Effect of Concanavalin A on Vesicular Stomatitis Virus Maturation

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SUMMARY

Addition of concanavalin A to BHK cell monolayers infected with vesicular stomatitis virus prevented the formation of mature virus particles. In these cells the virus glycoprotein (G) was inserted into the plasma membrane and the protein that is in close association with the ribonucleic acid, protein N, was found in the cytoplasm. At times when cells infected in the absence of the lectin were liberating virus into the supernatant medium, the M or matrix protein was found in association with the plasma membrane of the lectin-treated cells.

The removal of the lectin from the cells with \( \alpha \)-methyl-D-glucoside 3 h after infection was followed by the immediate release of mature virus particles. The rate of virus release from these cells was the same as that from cells infected in the absence of the lectin. Addition of cycloheximide, an inhibitor of protein synthesis, immediately after \( \alpha \)-methyl-D-glucoside treatment of the cells did not alter the rate of virus production, suggesting that the proteins required for virus synthesis were available in the lectin-treated cells and that virus assembly took place without further protein synthesis on removal of the lectin.

INTRODUCTION

In a recent review on the membrane structure of the lipid-containing viruses, Lenard & Compans (1974) suggested that virus lipids were arranged in the form of a bilayer. Such an arrangement is synonymous with the general structure of biological membranes and would require no gross re-orientation of the lipid structure when it leaves the cell to become part of the virus envelope. As no host-cell polypeptide has been detected in the envelope of these viruses it is considered that they derive their envelope from an area of the cellular membrane devoid of host-cell proteins but containing virus-specific glycoprotein. A variety of methods have been postulated by Compans & Caligiuri (1974) for the incorporation of the virus glycoproteins into the lipid bilayer but the site of synthesis, and the mode of insertion, of this protein into the cell membrane have still to be established.

It seemed that lectins might provide a means of interrupting the chain of events starting with the appearance of the virus glycoprotein on the outer surface of the cytoplasmic membrane of the cell and finishing with the incorporation of an area of the altered cellular membrane into the virus particle. The effect of concanavalin A (Con A) on the maturation of vesicular stomatitis virus (VSV) has been considered. The questions of whether the lectin would prevent the appearance of the virus glycoprotein on the cell surface, or whether this protein would be inserted into the plasma membrane but be unable to aggregate into the required configuration for virus formation have been partially answered. Further, these studies of the action of Con A on the cell surface have provided some details of virus maturation.
Table I. Effect of Con A on virus infectivity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Infectivity (ID₅₀/ml)</th>
<th>Mice</th>
<th>BHK cell monolayers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>1 × 10¹⁰</td>
<td>2 × 10¹⁰</td>
<td></td>
</tr>
<tr>
<td>Virus in Eagle’s medium + 10 mg/ml Con A*</td>
<td>3 × 10¹⁰</td>
<td>1 × 10¹⁰</td>
<td></td>
</tr>
<tr>
<td>Virus in 0.04 M-PO₄, pH 7.6 + 10 mg/ml Con A†</td>
<td>1 × 10¹⁰</td>
<td>2 × 10¹⁰</td>
<td></td>
</tr>
<tr>
<td>Virus diluted in PO₄ containing 200 µg/ml Con A</td>
<td>8 × 10⁸</td>
<td>1 × 10⁵</td>
<td></td>
</tr>
<tr>
<td>Virus diluted in PO₄ containing 200 µg/ml Con A and 0.1 M-α-methyl-D-glucoside</td>
<td>2 × 10¹⁰</td>
<td>1 × 10⁵</td>
<td></td>
</tr>
</tbody>
</table>

* Con A was added to the virus suspension prior to dilution in 0.04 M-PO₄, pH 7.6.
† Virus was diluted 1/1000 in PO₄ before addition of the Con A and the dilution was then continued in PO₄.

METHODS

Growth of virus. Strain Ind C (Indiana serotype) was used for all experiments. It was grown by infecting monolayers of BHK 21 cells in Roux flasks at an m.o.i. of 0.01 and then incubating in 25 ml Eagle’s medium at 37°C with gentle rocking, until the sheets left the glass. The medium, separated from the cell debris, provided the virus source.

Virus concentration and purification. Unfractionated virus suspensions were centrifuged for 1 h at 40000 g. The resultant pellet was resuspended before centrifuging for 2 h at 40000 g in a 15 to 45% sucrose gradient using the SW25 rotor of the Spinco ultracentrifuge.

Virus titration. Infectivity determinations were made either by intracerebral inoculation of 7 day-old mice with serial 10-fold dilutions of the virus or by plaque titration on BHK cell monolayers.

