Studies on the Structural Basis of the RNA Polymerase Activity of Newcastle Disease Virus Particles

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SUMMARY

Treatment of purified Newcastle disease virus (NDV) with Triton N101 to activate the virus particle RNA polymerase in the in vitro assay removed the glycoproteins and most of the phospholipid from the virus. The subvirus particles produced by the action of Triton N101 on virus had RNA polymerase activity, whereas purified NDV nucleocapsids did not. Apparently, either nucleocapsids did not contain the proteins essential for RNA polymerase activity, or the enzyme was denatured during the preparation of nucleocapsids. Incubation of several purified strains of NDV at 4 °C led to loss of polymerase activity which was regained on incubation at 37 °C. It is suggested that internal configurational changes in the virus particle were the most likely causes of these changes in enzyme activity on incubation at 4 °C and 37 °C.

INTRODUCTION

Newcastle disease virus (NDV) contains an RNA polymerase (Huang, Baltimore & Bratt, 1971) which may be assayed in vitro after treatment of the virus with the detergent Triton N101. Nothing is known about the structural basis of polymerase activity, and we have, therefore, commenced an investigation of the correlation between enzyme activity and the presence of the different virus proteins. The object of the experiments reported in this paper was to characterize the product(s) obtained by treatment of virus with the detergent. The product was found to be heterogeneous, for it was contaminated with whole virus and consisted of a range of particles which are heterogeneous with respect to chemical composition and size. However, since it was our object to characterize the virus preparations which possessed polymerase activity, no attempts at further purification were made. During the course of these experiments, it became clear that both enzyme activity, measured in the in vitro assay, and the ability to induce interferon depended on the conditions under which the purified virus had been stored.

METHODS

Materials. Triton N101, reduced glutathione, ATP, CTP, GTP and UTP were obtained from Sigma Chemical Company Ltd. [8-3H]-GTP (8.0 Ci/m-mol), [14C]-L-valine (225 mCi/m-mol) and [32P] (orthophosphate in dilute hydrochloric acid, pH 2 to 3, at 50 to 100 Ci/mg P) were supplied by the Radiochemical Centre, Amersham, Buckinghamshire. Cleland's reagent (A grade) (dithio-threitol) was supplied by Calbiochem., London, and
SDS (specially pure grade) by British Drug Houses, Poole, Dorset. Actinomycin D was given by Merck, Sharpe and Dohme Limited. Medium 199 was obtained from Wellcome Reagents Limited, and calf serum from Biocult Laboratories, Glasgow, Scotland.

Media and cells. Chick embryo cells were prepared from 11-day-old embryos as described by Waiters, Burke & Skehel (1967) using medium 199 plus 7% calf serum for cell growth and medium 199 plus 2% calf serum for cell maintenance.

Viruses. The HERTS 33, TEXAS, L, H and F strains of NDV were grown as described previously (Lomniczi, 1970; Lomniczi, Meager & Burke, 1971). NDV strain TEXAS labelled with $^{32}$P was prepared by the method of Barry & Bukrinskaya (1968). NDV strain TEXAS labelled with $^{14}$C-l-valine was prepared by infection of chick cells (2.5 x 10$^8$ cells/Petri dish) with NDV at high multiplicity for 1 h at 37 °C. The excess virus was removed and the cell monolayers washed twice with 10 ml medium 199 plus 2% calf serum containing 0.1 μg/ml actinomycin D (AMD). Then 25 ml medium 199 plus 2% calf serum containing 0.1 μg/ml AMD was added until 2.5 to 3 h after infection, when the medium was replaced with 15 ml of Earle’s saline (without glucose) containing 2% dialysed calf serum, 0.1 μg/ml AMD and 10 μCi [14C]-l-valine. The virus-containing medium was harvested after 24 h at 37 °C.

