Proteins of Four Biologically Distinct Strains of Newcastle Disease Virus[1] (35375)

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Our finding that cloned Newcastle disease virus (NDV) strains differ extensively in a number of virion properties which involve interaction with cell membranes—the ability to induce fusion (1, 2) and hemolysis, and elution potential—as well as in virulence and in the patterns of virus specific RNA which they induce (3), led us to wonder whether these differences might be correlated with differences in the proteins contained in their virions. It has recently been shown for another enveloped virus, herpes simplex virus, that strains which differ in the membrane alterations which they induce, differ in virion proteins and in the virus specific proteins induced during infection (4).

Recently three laboratories have shown that the vast majority of proteins contained in the NDV irion is virus specific rather than determined by the host cell (5–7). In these investigations, analysis of polyacrylamide gel electropherograms of radioactively labeled proteins has revealed that virions of two NDV strains contain three major protein peaks. In addition to these major peaks, as many as five minor protein bands can be observed when the gels are stained with Coomassie brilliant blue (6,7). The molecular weight estimates for the major proteins of the "Beaudette" strain were quite similar in two independent studies (5, 7), while those obtained for the proteins of the avirulent V-8 VRI/66 strain were somewhat lower (6). Such differences might be due to slight differences in technique, or more interestingly, might reflect genuine differences in the virus strains used.

We wish to report strain specific differences in the electrophoretic patterns of proteins of four strains of ND. These differences probably reflect variations in proportions of both minor and major proteins, rather than significant differences in molecular weights.

Materials and Methods. The four previously cloned (1, 5) NDV strains, NDV-AV, NDV-HP, NDV-L, and NDV-N, were grown in the allantoic sac of 11-day-old embryonated hen eggs and then were concentrated by centrifugation (8).

Lung cultures from 17-day-old chick embryos were prepared in 100-mm Falcon tissue culture plates (7) and incubated at 38° in a 5% CO_2 atmosphere in a medium consisting of Eagle's minimal essential medium (MEM), 2% calf serum, and 0.7 mg/ml of $NaHCO_3$. After 24 hr, or when confluent monolayers had formed, this medium was replaced by MEM containing only 10% of the usual amino acid concentrations, with the exception of glutamine which was present in the usual concentration.

Cultures were then infected at ~10–100 plaque-forming units/cell with either NDV-AV, NDV-HP, or NDV-L, or an equivalent number of egg infectious units (8) of NDV-N (which does not form plaques). Inocula were removed after 30-min incubation at 38° and 6 ml of the same medium containing 1–5

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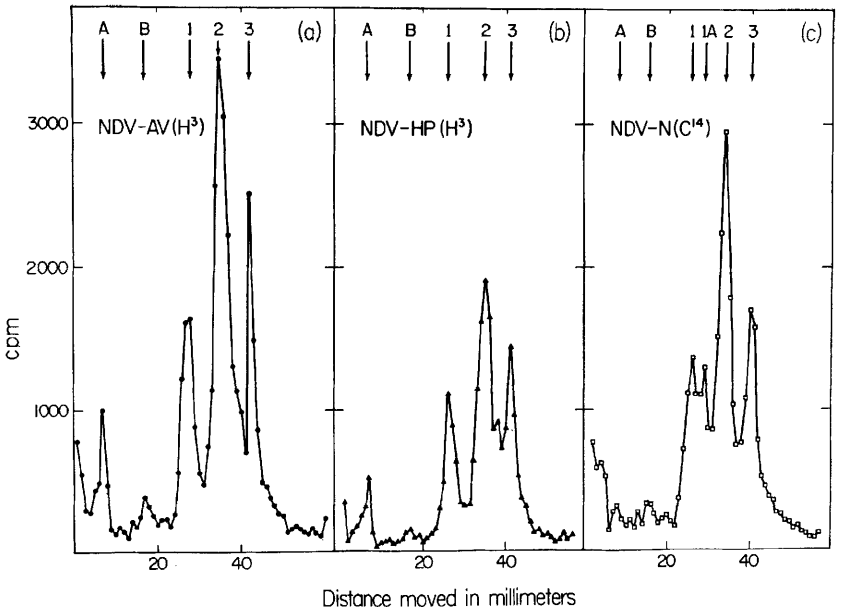

FIG. 1. Electropherograms of the proteins of three strains of NDV: Migration was from the origin at the left, toward the anode, at the right. Open and closed symbols are for ^{14}C and ^{3}H labeled proteins, respectively.

μCi/ml of either ^{3}H or ^{14}C labeled amino acid mixtures (New England Nuclear NET-250 and NEC-445) were added. Cultures were further incubated at 38° for 16–24 hr. At that time, the medium was removed and centrifuged at 6000 rpm for 10 min to remove cell debris. Virus was then purified from the supernatant by ammonium sulfate precipitation and density gradient centrifugation (7).