Radiolabelled cells. Infected BHK cell monolayers were incubated with 3⁵S-methionine (2 μCi/ml) in methionine-free Eagle’s medium.

Antisera. These were prepared against intact virus and against the ribonucleoprotein of the virus (Cartwright & Brown, 1972).

Complement fixation tests. Quantitative estimations of complement fixing activity were made by the method of Brooksby (1952) for foot-and-mouth disease virus. Suitably diluted cell fractions were allowed to react with excess antiserum in the presence of serially increasing amounts of complement and the 50% haemolytic end point calculated by the use of probits.

Polyacrylamide gel electrophoresis. The method described by Cartwright, Talbot & Brown (1970) was used.

RESULTS

Effect of concanavalin A on virus infectivity

Con A was added to unfractionated virus suspensions in Eagle’s medium or, to avoid possible inhibitory effects of the medium on the lectin, to the virus suspension after it had been diluted 1000-fold in 0.04 M-PO₄, pH 7.6. The two suspensions were further diluted in a 10-fold series in phosphate buffer and their infectivity compared with that of the original suspension. Initial concentrations as high as 10 mg/ml of Con A in virus suspensions containing 10⁷ to 10¹⁰ ID₅₀/ml did not affect the virus titre when assayed in suckling mice or BHK cell monolayers. Apparently under these conditions there was no aggregation or precipitation of the virus particles by the Con A, even when the ratio of lectin molecules to infective virus particles was greater than 10².

In contrast to the results described above, when virus dilutions were prepared in a
Effect of Concanavalin A on VSV maturation

Table 2. Production of virus components in BHK monolayers incubated with Con A

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sonicated cells</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount of 1/30 complement fixed (ml)</td>
<td>Infectivity (ID_{50}/ml)</td>
</tr>
<tr>
<td>3 h harvest</td>
<td>225</td>
<td>2 \times 10^6</td>
</tr>
<tr>
<td>3 h harvest, cells incubated with Con A after infection</td>
<td>225</td>
<td>2 \times 10^6</td>
</tr>
<tr>
<td>3 h harvest, cells in contact with Con A when virus added</td>
<td>NIL</td>
<td>3 \times 10^5</td>
</tr>
<tr>
<td>18 h harvest</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

* Sub-virus components, no virus particles detected.

phosphate diluent containing Con A at a concentration of 200 \mu g/ml and titrated in BHK cell monolayers there was a considerable loss of infectivity. This loss of infectivity was not observed when the virus solution was titrated intracerebrally in mice (Table 1). For example, the titre of the virus fell from 2 \times 10^{10} ID_{50}/ml to 2 \times 10^{5} ID_{50}/ml when titrated in BHK monolayers in the presence of 100 to 200 \mu g/ml lectin, but in mice the effect of the lectin on the virus titre was marginal.

Effect of Con A on BHK cell monolayers

BHK cell monolayers were incubated at 37°C for 30 min in Eagle’s medium containing 200 \mu g/ml Con A. The cells were then washed and infected at a m.o.i. of 10 before the medium containing the lectin was returned to the cells. The virus failed to infect these cells as there was no rise in virus titre and no complement fixing activity was found in the medium or in the sonicated cells.

Treatment of cells immediately after infection with 50 \mu g/ml lectin lowered the virus yield to less than 0.01% of that produced in cells infected in the absence of the lectin. When amounts above 100 \mu g/ml Con A were added to the monolayers after infection, no virus was detected but the sonicated cells contained complement fixing activity. The time of addition of the lectin to the cells to prevent virus formation could be as late as 1.5 h after infection.

Effect of Con A on the maturation of virus

The infectivity and complement fixing activity of sonicated cells and medium from infected monolayers and infected monolayers treated with Con A were compared (Table 2). In the monolayer infected with virus in the absence of the lectin, newly formed virus was liberated into the supernatant medium. The cells to which the lectin was added immediately after infection did not liberate virus into the supernatant medium but contained similar quantities of virus-specific proteins, when measured by complement fixation, as cells infected in the absence of Con A.

In case the rate of formation of complete virus had been retarded by the lectin, cells and medium were examined 18 h after infection. The results showed that the titre of the virus from cells infected in the absence of Con A was more than 1000-fold greater than the titre of the medium from cells incubated with the lectin for a similar period (Table 2). The infectivity of the medium from cells incubated in the presence of the lectin did not increase between 3 h and 18 h.

