Evidence for a New Antigen within the Adenovirus Capsid

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SUMMARY

Complement-fixation and radioactive-agglutination tests were performed on purified preparations of adenovirus type 5 using specific antisera against capsid antigens and an antiserum which can detect an antigen (the P antigen) in infected cells which has many of the properties of the T antigen associated with infection by the oncogenic adenoviruses. A serological reaction was detected between preparations containing intact virions and antihexon serum but not with antisera against fibres, penton bases and the P antigen. However, virus preparations which had been 'aged' at 4°C, and in which electron microscopic examination revealed the presence of many broken capsids, did react in complement fixation and radioactive agglutination with all the antisera. It was concluded that within the adenovirus capsid there is a hitherto undescribed protein related to the P antigen.

INTRODUCTION

In a previous communication, Russell et al. (1967a) reported that on infection of cells with adenovirus type 5, an antigen designated the P antigen could be detected before the appearance of the capsid antigens. This 'early' antigen has many of the properties of the T (or neo-) antigens associated with infection of cells by the oncogenic adenoviruses types 12 and 18. Furthermore, the experiments on the effect of heat on the anatomy of the adenovirus described in the preceding paper (Russell, Valentine & Pereira, 1967b) suggest that there is a labile protein within the capsid. Since the P antigen can be detected by serological methods it was important to ascertain if the suggested inner protein bore any antigenic relationship to the P antigen. One of the principal difficulties inherent in this type of investigation is to break open the capsid of the virus by means which would not degrade any possible labile protein. Thus, it has been shown that the adenovirus capsid can be ruptured by heat (Russell et al. 1967b) or by dialysis against bicarbonate buffer at pH 10.5 (Wilcox, Ginsberg & Anderson, 1963); these are methods which would have little likelihood of retaining the antigenicity of a labile protein. However, it was found that a relatively simple method sufficed to break open the capsid of the virus, allowing serological tests to be performed.

METHODS

The methods of preparing and purifying adenovirus type 5 (labelled with [3H]thymidine and unlabelled), preparing and testing antisera, complement fixation, electron microscopy and radioactive counting were described by Russell et al. (1967a, b).

Radioactive agglutination test. In the course of this work it became obvious that
a serological test more sensitive than complement fixation would be of considerable value in detecting antigenicity in the virus particle, and use has been made of the fact that the virus particles readily agglutinate in the presence of anti-viral sera (Valentine & Pereira, 1965). Since the aggregates of virus and antisera sediment much faster than the virus alone an indication of the extent of agglutination can be obtained by incubating antiserum with virus labelled with \(^3\)H and centrifuging at a speed which deposits aggregates but not the virus. The amount of radioactivity remaining in the supernatant fluid relative to suitable controls will indicate the degree of serological reaction between the virus and the antiserum.

The following conditions were finally adopted for this test: 0.1 ml. of virus labelled with \(^3\)H was added to 0.1 ml. of antiserum in a 0.8 ml. plastic centrifuge tube and incubated at room temperature for 1 hr. A small quantity of celite (Hyflo SuperCel—Johns Manville Inc.) was added to facilitate precipitation and the mixture was centrifuged at 2000 g for 15 min. at room temperature and 0.1 ml. of the supernatant fluid withdrawn by inserting a fine Pasteur pipette attached to an automatic syringe, taking care not to touch the sides of the tube. This supernatant fluid was then spotted on to a square of filter paper, dried and placed in 10 ml. of toluene scintillating fluid and counted in the Packard TriCarb scintillator.

**RESULTS**

**Complement-fixation tests**

A freshly prepared preparation of virus showing considerable opalescence was dialysed against phosphate buffered saline without calcium and magnesium (Dulbecco & Vogt, 1954), and a sample was tested in the standard complement-fixation test with

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Fresh</th>
<th>2 days at 4°</th>
<th>7 days at 4°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexon</td>
<td>*200 (pr.)</td>
<td>&gt; 1024</td>
<td>&gt; 1024</td>
</tr>
<tr>
<td>Fibre</td>
<td>&lt; 2</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Penton base</td>
<td>&lt; 2</td>
<td>128</td>
<td>92</td>
</tr>
<tr>
<td>P antigen</td>
<td>&lt; 2</td>
<td>16</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

* Reciprocal of the highest antigen dilution giving 50% fixation against optimal serum dilutions.

sera against hexons, penton bases, fibres and the P antigen. No complement-fixing antigen could be detected using the antisera against penton bases, fibres or the P antigen. There was a significant fixation against the hexon antisera but this had an unusually large prozone. No reaction could be obtained with the penton base or the fibre antisera, suggesting that either the antigenic sites are unavailable when the penton is incorporated into the virion or that the fixation reaction requires the presence of antigen aggregates.

The virus not used in the above test was stored at 4° for 2 days when a considerable change was noted in the degree of opalescence. When a sample of this virus was tested again significant complement fixation was obtained with all the antisera (Table 1). Thus, the hexon antiserum reacted to a much higher titre (with a smaller prozone), penton base and fibre antigens could be detected, and significant fixation was now
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recorded with the antiserum against the P antigen. After further storage of the virus at 4° the opalescence completely disappeared and a deposit formed. Electron microscopic analysis of this virus (see below) indicated that the viral capsid had broken down. Complement fixation at a later stage (after 7 days) showed fixation with antisera against hexons, fibres and penton bases but not with the antiserum against the P antigen (Table 1).

