Archives of Virology 66, 193—206 (1980)

Archives of Virology
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Structural Polypeptides of Canine Distemper Virus

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With 7 Figures

Accepted June 6, 1980

Summary

The structural polypeptides of two strains of canine distemper virus and the Lec strain of measles virus were analysed by SDS-polyacrylamide-slab-gel electrophoresis. One strain of canine distemper virus derived from a live vaccine (Convac, Dumex), contained six major structural polypeptides with mol. wt. of 85, 78, 59, 43, 41 and 34×10^3. The 85K polypeptide was glycosylated. It was interpreted to be equivalent to the 79K glycoprotein of the measles hemagglutinin.

The second strain, a rapidly growing variant of the Onderstepoort strain of canine distemper virus characterized by extensive syncytium forming cytopathic effects in tissue culture, contained the 59, 43, 41 and 34K polypeptides, but the 85 and 78K polypeptides were not present in detectable amounts. The 43K polypeptide was identified as cellular actin by limited proteolysis. By use of monospecific rabbit hyperimmune sera against each of the major structural polypeptides of measles virus, the 59, 41 and 34K structural polypeptides could be identified as nucleocapsid protein (NP), fusion (F) polypeptide, and the membrane (M) polypeptide, respectively. In neutralization tests with rabbit hyperimmune sera against each of the two strains, this Onderstepoort strain, which contained reduced amounts of the hemagglutinin glycoprotein, gave higher neutralization titers than the vaccine strain.

Introduction

Measles, canine distemper and rinderpest virus belong to the Morbillivirus genus of the paramyxovirus family (10). During recent years much information has been gathered about the electrophoretic gel pattern of measles virus proteins (3, 6, 9, 17, 28, 29, 31, 32, 33). Measles virus has been shown to contain seven major structural polypeptides, the large (L), hemagglutinin (HA), polymerase (P), nucleocapsid (NP), actin, fusion (F) and membrane (M) polypeptides. The F component includes two polypeptide chains, F_1 and F_2, which are linked by

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disulfide bridges, and only the F_2 polypeptide chain is glycosylated. In this respect measles virus differs from other studied members of the paramyxovirus family, in which both F_1 and F_2 are glycosylated (26).

Different strains of measles virus have been shown to be remarkably similar in the molecular weights of their polypeptides. The difference reported has concerned small variations in the size of the nucleocapsid, polymerase and membrane polypeptides in different strains of measles virus (7, 17, 24, 34, 35).

In contrast to measles virus, only few studies have appeared in the literature concerning the structural polypeptides of the serologically related canine distemper virus (CDV). WATERS and BUSSELL (32) and BUSSELL *et al.* (3) reported that the major structural polypeptides found in CDV are similar to those in measles virus. They studied the Onderstepoort strain of CDV. Recently HALL *et al.* (8) confirmed and extended these findings on the Onderstepoort strain of CDV. They found the following structural polypeptides with their molecular weights given in parenthesis;

L, a large polypeptide (MW ~200,000); H, a glycoprotein (76,000); P, a phosphorylated protein (66,000); NP, the nucleocapsid polypeptide (58,000); F, a glycoprotein consisting of the disulfide-linked polypeptides F_1 (40,000) and F_2 (23,000); and M, the membrane protein (34,000). In contrast to measles virus the F_1 polypeptide of CDV was reported to be glycosylated.

The aim of the present investigation was to study the characteristics of the structural polypeptides of CDV strains and to determine their possible function and localization in the virion. With the aid of monospecific sera against each of the well-known major structural polypeptides of the serologically related measles virus, attempts were made to identify the major structural polypeptides of CDV.

Materials and Methods

Viruses

Three strains of CDV were used. One strain was derived from the commercially available live Convac vaccine (Dumex). It will be referred to as the "Convac" strain of CDV. The second strain of CDV was a rapidly growing variant of the Onderstepoort strain, kindly provided by Dr. M. Appel, Cornell University, Ithaca, New York (15). In some experiments the distemper virus strain Rockborn (25) was used. All strains of distemper virus as well as the Lec strain of measles virus were propagated in Vero cells maintained in Eagle's minimal essential medium (MEM) containing 2 per cent fetal calf serum.