Purification of NDV. Virus was purified by a modification of the method of Sheaff, Meager & Burke (1972). Infected allantoic fluid was clarified by sedimentation at 11 000 g for 30 min at 4 °C. Virus was then pelleted by centrifuging at 50 000 g for 2 h at 4 °C, resuspended in 4% of the original volume with TN buffer (10 mm-tris, 30 mm-NaCl, pH 7.3 at 32 °C) by Dounce homogenization, and the resulting suspension layered on to 5 ml (20%, w/v) sucrose cushions containing TN buffer and centrifuged at 59 000 g for 45 min at 4 °C. The virus pellets were again resuspended in TN buffer, the suspension layered onto a linear 15 to 60% (w/v) sodium potassium tartrate gradient buffered to pH 7.3 with TN buffer and the virus centrifuged to equilibrium (70 000 g for 14 to 18 h at 4 °C). The opalescent band was diluted and virus re-pelleted at 59 000 g for 30 min at 4 °C. The pellet was re-suspended as before in a small volume of TN buffer and dialysed against TN buffer overnight at 4 °C. Concentrated virus was preferably stored at 4 °C, but glycerol (to 20%, v/v) was added if the virus was to be stored frozen at −70 °C. Virus grown on CEC monolayers was purified in a similar fashion.

Preparation of NDV subvirus particles. The method described by Meager & Burke (1972) was used, except that virus was incubated with 0.08% (v/v) Triton N101 for 1 h at 32 °C. Nucleocapsids were prepared by adding 0.5 ml 5% (v/v) Triton X-100 and 0.2 ml (10%, w/v) sodium deoxycholate (DOC) to 2 ml of concentrated virus suspension (5 to 10 mg virus protein/ml) and incubating at 41 °C for 2 h. The nucleocapsids were purified by centrifuging to equilibrium on a 20 to 65% (w/v) linear sucrose gradient containing D$_2$O buffered with 10 mm-tris, 30 mm-NaCl, pH 7.3 at 78 000 g for 14 h at 4 °C. The band sedimenting to a buoyant density of 1.27 g/cm$^3$ was collected, diluted 3 to 4 times with TN buffer, and incubated for 30 min at 4 °C with DOC at the same concentration as before. Nucleocapsids were then re-banded on a 20 to 65% sucrose-D$_2$O gradient as described and, after dilution with TN buffer, pelleted by centrifuging at 56 000 g for 45 min at 4 °C. The pellet was dispersed in a small volume of ice-cold TN buffer by gentle shaking and stored at 4 °C prior to use. The methods of de-Thé & O’Connor (1966) and Mountcastle et al. (1970) for isolation of nucleocapsids were also used for comparison.

Heat treatment of NDV. Purified virus was incubated at 37 °C for 48 h in TN buffer.

Interferon production and assay was carried out as described by Sheaff et al. (1972). Results are expressed in research reference units of chick interferon.
NDV RNA-dependent RNA polymerase assay was carried out as described by Sheaff et al. (1972).

**Protein determination.** Virus protein was determined by a modification of the method of Lowry et al. (1951), described by Oyama & Eagle (1956), with bovine serum albumin as standard.

**Electron microscopy.** Purified virus or Triton-treated particles freed from Triton by sedimentation through sucrose, were diluted and a small amount of each mixed with an equal volume of 3% phosphotungstic acid adjusted to pH 6.0. A drop of the mixture was then placed on a 400-mesh carbon-formvar-coated grid and excess fluid withdrawn. After drying, the grid was examined immediately in a Philips EM 300 (Hoyle & Almeida, 1971).

**Solubilization of virus structural proteins.** The structural proteins of NDV and subvirus particles were solubilized by treatment of purified suspension with 1% (w/v) SDS, 0.5 M-urea and 1% (v/v) 2-mercaptoethanol, incubating at 37 °C for 3 h, and dialysing overnight against 1000 vol. 0.005 M-tris + 0.038 M-glycine, pH 8.4, containing 0.1% SDS and 0.01% Cleland’s reagent (Morser, Kennedy & Burke, 1973).

**Analytical polyacrylamide gel electrophoresis.** Proteins were electrophoresed in 10% (w/v) acrylamide gels cast to a height of 90 mm in 6 mm internal diameter perspex tubes (Morser et al. 1973). Gels were overlaid with 0.1 ml of 0.1 M-reduced glutathione and pre-electrophoresed at 100 V for 2 h before use. After electrophoresis the gels were stained with Coomassie blue (Maizel, Summers & Scharff, 1970), destained by washing with 7.5% (v/v) glacial acetic acid in 5% (v/v) methanol and scanned at 570 nm in a Chromoscan densitometer (Joyce, Loebl and Company Limited, Gateshead, Durham). Virus glycoproteins were stained using the periodic acid-Schiff method described by Kobylka et al. (1972) and scanned using a Gilford 2000 spectrophotometer at 550 nm.