A further analysis by complement fixation was made on a different preparation of purified virus to test the effect of the presence of a chelating agent. The virus was removed from the final equilibrium density gradient and two equal volumes were dialysed against phosphate-buffered saline containing EDTA (0.05 mM) and against phosphate buffered saline containing calcium (1 mM) and magnesium (0.5 mM).

Fig. 1. Development of CF antigens on ‘ageing’ preparations of purified virus. O—O, Penton base; ×—×, P antigen. A. Virus dialysed against phosphate-buffered saline containing EDTA (0.05 mM). B. Virus dialysed against phosphate-buffered saline containing calcium (1 mM) and magnesium (0.5 mM).

A further analysis by complement fixation was made on a different preparation of purified virus to test the effect of the presence of a chelating agent. The virus was removed from the final equilibrium density gradient and two equal volumes were dialysed against phosphate buffered saline containing EDTA and against phosphate buffered saline containing calcium and magnesium for 4 hr at 4° with frequent changes of buffer. Complement-fixation tests were done with the virus immediately after dialysis (i.e. at 4 hr) and after 1, 2, 3 and 5 days storage at 4° (Fig. 1). The opalescence disappeared within 1 day from the virus which had been dialysed with the buffer containing EDTA; this also happened with the virus which had been dialysed against the buffer containing calcium and magnesium, but not until between the second and third day. The penton base antigen and the P antigen could be detected only after the virus had ‘aged’ at 4° for times consistent with there being significant degradation of the capsid as revealed by loss of opalescence from the virus suspension.
As in the previous tests antihexon serum gave a positive reaction at all times, whilst antifibre serum only reacted with degraded virus. The 'ageing' process was apparently accelerated in the presence of versene but was not inhibited by the presence of calcium and magnesium at the molarities tested.

**Radioactive-agglutination tests**

The efficacy of this test was determined by its ability to measure agglutination of the virus after incubation with graded dilutions of hyperimmune rabbit antiserum against adenovirus type 5. The control for this assay was the virus incubated with the preimmune rabbit serum at a dilution of 1/20. About 97% of the virus agglutinated with antiserum diluted from 1/10 to 1/160 (Fig. 2A). Tests with different preparations of labelled virus using antithexon sera gave similar results. Reaction with the P anti-
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serum was performed in a similar way using as control the virus incubated with the relevant preimmune rabbit serum. Fresh virus suspension under 1 day old was not agglutinated by P antiserum, whereas various ‘aged’ virus preparations reacted with this serum in a way analogous to that observed by complement fixation (Fig. 2, A to D).

**Morphological examination of ‘aged’ virus preparations**

The homogeneity of preparations of purified virus was checked by electron microscopy; only characteristic particles of adenovirus were seen (Pl. 1, fig. 1). In the preparations which had lost their opalescence considerable degradation of the capsids had occurred and free antigens and hexon shells could be readily identified (Pl. 1, fig. 2).

**DISCUSSION**

An antiserum reacting with ‘early’ extracts of adenovirus-infected cells also reacted in complement-fixation and agglutination tests with purified preparations of virus broken down by ageing in phosphate buffered saline at 4°C. Since no reaction was obtained with freshly prepared virus suspensions containing intact virions, it may be concluded that at least some of the antigen detectable as the P antigen is incorporated either into or within the viral capsid. The external features of the virion appear to be satisfactorily explained in terms of hexons and pentons (Valentine & Pereira, 1965) and it therefore seems more likely that the P antigen should reside inside the capsid. However, the antigen could be an integral part of the capsid which has not been morphologically recognized and which may only be revealed antigenically on ageing in a similar fashion to the fibre and penton base antigens. It should also be noted that since the P antigen need not be a single protein it does not necessarily follow that the ‘early’ protein is incorporated into the virion. The observation that the ageing process appeared to have been accelerated by the addition of a chelating agent but not inhibited by the presence of calcium and magnesium suggests that some other divalent cation or perhaps a polyamine may be important in retaining the integrity of the virus.

The function of the P antigen is at present largely conjectural but if it is a necessary component of the virion it could be argued that if insufficient (or faulty) P antigen is made during the infectious process all the viral DNA and capsid antigens produced would be unable to be assembled into mature virions—this would give rise to an excess of DNA and capsid antigen, a characteristic of adenovirus infection. Furthermore, since the incorporation of the P antigen (or a component of it) into the virion would be accompanied by its removal from the ‘soluble’ antigen pool in infected cell extracts, as the virus matured one might expect either a decrease in the complement-fixing activity of the P antigen or some indication of a change in its antigenic configuration. The results of the ‘three-dimensional’ complement-fixation tests carried out with the P antiserum on infected cell extracts described in a previous communication (Russell et al. 1967a) could be interpreted thus.

The similarity of the P antigen to the T (or neo-) antigens found in infection by oncogenic adenoviruses suggest that the latter could also be related to antigens which might be located within the virus capsid. This possibility is being examined.

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REFERENCES


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EXPLANATION OF PLATE

Electron micrographs of negatively stained preparations of purified virus.

The bar indicates 1000 Å.

Fig. 1. Purified virus preparation, freshly prepared.

Fig. 2. Purified virus preparation, after ‘ageing’ in phosphate buffered saline at 4° for 3 days.