Analysis of pathotype-specific structural features and cleavage activation of Newcastle disease virus membrane glycoproteins using antipeptide antibodies

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Peptides were synthesized, that correspond to cleaved and trimmed carboxyl termini of the F₂ polypeptide regions of fusion (F) protein precursors (F₀ proteins) in four different strains of Newcastle disease virus (NDV). These peptides differed only within the four carboxyl-terminal residues and represent F₀ polypeptides of virulent (AV), low-virulence (EG) and avirulent (V4 and WA) pathotypes of NDV. Polyclonal rabbit antisera against each peptide reacted with their corresponding monomeric F₂ polypeptides and F protein oligomers as analysed by immunoblotting of egg-propagated virions. Bidirectional cross-reactivity was observed between V4 and EG antisera and F₂ polypeptides which differ only by a single variation of lysine and arginine at position 3 from their carboxyl termini. The other two antisera (AV and WA) were specific for their corresponding F₂ polypeptides. All of these antisera were shown to react in a strain-specific manner with intact egg-propagated virions in an ELISA. A previously described antiserum, designed to target the haemagglutinin–neuraminidase (HN) protein precursor (HN₀ protein) of avirulent strains of NDV, has been shown to be specific for residual HN₀ protein of avirulent virions propagated in embryonated chicken eggs. Whereas the antiserum targeted at the carboxyl terminus of the V4 F₂ polypeptide did not react with F₀ proteins of cell culture-propagated strains in immunoblotting, antipeptide antibodies targeted at another region of the F₂ polypeptide and a segment of the F₁ polypeptide did react with the F₀ protein from infected cells. These data are consistent with inclusion of the terminal carboxylate of the F₂ polypeptides in the recognition determinants of the antibodies targeted at the carboxyl terminus of the V4 F₂ polypeptide. The antisera described herein are ideally suited to rapid immunochemical pathotyping of NDV isolates and immunochemical characterization of the sites of intracellular cleavage activation of F₀ and HN₀ proteins and may be useful for defining interactions involved in F protein folding.

Introduction

Avian paramyxovirus type 1, commonly known as Newcastle disease virus (NDV), exists as a number of strains which cause disease in poultry to varying degrees of severity (Waterson et al., 1967). This variation in virulence is determined in part by variation in the susceptibilities of the fusion (F) protein biosynthetic precursors (F₀ proteins) of the different strains to proteolytic activation in different cell types (Nagai et al., 1976, 1979, 1989; Klenk et al., 1977). Cleavage of this single chain F₀ protein results in formation of the active F protein which comprises disulphide-linked F₂ and F₁ polypeptides (Nagai et al., 1976; Scheid & Choppin, 1974, 1977). This cleavage occurs at the carboxyl terminus of the smaller F₂ polypeptide region of the F₀ protein and generates the amino terminus of the F₁ polypeptide (Scheid & Choppin, 1977; Gething et al., 1978; Scheid et al., 1978; Richardson et al., 1980). It is believed that generation of the hydrophobic amino-terminal sequence of the F₁ polypeptide enables it to participate directly in the fusion of viral and host cell membranes (Gething et al., 1978; Richardson et al., 1980).

Susceptibilities of the F₀ proteins to proteolytic activation appears to be dictated by the nature of the amino acids linking the carboxyl termini of their F₂ polypeptide regions to the amino termini of their F₁ polypeptide regions prior to cleavage (McGinnes & Morrison, 1986; Toyoda et al., 1987, 1989; Glickman et al., 1988; Gorman et al., 1988, 1990; Millar et al., 1988; Nagai et al., 1989). Two pairs of basic amino acids, separated by a single glutamine residue, serve as a cleavage activation signal at the carboxyl terminus of the F₂ polypeptide regions of F₀ proteins of virulent strains. With less virulent strains, the first basic amino acid of
each pair of basic residues in the cleavage motif is replaced by another type of amino acid. Protease(s) capable of cleavage at the motif of virulent strains appear to be distributed throughout a wider range of cells than those which can affect cleavage of the motif of less virulent strains (Nagai et al., 1976, 1979, 1989; Klken et al., 1977; Morrison, 1988; Morrison et al., 1985; Gorman et al., 1988, 1990). Thus, virulent strains can spread more extensively through the host than less virulent strains and cause more severe disease as a consequence.