Virus was disrupted by heating for 2 min at 90° in a solution consisting of 0.02 *M* NaCl, 0.02 *M* Tris (pH 7.4), 0.002 *M* EDTA, 0.003 *M* dithiothreotol, 0.02 *M* sodium thioglycolate, 0.6% (w/v) sodium lauryl sulfate, and 10% (v/v) glycerol. The mixture was layered on a 6-cm (6%) polyacrylamide gel (9) and subjected to a current of 5 mAp/gel, at 5 V/cm for 30 min, followed by 8 V/cm for 4 hr. Gels were sliced (7) and the slices were subjected to hydrolysis in 0.5 ml of concentrated NH_4OH for 3 hr, after which the ammonia was allowed to evaporate and 10 ml of scintillation fluid were added (10). Samples were counted in a Packard Tricarb liquid scintillation counter, and counts were corrected for crossover and quenching.

Results. The electropherograms of the proteins of NDV-AV, NDV-HP, and NDV-N were grossly similar to patterns obtained for other strains (5–7) but significant strain differences are easily discernible (Fig. 1). The patterns for NDV-AV and NDV-HP most closely resemble previously published patterns, in that they contain three major peaks designated as 1, 2, and 3, as well as several minor peaks including a shoulder on the leading edge of peak 2. The patterns for NDV-L are similar but the shoulder on the leading edge of peak 2 was never observed (not shown). The pattern for NDV-N also does not have the shoulder on the leading edge of peak 2, but contains a fourth major peak (1A) which lies between peaks 1 and 2. These patterns were consistently found with several preparations of each virus strain.

The accuracy of this technique is such that patterns obtained with different preparations and run on different days are nearly superimposable. However, it was felt that for more precise comparison it would be valuable to co-electrophorese proteins of different strains labeled with different isotopes. As might be expected from the accuracy in comparing

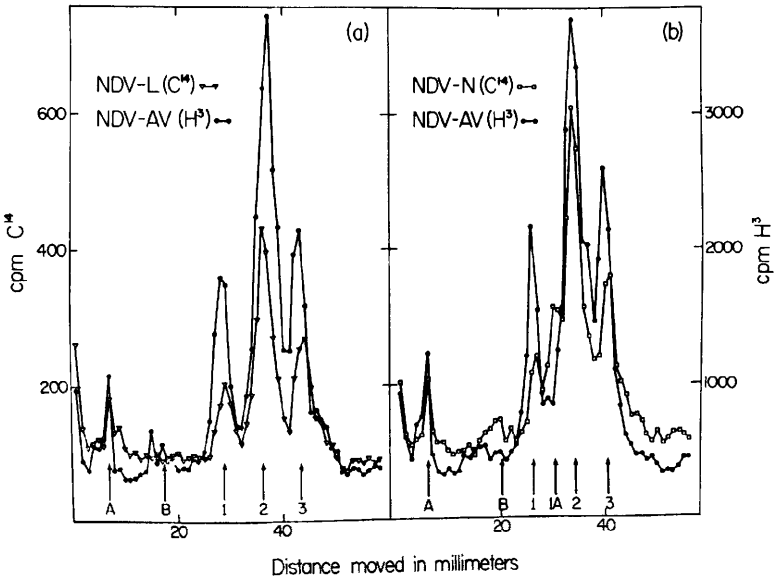

FIG. 2. Co-electrophoresis of proteins of different NDV strains. The migration direction and symbols are the same as shown in Fig. 1.

separately-run gels, co-electrophoresis of ^{14}C and ^{3}H labeled proteins from the same strains never showed more than one fraction difference in the positions of the major peaks. Coincidence of the major peaks of proteins obtained from strains NDV-AV and NDV-L can be seen (Fig. 2a) but as usual for NDV-L there is no shoulder on the leading edge of peak 2. Co-electrophoresis of the proteins of NDV-AV and NDV-N (Fig. 2b) shows the usual coincidence of the three major peaks, and in addition reveals the extra major peak for NDV-N, as well as the shoulder on the leading edge of peak 2 for NDV-AV.

We wanted to be able to compare directly the relative amounts of each protein peak associated with virions of each strain. We therefore calculated the percentage of total radioactivity associated with each peak (Table I) and then used these values and previously estimated molecular weights (6) to calculate the proportion (relative to peak 2) of polypeptides contained in the virions of each strain (Table II). The importance of this type of calculation can be clearly seen by comparing the relative values for peaks 1 and 3 in Tables I and II. Although peaks 1 and 3 usually contain similar amounts of radioactivity, the number of molecules of peak 3 protein is always 2–4× greater than the number of peak 1 molecules. In addition, it can be seen that for all strains, the amount of

TABLE I. Percentage of Radioactivity Associated with Virion Polypeptides.[a]

Electrophoretic peak	NDV-AV	NDV-L	NDV-HP	NDV-N
1	16.2	13.2	17.6	12.0
1A	—	—	—	10.2
2	39.0	32.8	43.9	29.5
3	16.8	19.0	20.3	20.2
A	6.1	7.7	4.9	4.2
B	5.1	5.2	2.8	6.1
Other[b]	17.0	21.1	13.9	14.5

[a] The values given are for the mean of three separate determinations and represent the percentage of total counts per gel associated with each peak. Since the amino acid mixtures used contain radioactivity for each amino acid proportional to the amino acid content of naturally occurring proteins, we have assumed that these values provide a reasonable measure of the amount of protein contained in each peak.

