphocytic choriomeningitis (LCM) virus by polyacrylamide gel electrophoresis (PAGE) revealed 12 components which, according to their apparent molecular weight and glycosylation status, were designated as p19, p25, p26, gp35, p38, gp44, gp60, p77, gp85, gp130, and p200. As shown by immunoprecipitation, they all bound to rabbit anti-LCM virus antibodies. Three proteins, namely gp35 (= 'GP-2'), gp44 (= 'GP-1') and p63 (= 'NP'), had previously been described by others as major constituents of the virion. Our results confirm this and suggest that gp60, p77, gp85, and p200 are further distinct structural proteins. In contrast, p25 and p38 appear to be cleavage or degradation products of p63; p19 and p26 seem to belong to gp60, which could be the monomeric form of a dimer, gp130. Peptide mapping by limited proteolysis revealed considerable overlapping of amino acid sequences among the major glycoproteins with one peptide being common to all. From the results of PAGE performed after external labelling of intact virions, we conclude that gp44, gp60, and gp85 (but not gp35) form the surface of the virus envelope. Analytical isoelectric focusing under non-reducing conditions has shown that the major glycoproteins appeared to consist of several components with different isoelectric points.

INTRODUCTION

Lymphocytic choriomeningitis (LCM) virus is of medical relevance and of great experimental value for virologists and immunologists alike (Buchmeier et al., 1980; Lehmann-Grube, 1981, 1982); yet its structural analysis is still at an early stage. We have investigated the chemical composition of the virion. In addition to the already known 'NP', 'GP-1', and 'GP-2' (Buchmeier & Oldstone, 1978; Buchmeier et al., 1978, 1981), we have detected a further four distinct proteins in association with purified infectious virus.

METHODS

Propagation and purification of infectious virus. The plaque-purified WE strain of LCM virus (Rivers & Scott, 1936; Popescu & Lehmann-Grube, 1976) was propagated under conditions known to favour infectious virus rather than interfering particles (Martinez Peralta et al., 1981), usually in L cells but also in secondary foetal fibroblastic cells from NMRI mice. Cells were infected at infectious multiplicities of 0·01. They were maintained with Eagle's minimum essential medium (Eagle, 1959) supplemented with 5% calf serum which had been treated prior to use with polyethylene glycol 40000 (Serva) (Gschwender et al., 1975). After incubation for 44 h at 37 °C, virus was concentrated by treatment with polyethylene glycol 40000 followed by centrifugation at 20000 g for 1 h (Gschwender et al., 1975). The sediment was resuspended in GNTE buffer (pH 7·5, containing 0·2 M-glycine, 0·2 M-NaCl, 0·02 M-Tris and 0·002 M-EDTA) and was put on a discontinuous Urografin (Schering AG, Berlin, Germany) gradient prepared by layering 10 ml of a 10% solution onto 6 ml of a 50% solution. After centrifugation for 3 h at 25000 rev/min in an SW27 rotor (Beckman), the virus on the Urografin cushion was collected from the side by piercing the tube. This fraction was adjusted to a Urografin concentration of 30% and placed in the appropriate position in a 0 to 40% Urografin gradient to be centrifuged to equilibrium in an SW50 rotor (Beckman) at 38000 rev/min for 18 h. Visible bands were collected, diluted fivefold with GNTE buffer, and centrifuged at 150000 g for 1 h. The pellet, representing the purified virus, was taken up in GNTE buffer. Because
of the thermal lability of this virus, the whole purification procedure was carried out at 0 to 4 °C. Infectious virus was monitored by plaque titration (Lehmann-Grube & Ambrassat, 1977). As a rule, final recovery was 40 to 50% of the starting material.

Our procedure for purifying radioative virus has recently been described (Martinez Peralta et al., 1981).