**RESULTS**

**Action of Triton N101 on purified NDV**

**Effect on infectivity and haemagglutinin titre**

NDV, strain Texas, was incubated in 0.08% (v/v) Triton N101 for 1 h at 32 °C, and the subvirus particles, together with any remaining virus particles, were deposited by sedimentation (Meager & Burke, 1972). The product had 0.01 to 0.10% of the infectivity and about 3% of the haemagglutinin titre of untreated virus.

**Characterization by electron microscopy of the subvirus particles produced by treatment with Triton N101**

The electron micrograph of the purified NDV suspension showed a homogeneous population of roughly spherical particles covered by fringes or spikes on their surfaces (Fig. 1a). However, the subvirus particles produced by treatment with Triton N101 showed considerable heterogeneity. The predominate feature was the appearance of electron dense aggregates of virus cores, many with their spikes removed. (Fig. 1b). There were also a few intact virus particles, some of them fused.
Sedimentation coefficients and buoyant density of the subvirus particles produced by treatment with Triton N101

The sedimentation characteristics of the subvirus particles, produced by Triton treatment, were found to be in keeping with the heterogeneity observed by electron microscopy. Using sucrose density gradients containing Triton N101 and [32P]-labelled NDV it was found that, after Triton N101 treatment, the subvirus particles sedimented as a broad band (Fig. 2a). Under the centrifuging conditions used (93 000 g for 1 h at 4 °C) the few remaining infectious virus particles in the suspension reached their equilibrium position, but not the subvirus particles. The majority of the subvirus particles had a $S_{20,w}$ value of approximately 440S calculated using ferritin, a macro-molecule of known sedimentation coefficient ($S_{20,w}$ of ferritin = 70S) as a marker (Martin & Ames, 1961). Using sedimentation conditions under which all particles reached equilibrium (78 000 g for 26 h at 4 °C), it was shown that the buoyant density of the majority of subvirus particles, as judged by [32P] label, was slightly greater than that of infectious virus (1.226 g/cm³ as compared to 1.210 g/cm³) (Fig. 2b).

Characterization of subvirus particles produced by treatment with Triton N101 by protein polyacrylamide gel electrophoresis

Fig. 3a illustrates the electrophoretic pattern of NDV strain TEXAS structural proteins after staining with Coomassie blue. It was found that at least seven protein species could be identified. Comparison with two other NDV strains, H and F, showed that six of these proteins (VP1, VP3, VP4, VP5, VP6 and VP7) were common to them all. Three of these protein species (VP3, VP4 and VP7) were present in relatively large amounts and have been described by others (Bikel & Duesberg, 1969; Evans & Kingsbury, 1969;
Fig. 2. Sedimentation of [32P]-labelled NDV treated with Triton N101 in (a) a 15 to 65 % (w/v) sucrose density gradient buffered to pH 7.3 with TN buffer and containing 0.08 % (w/v) Triton N101, and (b) a 15 to 60 % (w/v) sodium potassium tartrate gradient buffered to pH 7.3 with TN buffer. Radioactivity, O--O; infectivity, △--△; density, ■—■. Inset: sedimentation of untreated [32P]-labelled NDV in a 15 to 65 % (w/v) sucrose gradient buffered to pH 7.3 with TN buffer not containing Triton N101. (The infectivity of the subvirus particle preparation produced by Triton N101 was approximately 0.1 % of the untreated virus.)

Fig. 3. Polyacrylamide gel electrophoresis of (a) the proteins extracted from NDV strain TEXAS, and (b) the proteins extracted from subvirus particles produced by treatment of NDV with Triton N101. The gels were stained with Coomassie blue and scanned as described in Methods.
Fig. 4. Polyacrylamide gel electrophoresis of (a) the glycoproteins extracted from NDV strain TEXAS, and (b) the glycoproteins extracted from subvirus particles produced by treatment of NDV with Triton N101. The gels were stained using the periodate-Schiff method as described in Methods. An accurate diagram of the migration of NDV proteins as determined by subsequent Coomassie blue staining of each gel is also shown.

