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Precursor Protein for Newcastle Disease Virus

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The course of viral protein synthesis during infection of chicken embryo fibroblasts with Newcastle disease virus (NDV) L. Kansas has been followed by using sodium dodecyl sulfate polyacrylamide gel electrophoresis. Of the three major virion polypeptide molecular weight classes, I (78,400 daltons), II (53,500 daltons), and III (37,600 daltons), only II, having the same electrophoretic mobility as nucleocapsid polypeptide, appears to be the cleavage product of a precursor polypeptide PII (64,800 daltons) detected in NDV-infected cells after brief labeling with radioactive amino acids. Nucleocapsids were isolated from NDV-infected cells which had been pulse-labeled with radioactive amino acids or pulse-labeled and further incubated with unlabeled amino acids. Gel electrophoretic analysis of proteins derived from nucleocapsids showed that an increase in the period of incubation with unlabeled amino acids resulted in an increase in the amount of radioactivity in nucleocapsid protein. Polypeptide PII was not detected as a transient component of the isolated nucleocapsid fraction. These results are consistent with two interpretations. The product of PII cleavage is (i) nucleocapsid polypeptide, or (ii) a nonvirion or minor envelope polypeptide having the same electrophoretic mobility as nucleocapsid polypeptide.

Purified virions of the paramyxovirus Newcastle disease virus (NDV) contain three major polypeptides and at least three additional minor polypeptides, as revealed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE) (1, 7, 15). The major polypeptide of highest apparent molecular weight is a glycoprotein (I, 74,800 daltons) associated with both the hemagglutinin and neuraminidase activities of the virion (16). The second largest major polypeptide (II, 53,500 daltons) is the nucleocapsid protein (10, 14). The role of the third major protein (III, 37,600 daltons) is unknown, but it is probably a membrane protein (16). Neither II nor III is a glycoprotein. One of the minor virion polypeptide components is a glycoprotein with a mobility similar to nucleocapsid polypeptide II by SDS-PAGE. This glycoprotein is readily separated from nucleocapsid protein after virion disintegration with Triton X-100 and sodium deoxycholate (14).

Few studies have been made concerning the synthesis of proteins in cells infected with NDV (2, 12) and none has sought any short-lived polypeptide species that may play a role in virus maturation. This study was undertaken to determine the role of a novel nonvirion protein detected in pulse-labeled NDV-infected chick cells.

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MATERIALS AND METHODS

Virus. The L. Kansas strain of NDV used in this study was obtained from W. S. Robinson of Stanford University.

Cells. Chicken embryo fibroblast cultures were prepared from 10-day-old chicken embryos and grown to confluency in medium 199 IC (medium 199 modified to contain 2 μg of L-isoleucine per ml and supplemented with 5% dialyzed calf serum). Secondary cultures were made from these primaries in 199 IC medium in 60-mm diameter plastic dishes and used when confluent (approximately 2×10^7 cells per dish). Cells were grown and the experiments were conducted in medium 199 IC at 38 C in an atmosphere of 95% air and 5% CO_2.

Virus purification and assay. Virus was grown in 10-day-old embryonated eggs at 41 C for 48 h after inoculation of 10^3 PFU of NDV per egg. The chorioallantoic fluid was harvested and clarified by centrifugation at 10,000 × *g* for 10 min. Virus was pelleted by a 2-h centrifugation at 70,000 × *g* in the Spinco 42.1 fixed-angle rotor. The viral pellet was suspended and centrifuged to equilibrium (16 h) in a 65 to 16% preformed linear sucrose-D_2O gradient in TSE buffer (0.01 M Tris, 0.05 M disodium EDTA, pH 7.4) in a

Spinco SW41 rotor at 40,000 rpm. The virus band was removed, concentrated by centrifugation, and banded at equilibrium (16 h) in a 25 to 6% iodine equivalent preformed linear Renografin gradient in TSE buffer of pH 7.4 in a Spinco SW41 rotor at 40,000 rpm. The purified virus band was again pelleted, suspended in TSE buffer, and stored at -70 C.

Virus was estimated by plaque formation on chick primary monolayers after 2-day incubation under 1% agar-medium 199 IC, and observed by staining with neutral red solution.

