Inhibition of Murine Sarcoma Virus Induced Transformation by Adenovirus Structural Proteins

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SUMMARY

The purified fibre and hexon of adenovirus 12 inhibit the transformation in tissue culture of murine sarcoma virus (MSV-M) by as much as 80% and 70%, respectively, when they are added to cells 8 to 20 h before MSV-M infection. During a 12 h period, only about 6 to 8% of added radiolabelled viral proteins become associated with cells (or 1.0 μg protein bound/10^5 cells). No inhibition occurs when the proteins are added simultaneously with MSV-M or 90 min or 4 h after MSV-M. There is also a direct correlation between the extent of focus inhibition and the concentrations of viral proteins used. Concentrations of viral proteins used to inhibit cell transformation do not affect cell growth, but do reduce cell macromolecular synthesis.

Recent work from this laboratory has demonstrated that adenovirus type 2 fibre and hexon proteins can significantly inhibit transformation of hamster embryo cells by simian adenovirus type 7 (Long & Khoobyarian, 1973). The possibility that this inhibitory action of adenovirus proteins on virus transformation might be a general characteristic of these proteins was considered by studying their effect on transformation caused by a different group of viruses, namely oncornaviruses.

We describe here the inhibition of mouse sarcoma virus (MSV-M) induced transformation in normal rat kidney cells (NRK) by both fibre and hexon proteins. These proteins may be of potential interest in the analysis of steps involved in cell transformation by MSV-M.

Pools of MSV-M obtained from MSV-induced tumours in Balb/c mice were prepared by differential centrifugation (Moloney, 1956). Stock virus prepared in this manner titred 10^4 to 10^5 f.f.u./ml. A clonal line of NRK cells was grown in Eagle's MEM with 8% foetal calf serum (FCS) and 20 mm-glutamine.

For purification of adenovirus structural proteins, the procedure involving infection of HEp-2 cultures with adenovirus 12 (Ad12) and extraction of virus proteins from infected cultures, as well as the purification of these proteins by DEAE-cellulose column chromatography was essentially the same as that described previously (Long & Khoobyarian, 1973). The identity of virus proteins was confirmed by Ouchterlony immunodiffusion using monospecific antisera. The purity of virus proteins was monitored by polyacrylamide disc electrophoresis according to a modified method of Reisfeld & Small (1966) and which has been reported elsewhere (Abid & Khoobyarian, 1976). Both fibre and hexon proteins appeared as single bands, thus suggesting their relative purity.

To determine the percent binding of viral proteins to cells over a period of 12 h, concentrations of fibre or hexon (200 to 300 μg/ml) sufficient to inhibit MSV-M transformation by more than 60% were iodinated (sp. act. 857 and 923 ct/min/μg of fibre and hexon, respectively) by a modification of a published method (Phillips & Morrison, 1970). Briefly, virus proteins were mixed with 25 μg of lactoperoxidase (Calbiochem, Los Angeles, Calif.), 25 μl of 10^4 μM-NaI containing 25 to 50 μCi of ^125I (Amersham, Buckinghamshire, England)
Fig. 1. Dose-response curve of inhibition of MSV-M transformation by fibre and hexon. Each point represents the mean focus forming units of three cultures. Symbols: ○—○, hexon; ●—●, fibre.

and 10 µl of $5 \times 10^{-3}$ M-H$_2$O$_2$. The suspension was thoroughly mixed and incubated at room temperature for 10 to 15 min, and then passed through a Sephadex G-25 column so that protein-bound $^{125}$I could separately elute before $^{125}$I fractions. To remove the unbound $^{125}$I, the radiolabelled proteins were dialysed for 36 h against 0.01 M-phosphate buffer, pH 7.0, prior to use.

For radioactive labelling experiments, NRK cells (2.5 × 10$^5$/flask) were treated for different times with 200 µg each of fibre or hexon, or growth medium. The test materials were removed and monolayers washed with Earle's balanced salt solution. Cells were pulse labelled for 90 min at 37 °C with 10 µCi/ml each of $^3$H-thymidine (28 Ci/mmol) or $^3$H-uridine (28 Ci/mmol) or 5 µCi/ml of $^3$H-leucine (28 Ci/mmol, Amersham-Searle, Arlington Heights, Ill.) in 1 ml growth medium. After removing the labels, the cultures were washed twice, trypsinized, and then collected. Two ml of 5 % cold TCA was added to cells and incubated at 4 °C for 30 min. Cells were then washed three times with 5 % cold TCA and radioactivity in TCA insoluble fractions was determined. To solubilize any t-RNA bound leucine (Shatkin, 1969), leucine-labelled samples were heated at 90 °C for 20 min.