In addition to producing F₀ proteins with reduced susceptibilities to activation, some strains also produce a haemagglutinin–neuraminidase (HN) protein which requires proteolytic activation before these strains can attach to receptors on host cells (Garten et al., 1980; Nagai et al., 1976, 1979, 1980; Nagai & Klken, 1977). This HN protein precursor (HN₀ protein) has up to an additional 45 amino acids at its carboxyl terminus compared to HN proteins (McGimnes et al., 1987; Sato et al., 1987a; Gorman et al., 1988; Gotoh et al., 1988; Millar et al., 1988; Sakaguchi et al., 1989). Cleavage activation of the HN₀ protein of the progeny of these avirulent strains is also limited to a restricted range of host cells (Nagai et al., 1976, 1979, 1980, 1989; Nagai & Klken, 1977). Hence, different strains of NDV may be separated into three broad categories of virulence depending upon the susceptibilities of their F₀ proteins to proteolytic activation and whether or not they produce an HN₀ protein. Whereas virulent strains produce readily cleaved F₀ proteins, low-virulence and avirulent strains produce an F₀ protein with restricted cleavage properties and only avirulent strains produce an HN₀ protein. This categorization system correlates well with in vivo assays of virulence (Nagai et al., 1976, 1989).

Procedures for categorization and diagnosis of NDV isolates have generally been based on complex and protracted in vivo tests (Waterson et al., 1967). Variations in proteolytic activation of the membrane glycoprotein precursors of different strains offer the prospect of developing simpler, more precise and rapid assay systems for this purpose. Biosynthetic labelling of NDV proteins with radionuclides during propagation in cell culture has been shown to be useful for characterization of the susceptibilities of F₀ proteins to proteolytic activation and the nature of HN and HN₀ proteins (Nagai et al., 1976, 1979, 1980; Klken et al., 1977; Nagai & Klken, 1977). Antipeptide antibodies, targeted at regions either side of the cleavage activation sites of F₀ and HN₀ proteins, have been used to facilitate analysis of cell culture-propagated NDV isolates without the use of radioactivity (Gorman et al., 1992); however, even further improvement in the speed and ease of isolate characterization is desirable.

Direct structural studies have been performed on isolated F and HN proteins of representative strains from each of the categories of NDV (Schuy et al., 1984; Gorman et al., 1988, 1990). These studies clearly defined the molecular changes which result from cleavage activation of the respective F₀ and HN₀ proteins. It was demonstrated that the carboxyl termini of the F₀ polypeptides of the different F proteins varied according to the nature of the cleavage activation motif of their corresponding F₀ proteins (Gorman et al., 1990). In addition, the extent of cleavage of the sequence of amino acids unique to the carboxyl termini of HN₀ proteins of avirulent strains of NDV has been defined (Gorman et al., 1988).

The present study was undertaken to test the feasibility of production of antipeptide antibodies capable of detecting these differences in the fully processed carboxyl termini of the F₀ polypeptides of the various pathotypes of NDV. It was envisaged that such antibodies would be directly applicable to virions from allantoic fluid and thus further simplify and accelerate the process of pathotype analysis of NDV isolates. These antibodies were also expected to be applicable to defining aspects of cleavage activation and the molecular architecture of the F protein.

Methods

Viruses. Queensland or V4 (Simmons, 1967), Australia-Victoria or AV (Albiston & Gorrie, 1942), Eaves-Grimes or EG (Eaves & Grimes, 1978) and WA 2116 (Alexander et al., 1986) strains of NDV were propagated in 10-day-old embryonated specific pathogen-free chicken eggs as described previously (Gorman et al., 1988). Control allantoic fluid was obtained from identical eggs incubated for the same period without inoculation with virus. Propagation of these strains in BHK-21 cells and extraction of the infected cells have been described previously (Gorman et al., 1992).

