Antigenicity and Polypeptide Composition of Native and Heated Echovirus Type 7 Procapsids

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SUMMARY

The native procapsid (naturally occurring empty capsid) of echovirus type 7 (E7) possesses N-antigenicity, which is as highly strain-specific as the native virion. When the native procapsid is heated, its antigenicity is converted to H-antigenicity which is common among strains of E7 and thus type-specific. However, no difference was detected in the sedimentation rate (80S) and polypeptide composition (VP0, 1 and 3) of the native and heated procapsids.

INTRODUCTION

The native virions of picornaviruses possess N-antigenic reactivity. On heating at 50 °C, the virion loses its N-antigenicity and acquires a new antigenic determinant called H-antigenicity (LeBouvier, 1955; Schmidt & Lennette, 1956; Hummeler & Hamparian, 1958; Roizman et al. 1959; Hinuma et al. 1965). With poliovirus this antigenic conversion is accompanied by loss of VP4 (Breindl, 1971).

The native procapsid consists of three polypeptides, VP0, 1 and 3. It shows N-antigenicity (Halperen et al. 1964; Rowlands et al. 1975), H-antigenicity (Roizman et al. 1958) or both N- and H-antigenicity (Lonberg-Holm & Yin, 1973) according to the species of virus. However, little is known of the correlation of antigenicity with the polypeptide composition of the heated procapsid. In this report we describe the difference in antigenicity between the native and heated procapsids of echovirus type 7 (E7), which possess haemagglutinating activity. The relationship between the antigenicity and polypeptide composition of the E7 procapsids is also described.

METHODS

Cell cultures. The LLCMK2 continuous line of monkey kidney cells was cultivated in Roux bottles or 110 × 260 mm roller bottles using Eagle’s minimum essential medium (MEM) with 6% calf serum, 100 μg/ml streptomycin and 100 units/ml penicillin. Cells for virus plaque assay were grown in plastic dishes (Falcon, 60 × 15 mm).

Viruses. E7, prototype strain Wallace and strain 489, isolated in our laboratory and identified as E7 with the Schmidt pool serum for enterovirus identification, were used. Coxsackievirus type A9 (CA9), strain Bozek, type B5 (CB5), strain Faulkner, echovirus type 4 (E4), strain Pesascek and type 11 (E11), strain Gregory, were also used.

Preparation of procapsid and virion. Virus was inoculated on monolayers of LLCMK2 cells maintained in serum-free MEM at an m.o.i. of 50 to 70 p.f.u./cell and incubated at 37 °C for 6 h. For the preparation of radioactive virus antigens, 5 μCi/ml of 3H-leucine (53 Ci/
Table 1. Cross haemagglutination-inhibition tests between echovirus type 7 strains, Wallace and 489

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Procapsid Wallace</th>
<th>Virion Wallace</th>
<th>Procapsid 489</th>
<th>Virion 489</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wallace</td>
<td>512*</td>
<td>1024 (100000)†</td>
<td>32</td>
<td>16 (1500)</td>
</tr>
<tr>
<td>489</td>
<td>64</td>
<td>64 (1800)</td>
<td>512</td>
<td>512 (27000)</td>
</tr>
</tbody>
</table>

* HI antibody titres.
† Figures in parentheses show neutralization titres.

mmol, New England Nuclear Corp., Boston, U.S.A.) was added to the cultures 3 h after infection and the cultures were then incubated for an additional 3 h at 37 °C. Infected cells were harvested with a rubber policeman and disrupted with 15 strokes in a Dounce homogenizer. After removing cell debris by centrifugation for 5 min at 400 g, the supernatant was made to 1% sodium deoxycholate and 1% Brij 58 (Fernandez-Tomas & Baltimore, 1973). Then the virus antigens were precipitated with 8% polyethylene glycol 6000 in the presence of 0.5 M-NaCl. The preparation concentrated about 100-fold was mixed with a phosphate buffered saline (PBS)-saturated CsCl solution to a density of 1.33 g/ml and centrifuged in a Spinco SW50.1 rotor at 100000 g for 18 to 20 h at 4 °C. After centrifugation and fractionation, haemagglutination (HA) titres of each fraction were assayed. The procapsid fraction (1.29 g/ml) and the virion fraction (1.33 g/ml) were dialysed against PBS, and then centrifuged in 15 to 30% sucrose gradients in a Spinco SW27 rotor at 100000 g for 4 h at 4 °C. The procapsid (80S) and virion (160S) fractions were dialysed against Veronal-buffered saline (VBS) without calcium and magnesium, pH 7.2, concentrated with a single hollow fibre (MDA Scientific, Inc., Park Ridge, U.S.A.). All procedures for the preparation of virus antigens were carried out in the cold within 5 days after harvesting.