Preparation of Purified Virions

Vero cells were grown on 25 cm^2 plastic bottles. When the cell monolayer was confluent, the cells were infected with CDV or the Lec strain of measles virus (2) at a MOI of ≤ 0.01 $TCID_{50}$/cell. At the time when cytopathic effect started to appear, the cultures were washed twice with MEM with 2 per cent fetal calf serum and methionine reduced from 16 mg/l to 4 mg/l. The cultures were maintained in this medium for 4 hours, after which the medium was changed to 5 ml of the same medium containing 5 μCi/ml of [^{35}S]-methionine (Radiochemical Centre, Amersham, England). Three days later extracellular material was harvested and clarified four times at $1400 \times g$ for 5 minutes. The labelled virus material was then layered on a discontinuous sucrose gradient composed of 1 ml 65 per cent sucrose (w/w) and 6 ml 30 per cent sucrose layers in 0.01 M-phosphate buffer, pH 7.2, and centrifuged at 30,000 rpm for 45 minutes in an SW40 rotor. Labelled virions banding at the interphase between sucrose layers were collected and analysed by SDS-polyacrylamide-slab-gel electrophoresis.

The purification procedure of extracellular unlabelled virions followed that described for mumps virus (23).

SDS-Polyacrylamide-Slab-Gel Electrophoresis

The technique employed essentially followed that described by LAEMMLI (11). Electrophoresis was performed in 15 or 10 per cent polyacrylamide gels (0.18 per cent NN′-methylenebisacrylamide). The proteins were stained with Coomassie brilliant blue. The details of the procedure for polyacrylamide gel electrophoresis and scintillation autofluorography have been described previously (23). The molecular weights of the major structural polypeptides of measles and CDV were determined in 15 and 10 per cent polyacrylamide gels by comparison with adenovirus type 2 polypeptides (1) and reference standard proteins purchased from Bio-Rad, California. The molecular weight standard proteins were; phosphorylase B (94K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (21K) and lysozyme (14K).

Preparation of Isolated Polypeptides Used for Immunization

The major structural polypeptides of measles and CDV were prepared in the following way: purified virions were dissolved in 1 per cent SDS, 2 per cent β-mercaptoethanol and 10 per cent glycerol and heated to 70° C for 10 minutes. One ml of material containing one to two mg of viral protein was fractionated on a 15 per cent polyacrylamide slab gel with only one slot. After the run, the gel was stained and destained for 30 minutes each, rinsed in water and transferred to a water bath. The individual bands corresponding to the major structural polypeptides of measles and CDV were cut out with a razor blade and each band was re-banded on a new gel. After the second run the procedure of staining and destaining was repeated, and bands were cut out and fragmented. Thereafter the gel pieces were enclosed with 2 or 3 ml of electrolyte buffer used for electrophoresis (0.025 M Tris, 0.192 M glycine, 0.1 per cent SDS) in a dialysis bag which was placed in a 500 ml bath of electrolyte buffer and the polypeptides were electrophoresed out of the gel pieces at 50 V overnight, dialysed for three days against distilled water, which was changed every day, and was finally dialysed against 0.9 per cent NaCl. Before being used for immunization it was controlled that no free SDS was present by addition of red blood cells to a small portion of the material. No hemolytic activity was observed in the materials used for immunization.