Production of antipeptide antiserum. Peptides corresponding to carboxyl termini of the F₀ polypeptides of activated fusion proteins of the virulent AV, low-virulence EG and avirulent V4 and WA 2116 strains

**AV-** Cys-Lys-Val-Thr-Ser-Gly-Gly-Arg-Arg-Gln

**V4-** Cys-Lys-Val-Thr-Ser-Gly-Gly-Lys-Gln-Gly

**EG-** Cys-Lys-Val-Thr-Ser-Gly-Gly-Arg-Gin-Gly

**WA-** Cys-Lys-Val-Thr-Ser-Gly-Gly-Glu-Arg-Gln-Glu

Fig. 1. Peptides used for antibody production. AV, V4, EG and WA refer to the virulent Australia-Victoria, the avirulent Queensland, the low-virulence Eaves-Grimes and avirulent WA2116 strains of NDV, respectively. Amino acids presented in bold type at the carboxyl termini of these peptides represent the differences between the carboxyl termini of the F₀ polypeptides of these strains.
ELISA protocol. Indirect ELISAs were performed at 37 °C via a four-step protocol outlined below. Three washes with PBSA (pH 7.3) were performed between steps. Step 1: ascites fluid containing the HN protein-directed MAb was diluted 1:1600 in 0.05M carbonate–bicarbonate buffer pH 9.6 and incubated for 1 h in U-shaped wells of PVC microtitre plates (Tietertek, Flow Laboratories). Step 2: allantoic fluid, either undiluted or diluted in 0.5% (w/v) gelatin in PBSA, was incubated for 30 min with adsorbed antibody. Step 3: rabbit polyclonal antisera were diluted in 1% (v/v) non-infected allantoic fluid–PBSA, incubated for 1 h at 22 °C, dispensed into wells and incubated for 30 min. Step 4: bound polyclonal antibody was detected with sheep anti-rabbit IgG-horseradish peroxidase conjugate (Silenus Laboratories; cat. no. RAH; diluted 1:1000 in 1% v/v uninfected allantoic fluid–PBSA) in combination with 3,3',5,5'-tetramethylbenzidine substrate (Bos et al., 1981) and quantified at 450 nm.

Results

Specificity of F subunit polypeptide-directed antisera by immunoblotting

Positive antibody responses were achieved with each of the F subunit polypeptide-based synthetic peptides presented in Fig. 1 as judged by Western blot analysis of purified egg-propagated virions of each strain (Fig. 2). The antibody designed to target the F subunit polypeptide of the AV strain exhibited an intense band of immunoreactivity with a polypeptide species of M\(_f\) consistent with the reduced monomeric F subunit polypeptide (Fig. 2a). This immunoreactivity was specific for the AV strain. Specific immunoreactivity was also evident for species of much higher M\(_f\) in the AV virus sample which appear to represent non-disrupted oligomers of the F protein similar to those observed with respiratory syncytial virus (Arumugham et al., 1989; Collins & Mottet, 1991), Sendai virus (Sechoy et al., 1987) and NDV (M. E. Peebles, B. Newton, G. Raghu, F. Robey, C. Bencsics and C. Wang, unpublished).

Antisera directed at the F subunit polypeptide of the V4 strain reacted strongly with species with M\(_f\) values consistent with the monomeric F subunit polypeptide and F protein oligomers of the V4 strain (Fig. 2b). Some cross-reactivity was apparent for the V4-directed antisem with the monomeric F subunit polypeptide of the EG strain, albeit weaker than with the V4 F subunit polypeptide. Oligomeric F protein bands in the EG virus were barely detectable with the V4 strain-directed antibody. This antisem did not react with monomeric F subunit polypeptides or F protein oligomers of the AV or WA2116 strains. Reactivity of the EG F subunit polypeptide-targeted antisem was essentially a mirror image of the V4-targeted antisem (Fig. 2c). Immunoreactivity with this antisem was strongest for species with M\(_f\) values consistent with the monomeric F subunit polypeptide and F protein oligomers of the EG strain and much weaker reactivities were observed with the same species of the V4 strain.