Haslam, Cheyne & White, 1969). Mountcastle, Compans & Choppin (1970) have also described two minor protein species which were probably identical with VP5 and VP6. Treatment of virus with Triton N101 removed approximately 16 to 23 % of the total virus proteins, as estimated by colorimetric assay, within 30 min incubation at 32 °C. Prolonged incubation (up to 2 h at 32 °C) did not remove any more protein. Treatment of the virus, labelled with [14C]-valine, with Triton N101 showed that 24 % of the [14C] counts were released. Polyacrylamide gel electrophoresis of the structural proteins of the product showed that about 80 % of VP3 and about 15 % of VP4 had been removed (Fig. 3b). There was only 1-5 % decrease in the amount of VP7. Indirect evidence has shown that VP3 is associated with virus haemagglutinating activity (Evans & Kingsbury, 1969; Haslam et al. 1969), while more positive evidence was obtained by Inuma et al. (1971). In agreement with this, we found that the isolated subvirus particles contained only 3 % of the haemagglutinating activity of intact virus particles, and because of the predominance of spikeless particles as shown by electron microscopy (Fig. 1b) VP3 was implicated as a major component of the virus spikes. Mountcastle et al. (1970), using virus labelled with [3H]-glucosamine and [14C]-amino acids, have shown that NDV contains two glycoproteins, the faster migrating one of which was masked by the ribonucleoprotein (RNP) peak. We were unable to obtain satisfactory labelling of virus when it was grown in chick cells in the presence of [3H]-glucosamine and therefore used a periodic acid-Schiff stain to identify NDV glycoproteins on SDS-acrylamide gels (Kobylnka et al. 1972). This staining procedure was rela-
Table 1. Removal of radioactivity from $[^{32}P]$-labelled NDV strain TEXAS by Triton N101

<table>
<thead>
<tr>
<th>Incubation at 32 °C (min)</th>
<th>Total $[^{32}P]$ in virus or sub-virus particles (ct/min)</th>
<th>Total $[^{32}P]$ in supernatant fluid after isolation of subvirus particles (ct/min)</th>
<th>Total $[^{32}P]$ in virus/subvirus particles+supernatant fluid (ct/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2158</td>
<td>Not done</td>
<td>2158</td>
</tr>
<tr>
<td>30</td>
<td>756</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>60</td>
<td>742</td>
<td>1224</td>
<td>1966</td>
</tr>
<tr>
<td>120</td>
<td>818</td>
<td>1377</td>
<td>2195</td>
</tr>
</tbody>
</table>

Table 2. RNA polymerase activity of NDV strain TEXAS and the subvirus particle obtained by treatment with Triton N101

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>RNA polymerase activity (ct/min/mg virus protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Triton in assay</td>
<td>- Triton in assay</td>
</tr>
<tr>
<td>Virus</td>
<td>1125</td>
</tr>
<tr>
<td>Subvirus particle</td>
<td>700</td>
</tr>
</tbody>
</table>


tively insensitive and required up to 450 µg of virus protein/gel. The magenta coloured bands also faded quickly. It was found that at least three glycoproteins could be identified (Fig. 4a) in three strains of NDV (TEXAS, H and F). The relative positions of the glycoproteins were identified by restaining the gels with Coomassie blue. Two of the glycoproteins, VGP1 and VGP2, were probably the same as those identified by Mountcastle et al. (1971). VGP1 is probably identical with VP3, that is this major protein is a glycoprotein. However, VGP2 is not identical with VP4 (Mountcastle et al. 1971). The third glycoprotein (VPG3), which had the lowest mol. wt. and was present in the smallest amount, has not been described before. Its mobility was approximately the same as that of VP7. The bottoms of the gels were irregularly stained, probably due to the presence of glyco- or phospho-lipid which also reacts with periodic acid (Carraway et al. 1972). Treatment of the virus with Triton N101 reduced the amounts of all carbohydrate staining bands including the stain at the bottom of the gels (Fig. 4b), indicating that Triton N101 removed both the external virus glycoproteins and the phospholipid. The removal of VGP2, which runs with VP4, explains the loss of Coomassie blue staining from this part of the gel, while the loss of VGP3, which runs with VP7 caused an insignificant loss of Coomassie blue staining, probably because it was present in a very small amount. The removal of phospholipid was confirmed by treatment of virus labelled with $[^{32}P]$ with Triton N101. NDV strain TEXAS grown in eggs in the presence of $[^{32}P]$ contained approximately 25 % of radioactive label in particle RNA as estimated by alcohol precipitation of RNA after SDS-phenol extraction; the remaining 75 % of the $[^{32}P]$ label presumably being present as phospholipid. Triton N101 was shown to remove 63 % of the total $[^{32}P]$ label from NDV strain TEXAS during incubation at 32 °C for 30 min (Table 1) or 84 % of the phospholipid. Further incubation at 32 °C did not increase the amount of $[^{32}P]$ removed.