Polyacrylamide gel electrophoresis. Electrophoresis of radioactive polypeptides was performed in 13% acrylamide gels containing an acrylamide/bisacrylamide ratio of 130:1 (Lamb & Choppin, 1977). The discontinuous buffer system containing SDS described by Laemmli (1970) was used. Electrophoresis was carried out at room temperature for 16 h at constant voltage and an initial current of 0.75 mA/gel.

Preparation of hyperimmune sera. Immune sera were produced in guinea pigs weighing 400 to 600 g. The purified virion preparation was mixed with an equal volume of Freund’s complete adjuvant. Each animal received a total of 0.4 ml of the mixture by the foot-pad route. One month later the animals were inoculated intraperitoneally with 0.4 ml of aqueous antigen and bled a week later.

Serological tests. Neutralization and haemagglutination-inhibition (HI) tests were carried out as reported previously (Hasegawa, 1975). Complement fixation (CF) tests were performed in microplates using five 50% haemolytic units of complement.

Heating experiments. The virus antigen samples were heated in glass tubes in a water bath at a pre-set temperature. After the desired period of heating, the tubes were transferred to an ice bath.
Antigenicity of E7 procapsid

Fig. 1. The effect of heating on HA and CF activities of E7. Procapsid was heated at 40 °C (a), 45 °C (b) and 50 °C (c). Virion was heated at 45 °C (d), 50 °C (e) and 55 °C (f). ●—●, HA titre (a reciprocal of the highest dilution of either procapsid or virion with positive HA); ■—■—■, CF antigen titre to homologous antiserum; □—□, CF antigen titre to heterologous antiserum.

RESULTS

Antigenicity of the native procapsid

Cross HI and CF tests were carried out using antisera to two different E7 strains, Wallace and 489. The antisera used were highly strain-specific in neutralization tests (Table 1). The procapsid and virion fractions of the corresponding strains were used as antigens. The HI test showed that the antigenicity of the native procapsid was as strain-specific as that of the virion (Table 1). In a CF test the procapsid was also as highly strain-specific as the virion (data not shown).
Effect of heating on HA and CF antigenicity of the procapsid

The Wallace strain procapsid and virion were heated at 40 to 55 °C for 15 min. CF antigen titres were determined with both homologous (anti-Wallace) and heterologous (anti-489) sera (Fig. 1). HA activity and CF antigenicity of the procapsid were stable on heating at 40 °C (Fig. 1a). When heated at above 45 °C, there was a rapid fall of HA titres with the concomitant expression of the common CF antigenicity detected by heterologous antiserum (Fig. 1b, c). The CF antigen titres detected by homologous antiserum remained at the same level throughout the time at all temperatures used (Fig. 1a, b, c). However, the virion did not show the antigenic conversion until it was heated to 50 °C (Fig. 1d, e, f).

From the above experiments, the procapsid seemed more heat-labile than the virion. To determine whether or not the addition of 1 M-MgCl₂ stabilizes the procapsid, both procapsid and virion preparations were mixed with an equal volume of 2 M-MgCl₂ solution and heated at different temperatures. HA and CF activities were titrated after dialysing against VBS (pH 7.0) at 4 °C overnight to remove Mg ions. The addition of 1 M-MgCl₂ stabilized the antigenicity of the procapsid considerably. The HA activity did not decrease until it was heated at 55 °C and the common CF antigenicity was not produced at all temperatures used (data not shown). HA and CF activities of the virion were quite stable even after heating at 60 °C.