Radioactive labelling of virus. Infected cells were exposed to the radioactive isotope for the final 24 h of virus growth. Proteins were labelled by adding either 10 μCi/ml of 14C-labelled protein hydrolysate (59 mCi/mmol of D-[6-3H]glucosamine hydrochloride (30 Ci/mmol) at a concentration of 30 μCi/ml was incorporated into culture medium, in which fructose was exchanged for glucose, or 30 μCi/ml of D-[2-3H]mannose (16 Ci/mmol) were added. Radioactive tyrosine was incorporated by exposing infected cells to 30 μCi/ml of L-[3,5-3H]tyrosine (50 Ci/mmol). For the detection of sulphated viral components, [35S]potassium sulphate (25 to 40 Ci/mg) was added to the culture medium to a concentration of 30 μCi/ml.

For the external iodination of intact virions, chloramine T (Bruns et al., 1977), IODO-GEN (Pierce Eurochemie B.V., Rotterdam, Holland) (Fraker & Speck, 1978), or lactoperoxidase (Moore et al., 1974) were employed. Since in the chloramine T procedure a high iodine concentration is known to be deleterious to virus particles (Montelaro & Rueckert, 1975), we used relatively low quantities, and in each test 35 μg of virus was allowed to interact with 200 μCi of iodine-125 or iodine-131. Separation of labelled virions and labelled virus proteins was achieved, respectively, by ultracentrifugation in a 0 to 50% sucrose gradient in a Beckman SW41 rotor at 38000 rev/min for 1 h and by gel filtration on Sephadex G-50 (Pharmacia). Subsequently, virions were concentrated by ultracentrifugation and proteins by precipitation in the cold with 10% trichloracetic acid (TCA) followed by ethanol. For control purposes, ether-disrupted virus was iodinated by the chloramine T method. (All radiochemicals were purchased from Amersham Buchler.)

Immune precipitation of viral antigens. Anti-LCM virus antiserum was obtained from rabbits which had been immunized by repeated intravenous inoculation of LCM virus passaged once and propagated in primary rabbit kidney cells grown and maintained in Eagle's minimum essential medium supplemented with 5% heated rabbit serum. Radioactively labelled virus was purified and concentrated. It was lysed in 200 μl of TNE buffer (pH 7.4, consisting of 0.01 M-Tris–HCl, 0.01 M-NaCl, and 0.001 M-EDTA) containing in addition 2% Nonidet P40 (NP40) and 1 M-KCl (Buchmeier et al., 1978). Immunoprecipitation of the smaller polypeptides was more effectively achieved by use of the disruption solution proposed by Witte & Baltimore (1978). To a phosphate buffer consisting of 0.01 M-Na2HPO4, 0.01 M-Na2HPO4, and 0.1 M-NaCl at pH 7.5 were added 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium lauryl sulphate (SDS) (Witte & Baltimore, 1978). Both these solutions also contained ribonuclease (Merck) at a concentration of 1 mg/ml. Five μl of either antiserum or normal rabbit serum were added and the mixtures were kept for 18 h at 4 °C. Thereafter, 200 μl of 10% (v/v) formalin-fixed Staphylococcus aureus Cowan strain I (Kessler, 1975) were admixed and the precipitate that had formed after 2 h at 4 °C was washed and electrophoresed.

Hyaluronidase treatment of virions. Purified infectious virus was treated with hyaluronidase (Merck) as described by Pinter & Comans (1975) and repurified by banding in a sucrose gradient.

Electrophoresis. Analytical polyacrylamide gel electrophoresis (PAGE) was performed essentially according to Laemmli (1970). We used 10% cylindrical gels; slab gels were prepared either with 7.5% acrylamide or with 7.5 to 15%. 7.5 to 20% or 15 to 20% acrylamide gradients. Slab gels were always employed with 3% stacking gels. Labelled virions were purified and disrupted by heating for 1 min at 100 °C in the presence of 2% SDS and 5% 2-mercaptoethanol. In cylindrical gels the isoepotes were localized by cutting 1.5 mm slices and then determining their radioactivities with a model 'BF Gammaszint 5300' gamma counter (Berthold, Wildbad, F.R.G.). Slab gels were fixed and processed for fluorography as described by Bonner & Laskey (1974) and were exposed to X-ray films at -70 °C. As mol. wt. markers, the 14C-methylated protein mixture of Amersham Buchler was used.