Immune Precipitation

[^{35}S]-methionine labelled purified virions were used as antigen in immune precipitation experiments. The virions were labelled and purified as described above, the only exception being that the infected cultures were labelled with 20 μCi per ml of [^{35}S]-methionine. After purification the virions were mixed with SDS at a final concentration varying from 0.5 to 1 per cent and were boiled for 2 minutes. The material in the final antigen-antibody mixture was adjusted to a concentration of 0.1 per cent SDS, 1 per cent sodium deoxycholate (DOC), 1 per cent Triton X-100 in 0.15 M NaCl, 0.01 M Tris-HCl, pH 7.4. One to two hundred thousand counts per minute of [^{35}S]-methionine labelled purified virions were mixed at 0° C with rabbit hyperimmune sera which had been extensively absorbed with uninfected Vero cells. The volume of the reaction mixture was 0.5 ml. In most experiments the final antibody dilution was 1:125. After 2 hours at 0° C, immune complexes were precipitated by addition of 50 μl of a 1:1 slurry of *Staphylococcus aureus* protein A bound to Sepharose CL-4B suspended in 0.15 M NaCl, 0.01 M Tris-HCl, pH 7.4, at 0° C for 1 hour, with frequent vortexing of the beads. The Sepharose beads were pelleted in an Eppendorf microfuge (10,000 rpm for two minutes) and washed four times with fresh buffer as described above. A fifth wash in 1 ml of 0.01 M phosphate buffer, pH 7.2 was performed in order to remove the detergents. The beads were dried and dissolved in 120 μl of 1 per cent SDS, 2 per cent β-mercaptoethanol and 10 per cent glycerol. After 2 minutes of boiling the samples were counted and subjected to electrophoresis.

Peptide Mapping by Limited Proteolysis in SDS and Analysis by Gel-Electrophoresis

The technique used was essentially similar to that described by Cleveland *et al.* (5). The acrylamide concentration and acrylamide to bisacrylamide ratio in the analysis gel was the same as that used by Lamb and Choppin (13). Excised gel bands of purified viral polypeptides at an estimated protein concentration from 2 to 20 μg were degraded by proteolytic enzymes. The enzyme concentrations used were *Staphylococcus aureus* V8 protease, 1 μg in 20 μl of buffer (5), and papain 0.02 μg in 20 μl. After electrophoresis the gels were stained and destained.

Sera

Rabbits were immunized with isolated polypeptides by repeated intramuscular injections into the hind legs every four weeks with a 2 to 4 ml mixture consisting of equal volumes of antigen and Freund's complete adjuvant. Serum samples from the rabbits were taken 10 days after each booster. The method for preparation of rabbit hyperimmune sera against untreated purified virions followed that described in an earlier publication (19).

Serological Test

Complement fixation (CF) tests were performed as described in an earlier publication (18). The techniques used in neutralization (NT) and neutralization-enhancement (NE) tests in the absence (NT) and presence of anti-γ-globulin (NE) were performed as described previously (21). Final readings of the tubes were taken after two weeks with the Convac vaccine strain of CDV and after one week with the Onderstepoort strain. Mixed hemadsorption (MH) was used to measure antibodies against envelope components (20, 22). Monolayer cultures of Vero cells in milk bottles were infected with the Convac strain of CDV at a MOI of 0.01 $TCID_{50}$/cell. When cytopathic effect started to appear the bottles were used in experiments.

Results

The Polypeptide Pattern of Two Strains of CDV and the Lec Strain of Measles Virus

Six major structural polypeptides were detected in the Convac vaccine strain of CDV. The molecular weights of these polypeptides were 85K, 78K, 59K, 43K, 41K and 34K (Fig. 1). These polypeptides corresponded in location in the virion and function (see below) to the 79K, 72K, 60K, 43K, 40K and 36K polypeptides of measles virus (29, Fig. 1). The amount of the 43K polypeptide varied in different preparations of purified virions.

In some preparations additional CDV polypeptide band(s) were found located between the 41K and 34K polypeptides (Figs. 2, 3, 5, 6). These bands occurred irregularly. The origin of these polypeptides could not be defined. The polypeptide composition of the Onderstepoort strain of CDV differed from the vaccine strain (Fig. 1). The 59K, 43K, 41K and 34K polypeptides identified in the vaccine strain were present also in this strain, but prominent bands larger than the 59K polypeptide were never observed in the gels.

The structural polypeptides of the Convac vaccine strain were also analysed after immune precipitation with extensively Vero cell absorbed rabbit hyperimmune sera directed against this strain (Fig. 2). The 85K, 78K, 59K, 41K and 34K polypeptides, but not the 43K or minor bands were precipitated. This affirmed the identification of the former bands as virus- specific. The 59 and 34K polypeptides were identified as NP and M, respectively, by immune precipitation of CDV with monospecific rabbit hyperimmune sera directed against the corresponding measles polypeptides (data not shown).

