Comparison of Structural Polypeptides from Vesicular Stomatitis Virus (Indiana and New Jersey Serotypes) and Cocal Virus

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SUMMARY

The molecular size of the structural proteins of the Indiana and New Jersey serotypes of vesicular stomatitis virus (VSV) and of Cocal virus have been compared by co-electrophoresis in SDS-polyacrylamide gel. Virus polypeptides (VP) II, III and V of the Indiana serotype have different electrophoretic mobilities from the corresponding components of the New Jersey serotype. Cocal virus differs from VSV Indiana in the mobility of VP II and VP V, and from VSV New Jersey in the mobility of VP II and VP III. Mol. wt for VP II, III, IV and V of all three viruses have been determined by co-electrophoresis with in vitro labelled radioactive protein markers. In vitro labelling of VSV polypeptides did not affect their electrophoretic mobility.

INTRODUCTION

There are two major serological types of vesicular stomatitis virus (VSV) known as the Indiana and New Jersey serotypes. Most strains of VSV resemble one or the other serologically. The New Jersey serotype (VSV New Jersey) is distinct from the Indiana serotype (VSV Indiana) in the antigenicity of the coat protein, but the nucleoproteins of the two serotypes cross-react (Kang & Prevec, 1970). Temperature-sensitive (ts) mutants of wild-type strains of both serotypes have been isolated following mutagenesis and classified into complementation groups (Pringle, 1970a, b; Pringle, Duncan & Stevenson, 1970). Some of these mutants are able to induce synthesis of virus polypeptides under restrictive conditions (Wunner & Pringle, 1972 and in preparation), but no complementation has been observed in mixed infection experiments with ts mutants of the two serotypes in any combination (Pringle et al. 1971). This suggests that the strains are not closely related genetically and that the polypeptides coded by the Indiana and New Jersey virus genomes are not functionally interchangeable.

We now report that direct comparison of the structural proteins of VSV Indiana and VSV New Jersey, grown in BHK-21 cells under identical conditions, shows differences in the electrophoretic mobilities of corresponding polypeptides. Both serotypes in their turn are compared with Cocal virus (Jonkers et al. 1964), a rhabdovirus originally isolated from mites. Federer, Burrows & Brookesby (1967) showed that Cocal virus cross-reacts serologically with VSV Indiana, but not with VSV New Jersey, in neutralization and complement fixation tests, and therefore regard it as a subtype of VSV Indiana. Recent genetic complementation experiments in this laboratory (Pringle, unpublished) also suggest a relationship between Cocal virus and VSV Indiana.
METHODS

Virus. Wild-type strains of both the Indiana and New Jersey serotypes of VSV were obtained from the Animal Virus Research Institute, Pirbright, and have been described in detail previously (Pringle, 1970a, b; Pringle et al. 1971).

Cocal virus which had been propagated for six passages in Vero cells was obtained from Dr R. B. Somerville, Belvidere Hospital, Glasgow. This material was then passed three times in BHK-21, clone 13 cells (Macpherson & Stoker, 1962) and subsequently cloned by three successive single-plaque isolations. BHK-21 cells were used for propagation of all three strains.

Radioactive isotopes. Virus proteins were labelled with [14C]-protein hydrolysate (U) from Chlorella (54 mc/g. atom) plus [14C]-L-leucine (U) (331 mc/m-mole) or a mixture of fifteen [3H]-amino acids. D-Glucosamine-1-[3H] hydrochloride (3 c/m-mole) was used for the detection of glycoproteins. Protein markers for mol. wt determinations were labelled in vitro with [14C]-formaldehyde (15 mc/m-mole). All radioactive compounds were obtained from the Radiochemical Centre, Amersham, Buckinghamshire.

Protein markers for mol. wt determinations. The following proteins (purchased from Sigma Chemical Co.) were selected as markers in mol. wt determinations by polyacrylamide gel electrophoresis: phosphorylase a, bovine serum albumin Fraction V, catalase, l-glutamic dehydrogenase, ovalbumin, pepsin, carbonic anhydrase, a-chymotrypsinogen and lysozyme.