Quantification of proteins. Protein concentrations were measured by the method of Lowry et al. (1951) with bovine serum albumin as reference standard.

Phase separation of proteins. To enrich certain proteins, especially glycoproteins, the two-phase separation technique of Albertsson (1970) with dextran T500 and polyethylene glycol 6000 in buffer containing 1 M-NaCl, as employed by Bishop & Roy (1972), was used.

Estimation of molar ratios of structural proteins. Virus, labelled with 14C-protein hydrolysate or [3H]glucosamine, was purified and disrupted. Proteins were enriched by phase separation, subjected to PAGE, localized in the gel, and eluted electrophoretically as described by Cleveland et al. (1977). Radioactivities were then determined and molar ratios in the virion of non-glycosylated and glycosylated proteins were expressed as relative abundances taking a value of 1.0 for p63. Furthermore, the degrees of glycosylation were calculated by correcting the [3H]glucosamine counts for the relative abundances of each glycoprotein and expressing the values again relative to 1.0 for gp44 (Stern et al., 1982).

Peptide mapping by limited proteolysis. Proteins were phase separated and analysed essentially according to...
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Cleveland et al. (1977). Phases were subjected to PAGE. Radioactive bands were localized and cut out from the dried gel. The proteins were eluted electrophoretically and then precipitated with 20% TCA and ethanol. Thereafter, 1 μg of α-chymotrypsin (Sigma) in 0.2 ml buffer was added to the precipitate and enzymic cleavage of the viral proteins was allowed to proceed for 30 min at 37 °C, whereupon the peptides were again precipitated and analysed by electrophoresis in a 15 to 20% acrylamide gradient gel.

Analytical isoelectric focusing. To focus viral proteins and glycoproteins, a two-dimensional method was employed (O'Farrell, 1975). Purified virions, labelled with [35S]methionine or [3H]glucosamine, were disrupted by treatment for 45 min at room temperature with a buffer consisting of 2% NP40, 9.5 M urea, 2% alamines pH 3.5 to 4.0, and 5 μg/ml ribonuclease. Focusing was then carried out for 7 h at room temperature in 5% polyacrylamide gel containing 2% ampholytes and 8 M-urea in 12.0 cm × 0.5 cm glass tubes at an initial voltage of 100 V and a maximum power of 1.5 W. Thereafter, the gels were soaked for 1 h at room temperature in 2% SDS and 5% 2-mercaptoethanol before electrophoresis in a 7.5 to 15% acrylamide slab gel gradient as described above. The pH values attained during focusing were determined during gels run in parallel.

RESULTS

Structural polypeptides of the virus as revealed by PAGE

The majority of experiments to be reported were made with virus concentrated and purified from fluid of infected L cell cultures. However, essentially the same results were obtained when the virus had been propagated in foetal fibroblastic cells from NMRI mice. The number of virion-associated polypeptides appears to be considerably greater than initially thought, and to simplify their designation, we have adopted the terminology introduced for retroviruses by August et al. (1974). Hence, NP, GP-1, and GP-2 of Buchmeier & Oldstone (1978, 1979) become p63, gp44, and gp35. These three were also found by us and, in addition, we regularly saw nine further protein components when highly purified infectious virus was analysed by PAGE; these were named p19, p25, p26, p38, gp60, p77, gp85, p130, and p200 (Fig. 1 and 2). All 12 polypeptides were immunoprecipitated, although the autoradiographic bands thus obtained were always weak for p19, p25, p26, and gp130. Immunoprecipitated gp60 is not visible in Fig. 2. Presumably it is obscured by the heavy band of p63, but binding of antibody to gp60 is readily demonstrated by use of [3H]glucosamine-labelled virus (not shown). Sometimes, purified virus preparations contained a further glycoprotein of apparent mol. wt. 65 × 10^3 (see Fig. 1, lanes a and b). This gp65 has previously been shown to be a structural component of LCM virus interfering particles (Welsh & Pfau, 1972; Martinez Peralta et al., 1981) with which every virus preparation is inevitably contaminated (Martinez Peralta et al., 1981). Since the purification procedure employed in this study did not separate interfering particles from infectious virus, components of the former occasionally attained detectable levels. In the remainder of this report gp65 will be ignored unless it is evident in gels.

