Characterization of the Structural Proteins of Different Strains of Newcastle Disease Virus

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SUMMARY

The structural proteins of 14 strains of Newcastle disease virus (NDV) were examined on reduced polyacrylamide gels. Three major and seven minor virus proteins (VP) were found reproducibly, one of the major and one of the minor polypeptides being glycoproteins (VGP). The three major polypeptides had mol. wt. of 75,000 (VGP75), 55,000 (VP55) and 42,000 (VP42) and the minor polypeptides 180,000 (VP180), 110,000 (VP110), 55,000 (VGP55), 53,000 (VP53), 52,000 (VP52), 51,000 (VP51) and 49,000 (VP49). On polyacrylamide gel electrophoresis in a non-reduced system one of the minor components, VGP55, migrated to an apparently higher mol. wt. position (between VGP75 and VP55) with all strains of virus examined. Under the same conditions, it was found that VGP75 from some strains was either absent or present in greatly diminished amounts, and a new high mol. wt. glycoprotein appeared. By extraction of this high mol. wt. protein from non-reduced polyacrylamide gels, and electrophoresis of the reduced protein under reduced conditions the major component was found to be VGP75, but VP55 was also present. Amino acid analysis of the three major proteins from three strains of virus showed clear differences between the proteins of the different strains.

INTRODUCTION

A number of investigations with NDV have been concentrated on finding a property of the virus readily measured in the laboratory which is related to the virulence of the various strains. However, little work has been presented on the comparison of the structural polypeptides of strains of differing virulence. In a comparison of four strains of NDV by polyacrylamide gel electrophoresis Shapiro & Bratt (1971) found significant differences in the minor components. However, Alexander & Reeve (1972) were unable to show any significant differences between the polypeptides of any of the eight strains which they examined by polyacrylamide gel electrophoresis. The purpose of the work described in this paper was to examine the polypeptides of a number of strains of NDV by polyacrylamide gel electrophoresis, to isolate the major polypeptides in a pure form and to compare these by amino acid analysis. A number of strains with a broad spectrum of virulence were used, and it was hoped to relate any differences found to virulence.

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**METHODS**

**Materials.** These have been previously described (Meager & Burke, 1973; Morser, Kennedy & Burke, 1973).

**Virus strains.** (V, virulent; M, mesovirulent; A, avirulent.) La Sota (A) was supplied by the National Institute of Vaccine Control, Budapest, Hungary. F (A), Texas G.B. (V) and Herts 33 (V) were supplied by Dr T. H. Pennington, St Thomas's Hospital Medical School, London. Lamb Essex (V), Beaudette C (M), Field Pheasant (V), Queensland (A) and Warwick (V) were obtained from Dr P. Reeve, then of the Department of Virology, Postgraduate Medical School, DuCane Road, London. Strain Ulster 2C (A) was obtained from Dr J. B. McC Ferran of the Ministry of Agriculture, Stormont, Belfast, Northern Ireland. Essex 72 (V) (Northampton strain) was supplied by Dr W. H. Allan of the Ministry of Agriculture, Fisheries and Food, New Haw, Surrey.

**Virus growth.** All strains of virus were grown in ovo. Nine-day-old fertile eggs were inoculated with 0.1 ml of allantoic fluid (diluted with Medium 199 containing 2 % (v/v) calf serum) containing $4 \times 10^3$ to $2 \times 10^4$ p.f.u. for the plaque-forming strains (virulent and mesovirulent) and $3.2 \times 10^5$ to $4.1 \times 10^5$ EID$_{50}$ for the non-plaque-forming strains (avirulent). The eggs were incubated for 40 or 60 h, respectively before harvesting the allantoic fluid.

**The measurement of virulence.** The virulence of the 14 strains of NDV was assayed by mean death time (MDT), intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI), as described by Waterson, Pennington & Allan (1967) and by Dr W. H. Allan, Central Veterinary Laboratory, Weybridge, Surrey. The latter method was used only for differentiating strains of moderate virulence.

