The DNA Contained by Nuclear Polyhedrosis
Viruses Isolated from Four *Spodoptera* spp. (Lepidoptera, Noctuidae);
Genome Size and Configuration Assessed by Electron
Microscopy

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**SUMMARY**

The mol. wt. of the DNA from four nuclear polyhedrosis viruses isolated from *Spodoptera littoralis*, *S. exempta*, *S. exigua* and *S. frugiperda* were determined to be 84, 80, 68 and $74 \times 10^6$, respectively, by electron microscopy. The molecules were demonstrated to exist as double-stranded relaxed circular or supercoiled DNA, though linear forms of DNA were also observed.

**INTRODUCTION**

The nuclear polyhedrosis viruses (NPVs) are a group of viruses which are pathogenic to invertebrates. They cause lethal diseases in their insect hosts. The virus particles comprise enveloped nucleocapsids and are rod-shaped with dimensions of about $250 \times 50$ nm where nucleocapsids are singly enveloped. In some instances, more than one nucleocapsid can occur within the virus envelope. These virus particles, which contain double-stranded DNA, may be occluded in a large protein lattice or polyhedron (Tinsley & Harrap, 1977).

This laboratory is studying NPVs from four species of the genus *Spodoptera* to provide criteria whereby virus isolates may be characterized and distinguished from each other. These NPVs are potentially important biological control agents of the *Spodoptera* species which are important pests of a variety of crops throughout the world.

The four virus isolates used in this work share the general properties of the NPV group although they are serologically and biochemically different from each other (Harrap, Payne & Robertson, 1977; Kelly, 1977). The data reported here are concerned with the molecular weight and configuration of these NPV DNAs.

There have been several reports that the DNAs of NPVs are circular and supercoiled (Summers & Anderson, 1973; Harrap et al. 1977). This evidence has been obtained from caesium chloride-ethidium bromide gradients and sucrose gradient analysis. Two bands are observed in ethidium bromide gradients, one corresponding to supercoiled DNA and the other nicked circular and linear DNA (Radloff, Bauer & Vinograd, 1967). The molecular weights of NPV DNAs have been determined by neutral sucrose gradient sedimentation. There appear to be significant differences in the values obtained in different laboratories (Summers & Anderson, 1973; Harrap et al. 1977), although the molecular weights obtained by reassociation kinetics (Kelly, 1977) are generally comparable to those derived from neutral sucrose gradients by Harrap et al. (1977) in our laboratory.

In this paper, electron microscopy, which has been widely used to examine the properties
of DNA molecules (Banfield-Younghusband & Inman, 1974), has been used to examine the mol. wt. and structure of the DNAs contained by NPVs from four species of Spodoptera.

The NPVs used in this study were isolated from the following hosts: *S. frugiperda*, the fall armyworm from the Americas; *S. exempta*, the mystery armyworm found in Africa and Australia; *S. exigua*, the beet armyworm found in tropical and sub-tropical countries; and *S. littoralis*, the cotton leafworm found in Africa and the Mediterranean (Harrap et al. 1977; Kelly, 1977).

**METHODS**

*NPV production.* The virus isolates used were the same as reported by Kelly (1977). Viruses were grown in insects and purified in the manner described by Harrap et al. (1977). Third instar larvae were infected *per os* with a standardized suspension of purified polyhedra. The virus-infected larval cadavers were triturated in water and the polyhedra purified by low speed centrifugation. The polyhedra were dissolved by the addition of an equal volume of 0.1 M-Na₂CO₃ and then purified on 30 to 65 % (w/w) aqueous sucrose gradients at 90,000 g for 2 h. The virus bands were collected and pelleted from sucrose. The pellets were resuspended in water and stored at 4 °C.

*Extraction of the DNA.* Ten percent (w/v) SDS in ethanol:water (45:55, v/v) mixture was added to a suspension of virus particles (1 mg/ml) in 1×SSC (SSC = 0.15 M-NaCl, 0.015 M-sodium citrate). This solution was incubated at 37 °C for 60 min. The DNA was extracted seven or eight times with an equal volume of chloroform:n-butanol (3:1, v/v). The DNA was then precipitated from the aqueous phase with two vol. of 95 % ethanol. After resoiling, the DNA was dissolved in 20 mM-NaCl, 5 mM-EDTA.

*Preparation of ‘standard’ DNA.* Polyoma DNA was a gift from Beverly Griffin, ICRF, London. Bacteriophage T7 (obtained from Dr D. Bryant, Department of Botany, University of Oxford) was grown in *Escherichia coli B* thy⁻ mutants. The phage was harvested, and the DNA extracted as described by Avery & Kelly (1974).

