High C + G Content in Parapoxvirus DNA

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SUMMARY

The DNAs of eight parapoxviruses (four stomatitis papulosa viruses isolated from infected calves, a pseudocowpox virus isolated from a teat lesion of an infected cow and three orf viruses, one isolated from an infected sheep and two isolated from human infections) were analysed in CsCl gradients. The mole % of G+C was calculated from the buoyant density and found to be approx. 63 % for all virus isolates examined. Parapoxvirus DNA thus has by far the highest G+C content of all poxvirus DNAs so far examined.

Parapoxviruses form a group of morphologically clearly distinct viruses within the family Poxviridae. In contrast to the DNA of vaccinia virus (an orthopoxvirus) which is well characterized by its high mol. wt. of $122 \times 10^6$, (Geshelin & Berns, 1974), presence of cross-links at the termini (Berns & Silvermann, 1970; Geshelin & Berns, 1974) and a low G+C content of 36 to 37 % (Joklik, 1962a, b), very little is known about the genome of parapoxviruses. We have recently shown that the DNA of stomatitis papulosa virus has a mol. wt. of approx. $85 \times 10^6$ and that the ends are also cross-linked (Menna et al. 1979).

In order to characterize further the DNA of parapoxviruses we have calculated the mole % of G+C of the DNAs of several isolates of parapoxviruses from the buoyant density obtained in analytical CsCl gradients.

The following eight parapoxviruses were used in this study: four stomatitis papulosa viruses (from infected calves), three orf viruses (one isolate from an infected sheep and two isolates from human infections) and one pseudocowpox virus (isolated from a teat lesion of an infected cow). The buoyant densities of the DNAs of the parapoxviruses was compared to the DNAs from two members of the orthopoxvirus genus, vaccinia (strain Elstree) and rabbitpox virus (strain Utrecht). The two orthopoxviruses were grown on the chorioallantoic membrane of developing chick embryos and the virions purified as described by Joklik (1962a). The parapoxvirus isolates were all grown on bovine foetal lung cells (Goldsmith & Barzilai, 1975). After virus adsorption for 1 h at 37 °C the cultures were further incubated until 90 % of the cells showed a c.p.e. The cultures were then frozen and thawed and the cells and viruses pelleted by ultracentrifugation. Virions were purified essentially as described by Joklik (1962a) for orthopoxviruses. Since the parapoxviruses banded in the upper third of the sucrose gradients in preliminary experiments, the procedure was slightly modified. Virions were pelleted through a cushion of 36 % sucrose for 20 min at 26000 rev/min in a Beckman SW 40 rotor and banded in 25 to 40 % sucrose gradients for 25 min at 23000 rev/min, also in a Beckman SW 40 rotor. In order to isolate the DNA of orthopoxviruses and parapoxviruses, virions were collected from sucrose gradients, pelleted by centrifugation for 30 min at 25000 g and resuspended in 50 mm-tris-HCl, pH 7.8, 1 mm-EDTA, 27 % sucrose and 0.5 % SDS. The DNA was isolated by treatment of the virus suspension with 20 μg of proteinase K per 1 absorbance unit of purified virions (Joklik, 1962a) followed by two extractions with phenol-chloroform (1:1) and two extractions with chloroform-isoamylalcohol (24:1). The DNA was precipitated by the addition of 3 vol. of
Short communications

Fig. 1. Densitometer tracings of photographs of analytical CsCl gradients of vaccinia (a) and orf virus DNA (b). For experimental details see text.

Table 1. Density and G+C content of the DNAs of the parapoxvirus isolates and of vaccinia and rabbitpox virus

<table>
<thead>
<tr>
<th>Parapoxvirus spp.</th>
<th>Density (g/ml)</th>
<th>G+C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatitis papulosa virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolate No. 1</td>
<td>1.721</td>
<td>62.5</td>
</tr>
<tr>
<td>Isolate No. 2</td>
<td>1.716</td>
<td>57.6</td>
</tr>
<tr>
<td>Isolate No. 3</td>
<td>1.722</td>
<td>63.4</td>
</tr>
<tr>
<td>Isolate No. 4</td>
<td>1.721</td>
<td>62.5</td>
</tr>
<tr>
<td>Orf virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolate No. 1</td>
<td>1.723</td>
<td>64.5</td>
</tr>
<tr>
<td>Isolate No. 2</td>
<td>1.721</td>
<td>62.5</td>
</tr>
<tr>
<td>Isolate No. 3</td>
<td>1.722</td>
<td>63.4</td>
</tr>
<tr>
<td>Pseudocowpox virus</td>
<td>1.723</td>
<td>64.5</td>
</tr>
<tr>
<td>Orthopoxvirus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>1.696</td>
<td>36.8</td>
</tr>
<tr>
<td>Rabbitpox virus</td>
<td>1.695</td>
<td>36.2</td>
</tr>
</tbody>
</table>
absolute ethanol, pelleted and redissolved in 50 mM-tris-HCl, pH 7·8, 1 mM-EDTA. To the samples of purified virus DNAs (5 µg) diluted in 10 mM-tris-HCl, pH 7·4, 0·5 mM-EDTA (final vol., 0·8 ml) poly(dA).poly(dT) (1 µg, PL-Biochemicals, Milwaukee, Wis., U.S.A.) was added as a density marker (1·679 g/ml, Schildkraut et al. 1962). Sufficient CsCl was added to bring the starting density to 1·710 g/ml (determined refractometrically). Centrifugation was in a Beckman model E analytical ultracentrifuge using an AN-F rotor at 44,000 rev/min for a minimum of 16 h at 20 °C. Photographs were taken with u.v. optics and scanned. The densities of the virus DNAs were calculated with reference to poly(dA).poly(dT) and G+C contents were computed from these as described by Flamm et al. (1972).

Typical scans of two photographs of analytical CsCl gradients are shown in Fig. 1 (a and b). The DNA of the orf virus isolate (b) bands at a much higher density than the vaccinia virus DNA (a) analysed under the same conditions. The density of the vaccinia virus DNA is 1·696 g/ml corresponding to a G+C content of 36·8%. This value is in good agreement with that published previously (Joklik, 1962a, b). For the orf virus DNA a density of 1·723 g/ml was obtained corresponding to a G+C content of 64·5%. A summary of the densities of the various DNAs examined is shown in Table 1.

We consider that the parapoxviruses used in this study represent different strains of at least three species, firstly because the viruses used were isolated from various sources and secondly because restriction enzyme analysis of the DNAs of these viruses strongly suggests the occurrence of different species, even within the groups of stomatitis papulosa viruses and orf viruses examined (R. Wittek et al. unpublished data). Since the DNA from all the viruses studied has a G+C content of an average 63% we consider a high G+C content to be a general feature of parapoxvirus DNA.

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