Infection and pulse labeling of cells. Confluent chick monolayers in 60-mm diameter plastic dishes were infected with 50 PFU of NDV per cell. After adsorption in medium 199 IC for 30 min, unadsorbed virus was removed and the monolayer was washed twice with 2-ml portions of fresh medium. (Monolayers which had been infected in parallel were disaggregated with trypsin, and the cells were subsequently plated for PFU determinations. The results indicated that greater than 90% of cells had become infected with NDV under these conditions.) Fresh medium (2 ml per dish) was then added to the infected monolayers which were incubated further for 5.5 h prior to a 10-min pulse-labeling period with ^{3}H-isoleucine (0.5 ml, 50 μCi per dish) in medium 199 IC. Confluent chick monolayers were processed as above, but without infection with NDV, and were either labeled for 10 min with ^{3}H-isoleucine (0.5 ml, 50 μCi per dish) in medium 199 IC or labeled for 5 h with ^{14}C-isoleucine (2 ml, 12 μCi per dish) in medium 199 IC. After removal of radioactive medium, chick monolayers were either processed directly for SDS-PAGE or subjected to a further incubation for 10- or 30-min periods in medium 199 IC containing 200 μg of unlabeled isoleucine per ml.

After removal of medium, cell extracts were prepared for subsequent SDS-PAGE by quickly dissolving the radioactively labeled chick monolayer in 1 ml per dish of boiling 0.01 M sodium phosphate buffer (pH 7.2) containing 4% SDS and 5% 2-mercaptoethanol. The extracts were then boiled for 2 min.

Isolation of nucleocapsid. Nucleocapsids were extracted from intact virions, or from NDV-infected cells dislodged from dishes by 0.07% disodium EDTA alone, and purified by centrifugation through cesium chloride gradients as described by Mountcastle et al. (14). After centrifugation at 34,000 rpm in the Spinco SW50 rotor for 90 min, fractions were collected from the bottom of the tube, samples were taken for determination of radioactivity, and the remainder was boiled for 2 min in 0.01 M sodium phosphate buffer (pH 7.2) containing 4% SDS and 5% 2-mercaptoethanol. All operations subsequent to the pulse or pulse-chase incubations and prior to boiling in SDS solutions were performed between 0 and 5 C.

Sample preparation and polyacrylamide gel electrophoresis. Cell extracts boiled with SDS were dialyzed at room temperature for 48 h against four 2-liter changes of 0.01 M sodium phosphate buffer (pH 7.2) containing 0.1% SDS and 0.1% 2-mercaptoethanol. The dialyzed cell extracts were then boiled in gel sample buffer (final concentration: 2% SDS, 5% mercaptoethanol, 10% glycerol, 0.0625 M Tris-hydrochloride, pH 6.8) for 2 min. Bromophenol blue was added as tracking dye at a final concentration of 0.0001%. The discontinuous pH gel SDS electrophoresis system of Laemmli (11) was used throughout. *N,N'*-Diallyltartardiamide was used as a cross-linking agent, replacing *N,N'*-methylenebisacrylamide in equimolar amounts in the 10% acrylamide separating gels (3). Separating gels 10 cm long were formed in siliconized 6-mm inside diameter glass tubes and overlayered with water. A 0.2-ml 3% acrylamide, 0.08% *N,N'*-methylenebisacrylamide stacking gel was used. The final concentration of SDS in both gels was 0.1%. After gel polymerization and removal of the water overlay, samples of whole cell extracts (200 to 400 μliters, 80 to 160 μg of protein) were layered on top of the stacking gel. Samples were overlayered with reservoir buffer (11) and subjected to electrophoresis for 1 h at 1 mA per gel followed by electrophoresis at 2 mA per gel until the tracking dye was within 1 cm of the bottom of the gel (approximately 5 h).

Gel staining and fractionation. All gels were processed through a staining and destaining procedure (6) to allow comparison of stained egg-grown virion protein bands and molecular weight markers with the radioactive profiles of electrophoresed cell extracts. In addition, staining revealed the integrity of stained bands and removed any residual traces of unincorporated radioactive amino acids not removed by dialysis. After staining and destaining, the gels were frozen at -70 C for at least 1 h prior to slicing into 1.4-mm slices with a set of parallel mounted razor blades. Slices were completely dissolved by shaking for 30 min in 0.7 ml per slice of 2% periodic acid at 60 C in glass scintillation vials (3). Scintillation fluid (10 ml) containing 4 g of 2,5-diphenyloxazole, 50 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene, 250 ml of Triton X-100 and 750 ml of toluene was added to each vial for the estimation of radioactivity.

Protein. Protein was determined by the method of Lowry et al. (13) with bovine serum albumin as standard.

Chemicals and isotopes. ^{14}C-isoleucine (250 mCi per mmol) and ^{3}H-isoleucine (50 Ci per mmol) were obtained from New England Nuclear Corp., Boston, Mass; acrylamide, *N,N'*-methylenebisacrylamide and 2-mercaptoethanol were obtained from Eastman Organic Chemicals, Rochester, N.Y.; SDS and Coomassie Blue were obtained from Bio-Rad Laboratories, Richmond, Calif.; *N,N'*-diallyltartardiamide was obtained from Aldrich Chemical Co., Milwaukee, Wis.; and Triton X-100 was obtained from Rohm and Haas, Philadelphia, Pa. Renografin (meglumine diatrizoate) was obtained as a 36% iodine equivalent solution from E. R. Squibb and Sons, Inc., N.Y. Medium 199 modified to contain 2 μg of L-isoleucine per ml, and dialyzed calf serum were obtained from Gibco, Berkeley, Calif. Proteins for molecular weight calibrations were bovine serum albumin, Miles Laboratories, Inc., Kankakee, Ill.; myosin from K. H. Seraydarian, and ovalbumin and gamma globulin from D. I. Meyer, both at the University of California, Los Angeles.