**Virus purification.** Two types of gradient were used, both based on a mixture of glycerol, sucrose and tartrate (GST). Equal vol. of 60 % (w/v) sucrose in 0.1 M-tris-HCl, pH 7.3, 45 % (w/w) sodium potassium tartrate in the same buffer and glycerol were mixed to give the starting mixture. (a) The linear equilibrium gradient was made from a heavy component consisting of the above mixture and a light component consisting of the above mixture diluted with an equal vol. of 0.1 M-tris-HCl, pH 7.3. (b) The linear velocity gradient was made from a heavy component consisting of the above mixture diluted with twice its vol. of 0.1 M-tris-HCl, pH 7.3 and a light component diluted with seven times its volume of 0.1 M-tris-HCl, pH 7.3.

Virus was pelleted from clarified allantoic fluid by sedimentation at 21000 rev/min (46000 g$_{av}$) for 2.5 h at 4 °C in a 6 × 250 ml angle rotor. Resuspended virus was centrifuged to equilibrium on 58 ml equilibrium GST gradient (~ 10 mg of protein/gradient) for 12 to 16 h at 4 °C at 24000 rev/min (70000 g$_{av}$) in a 3 × 65 ml swing-out rotor. The collected virus bands were diluted with ice-cold 0.1 M-tris-HCl, pH 7.3 and pelleted at 24000 rev/min (70000 g$_{av}$) for 1 h in a 3 × 65 ml swing-out rotor. The resuspended virus was centrifuged on 60 ml GST velocity gradients at 24000 rev/min (70000 g$_{av}$) for 20 min at 4 °C in a 3 × 65 ml swing-out rotor, and the virus band was pelleted as above.

**Dansylation of virus proteins.** The procedure followed was a simplified version of that described by Talbot & Yphantis (1971). Ten % (w/v) SDS was added to purified virus in phosphate-buffered saline (PBS) (6 to 10 mg/ml) to give a final concentration of 3.3 % (w/v) SDS, and 50 µl of 5 % (w/v) dansyl chloride in acetone was added per ml. The mixture was immediately vigorously agitated and then boiled for 3 min at 100 °C. This material was used for polyacrylamide gel electrophoresis, or 10 % (v/v) 2-mercaptoethanol was added to give a final concentration of 1 % (v/v), and boiled for a further 90 s. The reduced or non-
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reduced material was then mixed with 1/5 vol. of 50 % sucrose and electrophoresed on the appropriate polyacrylamide gel system.

Polyacrylamide gel electrophoresis. The gel system used for much of the analytical and all the preparative electrophoresis contained 10 % (w/v) acrylamide, 0.27 % (w/v) N,N’-methylenebisacrylamide, 0.1 % (w/v) SDS, 0.5 M-urea, 3.37 M-tris-HCl (pH 8.9), 0.05 % (v/v) TEMED and 0.06 % (w/v) ammonium persulphate. The electrophoresis buffer consisted of 0.05 M-tris + 0.38 M-glycine + 0.1 % (w/v) SDS.

For the reduced polyacrylamide gel system samples were disrupted in the presence of 1 % (w/v) SDS + 1 % (v/v) 2-mercaptoethanol, and the electrophoresis buffer contained 0.02 % (w/v) reduced glutathionine. 2-mercaptoethanol and reduced glutathionine were excluded from the non-reduced polyacrylamide gel system.

Analytical polyacrylamide gels. Gels were cast to a height of 80 mm in 8 mm internal diam. glass tubes, and were pre-electrophoresed at 3 mA/gel (constant current) for 2 h before sample additions. Electrophoresis of virus proteins was performed initially at 1 mA/gel for 30 min and then at 3 mA/gel for 4.5 h unless otherwise stated.

Preparative polyacrylamide gel electrophoresis. Eight parallel 60 ml columns of 10 % (w/v) polyacrylamide were cast in 220 mm long cylindrical perspex running tubes, of internal diam. 18 mm, held equidistant from the centre of the top buffer tank. The lower cylindrical tank fitted directly to the top buffer tank to give a virtually continuous buffer system. The gels were pre-electrophoresed at 10 mA/gel for 12 h. Samples were initially electrophoresed at 2.5 mA/gel for 4 h and then electrophoresis was continued at 5 mA/gel or 10 mA/gel until adequate separation of the main virus structural components occurred. Four milligrams of protein was electrophoresed on each polyacrylamide gel, and either one or two of the gels contained dansylated virus proteins. To isolate individual virus proteins the areas parallel to dansylated proteins on the preparative polyacrylamide gels were excised.

