Rapid Purification of Lymphocytic Choriomeningitis Virus by Density Gradient Centrifugation in Colloidal Silica

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SUMMARY

Lymphocytic choriomeningitis virus was purified from cell culture fluid by density gradient centrifugation in colloidal silica. The specific infectivity increased 5000-fold and the recovery of infectivity was about 10%.

Attempts to purify lymphocytic choriomeningitis (LCM) virus in gradients of sucrose or salts of high density have not yielded satisfactory results, due to the high lability of the virus in these materials (Pfau, 1965; 1974). A considerable improvement was achieved by Gschwendner et al. (1975) by using a combination of column purification on controlled pore size glass (CPG) and centrifugation through gradients of amido-trizate (Gschwendner et al. 1975). A report on the successful application of gradients of colloidal silica for the purification of herpes viruses stimulated an attempt to use this material for the purification of LCM virus (Pertoft, 1970; Vahlne & Blomberg, 1974).

LCM virus, strain WE, was originally obtained from Professor W. Scheid, Cologne (Jochheim et al. 1957). Virus titrations were carried out by a quantal technique employing Vero cells in microtitre plates. Cytopathic effect was read on the fourth and sixth days after infection. Titres were calculated according to the method of Reed & Muench (1938), modified by Fazekas de St. Groth (1955) and were expressed in TCID50/ml.

For virus production Vero cells were grown to monolayers in roller bottles at 37 °C and 0.25 rev/min. The growth medium consisted of Eagle's minimal essential medium (MEM), supplemented with non-essential amino acids and 5% calf serum. Virus infection was carried out at a multiplicity of about 2.5 × 10^3 TCID50/ml and the infected cultures were incubated at 37 °C in the presence of maintenance medium consisting of MEM, non-essential amino acids and 2% heat-inactivated foetal calf serum, which was pre-treated with 4% polyethylene glycol (PEG) 40000 (Gschwendner et al. 1975). The extracellular virus was harvested at 40 h p.i. No cytopathic effect was visible at this time.

The virus was recovered from cell culture fluid after the addition of 4% PEG 40000 and 6% NaCl by centrifugation at 10000 g for 60 min at 4 °C. The sediment was resuspended in 10 ml of a buffer, pH 7.8, consisting of 0.01 M-tris, 0.1 M-NaCl, 0.001 M-EDTA and 0.1% (v/v) Haemaccel (Lehmann-Grube, 1971) and was cleared by centrifugation for 20 min at 4000 g. The supernatant was then subjected to two successive cycles of density gradient centrifugation through colloidal silica. The gradient solution for the first gradient consisted of 15% (w/v) SiO2 (Ludox HS 40, which was a gift from E. I. du Pont de Nemours & Co., Wilmington, Delaware), 5% (w/v) PEG 4000, 0.005 M-tris buffer, pH 7.8 and 0.1% (v/v) Haemaccel. Portions of virus suspension (1 ml) were layered on top of 11 ml of gradient solution in cellulose nitrate tubes and were spun for 70 min at 57000 g and 4 °C in the 65T angle head rotor of a Beckman centrifuge type L 65 B. Fractions were taken through a hole at the bottom of the tube and their density was determined gravimetrically. Peak infectivity was found at a density of 1.046 g/ml (Fig. 1a).

The second gradient consisted of three layers of SiO2 with concentrations of 10.8, 8 and
Short communications

Fig. 1. Density gradient centrifugation of LCM virus in colloidal silica. Centrifugation was performed at 57000 g for 70 min in the 65T angle head rotor at 4 °C. Infectivity (●—●) and density (○—○) of fractions are represented. (a) Centrifugation of cell culture virus, first cycle. (b) Centrifugation of cell culture virus, second cycle. (c) Centrifugation of mouse brain virus, second cycle.

5.2% (w/v) in the same buffer as in the first gradient. The virus sample was mixed homogeneously with the three layers of SiO₂. Centrifugation was carried out under the same conditions as above and peak infectivity was again obtained at a density of 1.048 g/ml (Fig. 1b). To remove SiO₂ from the fractions containing the virus, 0.38 M-NaCl was added, followed by centrifugation at 3000 g (Perotto, 1970). Virus recovery was usually in the range of 10% of the starting material.

In order to control the efficiency of the purification procedure, the specific infectivity was determined. Protein was measured according to Lowry et al. (1951) using bovine serum albumin as standard. Specific infectivity of the virus preparations increased by a factor of
Table 1. Representation of LCM virus purification in three experiments

<table>
<thead>
<tr>
<th>Purification step*</th>
<th>Volume (ml)</th>
<th>Total protein (µg/ml)</th>
<th>Specific infectivity (ID₅₀/mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>700</td>
<td>600</td>
<td>2.2 x 10⁷</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>3.50</td>
<td>420</td>
<td>395 x 10⁷</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1.52</td>
<td>66</td>
<td>1720 x 10⁷</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>0.76</td>
<td>22</td>
<td>10900 x 10⁷</td>
<td>17</td>
</tr>
<tr>
<td>1</td>
<td>800</td>
<td>800</td>
<td>1.0 x 10⁷</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>4.00</td>
<td>460</td>
<td>430 x 10⁷</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>2.00</td>
<td>36</td>
<td>3600 x 10⁷</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>0.88</td>
<td>24</td>
<td>4600 x 10⁷</td>
<td>13</td>
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<tr>
<td>1</td>
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<td>1200</td>
<td>6.7 x 10⁶</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>10.00</td>
<td>800</td>
<td>1250 x 10⁶</td>
<td>62.5</td>
</tr>
<tr>
<td>3</td>
<td>3.30</td>
<td>72</td>
<td>12500 x 10⁶</td>
<td>18.8</td>
</tr>
<tr>
<td>4</td>
<td>1.60</td>
<td>23</td>
<td>43500 x 10⁶</td>
<td>11.0</td>
</tr>
</tbody>
</table>

* 1, Starting material: infected tissue culture fluid; 2, PEG treatment; 3, first centrifugation in colloidal silica; 4, second centrifugation in colloidal silica.

5000. The results of the purification processes are shown by three typical experiments in Table 1.

As an additional method to control the degree of purification, mock infected Vero cells were labelled with ³H-uridine and ¹⁴C-protein hydrolysate (The Radiochemical Centre, Amersham, England). The cells were disrupted by five cycles of freezing and thawing and the radioactive lysates were added to the infected cell culture fluid before the purification procedure. Virus was purified from this mixture as indicated above. The distribution of radioactivity in the second gradient was determined by measuring samples with Scintigel (Roth, Karlsruhe, F.R.G.) in a liquid scintillation counter. In the three fractions containing most of the infectivity 0.06% of the added ³H and 0.16% of the ¹⁴C radioactivity were found.

Preliminary experiments were carried out using brains from LCM-infected NMRI mice (strain NMRI, Hannover, F.R.G.) as source of the virus. In these experiments 10% brain suspensions were centrifuged at 10,000 g for 1 h at 4 °C and the supernatants were treated in the same way as the cell culture fluids. Infectious virus from the mouse brains banded at the same density as the cell culture virus (Fig. 1c).

The advantage of this purification method is that it is simple and not time consuming. As a consequence of the exclusion effect in colloidal silica the virus has a much lower density in this material than in sucrose or amido-trizoate (Vahlne & Blomberg, 1974; Pertot et al. 1967).

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REFERENCES


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