In the experiment depicted in Fig. 1 (b), glycosylation was revealed by propagating virus in the presence of [3H]glucosamine. When radioactive mannose was employed, four glycoproteins formed bands but gp60 did not (not shown). In fact, this protein formed bands of low densities even after labelling the virus with glucosamine. When the virus had been grown in the presence of radioactive methionine (as in Fig. 1a) or protein hydrolysate (not shown), gp60 blended with the predominant p63 (NP). However, when the time of incubation was reduced to one growth cycle (16 h), two relatively dense radioactive bands were resolved, corresponding to p63 and a slightly smaller entity with apparent mol. wt. 60 × 10^3 (Fig. 1c). Probably gp60 is a glycoprotein whose carbohydrate moiety is either small or poorly labelled with glucosamine and not at all with mannose. The bands in Fig. 1 (a) designated p38 and p25 were not observed when virus had been labelled with [3H]glucosamine, were accentuated by treatment of virions with trypsin, and could be shown by limited-digest analysis to represent polypeptides related to p63 (not shown). Similarly, limited proteolysis (see below) revealed that p26 and p19 are probably cleavage products of gp60. On the other hand, gp130 appears to be an unreduced dimer of gp60, because when PAGE was performed under non-reducing conditions, the autoradiographic expression of the former was intensified, while that of gp60 was diminished.

The band below gp85 (Fig. 1a) with a mol. wt. 77 × 10^3 probably corresponds to a non-glycosylated polypeptide, because during phase separation it did not appear in the polyethylene
Fig. 1. Analysis by PAGE of the structural proteins of LCM virus. L cells were infected and virus was allowed to replicate for 44 h, the final 24 h in the presence of [35S]methionine (a) or [3H]glucosamine (b). It was purified and disrupted, and electrophoresis was performed with slab gels consisting of acrylamide at concentrations 7.5 to 20%. In the experiment depicted in lane (c), virus was electrophoresed after it had been grown for 16 h (one cycle) in the presence of [35S]methionine. The position of radioactive isotopes was determined by autoradiography.

glycol phase. We do not yet know whether it is a distinct entity or whether it is the protein portion of gp85 lacking carbohydrate.

A further polypeptide often found, with mol. wt. 100 × 10^3 (see the band above gp85 in Fig. 1a), also appeared in control experiments with uninfected cells. PAGE after immunoprecipitation of [35S]methionine-labelled virus revealed a band below that corresponding to p200 (Fig. 2, left side). It was only seen when Buchmeier's disruption solution was used; its origin is unknown.

The molar ratios for all 12 regularly resolved viral polypeptides, expressed as 'relative abundances' (Stern et al., 1982), have been calculated, and also their glucosamine contents (Table 1).

In addition to these proteins, in all PAGE runs performed with glucosamine-labelled virus we saw a heavy band on top of the separating gel (see Fig. 1b). This material was never detected when the labelling had been done with radioactive methionine of protein hydrolysate on the one hand or mannose on the other, and it was suspected to be a mucopolysaccharide. We therefore treated [3H]glucosamine- or [35S]sulphate-labelled virions with hyaluronidase prior to PAGE and found that it was highly sulphated and removable by the enzyme (see Fig. 3a to d). We conclude that this virion-associated substance is a sulphated polysaccharide, presumably of host cell origin.

Considerably more virion-associated polypeptides of LCM virus were found by us than had previously been published, and to rule out the possibility that the newly observed components
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Fig. 2. Immunoprecipitation of structural polypeptides of LCM virus. L cells were infected and labelled with \[^{35}S\]methionine as described for Fig. 1(a). Virus was purified and disrupted either with 2\% NP40 and 1 m-KCl in TNE buffer (left) or with 1\% Triton X-100, 0.5\% sodium deoxycholate, and 0.1\% SDS in phosphate buffer (right) and subsequently treated with rabbit anti-LCM virus antiserum (IS) or normal rabbit serum (NS) and Staphylococcus aureus. Conditions for electrophoresis and visualization of radioactive bands were as described for Fig. 1.