Preparation of protein for amino acid analysis. The excised regions of polyacrylamide gel containing single virus proteins were forced through a narrow orifice (i.e. the end of a 50 ml disposable syringe) with approx. a half vol. of extraction buffer (0.1 M-tris-HCl, pH 7.5 + 0.1 % (w/v) SDS). A further half vol. of extraction buffer was added and this mixture was vigorously stirred for 10 h at 37 °C. The acrylamide was centrifuged out of solution at 4000 rev/min in a 16 x 50 ml swing-out rotor for 10 min. The acrylamide was extracted with a further 2 x 1/2 vol. of extraction buffer for 2 h, the three supernatant fluids were pooled, and centrifuged at 4000 rev/min (~ 3500 g_w) for 10 min in a 4 x 50 ml swing-out rotor. The supernatant fluid was removed and the latter procedure was repeated before making the solution 80 to 90 % (v/v) with acetone. The precipitated protein was removed by sedimentation, and the pellet was washed twice with one-fifth vol. of 90 % (v/v) acetone. The pellet was finally suspended in the minimum vol. of distilled water and lyophilized over P_2O_5.

Amino acid analysis. Two milligram samples of dried protein were oxidized with 0.5 ml of fresh performic acid for 30 min at room temperature, and 1.5 ml of distilled H_2O was added to each sample, which was then lyophilized. The dried protein was thoroughly suspended in 2 ml of distilled H_2O and redried before hydrolysing in 3 ml of 6 M-HCl at 106 °C under vacuum. Samples of purified protein were hydrolysed in triplicate for 16, 34 and 60 h. Amino acid analyses were performed in a Biocal BC100 analyser.

Staining of proteins on polyacrylamide gels

Coomassie blue staining. The method used was essentially that described by Maizel, Summers & Scharff (1970) and gels were scanned at 570 nm using a Chromoscan densitometer (Joyce, Loeb & Co. Ltd., Gateshead, Durham).
Fig. 1. Polyacrylamide gel electrophoresis of the polypeptides of NDV strain Texas. Electrophoresis in 10% (w/v) SDS-gels was for 4.5 h at 3 mA/gel and the protein content of the applied samples was (A) 70 μg, (B) 140 μg, (C) 210 μg and (D) 280 μg. Virus proteins (VP) and virus glycoproteins (VGP) are shown with their mol. wt. as determined in this system.

Schiff’s periodate staining of carbohydrate moieties. The method used was as described by Kobylka et al. (1972) and gels were scanned at 550 nm in a Gilford 200 densitometer.

Estimation of mol. wt. of virus proteins. The reference proteins used were β-galactosidase (mol. wt. 130000), phosphorylase A (mol. wt. 95000), transferrin (mol. wt. 79000), bovine serum albumin (mol. wt. 68000), ovalbumin (mol. wt. 45000) and carbonic anhydrase (mol. wt. 34000). Mol. wt. of the virus proteins were calculated on a parallel 10% (v/v) polyacrylamide gel by the method described by Shapiro, Vinuela & Maizel (1967).

RESULTS

Characterization of the structural polypeptides of NDV Texas on polyacrylamide gels

Ten virus polypeptides were reproducibly found using the reduced electrophoresis system (Fig. 1). Three major polypeptides (VGP75, VP55 and VP42) and seven minor polypeptides
Table 1. *The structural polypeptides of NDV* (strain Texas)

<table>
<thead>
<tr>
<th>Virus polypeptide*</th>
<th>Total protein (%)</th>
</tr>
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<tbody>
<tr>
<td>VP180</td>
<td>2.3</td>
</tr>
<tr>
<td>VGP75</td>
<td>25.5</td>
</tr>
<tr>
<td>VP55 (+ VGP55)</td>
<td>31.2</td>
</tr>
<tr>
<td>VP53 + VP52</td>
<td>7.1</td>
</tr>
<tr>
<td>VP49</td>
<td>3.6</td>
</tr>
<tr>
<td>VP42</td>
<td>28.3</td>
</tr>
</tbody>
</table>

* The polypeptides were separated by gel electrophoresis, detected by Coomassie blue staining, and scanned on a Joyce–Loebl chromoscan using the integrator attachment.