The only species visualized as intense bands of
Fig. 2. Immunoblotting of SDS-PAGE-separated NDV F$_2$ polypeptides. Antisera used were designed to target the carboxyl termini of F$_2$ polypeptides of (a) virulent AV, (b) avirulent V4, (c) low-virulence EG and (d) avirulent WA2116 strains of NDV. Each lane contains approximately 12.5 μg of protein derived from purified AV, V4, EG and WA2116 virions. Arrowheads indicate the mobilities of monomeric F$_2$ polypeptides (F$_2$) and F protein oligomers (F$_n$).

Immunoreactivity with the antiserum directed at the F$_2$ polypeptide of the WA2116 strain were species with $M_r$ values consistent with the monomeric F$_2$ polypeptide and F protein oligomers (Fig. 2d) of the WA2116 sample. Intermediate $M_r$ species were visualized as faint nonspecific bands with the AV-, V4- and WA2116-targeted antisera. The $M_r$ values of these intermediate bands corresponded to the nucleocapsid and M proteins of the various strains (Fig. 2a, b and d) or the HN protein of the AV strain (Fig. 2d, lane AV).

Specificity of the HN$_0$ protein-directed antiserum

Previous experimentation demonstrated immunoreactivity with polypeptide species present in cell culture- and egg-propagated avirulent V4 virions using an antiserum
Characterization of NDV glycoproteins

Fig. 3. Immunoblotting of SDS–PAGE-separated NDV HN and HN₀ proteins. Proteins (approximately 12.5 μg) of purified V4 (lanes 1) and AV (lanes 2) virions were separated by SDS–PAGE and stained with Coomassie blue R-250 (Stain) or electrotransferred to nitrocellulose and probed with antisera targeted at the HN₀ proteins of avirulent strains (HN₀), a sequence common to HN proteins of most strains (HN) and a sequence common to M proteins of several strains (M).

Designed to detect a sequence unique to residues 593 to 613 of the HN₀ protein of avirulent strains (Gorman et al., 1992). This apparently represented the non-activated HN₀-protein of cell culture-propagated virions and a small proportion of the HN protein of virions grown in the endodermal cells of the chorioallantoic membrane. This antisera did not exhibit immunoreactivity with other cell culture-propagated strains which lack the coding capacity for production of an HN₀ protein. However, potential cross-reactivity with proteins in egg-grown virions of other strains was not investigated.

Data presented in Fig. 3 indicate that the HN₀ protein-directed antisera is specific for a protein species present in avirulent strains. When equivalent quantities of V4 and AV proteins from egg-propagated virions were subjected to SDS–PAGE (Fig. 3, Stain) only the V4 strain produced immunoreactivity with the antisera targeted at the sequence unique to the HN₀ protein (Fig. 3, HN₀). By comparison, protein species were detected in both strains by using an antisera directed to a sequence common to most HN proteins (Fig. 3, HN), which would also detect HN₀ protein. Similarly, an antisera directed to a sequence common to a variety of M proteins also detected protein species in both V4 and AV strains (Fig. 3, M). Egg-grown EG and WA2116 strains, which do not produce an HN₀ protein, also failed to react with the HN₀ protein-specific antisera (data not shown).

Differentiation between F₂ polypeptides by ELISA

Data presented in Fig. 4 demonstrate the feasibility of incorporation of an F₂ polypeptide-discriminating antipeptide antibody into a sandwich ELISA in combination with an HN protein-directed MAb. It is apparent that intact AV strain virions were adsorbed from allantoic fluid by the MAb raised with the V4 strain HN protein and were subsequently detected with the AV F₂ polypeptide-specific antipeptide antisera. Controls with uninfected allantoic fluid also produced significant background absorbance at the higher concentrations of antisera; however, this was reduced to negligible levels with dilution of the antisera. Titration of the antipeptide antisera directed at other cleavage activation motifs in the same assay, with MAb-trapped AV virions, provided evidence for the specificity of the assay (Fig. 5a). Although all antipeptide antisera produced significant absorbance values at high concentrations, these...
Fig. 5. Specificity of the F1 polypeptide targeted-antisera in the ELISA format. Allantoic fluids from embryonating eggs infected with the AV (a), V4 (b), EG (c) and WA2116 (d) strains of NDV were used in the ELISA in combination with antisera targeted at the AV (●), V4 (■) EG (●) or WA2116 (▲) F1 polypeptides. The AV allantoic fluid sample was used without dilution and the V4, EG and WA2116 samples were diluted 1:10, 1:5 and 1:5, respectively, in order to achieve haemagglutinin concentrations of between 50 and 100 units/aliquot. Antiserum concentrations are indicated on the abscissa and the values presented on the ordinate were corrected by subtraction of readings obtained with control allantoic fluid.
absorbances were diminished to negligible levels by quite moderate dilution. These high backgrounds with the unrelated antisera were not due to allantoic fluid as values for allantoic fluid controls had been subtracted from each antiserum dilution. Despite the high backgrounds at high antibody concentrations the AV F₂ polypeptide-targeted antiserum exhibited sufficient specificity to produce much higher absorbance values than the other antisera at dilutions of 1:400 or greater.