RNA polymerase activity of the subvirus particles produced by treatment with Triton N101

The RNA polymerase activity of subvirus particles relative to Triton N101 activated virus is shown in Table 2. On a ct/min/mg virus protein basis the RNA polymerase activity
was found to be approximately equivalent to that of Triton N101 activated virus when the subvirus particles were incubated in the \textit{in vitro} assay in the absence of Triton N101. The polymerase activity of the subvirus particles was inhibited if Triton N101 was present in the assay.

\textbf{RNA polymerase activity of virus nucleocapsids}

It has been reported previously (Meager & Burke, 1972) that NDV nucleocapsids retained some polymerase activity. However, the activity was extremely low as compared with the Triton N101 treated virus, and we have subsequently shown by protein polyacrylamide gel electrophoresis that the method of Mountcastle \textit{et al.} (1970) failed to give a product containing a single polypeptide. This could have been due to incomplete disruption of the concentrated virus or re-association of virus proteins with nucleocapsids on caesium chloride density gradient sedimentation. The Tween-ether disruption of NDV described by De Thé & O’Connor (1966) also did not yield a product containing a single polypeptide. In fact, it was found that even under the more severe conditions described in Methods that a preparation consisting only of ribonucleoprotein (VP4) could not be achieved. Polyacrylamide gel electrophoresis showed that VP5 did not appear to be removed at all, and that VP6 and VP7 were always present in small amounts (Fig. 5). These nucleocapsids had no RNA polymerase activity.
Decay of virus RNA dependent RNA polymerase on storage of purified virus

During the course of this work, it was noticed that purified strains of NDV rapidly lost RNA polymerase activity on storage at 4 °C (Fig. 6 and Table 3, columns 3 and 5). Concomitantly there was some loss of infectivity in these preparations, the virulent strains, HERTS 33 and TEXAS, being most unstable in this respect (Table 3, columns 2 and 4). The TEXAS strain of NDV showed 90 % reduction in infectivity and no RNA polymerase activity after being frozen at −70 °C. However, the addition of 20 % (v/v) glycerol to purified virus before freezing prevented the complete destruction of polymerase activity.

Effect of incubation at 37 °C on NDV infectivity, interferon inducing capacity and virus RNA polymerase

It has been previously shown that interferon production by NDV was associated with a reduced polymerase activity of the virus particle (Sheaff et al. 1972). It was therefore of interest to measure interferon production by strains which had been stored at 4 °C and had lowered polymerase activity. Kohno, Kohase & Shimizu (1969) have reported that when NDV was incubated in vitro at 37 °C for a few days it became capable of producing interferon. Several purified NDV strains which had been stored at 4 °C were assayed for infectivity, polymerase activity and tested as interferon inducers. Strains incubated at 37 °C for 48 h were also assayed for infectivity, interferon inducing ability and RNA polymerase activity at the same time. Some infectivity was lost on storage, and up to 90 % of the polymerase activity, but none of the strains which had been incubated at 4 °C were interferon inducers (Tables 3 and 4). However, it was found that all strains lost a considerable
Table 3. Decay of NDV infectivity and RNA polymerase activity on storage of different strains at 4 °C and ‘activation’ of polymerase activity after incubation of virus at 37 °C for 48 h