Isotopic labelling of virus. Monolayers of BHK cells were infected at 0.1 p.f.u./cell. After incubation for 2.5 hr in Eagle's medium with 1% calf serum, the monolayers were washed for 30 min. in medium without calf serum and added amino acids (AAF). At 3 hr after adsorption, [14C]-amino acids (4 #c/ml.), or [3H]-amino acids (8 #c/ml.) or [3H]-glucosamine (1-7 #c/ml.) were added in AAF and incubation continued for 40 min. at 31 °. Eagle’s medium containing 1% calf serum was then added, and incubation continued for a further 20 hr at 31 °.

Virus purification. The culture fluid was harvested and clarified by centrifugation at low speed. A virus pellet obtained by centrifugation at 31,500 g for 90 min. was resuspended in 0.04 M-sodium phosphate buffer (pH 7.6). The virus suspension was then clarified and layered on a 15 to 45% (w/v) sucrose gradient in the same buffer and centrifuged at 40,000 g (average) for 90 min. in a swinging-bucket rotor (SW 25, Spinco). The visible virus zone was collected by side puncture of the centrifuge tube.

Radioactive labelling of proteins in vitro. Virus proteins and protein markers were labelled with [14C]-formaldehyde according to the method of Rice & Means (1971). Briefly, 20 #l. of 0.04 M [14C]-formaldehyde was added to 200 #g. of protein in 0.2 ml. of 0.2 M-sodium borate buffer, pH 9.0, cooled in ice. This was followed in 30 sec. by four sequential additions of 5 #l. sodium borohydride (5 mg./ml.) and an additional 30 #l. of NaBH₄ solution after 1 min.

Preparations of proteins for polyacrylamide gel electrophoresis. Proteins of the purified virus were solubilized by incubation for 1 hr at 37 ° in 0.01 M-sodium phosphate buffer, pH 7.4 containing 1% (w/v) sodium dodecyl sulphate (SDS), 0.5 M-urea and 1% 2-mercaptoethanol or 0.1% dithiothreitol (DTT). Alternatively, virus proteins solubilized in 8 M-urea were first dissociated in acetic acid as described by Wagner, Schnaitman & Snyder (1969). [14C]-marker proteins and [14C]-virus proteins labelled in vitro were incubated at 37 ° for 1 hr in 1% SDS and 1% 2-mercaptoethanol or 0.1% DTT and then dialysed against 0.01 M-sodium phosphate buffer, pH 7.4, containing 1% SDS, 0.5 M-urea and 1% 2-
Polypeptides of VSV and Cocal virus

Fig. 1. Comparison of virus polypeptides from VSV Indiana and VSV New Jersey. Proteins were solubilized from purified virus which was grown in BHK cells and labelled with [3H]- or [14C]-amino acids as described in the text. The profiles shown were obtained by co-electrophoresis on 10 % SDS-polyacrylamide gels. Top left, [3H]- ( ) and [14C]- (-----) VSV Indiana. Bottom left, [3H]- ( ) and [14C]- (-----) VSV Indiana. Bottom right, [14C]- (-----) VSV Indiana and [3H]- (---) VSV New Jersey. Bottom right, [14C]- (-----) VSV Indiana and [3H]- (---) VSV New Jersey.

mercaptoethanol. All samples were heated to 90 to 100 °C for 1 min. before polyacrylamide gel electrophoresis. Sucrose was added to increase density and bromophenol blue was added as tracker dye.