Characterization of Antisera Directed Against Major Structural Polypeptides of CDV

Rabbits were immunized with four of the major structural polypeptides of CDV, the 85K, 59K, 41K and 34K polypeptides, isolated as described in Materials and Methods. Each antiserum reacted only with its corresponding antigen in immune precipitation (Fig. 3). The antibody titers of these sera were determined in different serological tests (Table 1). All sera reacted in complement fixation

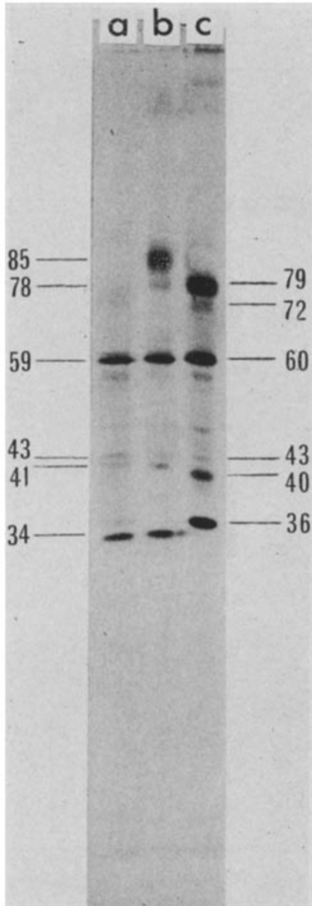

Fig. 1

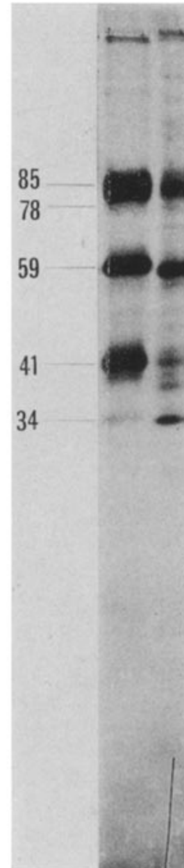

Fig. 2

Fig. 1. SDS-polyacrylamide-slab-gel electrophoresis of [^{35}S]-methionine labelled purified measles and CDV virions in a 15 per cent polyacrylamide gel. The following virus materials were included: the rapidly growing variant of the Onderstepoort strain of CDV obtained from Dr. M. Appel *(a)*, the Convac vaccine strain of CDV *(b)* and the Lec strain of measles virus *(c)*. The molecular weights of the major structural polypeptides of the three virus strains are indicated by their K values

Fig. 2. Immune precipitation of [^{35}S]-methionine labelled purified virions of the Convac strain of CDV with a rabbit hyperimmune serum directed against purified homologous virions analysed in a 15 per cent polyacrylamide gel: [^{35}S]-methionine labelled CDV virions (right) and precipitated polypeptides (left)

Table 1. *Determination of different antibody activities in four rabbit hyperimmune sera obtained by immunization with different preparations of purified CDV polypeptides*

Rabbit hyperimmune serum against	Antibody titers in serological tests		
	CF	MH[a]	NT
85K polypeptide	160	16	5
59K polypeptide	320	0	<5
41K polypeptide	160	16	<5
34K polypeptide	20	0	<5