[b] Counts not associated with a particular peak. These values are relatively high because backgrounds were subtracted on the basis of the conditions used for scintillation counting rather than by designating the lowest count per gel as background.

TABLE II. Relative Proportions of Polypeptides per Virion.[a]

Electrophoretic peak	Av estimated mol wt[b]	NDV-AV	NDV-L	NDV-HP	NDV-N
1	80,000	0.18	0.28	0.28	0.28
1A	(70,000)	—	—	—	0.27
2	54,000	1.0	1.0	1.0	1.0
3	38,000	0.64	0.68	0.72	1.1
A	170,000–185,000	0.05	0.08	0.04	0.05
B	120,000–130,000	0.07	0.08	0.03	0.11

[a] These values were calculated by dividing the percentage of radioactivity associated with each peak (Table I) by the estimated molecular weight and normalizing the resultant ratios with respect to peak 2 as unity. This was done using peak 2 rather than peak 1 as previously done (6) because it has previously been shown that peak 2 contains the nucleocapsid protein. The nucleocapsid protein is a more reasonable standard since the amount of this protein is probably less likely to vary from strain to strain than the amount of envelope protein.

[b] The values given were obtained from (6) since none of the other studies gave molecular weight estimates for peaks A and B. The molecular weight of peak 1A was arbitrarily chosen as 70,000 since it is slightly closer to peak 1 than to peak 2. Similar values would have been obtained for peaks 1, 2, and 3 had the molecular weight estimates given by the other investigators been used.

protein contained in minor peaks A and B is very small when compared to the amounts contained in the major peaks (Table II). Furthermore, as shown virions of NDV-N not only contain an extra protein peak (1A), but they also have a relatively high proportion of peak 3 protein (Table II), while virions of NDV-AV have relatively low amounts of peak 1 protein.

Discussion. One of the original goals of this study was to determine whether the virions of different NDV strains differ in their protein content. It has been found that virions of four biologically distinct strains show no difference in the electrophoretic mobilities of their three major portein peaks. Instead, these virions vary in the proportions and possibly the number of the various protein classes.

Previous studies have consistently found three major peaks and as many as five minor peaks (5–7). In this study, we have found three major peaks in the electropherograms of virion proteins of NDV-AV, NDV-L, and NDV-HP, but four major peaks in those from NDV-N. In addition, a shoulder on the leading edge of peak 2 has been consistently seen in the electropherogram of NDV-AV and NDV-HP.

Three possible explanations for these differences can be considered: (i) These proteins may be unique for these strains. (ii) They may be smaller or larger than proteins with similar activities which for other strains migrate under one of the major peaks. It is obvious that since this type of electrophoresis separates proteins mainly on the basis of size (11) each peak may contain more than one class of protein. (iii) Finally, these proteins may represent increased amounts of minor peaks which are normally found to migrate in these positions.

The fact that the proportions of the three usual major protein peaks vary from strain to strain shows that variation of the third type does exist and thus might account for the NDV-N extra major peak (1A) and the shoulder on the leading edge of peak 2 of electropherograms of NDV-AV and NDV-HP proteins. Since NDV strains are by definition all antigenically related, the first two possibilities involving unique proteins or different sized proteins seem less likely, but our data do not as yet allow us to distinguish between these three possibilities.

The second goal of this study was to determine whether possible strain specific differences in virion proteins might be correlated with strain specific differences in biological properties. Other investigators have assigned

the viral nucleocapsid protein to major peak 2 (5–7) while the hemagglutinin and neuraminidase have been tentatively assigned to peaks 1 and 3, respectively (5, 6). Differences in neuraminidase activity are presumably responsible for differences in elution rates. However, NDV-N, which elutes slowly (unpublished), has the highest proportion of peak 3, the putative neuraminidase peak, and the rapidly eluting strains, NDV-AV, and NDV-L (unpublished), have the lowest proportion of this peak. Thus, it seems unlikely that simple quantitative differences in neuraminidase content account for differences in elution rates.

NDV-N is the best inducer of hemolysis among the 12 NDV strains we have tested (Clavell and Bratt, unpublished) and an excellent inducer of fusion from without (Gallaher and Bratt, unpublished). It therefore seemed possible that peak 1A might be involved in one or both of these processes. However, this possibility seems improbable because NDV-HP, which lacks peak 1A, is also a good inducer of both hemolysis and fusion from without. In addition, viral envelope components other than proteins may play an important role in these processes as evidenced by the NDV requirement for the integrity of the envelope lipid for maintainance of the ability to hemolyze and fuse from without (12). Strain specific differences in viral lipids (13) may in fact play a role in determining strain specific differences in these two properties.

Thus, we have been unable to correlate strain specific differences in biological properties with differences in protein content. However, as has recently been done in the case of herpes simplex virus (4), we have been able to demonstrate that strain specific differences in NDV proteins do exist.

Summary. Virions of NDV show strain specific variation in the proportions of proteins which they contain.

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