Electrophoresis in SDS-polyacrylamide gel. Polyacrylamide gels of 5 %, 7.5 % and 10 % (w/v) total acrylamide were polymerized with a constant proportion (5 %) of N, N'-methylenebisacrylamide. The gels were cast in glass tubes of 7 mm internal diameter with 0.125 % tetramethylene diamine (TEMED) and 0.07 % ammonium persulphate in 0.1 M-sodium phosphate buffer (pH 7.4) containing 1 % (w/v) SDS, 0.5 M-urea and 0.0038 % K₃Fe(CN)₆. Electrophoresis was performed in Shandon electrophoresis apparatus with electrode buffer of 0.1 M-sodium phosphate (pH 7.4) containing 0.1 % SDS. A constant current of 2.5 mA/gel for 18 hr, 3 mA/gel for 16 hr or 6 mA/gel for 6 hr was applied to 5, 7.5 and 10 % gels, respectively. Then gels were immediately removed from their tubes and frozen on aluminium trays filled with solid CO₂. Stops were set at both ends of the gel to prevent linear expansion. The frozen gels were cut into slices 0.5 mm thick with a Mickle automatic gel slicer. Radioactivity was measured on glass fibre discs according to the
Fig. 2. Comparison of virus polypeptides from VSV Indiana and Cocal virus. Proteins of Cocal virus were prepared identically to VSV Indiana and VSV New Jersey and compared as described in the legend for Fig. 1. Top left, [3H]- (---) and [14C]- (-----) VSV Indiana. Bottom left, [3H]- (---) and [14C]- (-----) Cocal virus. Top right, [3H]- (---) VSV Indiana and [14C]- (-----) Cocal virus. Bottom right, [14C]- (-----) VSV Indiana and [3H]- (---) Cocal virus.

RESULTS

Co-electrophoresis of virus polypeptides

VSV Indiana and VSV New Jersey

Polyacrylamide gel profiles of mixed [3H]-labelled and [14C]-labelled virus polypeptides (VP) of the two strains are shown in Fig. 1. In the profiles of the heterologous mixtures (top and bottom right) VP II and VP III of the Indiana strain migrated faster while VP V migrated slower than the corresponding polypeptides of the New Jersey strain. There is no clear difference in migration of VP I and VP IV. The profiles of the homologous mixtures (top and bottom left) show that the radioactive label makes no difference to the mobilities of any of the virus polypeptides in SDS-polyacrylamide gel (Fig. 1 to 3).
Polypeptides of VSV and Cocal virus

Fig. 3. Comparison of virus polypeptides from VSV New Jersey and Cocal virus as described in the legends for Fig. 1 and 2. Top left, [3H]- (—) and [14C]- (— — — —) VSV New Jersey. Bottom left, [3H]- (——) and [14C]- (—— — —) Cocal virus. Top right, [3H]- (—) VSV New Jersey and [14C]- (— — — —) Cocal virus. Bottom right, [14C]- (— — — —) VSV New Jersey and [3H]- (— —) Cocal virus.

VSV Indiana and Cocal virus

Similar profiles of mixed [3H]-labelled and [14C]-labelled virus polypeptides from these two viruses are shown in Fig. 2. In the heterologous mixtures (top and bottom right) VP II of VSV Indiana migrated faster and VP V slower than the corresponding polypeptides of Cocal virus. Polypeptide III had the same electrophoretic mobilities for both viruses, and no clear-cut difference was detected in the migration of VP IV.

VSV New Jersey and Cocal virus

The profiles of mixed preparations of the [3H]-labelled and [14C]-labelled virus polypeptides of VSV New Jersey and Cocal virus in polyacrylamide gels are shown in Fig. 3. Polypeptide II of VSV New Jersey migrated faster whereas VP III migrated slower than the corresponding polypeptides of Cocal virus. In this comparison a difference in migration of VP IV was detectable whereas the electrophoretic mobilities of VP V were identical for the two viruses.
Fig. 4. Polypeptides of VSV Indiana labelled in vitro.
Whole virus was labelled with $^{14}$C-formaldehyde and reduced with sodium borohydride as described in the text. The profile compares by co-electrophoresis on 10% SDS-polyacrylamide gel the electrophoretic mobilities of $^{14}$C-labelled polypeptide (——) with $^{3}$H-VSV Indiana polypeptides labelled in vivo.

Glycosylation

$^{3}$H-glucosamine was used to label the structural glycoprotein of all three viruses. In each case, VP II was the only virus polypeptide to incorporate the carbohydrate precursor as shown previously for VSV Indiana by Wagner, Snyder & Yamazaki (1970) and Burge & Huang (1970).