[a] The sera were diluted 1:5 and the zones were measured in mm

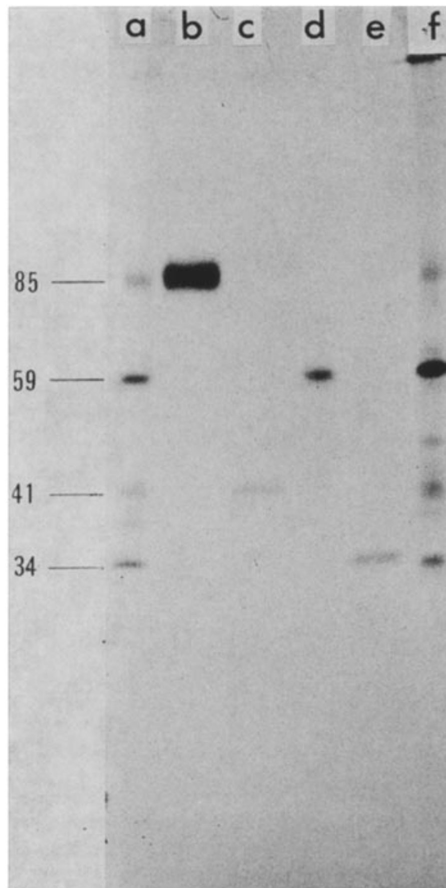

Fig. 3. Immune precipitation of [^{35}S]-methionine labelled purified virions of the Convac strain of CDV with rabbit hyperimmune sera directed against major viral structural polypeptides isolated from SDS-polyacrylamide gels. The following materials were included: purified CDV virions *(a, f)*, purified virions precipitated with antiserum directed against the 85K polypeptide *(b)*, 41K polypeptide *(c)*, 59K polypeptide *(d)* and 34K polypeptide *(e)*

tests. The antisera directed against the 85K and 41K polypeptides, but not the antisera directed against the 59K and 34K polypeptides, contained antibodies reacting with the surface of CDV infected Vero cells in the MH tests. This indicates that the former polypeptides build up envelope components of CDV. Of the four sera only the antiserum directed against the 85K polypeptide contained a low titer of NT antibodies.

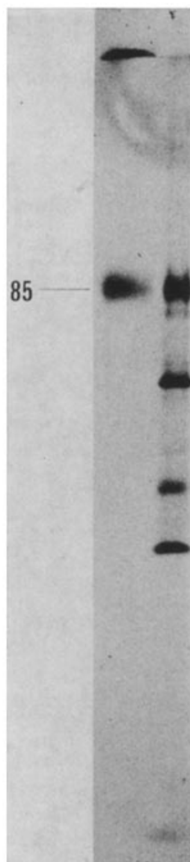

Fig. 4. Scintillation autofluorogram of [^{3}H]-glucosamine labelled purified virions of the Convac strain of CDV (left) and [^{35}S]-methionine labelled purified virions (right)

Identification of Two Envelope Peplomers in CDV

Cultures infected with CDV were labelled with [^{3}H]-glucosamine as described by Tyrrell und Norrby (29) and the virions were subsequently purified in the same manner as [^{35}S]-methionine labelled virions. The 85K polypeptide was glycosylated in the Convac vaccine strain of CDV (Fig. 4). This polypeptide therefore appears equivalent to the 79K hemagglutinin polypeptide in measles virus (29). The Onderstepoort strain of CDV was also labelled with [^{3}H]-glucosamine. Identification of a glycosylated structure in this strain was unsuccessful. This

might be due to low efficiency of labelling and/or relative paucity of a corresponding structure in this strain.

A second nonglycosylated envelope peplomer was identified in CDV. A rabbit hyperimmune serum directed against the 40K F_1 polypeptide of measles virus (Örvell and Norrby, in press) precipitated the 41K polypeptide of CDV (Fig. 5).

A small glycoprotein (SGP) could not be identified in [^{35}S]-methionine labelled extracellular CDV virions. However, immune precipitation of [^{35}S]-methionine and [^{3}H]-glucosamine labelled intracellular viral material (Örvell and Norrby, J. gen. Virol., in press) of the two CDV strains with a rabbit hyperimmune serum directed against whole virions detected a small glycoprotein (Fig. 6). The apparent