More precise CF reactivity of the procapsid and virion after heating was assayed in
Fig. 3. Sedimentation patterns and poly peptide composition of native and heated E7 procapsids labelled with H-lysine (53 Ci/mmol). (a) Sucrose gradient centrifugation of native (○), and heated particles, heated at 45°C for 15 min (△) and heated at 65°C for 15 min, (△). (b) SDS-polyacrylamide gel electrophoresis of native (○) and heated (○) procapsids. Electrophoretic migration is from left to right.
a chessboard titration. The Wallace procapsid and virion were heated at various temperatures from 45 to 60 °C for 15 min and examined by CF test using both homologous and heterologous antisera (Fig. 2). The procapsid became cross-reactive to heterologous antiserum after heating at 45 °C. The procapsid antigenicity with both homologous and heterologous antisera was slightly diminished after heating at above 60 °C (Fig. 2a). In contrast, the cross-reactivity of the virion increased after heating at 50, 55 and 60 °C (Fig. 2b).

Cross CF reactivity with other members of enterovirus

Cross-reactivity was examined among antigens of different types of enteroviruses. Cross chessboard CF tests were performed with both native and heated antigens of various types of enterovirus and their antisera. Anti-E7 serum was cross-reactive neither to the native nor heated antigens prepared from the prototype strains of E4, E11, CA9 and CB5. Furthermore, the native and heated antigens of E7 procapsid and virion were not reactive with anti-E11 and anti-CB5 sera. This shows that the common CF antigenicity after heating, detected by the hyperimmune guinea pig sera, is not intertypic but intratypic or type-specific.

Sedimentation patterns and polypeptide composition of the native and heated procapsids

To study the sedimentation rate of the heated procapsid, 3H-leucine labelled procapsid and virion were heated at 45 and 55 °C for 15 min and then sedimented through linear gradients of 15 to 30 % sucrose at 100000 g in a Spinco SW41 rotor for 4 h at 4 °C. The sedimentation rate of the procapsid heated at 45 and 55 °C was the same 80S as the control native procapsid (Fig. 3a).

The native and heated (55 °C) particles were examined for polypeptide composition by SDS-polyacrylamide gel electrophoresis. Both particles contained the same polypeptides, VPo, 1 and 3 (Fig. 3b).

DISCUSSION

The procapsid of E7 had the same N-antigenicity as the virion. Similar results have been reported in echovirus type 12 (Halperen et al. 1964) and foot-and-mouth disease virus (Rowlands et al. 1975). However, the antigenicity of the procapsids was found to be more heat-labile than the virion. The conversion of the N- to H-antigenicity readily took place on heating at 45 °C, while the virion antigenicity was not altered at this temperature (Fig. 2). Wallis et al. (1965) reported that 1 M-MgCl₂ stabilizes enteroviruses in infectivity by heating. We have now shown that 1 M-MgCl₂ stabilized the antigenicity of the E7 procapsid.

The H-antigenicity detected by hyperimmune guinea pig serum was intratypically common, and type-specific. This result was unexpected, since Schmidt et al. (1965) and Lonberg-Holm & Yin (1973) reported that heated picornavirions possess intertypically common (group-specific) antigenicity detected by monkey or human post-infection serum. This discrepancy may be due to the difference of the antibodies used; man suffers from repeated infections with human enteroviruses, whereas such viruses do not multiply in guinea pigs. Antibodies against the group-specific antigen on the surface of H particles may not be produced in guinea pigs. Recently, Hughes et al. (1977) showed by immune electron microscopy that human sera reacted with group-specific antigens of human picornaviruses, while guinea pig or rabbit antisera did not show any cross-reactivity.

With picornaviruses, treatments with acid, urea, alkali or heating induced inactivation and antigenic alteration of virions. Two models have been proposed to account for the results: the loss of VP₄ (Breindl, 1971; Katagiri et al. 1971) and the change in surface configuration resulting from a conformational alteration of the capsid proteins (Lonberg-Holm & Yin,
1973; Noble & Lonberg-Holm, 1973). Isoelectric focusing showed that capsids containing identical polypeptides were resolved into biologically active and inactive particles. Thus the isoelectric points and biological activities correlate with the conformation of the capsid (Mandel, 1971; Korant et al. 1975). Our results showed that the native and heated procapsids of E7 did not differ in sedimentation rate (80S; Fig. 3a) and polypeptide composition (both contained Vpo, 1 and 3; Fig. 3b), but that they had N- and H-antigenicities, respectively. When the procapsid was heated, HA activity was lost and concomitantly common CF antigenicity (H-antigenicity) appeared (Fig. 1b, c). These results suggest that the procapsid of E7 had altered in conformation and showed a new antigenicity on heating, and that the conformation rather than the composition of the E7 procapsid determined the antigenicity.

REFERENCES


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