The 36K polypeptide synthesized in Newcastle disease virus-infected cells possesses properties predicted for the hypothesized ‘V’ protein

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Newcastle disease virus (NDV) virions possess two proteins which react strongly with monoclonal antibody 688 following separation by high resolution two-dimensional (isoelectric focusing/SDS) PAGE and detection by Western blotting. One is the phosphorylated nucleocapsid-associated 53K [P (NAP)] protein, the other comigrates with the 36K protein detected by radiolabelling NDV-infected chick embryo fibroblasts. [35S]Cysteine/[3H]leucine dual-labelling experiments show that the 36K protein is very rich in cysteine compared to the P (NAP) protein. In the Beaudette C strain it comigrates on one-dimensional SDS-polyacrylamide gels with the matrix protein (M); however, it is resolved from the slower migrating M protein from the Ulster strain of NDV. The size, strain-specific isoelectric point, high cysteine content and antigenic relatedness to the P (NAP) protein suggest that the 36K protein is the ‘V’ protein of NDV, the counterpart of which is found in other Paramyxoviridae.

Newcastle disease virus (NDV) is a member of the genus paramyxovirus within the family Paramyxoviridae. Virions contain six virus genome-encoded proteins: nucleocapsid (NP) protein, the phosphorylated nucleocapsid-associated (P or NAP) protein, matrix (M) protein, fusion (F) protein, haemagglutinin–neuraminidase (HN) and the large polymerase (L) protein (Choppin & Compans, 1975; Samson, 1988). In addition several virus-encoded non-structural proteins have been detected in NDV-infected cells, in particular the 36K protein (Chambers & Samson, 1980, 1982; Collins et al., 1982), the detailed genetic origin of which remains in doubt. The P genes of many members of the Paramyxoviridae have now been analysed and shown to be capable of encoding several proteins. Some viruses produce a C protein, which is encoded by an mRNA molecule containing two overlapping reading frames, e.g. Sendai virus (Giorgi et al., 1983; Curran et al., 1986), parainfluenza virus 3 (Luk et al., 1986; Galinski et al., 1986; Spriggs & Collins, 1986), measles virus (Bellini et al., 1985) and canine distemper virus (Barrett et al., 1985). In addition, or alternatively, some viruses produce a cysteine-rich V protein translated from a P gene-encoded mRNA. Either the V mRNA or the P mRNA contains an extra guanosine residue(s) not present in the virion RNA, thereby providing access to a second reading frame, e.g. simian virus 5 (SV5) (Thomas et al., 1988), measles virus (Cattaneo et al., 1989), mumps virus (Takeuchi et al., 1988, 1990; Paterson & Lamb, 1990; Elliot et al., 1990), Sendai virus (Vidal et al., 1990) and parainfluenza virus 2 (Southern et al., 1990), and also proposed by Paterson et al. (1989) and Cattaneo et al. (1989) for canine distemper virus, parainfluenza virus 3 and NDV. In contrast, McGinnes et al. (1988) have proposed alternative in-frame, internal initiation sites to account for P gene-encoded polypeptides in NDV.

We decided to determine whether the NDV 36K polypeptide was antigenically related to the P protein and whether it was cysteine-rich, a property common to all V proteins predicted by Paterson et al. (1989) and by Cattaneo et al. (1989).

Preliminary Western blotting experiments with purified virions from three strains of NDV, one mesovirulent (Beaudette C) and two avirulent (D26 and Ulster), showed that proteins of approximately 53K and 35K to 40K from both the Beaudette C and Ulster (but not the D26) strains reacted strongly with monoclonal antibody (Mab) 688, which had previously been shown by Russell et al. (1983) to react with the Ulster strain P (data not shown). To characterize the smaller anti-P protein MAb-reacting protein further, chick embryo fibroblasts (CEF s) were labelled with [3H]leucine and [35S]cysteine after mock infection or infection with NDV strains Beaudette C and Ulster. Fig. 1 shows that a [35S]cysteine-labelled protein (∇, lane 6) seen in NDV strain Ulster-infected CEFs migrates slightly faster on SDS-polyacryl-
Fig. 1. Fluorograph of \([^{3}H]leucine\)- (lanes 1, 2 and 3) and \([^{35}S]cysteine\)– (lanes 4, 5 and 6) labelled polypeptides synthesized in CEFs, separated on an SDS 7.5 % polyacrylamide : 0.2 % bisacrylamide gel. Lanes 1 and 4, uninfected; lanes 2 and 5, infected with NDV strain Beaudette C; lanes 3 and 6, infected with NDV strain Ulster. H\(_{N_{0}}\), uncleaved precursor to HN; M, matrix protein (u, Ulster; c, Beaudette C); \(\nabla\), cysteine-rich protein.

amide gels (Laemmli, 1970) than the M protein (uM) produced by this strain, but is not resolved from the faster migrating M protein (cM) produced by the Beaudette C strain. This protein is not seen as readily in the \([^{3}H]leucine\)-labelled NDV strain Ulster-infected sample (lane 3), suggesting that it may be a relatively cysteine-rich protein.