As some complement fixing activity accumulated in the medium of the lectin-treated cells
Table 3. Complement fixation activity of cell fractions with antiserum raised to virus and virus ribonucleoprotein

<table>
<thead>
<tr>
<th>Sample</th>
<th>Complement fixed by</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virus antiserum*</td>
<td>Ribonucleoprotein antiserum†</td>
<td></td>
</tr>
<tr>
<td>Infected cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>3·6‡</td>
<td>NIL</td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) N protein</td>
<td>74·0</td>
<td>65·0</td>
<td></td>
</tr>
<tr>
<td>(b) RNP</td>
<td>24·7</td>
<td>20·0</td>
<td></td>
</tr>
<tr>
<td>Con A cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>4·6</td>
<td>NIL</td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) N protein</td>
<td>73·0</td>
<td>65·0</td>
<td></td>
</tr>
<tr>
<td>(b) RNP</td>
<td>21·5</td>
<td>18·7</td>
<td></td>
</tr>
</tbody>
</table>

* This measures the reaction with proteins G & N.
† This measures the reaction with protein N only.
‡ The figures represent the total units of complement fixed by a BHK cell monolayer containing approx. 10⁴ cells.

18 h after infection, this material was examined by labelling BHK cell monolayers with ³⁵S-methionine and then incubating for 18 h in the presence or absence of the lectin. The contents of the supernatant medium were concentrated by ultracentrifuging at 40000 g for 1 h before separation by sucrose density centrifugation. The control cells produced a peak of ³⁵S-virus. The medium from the Con A treated cells did not contain ³⁵S at the virus position, and radioactivity was associated only with fractions at the top of the sucrose density gradient. This experiment showed that no virus particles were formed in the lectin-treated cells even after 18 h incubation but some sub-viral components, capable of reacting in complement fixation tests with virus antiserum, were liberated from the cells towards the end of the incubation period.

Localization of the virus products synthesized in the presence of Con A

To localize the virus components formed in the lectin-treated cells, the membrane and cytoplasmic sap were separated by the method of Atkinson & Summers (1971) for the preparation of HeLa cell membranes. Fractions from sucrose gradients containing the separated membranes and cytoplasmic constituents were reacted in complement fixation tests with antiserum to the complete virus particle (this serum contains antibodies to the glycoprotein and to the ribonucleoprotein [RNP] of the virus), and with antiserum which reacts only to the RNP. The preparation of these antisera and their reactions with the virus components have been described in detail by Cartwright & Brown (1972).

The membranes from infected cells incubated in the presence of Con A reacted in complement fixation tests with antiserum to the whole virus but not with antiserum to the virus RNP. This reaction with the glycoprotein antibody demonstrates that the mechanism for the transport of the virus glycoprotein to the cell surface is unimpaired in the presence of the lectin.

The cytoplasmic fractions reacted with virus antiserum and with antiserum to the RNP. As the reaction with the RNP antiserum was almost as great as with total virus antiserum, the cytoplasmic fractions appear to contain a preponderance of the N protein.

When the plasma membranes and cytoplasm of infected cells are separated as described,
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Fig. 1. Polyacrylamide gel electrophoresis of infected BHK cells incubated in the presence of Con A and labelled with 35S-methionine. The autoradiographs of the dried gels were scanned on a Joyce-Loebl densitometer. The positions of the virus proteins G, N and M are indicated. (a) Separated plasma membranes; (b) cytoplasmic contents.

Identification of virus proteins by polyacrylamide gel electrophoresis

Cell monolayers which had been infected at a m.o.i. of 10 for 5 min at 37°C were washed before continuing the incubation in methionine-free Eagle’s medium containing 35S-methionine (2 μCi/ml) or in the 35S-methionine medium containing 200 μg/ml Con A. Three hours after infection, at a time when the cells infected in the absence of the lectin
were producing mature virus, the membranes and cytoplasm of the lectin-treated cells were separated and their proteins identified by polyacrylamide gel electrophoresis. The M protein was found in association with the membrane fraction whilst the N protein was present in the cytoplasm (Fig. 1).

When $^3$H-glucosamine was used to radiolabel infected cells incubated in the presence of the lectin, polyacrylamide gel electrophoresis of the cell proteins demonstrated the incorporation of $^3$H-glucosamine into a membrane protein that co-ran with the virus glycoprotein.

**Reversal of the lectin effect**

The rate of virus formation in the cells, after removal of Con A, was compared to the rate of formation in cells infected in the absence of the lectin. Duplicate monolayers were infected at a m.o.i. of 10. After 5 min the cells were washed three times and then incubated in Eagle's medium or in Eagle's medium containing 200 $\mu$g/ml Con A. After 2 h incubation at 37°C the cells which were incubated in the absence of the lectin were beginning to liberate progeny virus. Medium from these cells (0.1 ml) was removed at 15 min intervals and titrated in suckling mice. The results in Fig. 2 show that the virus titre in the supernatant medium increased from $1 \times 10^4$ p.f.u./ml at 2 h after infection to $1 \times 10^7$ p.f.u./ml 75 min later.