Fig. 2. Polyacrylamide gel electrophoresis of NDV strain Texas under (A) reducing and (B) non-reducing conditions. Gels A1, A3, B2 and B4 contained 70 µg of virus protein and gels A2, A4, B1 and B4 140 µg of virus protein. Electrophoresis at 3 mA/gel was for 2 h with gels A1, A2, B3 and B4 and 4 h with gels A3, A4, B1 and B2. a, VP180; b, VP49; c and d, new protein bands on non-reduced electrophoresis system.
(VP180, VP110, VGP55, VP53, VP52, VP51 and VP49) were reproducibly found. VP53 and VP52 could only readily be distinguished by the naked eye. Carbohydrate staining of the virus polypeptides showed that VGP75 is a glycoprotein, and a peak of staining was also found associated with VP55, which is the major structural polypeptide of the nucleocapsid (Mountcastle et al. 1970; Meager & Burke, 1973). The isolated nucleocapsid does not contain carbohydrate (Mountcastle et al., 1970), and hence VGP55 was identified as a glycoprotein co-migrating with the nucleocapsid protein (VP55) on reduced polyacrylamide electrophoresis. VGP75 is the virus protein responsible for haemagglutinin and neuraminidase activities (Scheid & Choppin, 1973). The latter workers also suggested that the lowest mol. wt. structural polypeptide (VP42) had a matrix function within the lipid membrane. With low loadings of virus protein on the polyacrylamide gel (A) VP53 and VP52 were seen as two species, while with higher loadings of virus (D) VP110 and VP51 were more readily seen. As seen in Table 1, VGP75, VP55 (+VGP55) and VP42 comprised 25.5%, 31.2% and 28.3% of the total virus protein.

Reaction of disrupted and reduced polypeptides of NDV strain Texas with excess iodoacetamide produced no alterations in the pattern of polypeptides on polyacrylamide gel electrophoresis. It was concluded that any disulphide bridges present were broken by reduction, and that none were able to reform.

To determine whether any of the virus polypeptides were held together by disulphide bridges in their natural state, the solubilized virus was fractionated by polyacrylamide gel electrophoresis under non-reduced conditions, i.e. with no reductant present in the virus disruption mixture or in the electrophoresis buffer. Fig. 2 shows the results obtained under reduced (Fig. 2A) or non-reduced (Fig. 2B) conditions. Under non-reduced conditions the amounts of VP180 (a) and VP49 (b) were considerably decreased. Two polypeptides not apparent in the reduced system were present on the non-reduced gels. The first was a faint band of staining material between VGP75 and VP55 (c), and the second was a diffuse band below VG42 (d). The latter polypeptide is more apparent as a discrete band with shorter electrophoresis times (gels B3 and B4). Comparison of the carbohydrate staining pattern of the glycoproteins of NDV strain Texas on polyacrylamide gels under reduced and non-
Fig. 4. Pictorial representation of the virulence rating of the freshly characterized strains of NDV used in this study. The strains shown are: avirulent F (a), B1 (b), Queensland (c) and La Sota (d); mesovirulent L (e), H (f) and Beaudette C (g); virulent Warwick (h), Herts (i), and Field Pheasant (j), Lamb Essex (k) and Texas (l). Ulster is not shown as it caused no deaths in embryonated eggs. Essex 72 (not shown) was the most recent isolate and is known to be virulent.

reduced conditions (Fig. 3) showed that the appearance of the minor component between VGP75 and VP55 was associated with the disappearance of VGP55 from its usual position, and that the carbohydrate staining was coincident with this new polypeptide.

Comparison of the polypeptides of 14 strains of NDV

The 14 strains of virus (including strain Texas) were chosen for this study on the basis of their broad spectrum of virulence. Fig. 4 shows the virulence range of the strains, assessed on the basis of ICPI and MDT. The avirulent strains form a discrete group, but the mesovirulent and virulent strains are more closely related.