The HN protein MAb was also found to adsorb V₄, EG or WA2116 virions from allantoic fluids of embryos infected with those strains. The F₂ polypeptide-targeted antisera exhibited specificities in the ELISA system similar to those observed by immunoblotting. The V₄ F₂ polypeptide-targeted antiserum reacted strongly with V₄ virions over a broad dilution range (Fig. 5b) with a downward curvature in the plot at high antiserum concentrations. This downward curvature was apparently a consequence of subtraction of substantial control allantoic fluid values at high antiserum concentrations. The EG F₂ polypeptide-targeted antiserum also exhibited strong reactivity with the V₄ virions in a manner unrelated to high background values. However, this reactivity was diminished more rapidly upon dilution than was the homologous reactivity (Fig. 5b). Neither the AV- nor the WA2116-targeted antisera exhibited specific reactivity with trapped V₄ virions (Fig. 5b).

Trapped EG virions also reacted with both EG and V₄ F₂ polypeptide-targeted antisera but not the AG and WA2116 F₂ polypeptide-targeted antisera (Fig. 5c). The heterologous reactivity of the V₄ F₂ polypeptide-targeted antiserum with EG virions was minimized with less dilution than required to minimize homologous reactivity (Fig. 5c). Cross-reactivities between the V₄ and EG systems (Fig. 5b and c) are consistent with observations made above by immunoblotting analysis (Fig. 2). Trapped WA2116 strain virions reacted specifically only with the antipeptide antiserum that was designed to target the cleavage activation motif of the F₀ protein of this strain. Only a residual amount of cross-reactivity was observed at high concentrations of non-homologous antisera (Fig. 5d).

Reactivities of antipeptide antibodies with F₀ proteins

The antiserum targeted at the carboxyl terminus of the F₂ polypeptide of the V₄ strain of NDV was found not to react with F₀ proteins present in cell culture-propagated V₄, EG and WA2116 strains or in purified egg-propagated V₄ virions (Fig. 6a). This antiserum reacted strongly with F protein oligomers and monomeric F₂ polypeptides in purified egg-propagated V₄ virions but did not react with corresponding polypeptides in cell culture extracts (Fig. 6a).
Evidence for the production of F₀ proteins in infected BHK-21 cells was obtained by probing with an antiserum targeted at an internal region of the F₂ polypeptide (Fig. 6b). This antiserum targeted a 25 amino acid segment of the F₂ polypeptide located adjacent to, but not including, the carboxyl terminus of the F₂ polypeptide. Monomeric F₂ polypeptides of the cell culture-propagated AV strain and egg-propagated V4 virions were detected with this antiserum. It also demonstrated the presence of F₀ proteins in cell-propagated V4, EG and WA2116 strains and egg-propagated V4 virions. F protein oligomers were also shown by this antiserum in cell culture- and egg-propagated V4 samples.

Further evidence for the presence of uncleaved F₀ protein in extracts of cells infected with V4 and WA2116 strains and purified chorioallantoic membrane-propagated V4 virions (Fig. 6c) was obtained using an antiserum targeted at the F₁ polypeptide region of the F₀ protein. Failure to detect F₀ protein or F₁ polypeptide in the cell culture-propagated EG sample (Fig. 6c; lane EG) has been reported previously (Gorman et al., 1992) and is believed to result from variation of the EG strain in the sequence targeted by the antiserum. This antiserum also demonstrated that the F₀ protein of the cell culture-propagated AV strain and the majority of the F₀ protein of purified chorioallantoic membrane-propagated V4 virions were activated by cleavage.