The second monolayer was incubated in the presence of Con A for 3 h before washing with 0.1 M-α-methyl-D-glucoside. Incubation of the monolayer was continued in Eagle’s medium and samples of the medium were removed at 15 min intervals. After removal of the lectin, the rate of virus formation paralleled that found in the control (Fig. 2). Further experiments showed that infected cells incubated for periods up to 18 h in the presence of the lectin were capable of producing infectious virus immediately after its removal.
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Reversal of the lectin effect in the presence of cycloheximide

No virus proteins were synthesized when cycloheximide (50 μg/ml) was added to BHK cells immediately after infection. When infected cells were incubated for 3 h in the presence of Con A and then washed to remove the lectin, the addition of Eagle's medium containing cycloheximide did not prevent the cells from producing progeny virus. The rate of virus production in the presence of cycloheximide was equal to that of infected cells releasing virus at the end of the lag phase (Fig. 2). The removal of the lectin and the addition of cycloheximide to infected cells 1.5 h after infection, that is, at a time before the end of the lag phase, produced less than 1% of the virus liberated by cells having contact with the lectin for 3 h prior to cycloheximide treatment.

DISCUSSION

The infectivity of VSV is not reduced when virus suspensions containing 10 mg/ml of Con A are diluted in a 10-fold series and titrated in suckling mice or BHK cell monolayers. This result suggests that no permanent aggregation takes place between the virus and the lectin. In contrast, Con A has a marked effect on BHK cell monolayers. Concentrations as low as 50 μg/ml prevent infection, presumably by blocking the glycoprotein receptors of the cell and thus preventing virus attachment. The separated plasma membranes and cytoplasm of these cells from 3 to 18 h after addition of the virus did not react in complement fixation tests with virus antiserum.

Addition of the lectin to the monolayer after infection prevents the formation of infectious virus but sub-viral particles are produced. The major antigen, glycoprotein G, appears on the surface of the cell and the nucleoprotein N is detected in the cytoplasm. The concentrations of these components are similar to those found in cells infected in the absence of Con A.

Polyacrylamide gel electrophoresis examination of the membranes of infected cells incubated in the presence of 3H-glucosamine and Con A, revealed that the virus glycoprotein, detected by complement fixation tests and PAGE, contained glucosamine. Similar cells labelled with 35S-methionine showed that, 3 h after infection, all the detectable M protein was in association with the plasma membrane. At this time the N protein and the RNP complex were only detectable in the cytoplasm.

A report by Meier-Ewert & Compans (1974) showed that the M protein of the WSN strain of influenza, grown in BHK cells, is both synthesized and incorporated at late times after infection. More recently, Nagai, Ogura & Klenk (1976) have shown that the M protein of Newcastle disease virus (NDV) is incorporated very rapidly after synthesis and its incorporation is one of the final steps in virus envelope assembly. It has been suggested previously (Cartwright, 1973) that the formation of the M protein of VSV is also a late event in virus assembly. The finding of M protein in the plasma membrane of cells infected in the presence of Con A suggests that the later stages of virus synthesis have been reached in these cells.

Evidence obtained by Nagai et al. (1975) demonstrated that NDV proteins show random distribution in the cell membrane prior to their aggregation into patches. The question arises whether patch formation is a pre-condition for the attachment of the M protein or whether the insertion of the M protein promotes the aggregation of the virus glycoproteins. In the case of VSV and the lectin-treated cells, attachment of M protein to the membrane does take place without the formation of the virus. Although the pre-formation of patches cannot be ruled out in the lectin-inhibited system it seems likely that the lectin will have reacted with
the cell membrane glycoproteins, limiting their mobility. This restriction on the movement of the cellular glycoproteins could be a factor preventing the virus glycoproteins forming patches.

When the lectin is removed from the cells 3 h after infection, the rate of virus production is indistinguishable from that in infected cells incubated in the absence of the lectin. Removal of the lectin from the cells as late as 18 h after infection still results in the immediate production of infectious virus. This final stage of virus assembly is not inhibited by the addition of cycloheximide, suggesting that all the virus structural proteins are available for assembly.

These results show that the addition of Con A to VSV infected BHK cells does not prevent the insertion of the virus-specific glycoprotein into the plasma membrane. The reaction of the lectin is probably with the cellular glycoproteins; this association prevents the virus glycoproteins forming a suitable rearrangement to allow the final assembly of the virus particle although the required sub-structures are available.

REFERENCES


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