*Electron microscopy.* DNA was spread by a modification of the technique described by Inman (1973). Thirty μl of DNA (0.2 μg) and 5 μl of polyoma DNA (0.05 μg) in 20 mM-NaCl and 5 mM-EDTA were added to 15 μl of a solution comprising 65 mM-sodium carbonate, 37 % (v/v) formaldehyde, 10-2 mM-EDTA and 46 mM-hydrochloric acid at pH 8.0. Fifty μl of formamide and 10 μl (1 mg/ml) cytochrome c solution were then added. Fifty μl of this DNA-containing phase were run down a clean stainless steel slide on to a hypophase of double glass-distilled water. The DNA was picked up from the hypophase on carbon coated (200 mesh) copper grids. The grids were fixed in absolute ethanol and then rotary shadowed, first with platinum and then palladium:gold (40:60) to improve the contrast. The grids were examined with an AE1 EM6B microscope. The magnification was calibrated using a grating carbon replica grid of 2160 lines/mm (EM Scope Laboratories, London) at each magnification, each time the microscope was used. Care was taken to minimize lens hysteresis effects. Twenty-five to thirty well spread circular molecules of DNAs and the same number of ‘standard’ DNAs were measured.

The plates were enlarged fourfold using a photographic enlarger, traced on to paper, and then measured using a Hewlett Packard 9821A calculator and digitizer.

**RESULTS**

All four preparations of NPV DNAs appear to consist of supercoiled, circular and linear double-stranded molecules. Two supercoiled molecules of *Spodoptera littoralis* NPV DNA
Fig. 1. Supercoiled DNA of *S. littoralis* NPV. A partially relaxed form (a) and a highly supercoiled form (b) are demonstrated. In (a) the arrows indicate supercoiled regions of the molecule as labelled by S and relaxed DNA as indicated by R.
Fig. 2. A relaxed circular DNA molecule from *S. littoralis* NPV. The arrows indicate polyoma DNA used as an internal standard.

are shown in Fig. 1, one is completely supercoiled and the other contains regions of non-supercoiled or relaxed DNA. A representative relaxed circular DNA molecule from each of the four isolates is shown in Fig. 2, 3, 4, 5. No single-stranded DNA was observed under the spreading conditions used here where single-stranded regions of greater than about 60 base pairs would have been apparent (Inman, 1967).

Under these spreading conditions, the molar linear density of DNA was found to be $2.03 \times 10^{10}$ daltons/cm. This was calculated from the equation $M = M'L$, where $M$ is the theoretical mol. wt. (in this case $25.1 \times 10^6$ for T7 as reported by Lang, 1970), $L$ is the length of the DNA molecule (found here to be 12.32 $\mu$m – Table 1) and $M'$ is the molar linear density. Using the value $2.03 \times 10^{10}$ daltons/cm, the mol. wt. of polyoma DNA was found to be $3.37 \times 10^6$ when polyoma DNA was co-spread with T7. This mol. wt. is similar to
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published values of \(3.0 \text{ to } 3.6 \times 10^6\) (Weil & Vinograd, 1963; Griffin, Fried & Cowie, 1974).

Subsequently this standardized polyoma DNA was co-spread with NPV DNA. Only intact relaxed circular molecules were measured for mol. wt. determinations since it was considered that these represented whole molecules. Linear molecules have also been measured and these were found to be not significantly larger or smaller than the circular molecules. The distribution of molecular lengths for each sample is represented by the histogram in Fig. 6. Equal numbers of polyoma and NPV DNA were measured for each sample and the mol. wt. calculated. The differences in mean molecular length of the polyoma DNA, measured with the NPV DNA, never exceeded twice the standard error \((\pm 0.02 \mu m)\) of the mean of the T7-standardized polyoma DNA. This indicated that the mean molecular lengths of the different NPVs were comparable. The coefficients of variation listed in Table 1 and shown in Fig. 6 for the four NPV DNAs arise mainly from errors described by Lang et al. (1967) and Lång (1970), i.e., tracing and measuring of the enlarged images of the molecules may be the cause of the major part of this variation although some natural variation in molecule size may occur.

Fig. 3. A relaxed circular DNA molecule from \(S. \text{ exigua}\) NPV. The arrows indicate polyoma DNA used as an internal standard.
Fig. 4. A circular DNA molecule from Spodoptera exempta NPV. The arrows indicate polyoma DNA used as an internal standard.