**Discussion**

Design of the antipeptide antibodies described in the present report was dependent upon complementary determinations of sequences of genes which encode F₂ proteins (McGinnes & Morrison, 1986; Toyoda et al., 1987, 1989; Glickman et al., 1988; Millar et al., 1988; Nagai et al., 1989; Sato et al., 1987b) and HN and HN₀ proteins (McGinnes et al., 1987; Sato et al., 1987a; Gorman et al., 1988; Gotoh et al., 1988; Millar et al., 1988; Sakaguchi et al., 1989) of different pathotypes of NDV and direct chemical analysis of the structures of the mature proteins (Gorman et al., 1988, 1990). The synthetic peptides used to raise specific antisera (Fig. 1) to different F₂ polypeptides represent the carboxyl termini of the longest possible forms of the F₂ polypeptides of the virulent AV and low-virulence EG strains which are representative of these two pathotypes (Gorman et al., 1990). These F₂ polypeptides are also cleaved and trimmed to produce sequences three amino acids shorter. A ratio of 1:1 applies for the two cleavage forms of these strains (Gorman et al., 1990). Avirulent V4 and WA2116 F₂ polypeptides each have only one major form of carboxyl terminus represented by the sequences presented for these strains in Fig 1 (Gorman et al., 1990).

Utilization of these sequences to obtain maximal structural difference between the synthetic peptides has apparently enabled production of antisera capable of differentiating between the cleaved F proteins of virulent strains and those of low-virulence and avirulent strains. This appears to involve recognition of differences in the four carboxyl-terminal residues of these sequences which is an indication of the presence of highly specific antibodies in these antisera. Differentiation between the F proteins of low-virulence and avirulent strains is not as clear-cut but cross-reactivity of antisera primarily targeted at V4 and EG strains with counterpart EG and V4 F₂ polypeptides, respectively, is not surprising. There is only one site of amino acid variation between these F₂ polypeptides in the region targeted by the antisera, position 3 from the carboxyl termini (Gorman et al., 1990), and the differing amino acids, lysine and arginine, have similar physicochemical properties. It is apparent that the V4 and EG F₂ polypeptide-targeted antisera contain a population of antibodies which are independent of a specific amino acid side-chain at the position of the Arg/Lys variation and a population of antibodies which include side-chain specificity at this position in the recognition site(s). The avirulent WA2116 sequence is atypical of cleavage activation motifs of F₀ proteins of NDV strains (Gorman et al., 1990) and thus can be uniquely identified.

The most critical factor in assessing the pathotype potential of an isolate is whether or not it has an F₀ protein cleavage activation motif typical of a virulent strain such as the AV strain. The approach presented herein is able to achieve this objective in both immunoblotting and ELISA formats. The nature of the requirement for activation of haemagglutinin and neuraminidase functions (i.e. whether or not a strain produces an HN₀ or HN protein as a primary gene translation product) is relevant to subcategorization between low-virulence and avirulent strains of the lentogenic group of NDV. As demonstrated herein, this is also feasible for egg-propagated virus with the immunoblotting format but problems could arise with analysis if the extension on the carboxyl terminus of the HN₀ protein, which is the structural feature distinguishing between the HN₀ protein and HN protein phenotypes, exhibits substantial variation between avirulent strains. However, the HN₀ proteins of avirulent strains that have been sequenced to date, including V4 (Gorman et al., 1988), D26 (Sato et al., 1987a; Gotoh et al., 1988) and Ulster (Millar et al., 1988), lack substantial variation (Sakaguchi et al., 1989). HN proteins of some low-virulence strains have slightly longer carboxyl termini than HN proteins of virulent strains (Della-Porta et al., 1991). However, the transition point at which additional carboxyl-terminal extension of the HN protein causes diminution in virulence has not
been determined. The HN\textsubscript{0} protein-specific antiserum has not yet been assessed for its applicability in ELISA.