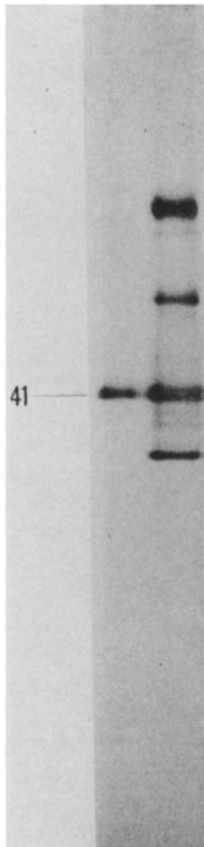

Fig. 5

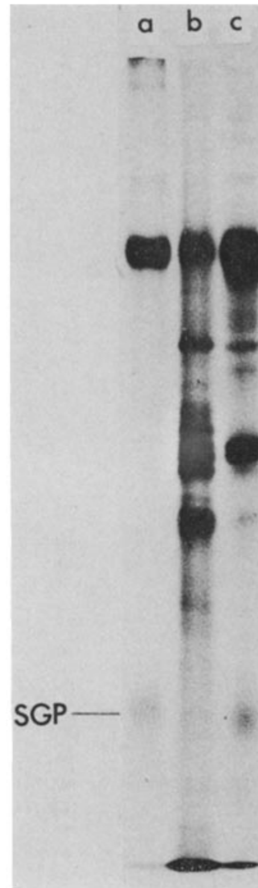

Fig. 6

Fig. 5. Immune precipitation of the Convac strain of CDV with an antiserum directed against the F_1 polypeptide of measles virus. The 41K polypeptide (left) of [^{35}S]-methionine labelled purified CDV (right) was precipitated

Fig. 6. Immune precipitation of [^{35}S]-methionine *(c)* and [^{3}H]-glucosamine *(a)* labelled intracellular Convac canine distemper viral antigen with a rabbit hyperimmune serum directed against purified homologous virions. A small glycoprotein *(SGP)* which could not be identified in [^{35}S]-methionine purified extracellular virions *(b)*, was detected

molecular weight of the precipitated polypeptide in 15 per cent gels was 23K. This polypeptide was not precipitated when [^{35}S]-methionine and [^{3}H]-glucosamine labelled uninfected cell lysates were used as antigen in immune precipitation. The polypeptide is therefore equivalent to the F_2 glycoprotein described in other paramyxoviruses. In some experiments this polypeptide was also found in [^{3}H]-glucosamine labelled purified virions.

Cross-Neutralization Tests

As described above the relative amounts of the H polypeptide differed in the two strains. In the rapidly growing variant of the Onderstepoort strain the F polypeptide was the dominating peplomer in the envelope. However, a HA polypeptide must be present also in this strain in order to allow the virus to adsorb to and infect cells. The cytopathic effects of the two strains of CDV were different. The rapidly growing Onderstepoort strain produced large syncytia in tissue cultures, whereas the vaccine strain produced mainly round cells and only small syncytia. This may indicate that the major route of spread in tissue culture were different. The Onderstepoort strain of CDV may spread predominantly by fusion of cells in tissue culture. The two strains of CDV were used in neutralization tests with rabbit hyperimmune sera directed against different strains of CDV (Table 2). The rapidly growing Onderstepoort strain gave significantly higher neutralization (NT) antibody titers than the vaccine strain in rabbit hyperimmune sera directed against distemper virus.

Table 2. *Cross-neutralization with rabbit hyperimmune sera directed against different strains of purified whole distemper virus*

	Antigen			
	CDV, Onderstepoort strain		CDV, Convac strain	
Rabbit hyperimmune serum against	NT	NE	NT	NE
CDV, Onderstepoort strain, 1[a]	2,560	5,120	320	640
CDV, Onderstepoort strain, 2	1,280	2,560	160	640
CDV, Convac strain, 1	10,240	20,480	2,560	5,120
CDV, Convac strain, 2	10,240	20,480	2,560	5,120
CDV, Rockborn strain, 1	5,120	10,240	1,280	2,560
CDV, Rockborn strain, 2	5,120	10,240	640	2,560

[a] 1 and 2 represent different rabbit hyperimmune sera

Demonstration of a 43K Actin Molecule in Purified Preparations of CDV

The relative amounts of the 43K polypeptide varied in different preparations of purified virions. It was not precipitated by a rabbit hyperimmune serum against purified distemper virus (Fig. 2). The 43K polypeptide comigrated with cellular

actin and was the only major CDV polypeptide that was heavily labelled when the virus was grown in Vero cells prelabelled with [^{35}S]-methionine. Similar results were obtained in a previous study on measles virus (29). In order to investigate further if the 43 K polypeptide in CDV and measles is cellular actin, the 43 K polypeptide from uninfected Vero cells was cut out from the gels and was compared with purified rabbit cell actin (27), kindly provided by Dr. Rigmor Torstensson, National Bacteriological Laboratory. The cleavage patterns of purified rabbit cell actin and the 43 K Vero cell polypeptide, obtained by limited proteolysis with *Staphylococcus aureus* V8 protease and papain were almost identical (data not shown). Thereafter the cleavage pattern of the 43 K actin polypeptide from uninfected Vero cells, and the 43 K polypeptide of purified measles and CDV were compared. As can be seen from Fig. 7 the cleavage pattern obtained with the three polypeptides was identical.