Table 1. Estimation of the relative abundance of the proteins and calculation of the glucosamine content per glycoprotein

<table>
<thead>
<tr>
<th>Protein</th>
<th>Relative abundance (A)*</th>
<th>Glucosamine content (C)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>p200</td>
<td>0.04</td>
<td>–</td>
</tr>
<tr>
<td>gp130</td>
<td>0.02</td>
<td>0.58</td>
</tr>
<tr>
<td>gp85</td>
<td>0.08</td>
<td>0.49</td>
</tr>
<tr>
<td>p77</td>
<td>0.08</td>
<td>–</td>
</tr>
<tr>
<td>p63</td>
<td>1.00</td>
<td>–</td>
</tr>
<tr>
<td>gp60</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>gp44</td>
<td>0.23</td>
<td>1.00</td>
</tr>
<tr>
<td>p38</td>
<td>0.15</td>
<td>–</td>
</tr>
<tr>
<td>gp35</td>
<td>0.22</td>
<td>0.14</td>
</tr>
<tr>
<td>p26</td>
<td>0.05</td>
<td>–</td>
</tr>
<tr>
<td>p19</td>
<td>0.08</td>
<td>–</td>
</tr>
<tr>
<td>p19</td>
<td>0.08</td>
<td>–</td>
</tr>
</tbody>
</table>

* A = (ct/min of band \times\ mol. wt. of p63)/(mol. wt. of band \times\ ct/min of p63).
† C = (ct/min of band \times\ A of gp44)/(A of band \times\ ct/min of gp44).

were of cellular origin, a control experiment was performed as follows. Uninfected L cells were grown in the presence of \[^{35}S\]methionine and \[^{3}H\]glucosamine under conditions identical to the ones employed to label virus. They were frozen and thawed once (to mimic the slight cytopathic effect usually observed in LCM virus-infected L cells in the log phase of growth) and non-labelled virus was added. The latter was then purified and subjected to PAGE exactly as described. Not surprisingly, several bands appeared but none migrated to a region corresponding to polypeptides presumably derived from the virion, except the above-mentioned material with mol. wt. 100 \times 10^3, which is presumably a cellular contaminant.
Fig. 3. Characterization of a virion-associated high molecular weight component. Cells were infected and labelled with $[^3H]glucosamine (a and b)$ or $[^35S]potassium sulphate (c and d)$. Virus was purified and treated with hyaluronidase. Electrophoresis of disrupted virus was performed with 7.5% acrylamide slab gels. Radioactive bands were visualized by autoradiography.

**Position of proteins in the virion**

The proteins forming the viral envelope were determined by iodination of intact virions followed by PAGE. Since interpretation of the results is critically dependent on integrity of the virions during labelling, we employed three procedures known to differ in their effects on biological membranes. The results were closely similar. The glycoproteins gp85, gp60, and gp44 were iodinated, but not gp35. Surprisingly, a non-glycosylated protein, p19, also associated with radioactive iodine, indicating that it was positioned on the surface of the virion. Of a number of experiments with similar results, one in which virus was labelled in parallel by use of either chloramine T or lactoperoxidase is depicted in Fig. 4(a). Fig. 4(b) illustrates the pattern obtained when the virus was first disrupted and then iodinated. There are peaks corresponding to gp85, p63, and gp44, but not to p19; presumably the concentration of the latter was too low. Significantly, gp35 was also labelled. The conclusion that absence of a corresponding peak in Fig. 4(a) is due to inaccessibility rather than inability of this glycoprotein to incorporate the label is strengthened by the results of experiments (not shown) demonstrating that gp35 is relatively rich in tyrosine, known to be the amino acid that interacts with iodine. [Our reason for assuming that the high peak in Fig. 4(a) represents gp60 rather than the nucleoprotein p63 will be presented in the Discussion.]
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Fig. 4. Determination of the polypeptides of LCM virus accessible to external iodination. (a) Infectious virus was purified and intact particles were iodinated with chloramine T (○) to add $^{125}$I or lactoperoxidase (△) to add $^{131}$I. The virions were then disrupted and electrophoresed in cylindrical 10% acrylamide gels which were subsequently cut into 1.5 mm slices whose radioactivity was then determined. (b) Purified virus was disrupted before iodination with the chloramine T method. Electrophoresis was performed as described for (a).