To determine whether the cysteine-rich protein detected in NDV-infected CEFs was a novel NDV protein and whether it comigrated with the anti-P MAb-reactive 35K to 40K protein, a high resolution two-dimensional isoelectric focusing/SDS-PAGE (IEF/SDS-PAGE) (O’Farrell, 1975) system was used as described previously (Samson et al., 1981).

CEF monolayers were infected with the Ulster strain of NDV and separately labelled with \([^{3}H]leucine\) and \([^{35}S]cysteine\) 5 h post-infection for a period of 3 h. Radioactive monolayers were then extracted into IEF sample buffer and similar amounts of \(^{3}H\) and \(^{35}S\) extracts were mixed together with purified, egg-grown (non-radioactive) NDV strain Ulster virions. The mixture was then applied to duplicate first dimension IEF gels in 2.5 mm internal diameter, 13 cm long glass tubes and samples were electrophoresed at 150 V for 18 h. Gels were then removed, shaken in equilibration buffer and electrophoresed in the second dimension (SDS–PAGE) on 12.5 % (w/v) polyacrylamide : 0.5 % diallyltartardamide gels. One slab gel was stained with Coomassie blue prior to fluorography and for subsequent scintillation spectrometry (Fig. 2a), the other slab gel was Western-
Table 1. \([^{35}S]Cysteine/[^3H]leucine values of NDV proteins\)

<table>
<thead>
<tr>
<th>NDV protein</th>
<th>Predicted number of amino acids*</th>
<th>([^{35}S]Cysteine/[^3H]leucine values</th>
<th>Measured†</th>
</tr>
</thead>
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<tr>
<td>HN80</td>
<td>Leucine 49, Cysteine 14</td>
<td>0.286†</td>
<td>0.286</td>
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<tr>
<td>F0</td>
<td>Leucine 65, Cysteine 11</td>
<td>0.169</td>
<td>0.17</td>
</tr>
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<td>P</td>
<td>Leucine 27, Cysteine 1</td>
<td>0.037</td>
<td>0.05</td>
</tr>
<tr>
<td>V</td>
<td>Leucine 9, Cysteine 7</td>
<td>0.77</td>
<td>0.56</td>
</tr>
<tr>
<td>36K</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Amino acid composition data predicted from the gene sequences.
NDV Ulster HN0 and F0 protein data taken from Millar et al. (1988); P/V protein data from I. B. Vicpd & P. T. Emmerson (personal communication).
† These values were measured by radioisotopy.
‡ The actual \([^{35}S]Cysteine/[^3H]leucine ratios were normalized to the HN0 predicted value.

blotted onto nitrocellulose, and the blot was incubated with anti-P MAb 688 and subsequently developed with anti-mouse peroxidase conjugate and 4-chloro-1-naphthol (Samson, 1986). The stained nitrocellulose blot was photographed (Fig. 2b) and Xeroxed prior to being treated with 2,5-diphenyloxazole (PPO)/toluene (2.5 g PPO dissolved in 10 ml toluene). This treatment permitted subsequent fluorography of the blotted radioactive proteins (Fig. 2c).

The Xerox copy of the stained nitrocellulose blot was aligned with the fluorogram of the blot using radioactive ink guide marks. There was perfect correspondence between fluorographed blot and stained blot for the pair of 36K protein spots and for the major radiolabelled P protein spot with the most basic (furthest on the right) P protein spot detected by antibody staining. [Migration in the first dimension was from anode (acidic) left, to cathode (basic) right.] The trail of MAb 688-reactive spots derives from the charge microheterogeneity of the mature virion P protein due to increasing levels of phosphorylation (Chambers & Samson, 1980).

Fig. 2(b and c) show that the NDV-induced protein (other than the P protein) which comigrates with the anti-P MAb 688-reactive species is the 36K protein first described by Chambers & Samson (1980).

The fluorographed duplicate gel (Fig. 1a) was then used as a source of [^3H]leucine and \([^{35}S]Cysteine dual-labelled virus proteins which were excised from the gel and counted in a scintillation counter. If the 36K protein is indeed the hypothesized NDV V protein it should have a far higher cysteine to leucine value than the P protein, from whose gene sequence it is thought to be derived (Paterson et al., 1989). If, however, the 36K protein is derived entirely from the same reading frame as the P protein, as favoured by McGinnes et al. (1988), then no great disparity between the cysteine to leucine values of the P and 36K proteins would be expected.