<table>
<thead>
<tr>
<th>NDV strain</th>
<th>Initial infectivity (p.f.u./ml)</th>
<th>Initial polymerase activity (ct/min/mg virus protein)</th>
<th>Infectivity after 7 days storage at 4 °C (p.f.u./ml)</th>
<th>Polymerase activity after 7 days storage at 4 °C (ct/min/mg virus protein)</th>
<th>Polymerase activity after incubation at 37 °C for 48 h (ct/min/mg virus protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERTS 33</td>
<td>$3 \times 10^{11}$</td>
<td>$10 \times 10^{3}$</td>
<td>$1 \times 10^{11}$</td>
<td>$0 \times 10^{3}$</td>
<td>$4.5 \times 10^{3}$</td>
</tr>
<tr>
<td>TEXAS</td>
<td>$5 \times 10^{11}$</td>
<td>$21 \times 10^{3}$</td>
<td>$4 \times 10^{11}$</td>
<td>$3 \times 10^{3}$</td>
<td>$2 \times 10^{3}$</td>
</tr>
<tr>
<td>L</td>
<td>$2 \times 10^{11}$</td>
<td>$2 \times 10^{3}$</td>
<td>$1 \times 10^{11}$</td>
<td>$0 \times 10^{3}$</td>
<td>$2 \times 10^{3}$</td>
</tr>
<tr>
<td>H</td>
<td>$2 \times 10^{11}$</td>
<td>$8 \times 10^{3}$</td>
<td>$2 \times 10^{3}$</td>
<td>$2 \times 10^{3}$</td>
<td>$7 \times 10^{3}$</td>
</tr>
<tr>
<td>F</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 4. Effect of incubation for 48 h at 37 °C on infectivity and interferon inducing capacity of different NDV strains

<table>
<thead>
<tr>
<th>NDV strain</th>
<th>Infectivity of virus stored at 4 °C (p.f.u./ml)</th>
<th>Interferon inducing capacity of virus stored at 4 °C (interferon units)</th>
<th>Infectivity of virus incubated at 37 °C for 2 days (p.f.u./ml)</th>
<th>Interferon inducing capacity of virus incubated at 37 °C for 2 days (interferon units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERTS 33</td>
<td>$4 \times 10^{10}$</td>
<td>15</td>
<td>$1 \times 10^{8}$</td>
<td>170</td>
</tr>
<tr>
<td>TEXAS</td>
<td>$2 \times 10^{11}$</td>
<td>$&lt; 10$</td>
<td>$8 \times 10^{3}$</td>
<td>$&lt; 10$</td>
</tr>
<tr>
<td>L</td>
<td>$2 \times 10^{11}$</td>
<td>$&lt; 10$</td>
<td>$5 \times 10^{3}$</td>
<td>$&lt; 10$</td>
</tr>
<tr>
<td>H</td>
<td>$1 \times 10^{11}$</td>
<td>$&lt; 10$</td>
<td>$4 \times 10^{3}$</td>
<td>1600</td>
</tr>
<tr>
<td>F</td>
<td>—</td>
<td>$&lt; 10$</td>
<td>—</td>
<td>$&lt; 10$</td>
</tr>
</tbody>
</table>

* The F strain does not plaque.

Table 5. RNA polymerase activity of NDV strain TEXAS and its subvirus particle after (a) storage at 4 °C throughout, (b) storage at 4 °C followed by incubation for 48 h at 37 °C

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>RNA polymerase activity (ct/min/mg virus protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus stored at 4 °C throughout</td>
<td>1125</td>
</tr>
<tr>
<td>Virus incubated for 48 h at 37 °C</td>
<td>6900</td>
</tr>
<tr>
<td>Subvirus particle isolated from virus stored at 4 °C</td>
<td>1462</td>
</tr>
<tr>
<td>Subvirus particle isolated from virus incubated at 37 °C</td>
<td>17850</td>
</tr>
</tbody>
</table>

Triton N101 was added to the assay when virus was used as a source of enzyme, but not when the subvirus particle was used.