Peptide mapping of glycoproteins

As a first step, glycoproteins were enriched by a two-phase separation procedure. Similar to the observations of Vezza et al. (1977) for the arenavirus Pichinde virus, all glycoproteins, but also p26 and p19, were found predominantly in the polyethylene glycol phase. The efficiency of separation depended on the pH. While the yields of gp85, gp60, p26, and p19 were best at high pH values (pH 8), gp35 and gp44 accumulated preferentially at low pH (pH 4). This statement is based on a number of experiments with similar results.

Glycoproteins (and p26 and p19, but not gp130, which was present in too low quantities) were
Fig. 5. Limited-digest peptides of glycoproteins and p19 and p26 of LCM virus. Labelled viral proteins enriched by two-phase separation in the polyethylene glycol phase at pH 8.0 were separated by PAGE. The contents of individual bands were subjected to limited digestion with chymotrypsin, and peptides thus obtained were separated on a 15 to 20% polyacrylamide gel and subsequently visualized by fluorography.

partly digested with chymotrypsin and subsequently compared by PAGE. One of two similar experiments with essentially identical results is presented in Fig. 5. Multiple fragments were obtained, of which several appeared to be represented in more than one protein. One peptide was found in all glycoproteins thus analysed. Others were shared by gp85 and gp60 and by gp85, gp60, and gp44, respectively. The non-glycosylated structural components p26 and p19 appeared to be related with the glycoproteins in that they contained fragments also released from gp60.

Isoelectric points of structural proteins of the virus

At the beginning of this work we had tried to isolate the viral proteins by isoelectric focusing in columns. These attempts were not successful, because the glycoproteins especially had multiple isoelectric points extending over large areas of the pH gradient. For analytical purposes, a two-dimensional procedure was adopted in which isoelectric focusing employing a pH gradient in the range 3.5 to 10.0 was followed by PAGE at an angle of 90°. In Fig. 6, two-dimensional slab gels are presented, which were performed with virus whose structural proteins (Fig. 6a) and glycoproteins (Fig. 6b) had been labelled with radioactive methionine and glucosamine, respectively. PAGE in the second dimension revealed that during focusing (under non-reducing conditions) gp35 and gp85 migrated together, probably as complexes. (Less likely is the possibility that they have similar isoelectric points.) Fig. 6 also discloses that individual glycoproteins had settled at more than one position, meaning that their isoelectric points were not uniform. Thus, gp44 had migrated to at least 13 spots corresponding to isoelectric points ranging from pH 5.8 to 8.5, and gp35 and gp85 to 12 spots between pH 4.5 and 8, of which eight were heavy and four light.

Of gp60, five small spots or two faint ones were seen when the virus had been labelled with [35S]methionine or [3H]glucosamine, respectively, indicating that this protein was only partially glycosylated. Similarly, of the two spots formed by gp65 (the major glycoprotein of interfering particles) only one appeared to contain carbohydrate. The faint spot seen below gp130 (see Fig. 6b) could be a dimer of gp65.

As to the non-glycosylated viral polypeptides, these too were found to consist of subclasses
Fig. 6. Two-dimensional separation of structural non-glycosylated (above) and glycosylated (below) polypeptides of LCM virus by sequential application of isoelectric focusing (horizontal) and PAGE (vertical). Virus grown in the presence of [35S]methionine or [3H]glucosamine, respectively, was purified and disrupted with NP40 in the presence of urea (but absence of 2-mercaptoethanol). Proteins thus obtained were focused in polyacrylamide gel containing urea. Subsequently, they were reduced and electrophoresed. Radioactivity was localized by autoradiography. Large and small arrows point to the positions of gp60 and gp65, respectively.

with different isoelectric points. The nucleoprotein (p63) stretched over a large part of the gradient; it was concentrated at pH 5·7 and pH 7·7.