The findings of this study indicate that antisera produced by the currently described approach will enable routine characterization of field isolates of NDV in a more rapid manner than the traditionally employed \textit{in vivo} assays (Waterson \textit{et al}., 1967) and with diminished animal welfare concerns. However, it will be necessary to evaluate them with a larger panel of defined NDV isolates and field isolates before this can be established with more confidence and these antisera are adopted as tools for rapid pathotyping of NDV isolates on a routine basis. This evaluation will form the next phase in the development of these antisera for routine exploitation in NDV diagnosis and pathotyping. Other investigators have proposed the use of PCR-based amplification of NDV F and HN protein genes for pathotyping purposes (Jestin \& Jestin, 1991). However, this is far from being a universal or technically simple approach. The possibility of mixed infections (e.g. coexistence of virulent and avirulent strains) is a limitation to the PCR approach which demands the use of oligonucleotide sequencing of PCR products in order to define fully the cleavage activation motifs of F\textsubscript{0} proteins of all NDV strains potentially present in any given sample. The PCR approach is predicated on knowing the RNA sequences of relevant gene segments in advance so that appropriate oligonucleotide primers are used assuming that all genes will be amplified to equivalent extents. The present panel of antibodies is able to identify all F\textsubscript{0} protein cleavage activation motifs thus far defined for NDV and is able to detect the presence of a virulent strain despite the coincidental presence of low-virulence or avirulent strains. Moreover the immunochemical approach is technically simpler and more robust than PCR and oligonucleotide sequencing even in a Western blot format. The nucleic acid and immunochemical approaches may have complementary roles in characterization of NDV isolates. Immunochemical analysis is well suited to initial identification and routine screening on a large scale. On the other hand nucleic acid analysis may be best suited to detailed isolate characterization.

Demonstration of the reactivities of the F\textsubscript{2} polypeptide-targeted antisera in an ELISA format has fundamental implications regarding the structure of the active F protein. This finding indicates that the carboxyl terminus of the F\textsubscript{2} polypeptide is on the surface of the activated F protein. Failure of the carboxyl-terminal specific F\textsubscript{2} polypeptide antibodies to react with uncleaved F\textsubscript{0} proteins indicates that these antibodies have recognition determinants which include the carboxylate functionality as well as strain-specific amino acids (Fig. 1) at the carboxyl termini of the fully processed F proteins. These properties of the carboxyl-terminal specific F\textsubscript{2} polypeptide-targeted antisera will make them very useful reagents for further defining intracellular site(s) of activation of F\textsubscript{0} proteins by using an immunohistochemical approach. Similarly, the HN\textsubscript{0} protein-specific antibodies should also facilitate definition of the process of activation of this precursor.

The present data also provide insights into the folding of the F protein oligomers. Observation that the carboxyl-terminal specific F\textsubscript{2} polypeptide-targeted antibodies react with F protein oligomers of SDS-disrupted and mercaptoethanol-reduced virions suggests that the F\textsubscript{1} and F\textsubscript{2} polypeptides of the oligomers are held in association by forces other than the disulphide bond believed to exist between the two polypeptides (Nagai \textit{et al}., 1976; Scheid \& Choppin, 1977). Moreover, this may be an indication that the interaction between the F\textsubscript{1} and F\textsubscript{2} polypeptides is so tight that the disulphide bond is protected from reduction. These possibilities are consistent with the findings (Wang \textit{et al}., 1992) that temperature-sensitive mutations in a potentially interactive amphipathic \(\alpha\)-helix motif adjacent to the amino terminus of the F\textsubscript{1} polypeptide (Chambers \textit{et al}., 1990) of the AV strain of NDV may be compensated for by a mutation in the F\textsubscript{2} polypeptide. This motif of the F\textsubscript{1} polypeptide has a cysteine residue in close juxtaposition, believed to be involved in a disulphide bond with the F\textsubscript{2} polypeptide and it is thought that the helix interacts with the F\textsubscript{2} polypeptide through non-covalent forces (Wang \textit{et al}., 1992). Further work is in progress in order to evaluate the significance of this observation.

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