amount of infectivity during incubation at 37 °C, but only two strains, HERTS 33 and H, became capable of inducing interferon (Table 4). The only strain which induced high titres of interferon (strain H) was also the one that at low m.o.i. produced low titres of interferon without any treatment (Lomniczi & Burke, 1971). When RNA polymerase activity of these preparations was assayed it was found that, in all strains, activity was increased by incubation at 37 °C after prior incubation at 4 °C (Table 3). In some instances (i.e. the H and F strains) polymerase activity was higher than that of virus assayed immedi-
Structural basis of NDV polymerase

ately after purification. In general, the longer the virus was left at 4 °C before incubating at 37 °C for 48 h, the smaller the 're-activation' of RNA polymerase (Table 3). The change in activity was not due to any loss of virus structural proteins, since incubation of the virus at 37 °C for 48 h did not alter the protein polyacrylamide gel electrophoretic pattern of the virus. The polymerase activity of the subvirus particle isolated from virus preparations which had been activated by incubation at 37 °C was higher than that of the subvirus particle incubated throughout at 4 °C (Table 5). Since Triton N101 treatment removes most of the surface components, this suggests that the increase in polymerase activity on incubation at 37 °C is due to an effect on the internal components of the virus.

DISCUSSION

The action of Triton N101 on NDV was rapid and limited. Examination of electron micrographs and the sedimentation coefficients showed that the subvirus particles produced by the action of Triton N101 were heterogeneous in their morphology. They ranged from completely stripped particles to apparently intact virus particles. It was also shown that no more protein or [32P] label (phospholipid) could be removed from virus particles after 30 min incubation at 32 °C.

Protein polyacrylamide gel electrophoresis showed that all NDV glycoproteins were substantially removed by Triton N101. Only one of these glycoproteins (VP3) was a major component of untreated virus, and this has been shown to be associated with virus haemagglutinating activity (Evans & Kingsbury, 1969; Haslam et al. 1969; Inuma et al. 1971). The other two glycoproteins, the smaller of which has not been described before, can only be present in minor amount, for only small differences were observed in the peak areas on stained gels of either VP4 (15 %) or VP7 (1.5 %), their positions of electrophoretic mobility, after removal of the glycoproteins by detergent treatment. Therefore, the action of Triton N101 in activating NDV RNA polymerase activity probably consisted of removal of the virus envelope, comprising the protein surface projections (spikes) and a phospholipid layer. This yielded subvirus particles that were permeable to the RNA polymerase substrates and co-factors, thus revealing enzyme activity. In agreement with this interpretation, it was found that the isolated subvirus particles produced by Triton N101 treatment could be assayed for polymerase activity without further addition of Triton N101. Since polymerase activity was shown by particles which contained no surface proteins, it follows that the virus envelope plays no part in determining RNA polymerase activity.

Like many other enzymes, NDV RNA-dependent RNA polymerase activity was unstable. Previous work (Sheaff et al. 1972) showed that heating NDV at 56 °C caused loss of polymerase activity at the same rate as loss of infectivity. In this paper we found that both RNA polymerase activity and infectivity were also unstable at 4 °C. However, on incubation at 37 °C, infectivity fell but polymerase activity rose. The rate of decay of RNA polymerase activity at 4 °C was approximately the same in all NDV strains tested, but 're-activation' of polymerase activity by incubating virus at 37 °C for 48 h was not. Two NDV strains, 11 and 1, after incubation at 4 °C and then at 37 °C, were shown to have polymerase activities greater than those assayed immediately after purification. This information, coupled with the variation in initial polymerase activities found in different NDV strains, suggest the presence of control factors for NDV RNA polymerase. These control factors might determine the characteristic polymerase level of each NDV strain, and be affected by incubation at 37 °C so that an elevated polymerase activity results. The rise in polymerase activity accompanied by a fall in infectivity suggests that the product of the RNA poly-
merase, after incubation at 37 °C, might be different from normal. This is now under investigation. No simple explanation can yet be given why two of five NDV strains tested became interferon inducers after incubation at 37 °C.

Since the subvirus particles produced by Triton N101 treatment had polymerase activity, they clearly contained the RNA polymerase enzyme complex. However, the proteins which make up the RNA polymerase enzyme remain unidentified. NDV nucleocapsids apparently did not have RNA polymerase activity. This could be due to loss of enzyme or enzyme co-factors during purification, denaturation of the enzyme by detergent or incomplete removal of deoxycholate, or aggregation of nucleocapsids to form impermeable clumps.

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REFERENCES


Structural basis of NDV polymerase


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