The mol. wt. of the Spodoptera NPV DNAs were found to be $79.6 \times 10^6$, corresponding to a length of $39 \mu m$ for S. exempta NPV DNA, and $84.5 \times 10^6$, corresponding to a length of $41 \mu m$ for S. littoralis NPV DNA. S. exigua NPV DNA and S. frugiperda NPV DNA had smaller genomes with lengths of $33.5 \mu m$ and $36 \mu m$ and mol. wt. of 68 and $74 \times 10^6$, respectively (Table 1 and Fig. 6).

DISCUSSION

Some supercoiled molecules were observed for each of the four NPVs. The photographs of supercoiled molecules shown here (Fig. 1) are similar to those of mitochondrial DNA reported by Dawid & Wolstenholme (1967). It is possible that minor variations in the environment of the DNA outside the virus particle induce either supercoiling or relaxation of the DNA molecule. However, our observations of the different forms of NPV DNA (i.e. supercoiled, relaxed circular and linear) support caesium chloride-ethidium bromide gradient analysis reported by Summers & Anderson (1973) and Harrap et al. (1977) for NPVs isolated from species of Spodoptera. They are also consistent with evidence of the
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Fig. 5. A relaxed circular DNA molecule from *S. frugiperda* NPV.

Table 1. Molecular weights of the DNAs of four NPV isolates

<table>
<thead>
<tr>
<th>DNA</th>
<th>No. molecules counted</th>
<th>Mean length (µm) and s.d.</th>
<th>Coefficient of variation (%)</th>
<th>Mol. wt. × 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7</td>
<td>30</td>
<td>12.31 ± 0.74</td>
<td>6.0</td>
<td>25.10</td>
</tr>
<tr>
<td>Polyoma</td>
<td>25</td>
<td>26.65 ± 0.07</td>
<td>4.2</td>
<td>33.36 ± 0.14</td>
</tr>
<tr>
<td><em>Spodoptera exempta</em></td>
<td>24</td>
<td>39.34 ± 2.20</td>
<td>5.6</td>
<td>79.6 ± 4.5</td>
</tr>
<tr>
<td><em>Spodoptera littoralis</em></td>
<td>25</td>
<td>41.51 ± 2.20</td>
<td>5.3</td>
<td>84.5 ± 4.2</td>
</tr>
<tr>
<td><em>Spodoptera exigua</em></td>
<td>26</td>
<td>33.48 ± 1.97</td>
<td>5.9</td>
<td>68.2 ± 4.0</td>
</tr>
<tr>
<td><em>Spodoptera frugiperda</em></td>
<td>25</td>
<td>36.23 ± 3.67</td>
<td>10.1</td>
<td>73.8 ± 7.5</td>
</tr>
</tbody>
</table>

* S.d. as a percentage of the mean.

Fig. 6. Histograms showing the length distribution of the DNA molecules measured for mol. wt. determination. (a) *Spodoptera littoralis* NPV DNA, (b) *S. exempta* NPV DNA, (c) *S. frugiperda*, and (d) *S. exigua*. 
physical structure of other NPV DNAs (Kok et al. 1968; Summers & Anderson, 1973), granulosis virus DNA (Brown, Bud & Kelly, 1977) and Oryctes virus DNA (Revet & Monsarrat, 1974).

The mol. wt. of S. frugiperda, S. exigua, S. exempta and S. littoralis NPV DNAs described here are significantly different from each other. These values are consistent with the results obtained by reassociation kinetics for three of the viruses (Kelly, 1977), i.e. for the S. exempta isolate, the NPV DNA was found to have a mol. wt. of $82.5 \pm 4.6 \times 10^6$, that of S. exigua was $65.4 \pm 3.5 \times 10^6$ and that of S. frugiperda was found to be $67.8 \pm 4.3 \times 10^6$.

The mol. wt. value of $84 \times 10^6$ for S. littoralis NPV DNA determined by electron microscopy is higher than the value determined by reassociation kinetics which is $64 \times 10^6$. It is not clear why this is so. However, the mol. wt. of S. littoralis DNA, as determined by sucrose gradient analysis is $84 \times 10^6$ (Harrap et al. 1977) and thus is comparable with results derived by electron microscopy.

The coefficient of variation of the mol. wt. derived from the molecular lengths of both standards and NPV DNA fell in the range of 4-2 to 6-0 % (with the exception of S. frugiperda DNA which was slightly more variable over the sample of molecules chosen); this indicates that the determinations are directly comparable.

The large circular genomes derived from purified virus particles appear to be a distinguishing feature of baculovirus DNAs. The NPVs isolated from Spodoptera spp. are typical in this respect. Circular DNA of this size has not been described for vertebrate viruses or bacteriophages. It is hoped that further study will reveal more information about these molecules. We are continuing to look at the genome by means of partial denaturation mapping.

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REFERENCES


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