Samples (200 μg of protein) of each of the 14 strains of virus were disrupted and electrophoresed under reduced conditions. To investigate the role of disulphide bands the virus polypeptides were also fractionated by polyacrylamide gel electrophoresis under non-reduced conditions. From Fig. 5 and 6 it is evident that the polypeptides from the 14 strains are not significantly different when extracted and electrophoresed under reduced conditions, small differences apparent in the virus particle components present between VP180 and VGP75 being attributed to the photography rather than real differences between the strains.
Fig. 5, 6. Polyacrylamide gel electrophoresis of 200 μg of protein extracted from each of a variety of strains of NDV under non-reduced and reduced conditions. Electrophoresis on 10% (w/v) gels was for ~4.5 h at 3 mA/gel. Strains shown are 1, Beaudette C; 2, Ulster; 3, Warwick; 4, F; 5, B1; 6, Herts; 7, Texas; 8, L; 9, La Sota; 10, Queensland; 11, Field Pheasant; 12, H; 13, Lamb Essex; 14, Essex.

However, under non-reduced conditions a number of significant differences were apparent both between reduced and non-reduced electrophoresis of the virus polypeptides and also between individual strains. With all the strains electrophoresed under non-reduced conditions, the changes described with NDV strain Texas were apparent. A minor species appeared between VGP75 and VP55 and the sharp band of high mol. wt. (VP180) on reduced gels was markedly diminished, and stained material was present at the origin. VP42 spread, either to give a new protein band or to form a broad diffuse band on the leading edge of this protein, although there was some variability between strains in the spread of VP42. Some decrease in the amounts of the virus particle component VP49 was also apparent. The only major difference between the strains was that VGP75 was considerably decreased with some strains on non-reduced gel electrophoresis. This was associated with the appearance of a high mol. wt. polypeptide. These strains were Ulster, Warwick, Herts, Queensland, Field Pheasant, H, Lamb Essex and Essex, a list including virulent, mesovirulent and avirulent strains. No differences in the mol. wt. of any of the virus polypeptides could be distinguished under reduced or non-reduced conditions.

The proteins of a further seven strains (Herts, L, B1, Ulster, Beaudette C, Warwick and F) were stained for carbohydrate after polyacrylamide gel electrophoresis. With these seven strains VGP75 and the band present between VGP75 and VP55 under non-reduced conditions both stained for carbohydrate. However, with strains Herts, Ulster and Warwick carbohydrate staining associated with VGP75 was considerably reduced under non-reduced
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Conditions, and was associated with the new high mol. wt. polypeptide. This is shown for strain Ulster in Fig. 7.

In addition, carbohydrate was associated with VP180 of Ulster, Beaudette C, Warwick and F when these strains were electrophoresed under reduced conditions. The fact that this protein was not associated with carbohydrate in all of the strains examined suggested that it may be a mixture of glycosylated and non-glycosylated polypeptides. When virus was dis-
Fig. 7. Densitometer traces of the structural polypeptides of NDV strain Ulster electrophoresed under reduced (R) and non-reduced (N) conditions. Electrophoresis was for 4.5 h at 3 mA/gel and each gel contained 200 μg of protein. —, Coomassie blue stain, ——, periodic acid-Schiff's stain.

Fig. 8. Electrophoresis of NDV polypeptides, which have been reacted with dansyl chloride under reduced and non-reduced conditions. Electrophoresis of 150 μg of dansylated protein was at 1 mA/gel for 0.5 h and the 4 mA/gel for 4 h. Gel 1, non-reduced strain Texas; gel 2, non-reduced strain Ulster; gel 3, reduced strain Texas. The glycoprotein between VGP75 and VP55 (B) is strongly dansylated. With non-reduced NDV strain Ulster the high mol. wt. glycoprotein is apparent at A.
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rupted under more severe conditions and electrophoresed on a high urea polyacrylamide gel system VP180 was considerably diminished. However, VP180 is resistant to boiling in SDS or SDS plus reductant, and many other published profiles of the proteins of the virus show a peak in this region. It is possible that VP180 is an artefact.

A further comparison of the polypeptides of the different strains was performed by mixing 50 μg samples of them in various combinations, and electrophoresis under different conditions. All proteins co-migrated and no new bands appeared.