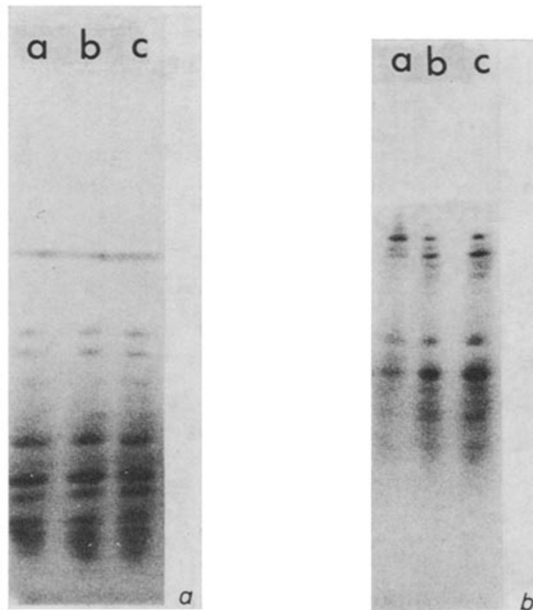

Fig. 7. Peptide mapping by limited proteolysis in SDS and analysis by gel electrophoresis of the 43 K polypeptide. The 43 K polypeptide of uninfected Vero cells *(a)*, measles *(b)*, and CDV *(c)* was degraded with 1.0 μg of *Staphylococcus aureus* V8 protease in 20 μl of buffer (Fig. 7a) and 0.02 μg of papain in 20 μl (Fig. 7b). The uppermost band observed in Fig. 7a originated from a polypeptide of the enzyme

Discussion

In the present investigation six major structural polypeptides were demonstrated in CDV. These polypeptides corresponded to previously identified polypeptides in measles virus (Örvell and Norrby, in press). A large polypeptide with a molecular weight of 200,000 daltons has been identified in measles virus (6, 28,

33) and also in other paramyxoviruses. The L polypeptide occurs only in low concentrations in the virus particle. Recently HALL *et al.* (8) demonstrated that a L polypeptide exists also in CDV. In this study the L polypeptide was not identified.

Paramyxoviruses contain two envelope peplomers. The larger is associated with hemagglutinating and neuraminidase activity in members of the paramyxovirus genus of paramyxoviruses. In members of the morbillivirus genus neuraminidase activity does not occur. Hemagglutinating activity has only been demonstrated in the case of measles virus. The 79K polypeptide in measles virus is responsible for adsorption to cells and hemagglutinating activity. The corresponding glycosylated polypeptide in the Convac strain of CDV is the 85K polypeptide, although, no hemagglutinating activity has been demonstrated in CDV products. This polypeptide is considerably larger than in measles virus. The F_1 polypeptide of measles and the two strains of CDV studied here had a similar molecular weight and were not glycosylated. HALL *et al.* (8) recently reported that the F_1 polypeptide of CDV is glycosylated. The different results obtained in the two studies can not be explained at present. The F_2 polypeptide of paramyxoviruses has been reported to contain a relatively low amount of methionine (26). This may explain the failure to detect this polypeptide in [^{35}S]-methionine labelled preparations of purified virions. The molecular weight of the F_2 polypeptide corresponded to that reported by HALL *et al.* (8).