To test for the inhibition of cell transformation by virus proteins, NRK cells in 25 cm$^2$ plastic flasks (2 × 10$^5$ cells/flask) were treated with fibre, hexon, or growth medium for designated times at 37 °C in a 5 % CO$_2$ incubator. Then the proteins were removed and monolayers washed prior to infection with MSV-M (0.03 to 0.05 f.f.u./cell) for 90 min. Without removing the virus inoculum, 4.5 ml of Dulbecco's modified Eagle's medium containing 5 % FCS was added to each culture flask. Three days later the culture medium was replaced by 5 ml of fresh medium and the morphologically transformed foci were counted 6 to 7 days p.i. Percentage focus inhibition was expressed on the basis of focus forming units in control cultures. Up to 80 % inhibition in transformed foci with fibre (325 µg/ml) or 70 % with hexon (300 µg/ml) was observed over periods of 8 to 20 h. However, when proteins were added simultaneously with, or 90 min or 4 h after, MSV infection, virtually no inhibition in foci was noted, suggesting that inhibition occurred most probably at sites other than the cell surface. To
Table 1. Radioisotope uptake after treatment with fibre or hexon*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>³H-thymidine (h)</th>
<th>³H-uridine (h)</th>
<th>³H-leucine (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control fluids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibre (200 µg/ml)</td>
<td>7988</td>
<td>7001</td>
<td>7204</td>
</tr>
<tr>
<td>% inhibition</td>
<td>27</td>
<td>28</td>
<td>47</td>
</tr>
<tr>
<td>Hexon (200 µg/ml)</td>
<td>18310</td>
<td>23850</td>
<td>16475</td>
</tr>
<tr>
<td>% inhibition</td>
<td>31</td>
<td>64</td>
<td>83</td>
</tr>
</tbody>
</table>

* Values represent average mean of 3 replicate cultures.
† N.T. = not tested.

To establish a dose-response relationship, monolayers of NRK cells were pre-treated with increasing doses of fibre or hexon for 12 h before they were infected with MSV-M (< 1.0 f.f.u./cell). The degree of inhibition in foci increased proportionately to the concentrations of viral proteins used (Fig. 1). Increasing the concentrations of viral proteins beyond 300 µg/ml did not increase their inhibitory activity. Furthermore, when NRK cells (3 to 6 x 10⁵ cells/plate) were treated with either ¹²⁵I-fibre or ¹²⁵I-hexon (60,000 ct/plate) for 12 h at 37 °C, approx. 7.5 to 8.7 % of added fibre or 6 to 7.8 % of hexon remained associated with 3 x 10⁵ cells (or 1.0 µg protein bound/10⁵ cells), a finding which did not differ significantly from that of Levine & Ginsberg (1967), who found 0.4 µg of fibre associated with 10⁶ KB cells.

Studies of the effect of fibre or hexon on macromolecular synthesis of NRK cells showed an increased inhibition (27 to 47 %) of thymidine uptake during the period of 2 to 12 h (Table 1). The uridine and leucine uptakes were inhibited by up to 32 and 30 %, respectively, by 12 h. Over the same period of time, a similar concentration of hexon was more effective, as it reduced the incorporation of ³H-thymidine by 83 %, ³H-uridine by 53 % and ³H-leucine by 37 % into DNA, RNA, and protein, respectively. However, there was a discrepancy between these results and those on the effect of virus proteins on cell growth. For example, when NRK monolayers were treated with fibre or hexon (200 to 300 µg/ml) for 12 h at 37 °C and then washed, trypsinized, diluted, and plated in 60 mm plastic dishes (1000 cells/dish) in MEM containing 10 % FCS and 20 mM-NaHCO₃, virtually no inhibitory effect on cell growth was noted, as plating efficiencies for control cells, fibre-treated, and hexon-treated cells were 3.0, 2.85, and 2.28 %, respectively. Since cell colonies were scored 10 days after the cells were plated, it is conceivable that during this period the treated cells synthesized sufficient amounts of metabolic compounds to allow them to survive and to regain their growth potentialities even though their macromolecular synthesis was reduced.

We believe that inhibition occurs through the blockage of intracellular DNA and RNA synthesis, since both the fibre and hexon proteins reduced DNA and RNA synthesis probably through a mechanism similar to that reported by Levine & Ginsberg (1968). It is possible that these virus proteins inhibit MSV transformation by inhibiting the RNA-directed DNA polymerase of parental virus and thus inhibit synthesis of proviral DNA, or perhaps inhibit the integration of virus DNA into host cell DNA. These possibilities are now under consideration.
Short communication

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REFERENCES


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