Extraction of virus polypeptides from polyacrylamide gels

To investigate the relationship between the virus polypeptides separated by reduced and non-reduced electrophoresis, and also to prepare pure virus polypeptides, the preparative electrophoresis system described in the Methods section was developed. By dansylation of the virus polypeptides it was possible to localize their position on polyacrylamide gels. Fig. 8 shows the dansylated protein of strains Ulster and Texas electrophoresed under non-reduced conditions (1 and 2). Only strain Texas is shown under reduced conditions (3) since all the strains were identical under these conditions. The glycoprotein between VGP75 and VP55 in non-reduced gels was found to dansylate very strongly with all strains of virus examined. Satisfactory resolution was obtained when the dansylation and electrophoresis procedure was scaled up to the preparative level, and by extraction of areas of acrylamide in parallel gels it was possible to obtain pure virus polypeptides. The dansylated protein containing gel(s) were lined up with the unreacted protein gels and the following regions were excised: a broad slice around VP42, a more narrow slice within the leading and tailing edge
Table 2. Amino acid composition of the major structural polypeptides of three strains of NDV (Ulster, Texas and Beaudette C)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Ulster VP42</th>
<th>Texas VP42</th>
<th>Beaudette C VP42</th>
<th>Ulster VP55</th>
<th>Texas VP55</th>
<th>Beaudette C VP55</th>
<th>Ulster VGP75</th>
<th>Texas VGP75</th>
<th>Beaudette C VGP75</th>
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<tr>
<td>Cysteic acid</td>
<td>2.03</td>
<td>1.58</td>
<td>1.73</td>
<td>1.61</td>
<td>1.43</td>
<td>0.84</td>
<td>2.84</td>
<td>3.21</td>
<td>3.44</td>
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<tr>
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<td>7.57</td>
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<td>6.9</td>
<td>8.85</td>
<td>11.31</td>
<td>9.90</td>
</tr>
<tr>
<td>Glycine</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>Valine</td>
<td>9.02</td>
<td>8.16</td>
<td>1.88</td>
<td>5.19</td>
<td>6.32</td>
<td>2.35</td>
<td>7.27</td>
<td>5.80</td>
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<tr>
<td>Isoleucine</td>
<td>4.75</td>
<td>4.37</td>
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<td>3.75</td>
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<td>2.15</td>
<td>4.86</td>
<td>5.59</td>
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<td>Leucine</td>
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<td>8.46</td>
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<td>9.34</td>
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<td>ND</td>
<td>ND</td>
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<tr>
<td>Phenylalanine</td>
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<td>2.84</td>
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<td>3.58</td>
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<tr>
<td>Lysine</td>
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<td>1.00</td>
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<td>1.31</td>
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<td>8.28</td>
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<td>6.18</td>
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<td>5.07</td>
<td>7.39</td>
<td>6.41</td>
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<td>Arginine</td>
<td>4.87</td>
<td>5.17</td>
<td>5.75</td>
<td>5.18</td>
<td>6.59</td>
<td>5.88</td>
<td>4.51</td>
<td>5.35</td>
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<tr>
<td>Methionine†</td>
<td>3.66</td>
<td>3.04</td>
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<td>5.59</td>
<td>7.91</td>
<td>4.33</td>
<td>3.41</td>
<td>3.99</td>
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<td>ND</td>
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</table>

Values are expressed as moles of amino acid per 100 mol recovered. The figures are means of triplicate determinations and corrections were made for the loss of labile amino acids (e.g. serine and threonine) during hydrolysis.

* ND, Not done.
† Estimated as the sulphone.

of VP55 and a broad slice including the tailing edge of VGP75 but excluding its leading edge. When samples of the extracted proteins were electrophoresed on reduced gels they were normally found to be uncontaminated. When the isolated major polypeptides (VGP75, VP55 and VP42) from a number of strains were stained for carbohydrate only VGP75 was found to be a glycoprotein. No carbohydrate was associated with isolated VP55 when electrophoresed under either reduced or non-reduced conditions. This provides further evidence that VP55 is no longer associated with VGP55 in the non-reduced system. When the protein between VGP75 and VP55 was extracted from non-reduced preparative gel and reduced, it migrated to a position coincident with that of VP55. This was also demonstrated by electrophoresis of mixed samples of purified VGP75 and the protein between VGP75 and VP55.