Mol. wt determinations

Suitable protein markers were labelled in vitro with $^{14}$C-methyl groups from formaldehyde as described in Methods. The electrophoretic mobilities of these proteins were unaltered by the in vitro labelling procedure when compared by co-electrophoresis in SDS-polyacrylamide gel with unlabelled protein stained with Coomassie blue (W. H. Wunner & I. W. Halliburton, personal communication).

The effect of in vitro labelling of virus particles was investigated as a further criterion for exact comparison of $^{14}$C-protein markers and virus proteins. All four major polypeptides of VSV Indiana were labelled by reductive alkylation and migrated identically in SDS-
Polypeptides of VSV and Cocal virus

Fig. 5. SDS-polyacrylamide gel electrophoresis. Electrophoretic mobilities of the protein markers used in this study are shown as a function of their respective mol. wt in three different SDS-polyacrylamide gels. Left, 5%; middle, 7.5%; right, 10% total acrylamide (T). Further details of gel composition, conditions for electrophoresis and discussion of the plots are given in the text.

Fig. 6. Co-electrophoresis of virus polypeptides (-----) and mol. wt markers (---). [3H]-polypeptides of VSV Indiana (left) and VSV New Jersey (right) were mixed with in vitro labelled phosphorylase a (94,000, a), BSA Fraction V (68,000, b), catalase (57,500, c), ovalbumin (43,000, d), pepsin (35,000, e), α-chymotrypsinogen (25,700, f) and lysozyme (14,300, g). Values for a, b, d, e, f and g were taken from Weber & Osborn (1969); c from Klotz & Darnall (1969). The profiles were obtained on 10% SDS-polyacrylamide gel.

polyacrylamide gel with the corresponding virus polypeptides labelled in vivo (Fig. 4). The additional polypeptides labelled in vitro were also observed in similar gels stained with Coomassie blue and may be contaminants of cellular origin.

Estimates of mol. wt of the virus polypeptides from VSV Indiana, VSV New Jersey and, Cocal virus were made by co-electrophoresis in 5, 7.5 and 10% (total) SDS-polyacrylamide gels. The relationship of mol. wt to electrophoretic mobilities in these gels is shown in Fig. 5. Migration of the virus polypeptides was compared in the same gel with the protein markers
Table I. Mol. wt (× 10⁻³) of VSV and Cocal virus polypeptides determined by polyacrylamide gel electrophoresis

<table>
<thead>
<tr>
<th>Virus</th>
<th>% polyacrylamide gel</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSV</td>
<td>10</td>
<td>60 (60 to 60)</td>
<td>44-6 (44-2 to 45)</td>
<td>38-4 (38-1 to 38-6)</td>
<td>28-2 (28 to 28-3)</td>
</tr>
<tr>
<td>Indiana</td>
<td>7.5</td>
<td>61 (60-9 to 61)</td>
<td>45-6 (45-2 to 46)</td>
<td>37-8 (37-1 to 38-6)</td>
<td>28-3 (27-8 to 28-9)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>63-3 (62-5 to 64-5)</td>
<td>45-6 (45 to 46)</td>
<td>—</td>
<td>28-8 (28-2 to 29)</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>61-4</td>
<td>45-3</td>
<td>38-1</td>
<td>28-4</td>
</tr>
<tr>
<td>VSV</td>
<td>10</td>
<td>63</td>
<td>47</td>
<td>39</td>
<td>25-9</td>
</tr>
<tr>
<td>New Jersey</td>
<td>7.5</td>
<td>63-3 (63 to 63-5)</td>
<td>46-9 (46-5 to 47-2)</td>
<td>39 (38 to 40)</td>
<td>25-9 (25-8 to 26)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>61-9 (61-2 to 62-5)</td>
<td>46-6 (46-5 to 46-8)</td>
<td>—</td>
<td>26-7 (26-5 to 27)</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>62-7</td>
<td>46-8</td>
<td>39</td>
<td>26-2</td>
</tr>
<tr>
<td>Cocal</td>
<td>10</td>
<td>65-2 (64-5 to 66)</td>
<td>44-7 (44 to 45-4)</td>
<td>37-7 (37-5 to 37-9)</td>
<td>26-6 (26-5 to 26-7)</td>
</tr>
</tbody>
</table>

