Polypeptides of Simian Rotavirus (SA-11) Determined by a Continuous Polyacrylamide Gel Electrophoresis Method

(Accepted 20 December 1978)

SUMMARY

Simian rotavirus (SA-11) isolated from infected African green monkey kidney cells was separated into two virus fractions in a CsCl density gradient and their proteins analysed on a continuous phosphate buffered polyacrylamide gel electrophoresis system. One peak (buoyant density 1.37 g/ml) contained double capsid virus particles which were radioimmunoassay (RIA)- and haemagglutinin (HA)-positive and yielded eight polypeptides whose mol. wt. ranged from 48,000 to 128,000. The second peak (buoyant density 1.39 g/ml) which contained 70% single capsid particles and was RIA-positive but HA-negative, yielded only five polypeptides. The three polypeptides missing in the second peak are associated presumably with the outer capsid of SA-11 virus particles and one or more of these is assumed to be the HA of SA-11 rotavirus.

Rotaviruses have an eleven segment double stranded RNA genome (Newman et al. 1975; Rodger et al. 1975; Kalica et al. 1976, 1978b; Schnagl & Holmes, 1976; Todd & McNulty, 1976; Obijeski et al. 1977) which presumably codes for at least eleven virus polypeptides. Complete rotavirus particles from various species have been shown by polyacrylamide gel analysis to contain from five to ten structural polypeptides. Newman et al. (1975) and Bridger & Woode (1976) used a phosphate buffered continuous gel electrophoresis system and resolved five polypeptides for calf rotavirus. Rodger et al. (1977) used a discontinuous tris-glycine buffered method described by Laemmli (1970) and were able to resolve eight or nine structural polypeptides for human, calf and simian rotaviruses. Todd & McNulty (1977), using a similar discontinuous system, reported ten structural polypeptides for lamb rotavirus. Obijeski et al. (1977) compared a continuous with a discontinuous system for fractionation of human rotavirus and resolved ten structural polypeptides with each system; however, their starting material came from pooled stool specimens and therefore may reflect a falsely high number of polypeptides.

Using a continuous phosphate buffered gel system with calf and human rotaviruses derived from calf faecal material we have previously observed only five structural polypeptides (A. R. Kalica & T. S. Theodore, unpublished observations). However, the composition of our starting sample with respect to the concentration of complete virus particles may not have been sufficient to permit detection of outer capsid polypeptides. Since the discontinuous gel systems are reputed to be more sensitive and give higher resolution (Maizel, 1971), it has also been suggested that the use of a continuous gel system might explain the detection of fewer rotavirus polypeptides (Rodger et al. 1977).

Recently, we used the simian rotavirus (SA-11) as a model for study since it grows well in cell culture and possesses a haemagglutinin (HA) associated with its outer capsid. The marker provides a practical assay for double capsid (SA-11) virus particles (Kalica et al. 1978a). When subjected to buoyant density fractionation, several populations of virus
particles were obtained from African green monkey kidney cell culture fluid infected with SA-11 (Kalica et al. 1978a). One of these peaks with a buoyant density of 1.37 g/ml in CsCl contained approx. 90% particles with double capsids and was RIA- and HA-positive. A second peak contained 70% particles with single capsids, was RIA-positive and HA-negative and had a buoyant density of 1.39 g/ml. Both of these peaks contained little or no contaminating debris as determined by electron microscopy (EM).

In the present study, gel electrophoresis in a continuous gel system as described by Weber & Osborn (1969) was carried out on both peak fractions to compare the number of polypeptides present in single and double shelled SA-11 rotavirus particles and to determine the level of sensitivity of this continuous gel system for detecting polypeptides from particles with double capsids (determined by both EM and HA activity). The fractions were dialysed against several changes of 0.01 M-sodium phosphate buffer, pH 7.0, then treated with sodium...
Table 1. Molecular weight estimates of simian rotavirus (SA-11) polypeptides by a continuous phosphate buffered gel electrophoresis system

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Mol. wt. × 10^-9</th>
<th>Location on virus particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>128</td>
<td>Inner capsid</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>Inner capsid</td>
</tr>
<tr>
<td>3</td>
<td>115</td>
<td>Inner capsid</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>Outer capsid</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>Outer capsid</td>
</tr>
<tr>
<td>6</td>
<td>87</td>
<td>Inner capsid</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>Outer capsid</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>Inner capsid</td>
</tr>
</tbody>
</table>

dodecyl sulphate (SDS) and mercaptoethanol (1% final concentration each) and boiled for 2 min. The samples were loaded on to 7.5% acrylamide disc gels (65 mm x 6 mm) and electrophoresed at a constant current of 8 mA/gel for approx. 4 to 5 h (until the tracking dye reached the end of the gel). The gels were pre-fixed in methanol–acetic acid–water (50:10:40) for 3 h or overnight, stained for 30 min with 0.25% Coomassie brilliant blue (dissolved in methanol–acetic acid–water, 45:9:46), destained by simple diffusion in methanol–acetic acid–water (5:7.5:87.5) and scanned on a 20 cm Gilford gel scanner at 550 nm. Mol. wt. determinations were made on 7.5 acrylamide gels according to the method of Weber & Osborn (1969). Bovine serum albumin, catalase, pepsin, DNase, RNase, trypsin, lysozyme and cytochrome c were used as standards.

Fig. 1(a) is a densitometer tracing of a gel from the lower density peak (1.37 g/ml) in which 90% of the particles had double capsids and produced eight distinct polypeptide bands. The tracing in Fig. 1(b) is that produced from electrophoresis of the peak of more dense particles (1.39 g/ml) with single capsids. Five distinct polypeptide bands were present in this gel. The estimated mol. wt. of the SA-11 polypeptides ranged from 48,000 to 128,000 (Table 1). Three of the eight polypeptides (110,000, 95,000 and 67,000) are missing from the peak of single capsid particles (Fig. 1b) and are assumed therefore to be outer capsid components. Furthermore, one or more of these three outer capsid polypeptides can be assumed to be the haemagglutinin of the simian rotavirus.

The continuous gel system employed in this study appears to be comparable to the more commonly used discontinuous tris-glycine gel systems of Rodger et al. (1977) and Todd & McNulty (1977), since the eight polypeptides resolved for the SA-11 double shelled particles observed in the present study compare favourably with the eight to ten polypeptides reported by these workers for calf, human, simian and lamb rotaviruses. It would appear that resolution of fewer polypeptides, observed in our unpublished studies and reports by others (Newman et al. 1975; Bridger & Wood, 1976) using continuous gel systems, is probably due more to a low concentration of particles with double capsids in the starting material than to the gel system employed. Our finding of three outer capsid polypeptides agrees well with published results from other studies (Rodger et al. 1977; Todd & McNulty, 1977).

We thank Mr T. J. Popkin for photographic assistance.

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REFERENCES


(Received 9 October 1978)