The high mol. wt. glycoprotein obtained with strains Ulster, Herts, Warwick, etc., under non-reduced conditions was extracted from both the marker gel containing dansylated proteins and the parallel unlabelled gels. Polyacrylamide gel electrophoresis of the extracted protein showed that in all cases it was contaminated with other virus proteins. In Fig. 9 the extracted high mol. wt. glycoprotein from a non-reduced preparative gel containing the dansylated protein of Ulster is shown. Under non-reduced conditions (1) with high loadings of protein, the complexed glycoprotein is apparent and also a small amount of VGP75. Under reduced conditions VGP75 is the predominant species, but VP55 is also present along with traces of other virus proteins (VP51 + 52, VP49 and VP42). No carbohydrate was found associated with VP55, thus excluding the possibility that this protein was VGP55. It was concluded that the high mol. wt. glycoprotein consisted of VGP75 together with VP55.
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Fig. 10. Electrophoresis of a sample of the material extracted from the top of a non-reduced preparative gel containing the dansylated protein of NDV strain Ulster (1) in parallel with 150 μg of NDV strain Ulster (2). The gel system was reduced, and electrophoresis was for 0.5 h at 1 mA/gel and 4 h at 3 mA/gel.

Amino acid analysis of virus polypeptides

Pure virus polypeptides, extracted from preparative polypeptide gels, were oxidized with performic acid, hydrolysed and the amino acid composition determined as described in the Methods section. The amino acid composition of VGP75, VP55 and VP42 of NDV strains Ulster, Texas and Beaudette C is shown in Table 2. Except for VGP75 of strain Ulster which was isolated from reduced preparative gels, all the proteins were extracted from non-reduced gels. Tyrosine was destroyed during the performic acid oxidation and tryptophan was lost during hydrolysis, so values for those amino acids are not shown. Methionine was converted to the sulphone derivative and appeared on the analysis charts between aspartic acid and threonine with good separation from both (Hirs, 1967). Glycine was not determined as small quantities of this amino acid, originating from the electrophoresis buffer, were present in the extracted polypeptides.

As seen from Table 2, the valine content of all three polypeptides of Beaudette C was...
remarkably low, and in general there were fewer differences between strains Texas and Ulster than between Ulster and Beaudette C or Texas and Beaudette C. The total acidic amino acids (aspartic and glutamic) were high in all three proteins. The major structural protein of the nucleocapsid (VP55) contained a relatively small proportion of the basic amino acids (lysine + histidine + arginine = 12.4 to 13.8), whereas the figure for the nucleocapsid from the group A togavirus, Semliki Forest virus, was 20.4 (Kennedy & Burke, 1972). However, the total for three strains of influenza virus nucleoprotein was only 14.8 to 15.3 (Laver & Baker, 1972). These authors found that the virus nucleoprotein was rich in acidic amino acids (aspartic and glutamic acid = 21.9 to 23.9). This is comparable to the values from Table 2 of 20.3 to 24.7.

**DISCUSSION**

Polyacrylamide gel electrophoresis of 14 strains of NDV under reduced conditions failed to reveal any differences between the strains. However, by electrophoresis under non-reduced conditions the strains could be divided into two groups, i.e. those which exhibited a high mol. wt. glycoprotein and little or no VGP75 and those which had a normal VGP75. As the two groups of virus both possess members of differing virulence this phenomenon is not virulence related. Extraction of this protein and electrophoresis under reduced conditions showed that it was a complex containing several virus proteins. Its origin is unknown, but it clearly shows that differences exist between the polypeptides of the different strains.

The spreading of VP42 on electrophoresis under non-reduced conditions is attributed to the presence of internal disulphide bridges. On reduction such bridges are broken and the molecule would take up a more open conformation, thus giving it an apparently higher mol. wt. on non-reduced gels. VP180, which is probably a complex, was found to be retained at the top of the gel of non-reduced polyacrylamide gels (Fig. 10).

The amino acid analyses on VGP75, VP55 and VP42 of NDV strains Ulster, Texas and Beaudette C clearly showed differences between the three strains and between the individual polypeptides of each strain. The valine content of all three polypeptides of Beaudette C was remarkably low. There was no significant difference in the cysteic acid contents of VGP75 between those strains in which the high mol. wt. protein was formed (Warwick and Ulster) and those where it was not (Texas). The low content of basic amino acids in the major structural polypeptide of the nucleocapsid was unexpected. However, a low content of basic amino acid in a protein does not necessarily mean that it is not associated with nucleic acid, as any basic amino acid involved in interaction with nucleic acid would be localized into the area which is exposed to the nucleic acid.

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**REFERENCES**


Proteins of NDV


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