DISCUSSION

Although the LCM virus was among the first animal viruses to be investigated experimentally (Traub, 1936, 1939), for a long time the virion remained largely unexplored, the main reason
being the difficulty of purifying it. Experience with related agents such as Pichinde virus (Rawls & Leung, 1979) and the application of advanced methodology have led to greatly improved procedures; the principles of our approach and the criteria of purity have recently been published (Gschwender et al., 1975) and were proved by us electrophoretically. When radioactively labelled virus thus prepared was disrupted and subjected to PAGE, autoradiography revealed a limited number of distinct bands, of which 12 were regularly seen; according to their mol. wt. and glycosylation status, they were designated p19, p25, p26, gp35, p38, gp44, gp60, p63, p77, gp85, gp130, and p200. Since they bound LCM-viral antibodies, we are convinced that they represent structural components of the virion. With respect to gp35, gp44, and p63, our observations fully confirm the work of Buchmeier and his colleagues (Buchmeier & Oldstone, 1978, 1979; Buchmeier et al., 1978), who detected them consistently and found them to be relatively plentiful in highly purified preparations they also showed that these proteins combined with LCM virus-specific antibodies. In the same category fall gp60, p77, gp85, and p200, and the question may be asked why these have not been seen previously. With respect to gp60, we too had initially overlooked it. Its presence was suspected from a weak band appearing in PAGE after labelling the virus with glucosamine (but not mannose). Later, we observed a distinct entity in the neighbourhood of p63 when [35S]methionine-labelled virus was harvested after 16 h (one cycle). Separation of both proteins was achieved by a two-phase procedure with p63 appearing in the dextran phase and gp60 in the polyethylene glycol phase. Two-phase separation followed by PAGE also allowed an assessment of the relative quantities. Taken together, our data indicate that gp60 is a major structural glycoprotein whose carbohydrate moiety is either small or of unusual composition. With respect to gp85, Buchmeier et al. (1978) have described heterogeneity in the region of GP-1 ranging from a skew towards the high mol. wt. side to peaks corresponding to an apparent mol. wt. of over 63,000. Perhaps it is this component which, because of methodological differences, migrated in our experiments as a distinct glycoprotein with apparent mol. wt. 85 x 10^3.

Non-glycosylated proteins with mol. wt. 77 x 10^3 and 200 x 10^3 (corresponding to our p77 and p200) have been detected in other arenaviruses. A protein of mol. wt. 77 x 10^3 to 79 x 10^3 has been described by Compans et al. (1981) as part of the virion of Tacaribe virus, and a polypeptide ('L') with mol. wt. 200 x 10^3 was found in association with the virion of Pichinde virus and could be immunoprecipitated from Pichinde virus-infected BHK21 cells (Harnish et al., 1981). The function of the L protein is unknown, but Harnish et al. (1981) have considered the possibility that it is an RNA polymerase.

Five further components resolved by PAGE of highly purified infectious LCM virus are probably not proteins sui generis. Thus, p38 and p25 appear to be cleavage or degradation products of p63. Perhaps they correspond to NP38 and NP28, respectively, found by Harnish et al. (1981) in association with Pichinde virus. As to p19 and p26, they are probably derived from gp60. Whether in the virion they are distinct components, we do not know. Indeed, we are not certain whether gp60 exists as such or as a dimer (gp130). The close relationship between p19, p26, gp60, and gp130 is also indicated by our finding (unpublished) that these four viral components are the only ones that were found to be covalently bound to lipids.

The structural relevance of the mucopolysaccharide is unknown. Since it is easily removed from the virus particle by treatment of the latter with hyaluronidase, this compound is probably associated with the viral envelope. Interestingly, incubation with the enzyme increased the infectious titre by up to 10-fold (unpublished).