A cork borer was used to remove 4 mm diameter discs from regions of the dried fluorographed gel which corresponded to various NDV proteins (i.e. HN0, F0, P and 36K). These gel discs were dissolved overnight in 3% (w/v) periodic acid (Anker, 1970). The solubilized gel discs were transferred to vials which had been precounted with scintillation fluid and the amounts of [^3H]leucine and \([^{35}S]Cysteine measured after correcting for background and spillover.

In Table 1 the amounts of \([^{35}S]Cysteine and [^3H]leucine observed in each protein sample are expressed as cysteine/leucine values and are compared with the cysteine/leucine values predicted from the amino acid content deduced from the gene sequences. To make this comparison, observed and expected values were normalized to that of the HN0 protein. There was excellent agreement between the predicted and observed values for the F0 protein and reasonable agreement for the P protein (0.037 compared to 0.05). (It should be pointed out that the P protein is particularly cysteine-poor, having only one predicted cysteine residue.) The expected cysteine/leucine value for the NDV V protein (using the scheme proposed by Paterson et al., 1989) is 0.77; a value of 0.56 was found for the 36K spots. Clearly the 36K protein is very cysteine-rich compared to the P protein when measured radioisotopically; in this experiment the 36K protein was found to be over 10 times as rich in cysteine residues as the P protein (0.56/0.05) on a per leucine residue basis.

The predicted size of the NDV P (NAP) protein (42K) is approximately 11K less than that observed (53K) and a similar size difference is found between the predicted size of the NDV V protein (25K) and the 36K protein. Anomalous electrophoretic mobility is not uncommon among such phosphoproteins and has been reported for Sendai virus proteins (Giorgi et al., 1983).

We had hoped to exploit the Beaudette C strain temperature-sensitive mutant (ts172), which has a P (NAP) protein with an altered isoelectric point (Samson et al., 1981), to establish a common gene origin for both P (NAP) and 36K proteins; however, no change in isoelectric point was observed for the 36K protein (not shown). This lack of a charge-shift in the 36K protein, despite a shift in the P (NAP) protein, can be explained if the ts172 lesion affects an amino acid beyond the P/V frameshift at amino acid position 142 (see Fig. 3).

Our observations lead us to the conclusion that the 36K protein described by Chambers & Samson (1980) and by Collins et al. (1982) is the NDV V protein predicted by Paterson et al. (1989) and by Cattaneo et al. (1989).
Other studies have shown that a 36/38K protein is related to the P protein, either using tryptic peptide analysis (Collins et al., 1982) or, more recently, using immunoprecipitation with an MAb (McGiness et al., 1988). We have confirmed this relatedness using a different MAb and Western blot analysis of highly resolved proteins.

Initially it was concluded that the 36K protein was non-structural (Chambers & Samson, 1982). Now, using the highly sensitive Western blot:MAb detection system, the 36K protein has been shown to be present in purified virions. Paterson et al. (1989), also using MAbs and Western blot analysis, reported that the SV5 V protein is a structural protein. Most recently, Takeuchi et al. (1990) detected the V protein in mumps virions using antisemum against a synthetic peptide. Simpson et al. (1984) had shown earlier that the mumps virus NS1 (now V) protein was particularly cysteine-rich.

Our estimates of the relative amounts of cysteine to leucine for the 36K protein compared to that for the P protein are inconsistent with the proposal by McGiness et al. (1988) that the 36/38K protein is derived entirely from within the same reading frame that encodes the P protein [by using, for example, alternative internal in-(P protein)-frame AUG start codons]. However, our findings are consistent with the proposal put forward by Patterson et al. (1989) and Cattaneo et al. (1989) that V proteins of the Paramyxoviridae are made up of an N-terminal portion of the P protein sequence (this portion in NDV containing the epitope common to P and V proteins recognized by MAb 688) and a C-terminal region read in a different reading frame [due to the insertion of extra G residue(s)], this other reading frame encoding a particularly cysteine-rich moiety (see Fig. 3).

The functions of V proteins of the Paramyxoviridae have not been elucidated. It has been shown to be a virion protein in the case of SV5, mumps virus and NDV, and co-immunoprecipitates with the L, NP and P proteins (SV5 and mumps virus). Together with the observation that the highly conserved cysteine-rich motif resembles zinc-binding proteins, these properties strongly suggest a role for the V protein in RNA binding in transcription/replication in these viruses. Further experiments will be conducted to determine whether the NDV 36K/V protein is a zinc-binding and/or RNA-binding protein.

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References


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