* Results of 2 to 5 determinations. Range shown in brackets.

using two isotopes. Typical profiles in 10 % SDS-acrylamide gel for the [³H]-labelled VSV Indiana and VSV New Jersey polypeptides are shown in Fig. 6. The average value and the range of several runs in 5, 7.5 and 10 % SDS-polyacrylamide gels for all three viruses are given in Table 1. Only three of the major peaks of radioactivity associated with virus polypeptides were resolved in the 5 % gels; VP III and VP IV were only separated in the 7.5 % and stronger gels which contained 5 % bisacrylamide. Therefore values were obtained for only three of the four virus polypeptides of VSV when estimating the mol. wt from relative electrophoretic mobilities according to the linear relationship in 5 % gels (Shapiro, Viñuela & Maizel, 1967). The resolution and sharpness of peaks in the 10 % gels was superior to both 7.5 and 5 % gels and the accuracy of the mol. wt estimations was improved although the relationship is no longer linear with relative electrophoretic mobility. Resolution of the virus polypeptides of Indiana VSV solubilized in 8 M-urea with or without 1 % SDS and 0.1 DTT and their electrophoretic mobilities were identical to the virus polypeptides of Indiana VSV shown in Fig. 6.

**DISCUSSION**

A direct electrophoretic comparison of the structural polypeptides of the Indiana and New Jersey serotypes of VSV reveals that all three major polypeptides have different molecular sizes. One of these differences was observed by Wagner *et al.* (1969). VSV Indiana and the serologically related Cocal virus compared under similar conditions differ in at least two of the three major structural polypeptides of the particles as do VSV New Jersey and Cocal virus. VP III of VSV Indiana and Cocal virus, and VP V of VSV New Jersey and Cocal virus are of similar molecular size.

The detected differences in mol. wt between the corresponding virus polypeptides range from 3800 for the glycoproteins (VP II) of VSV Indiana and Cocal virus to 1300 for VP IV of VSV New Jersey and Cocal virus. The size of the carbohydrate moiety in VP II of the three viruses has not been measured. However, it is unlikely that a large proportion of the difference between the three viruses grown under identical conditions in BHK cells is due to the virus polysaccharide (Burge & Huang, 1970). The small differences in VP IV of all three viruses shown in Table 1 may be real, although these could not be clearly resolved by mixing experiments.
Polypeptides of VSV and Cocal virus

The mol. wt values for VSV Indiana structural polypeptides do not precisely agree with previously published results (Wagner et al. 1969; Burge & Huang, 1970; Cartwright, Talbot & Brown, 1970; Mudd & Summers, 1970). In particular, VP II and VP III are smaller polypeptides than estimated by the above-mentioned authors. The reason for this discrepancy is not immediately clear. Estimates we have made, however, are reproducible in gels of different acrylamide–bisacrylamide ratios including the gel composition used by Wagner et al. (1969), Cartwright et al. (1970) and Mudd & Summers (1970). In addition, the electrophoretic mobility of the virus proteins is not altered by solubilization in 8 M-urea.

The pattern of radioactivity observed for the major VSV polypeptides of particles labelled in vitro shows that VP II is labelled preferentially. Reductive alkylation with [14C]-formaldehyde depends on available protein amino groups which are converted to the dimethyl amino derivative (Rice & Means, 1971). Electron microscopy (unpublished) indicates that the virus particle structure is unaltered in the brief exposure to cold sodium borate buffer (pH 9.0) prior to the labelling reaction. Under these conditions the preferential attachment of [14C]-formaldehyde to VP II is consistent with the observation that VP II is a surface projection (Cartwright, Smale & Brown, 1969).

In conclusion, our findings support the suggestion that Cocal virus is a serological variant of VSV. We propose therefore that this virus be renamed VSV Cocal serotype, since Federer et al. (1967) have shown that it can produce vesicular lesions experimentally in domestic animals.

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REFERENCES


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