As in all arenaviruses so far described, the genome of the LCM virus is contained in two molecules of single-stranded RNA (Pedersen, 1979; Rawls & Leung, 1979). The combined coding capacity has not been estimated for LCM virus, but should be closely similar to that of Pichinde virus, which is assumed to be capable of directing the synthesis of proteins corresponding to a total mol. wt. of 410,000 (Harnish et al., 1981). We do not know how much the carbohydrate portions of the glycoproteins contribute to their apparent molecular weight but a priori it is likely that the sum of the protein moieties of the virion as found by us is in excess of the theoretical coding capacity, even if we ignore further as yet unknown non-structural proteins. Hence, our results showing overlapping of limited-digest peptides among individual
glycoproteins were not surprising. Initially, Buchmeier & Oldstone (1979) had found a simple precursor–cleavage product relationship between a cell-associated glycoprotein (‘GP-C’) and the structural glycoproteins gp35 (GP-1) and gp44 (GP-2), and the latter two were assumed to be distinct entities. More recently, however, Buchmeier and his colleagues (Buchmeier et al., 1981), investigating a panel of monoclonal antibodies directed against structural components of the LCM virus, found no fewer than three that bound equally well to both glycoproteins, and the possibility of identical epitopes was considered. Our results point in the same direction; they indicate that one peptide is shared not only by gp35 and gp44 but by all glycoproteins which we have analysed with this method. Other peptides, too, were enzymically released from more than one glycoprotein. We cannot, at present, offer a satisfactory interpretation. Probably all viral glycoproteins have one or more common precursor(s) its composition as well as relationship with GP-C we hope to reveal by immunoprecipitation of viral polypeptides from infected cells, in combination with nearest-neighbour analysis of structural proteins.

The latter method is also employed by us to learn more about the architecture of the virion. Our present results indicate that, besides gp44, gp60 and gp85 participate in the formation of the viral envelope. Presumably gp35 and gp130 belong to a deeper layer. The PAGE profile obtained after iodination of the intact virions always showed a high peak in a region corresponding to gp60. Initially, we thought that this activity was associated with p63, and when it remained after we had convinced ourselves that few damaged virions had been present during external labelling, we even considered the possibility that p63 (or a part of it) contributed to the viral envelope. This (a priori unlikely) assumption was dropped when it could be established that the virion contained a glycoprotein of apparent mol. wt. 60 × 10^3. Furthermore, after iodination of intact interfering particles, which do not contain gp60 (Martinez Peralta et al., 1981), PAGE did not reveal radioactivity above the background in the region corresponding to 60 × 10^3 daltons (unpublished). The conclusion that gp60 is part of the envelope (and distinct from p63) is strengthened by the observation that it, together with all other components that are externally accessible to iodine, could be selectively removed from the virion by its treatment with bromelain (Buchmeier et al., 1978; and unpublished results). As has been pointed out, p19 and p26 seem to be parts of gp60. Since p19 could be externally labelled with iodine but p26 could not, we think that the former is the external portion of gp60, and since all appear to be covalently bound to the viral lipids, gp60 is probably anchored with its p26 portion in the viral envelope.

Our inability to detect gp35 on the viral surface is in keeping with the finding of Buchmeier & Oldstone (1979) who could not iodinate this glycoprotein on infected cells; since the LCM virus emerges by budding, it is (at least temporarily) a component of the plasma membrane. More recently, Buchmeier mentioned predominant attachment of label to gp44 (GP-1) during iodination of intact particles (Buchmeier et al., 1981), and by use of monoclonal antibodies he and his colleagues found that those specific for gp44 neutralized viral infectivity more efficiently than the one directed against gp35 (Buchmeier et al., 1981).

Our motive in employing the method of isoelectric focusing was twofold: we wanted to take a first step in the direction of biochemically characterizing the structural proteins of the LCM virus and had, furthermore, hoped to achieve their separation for preparative purposes; we were only partly successful. All major glycoproteins were found to comprise multiple components with differing isoelectric points, an observation closely resembling the one made by Compans et al. (1981) for the related Tacaribe virus. The same method revealed that gp35 and gp85 migrated to the same pH. Perhaps they form complexes already in the virion. If this should turn out to be the explanation, analysis of the complexes may become of help in our attempts to understand the architecture of the virion.

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Structural proteins of LCM virus


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