Strain variation of the molecular weights of the measles virus polypeptides have been well documented in the case of the M polypeptide (7, 34, 35) and a small variation in the migration of the NP polypeptide has also been demonstrated (17). Of the different strains that have been investigated (7), no M polypeptide has been shown to migrate faster than the M polypeptide of the Lec strain of measles virus. The two strains of CDV studied here had a significantly smaller M polypeptide than the Lec strain of measles virus. Although the size of the M polypeptide was identical in the two CDV strains presented in this investigation, the Rockborn strain of CDV has a significantly smaller M polypeptide (ÖRVELL, unpublished data). The size of the P polypeptide has been reported to differ in measles virus strains (7, 24), but no pronounced differences in the size of the H polypeptide of different strains of measles virus has been reported. In the case of CDV pronounced differences appear to exist in the size and relative concentration of the H polypeptide. HALL *et al.* (8) found a molecular weight of 76,000 and 66,000, respectively, for the H and P polypeptides in the Onderstepoort strain of CDV. The molecular weight of the corresponding polypeptides of the Convac strain of CDV studied in the present investigation were 85,000 and 78,000, respectively. Although no direct comparison between the strain used by HALL *et al.* and the Convac strain of CDV, these pronounced differences in molecular weight determinations reflect a real difference in the migration of the two polypeptides as a comparison to the structural polypeptides of measles virus was made in both studies. The Onderstepoort strain of CDV presented in this investigation did not contain any major structural polypeptides larger than the NP polypeptide that could be assigned to represent the H polypeptide. The possibility that virions of the Onderstepoort strain were not intact virions but rather incomplete particles lacking the H polypeptide was investigated. Ratios of infectivity to protein content were studied in different

purified virus preparations of the two CDV strains. No significant differences were recorded between the two strains. These results indicated that intact virions of the Onderstepoort strain had been analysed.

The rapidly growing variant of the Onderstepoort strain of CDV was obtained by passage at a dilution of 10^{-4} in Vero cells (15). In measles virus, two variants of the Edmonston strain have been established (4). The two variants of measles virus, designated as the DP (diluted passage) and UP (undiluted passage) were obtained by means of serial passage in HeLa cell cultures with diluted and undiluted inoculum, respectively. The DP strain induces a rapidly evolving cytopathic effect (CPE) characterized by large syncytia, which in short time leads to a complete destruction of the cultures. In contrast, the UP strain induces a delayed and slowly developing CPE with only small syncytia. The polypeptide composition of the rapidly growing variant of the Onderstepoort strain of CDV appears to be unique among paramyxoviruses. It must, in order to effectuate its functions in adsorption and replication, contain a polypeptide responsible for adsorption. This polypeptide appears to be present in such a small amount on the envelope of extracellular virions that it may escape identification.

The differences in relative concentration of the two envelope structures may be expected to result in a different biological behaviour of the two strains. Significantly higher NT antibody titers were recorded in rabbit hyperimmune sera directed against CDV when the rapidly growing Onderstepoort strain was used in NT tests. Lower amounts of antibodies were therefore required to neutralize the infectivity of this strain. Although alternative explanations may be possible, this finding can be interpreted to reflect the polypeptide pattern, in as much as this strain contains less of the H protein. It has been shown that the fusion polypeptide is responsible for penetration of virions into cells and cell to cell spread in tissue culture (16). The cytopathic effect of the rapidly growing strain was characterized by large syncytia, which would indicate that this strain may spread from cell to cell in the cultures. The mode of spread of the two strains in cell culture, that is, extracellularly, or from cell to cell, warrants further studies.

The presence of actin has been demonstrated in measles and Sendai virus (12, 14, 17, 29). The best proof that actin is incorporated into paramyxoviruses would come from peptide mapping and by direct comparison of the polypeptide in virions and uninfected cells. By the use of this technique actin has been identified in Sendai virus (14), and mumps virus (ÖRVELL, unpublished data). The finding of actin also in measles and distemper virus makes it possible that incorporation of cellular actin is a generalized phenomenon during maturation of paramyxoviruses. Actin is an internal polypeptide in mumps and measles virus (23, 29). Recent findings suggest that actin filaments may mediate the communication between viral nucleocapsids and the cell membrane during maturation of paramyxoviruses (30).

Acknowledgements

The excellent technical assistance of Mrs. Anita Bergström is gratefully acknowledged. This work was supported by a grant from the Swedish Medical Research Council (Project no. 16x.116).

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Received April 23, 1980