RESULTS

Protein labeling pattern of NDV-infected and uninfected chick cells. Preliminary studies of NDV-infected chick monolayers labeled for 2-h periods at different times after infection indicated that host protein synthesis inhibition was insufficient to avoid the use of a dual labeling technique to distinguish differences between infected versus uninfected cells. A similar approach to studying NDV-induced proteins has been employed by other workers (2, 12). Electrophoresis of NDV-infected cells labeled for 2 h with ^{3}H-isoleucine and uninfected cells labeled for 8 h with ^{14}C-isoleucine revealed the presence of three major peaks of excess ^{3}H corresponding to the positions of the three major virion polypeptides. The relative amounts of these three peaks remained essentially constant between 2 and 10 h postinfection (data not shown).

Figures 1a and b show the dual labeling patterns of an extract of uninfected chick cells labeled for 5 h with ^{14}C-isoleucine subjected to electrophoresis with extracts from uninfected cells pulse incubated for 10 min with ^{3}H-isoleucine. In Fig. 1a, cells were immediately processed for SDS-PAGE after the radioactive labeling period. In Fig. 1b, cells were subjected to a 30-min chase incubation in medium 199 IC containing 200 μg of unlabeled isoleucine per ml before processing for SDS-PAGE. The patterns of ^{3}H and ^{14}C radioactivity within each gel imply that the two isotopes are similarly incorporated and that polypeptide cleavage is not a general mechanism for protein maturation in uninfected chick cells. A similar conclusion for other cell systems has been reached previously (9). Differences in ^{14}C and ^{3}H patterns were completely eliminated when both isotopes were incorporated for the same time period (5 h). Differences in the labeling patterns of pulsed and pulsed-chased cells are significant and probably reflect dissimilar incorporation and turnover rates previously demonstrated for cellular proteins (17). The dual labeling patterns of extracts of cells infected with NDV and subjected to a 10-min pulse incubation with ^{3}H-isoleucine subjected to electrophoresis with extracts of uninfected cells labeled for 5 h with ^{14}C-isoleucine are shown in Fig. 2. Four major peaks of excess ^{3}H can be discerned. The mobilities of three of these correspond to the mobilities of the three major egg-grown virion protein classes, I, II, and III (listed in order of decreasing molecular weight). The novel peak, PII, located between I and II, and also peak II

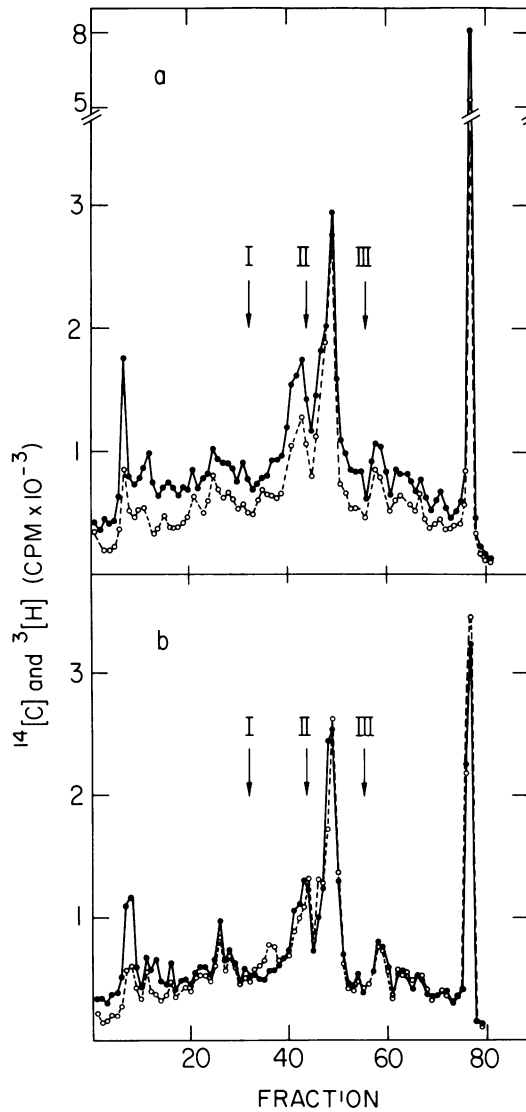
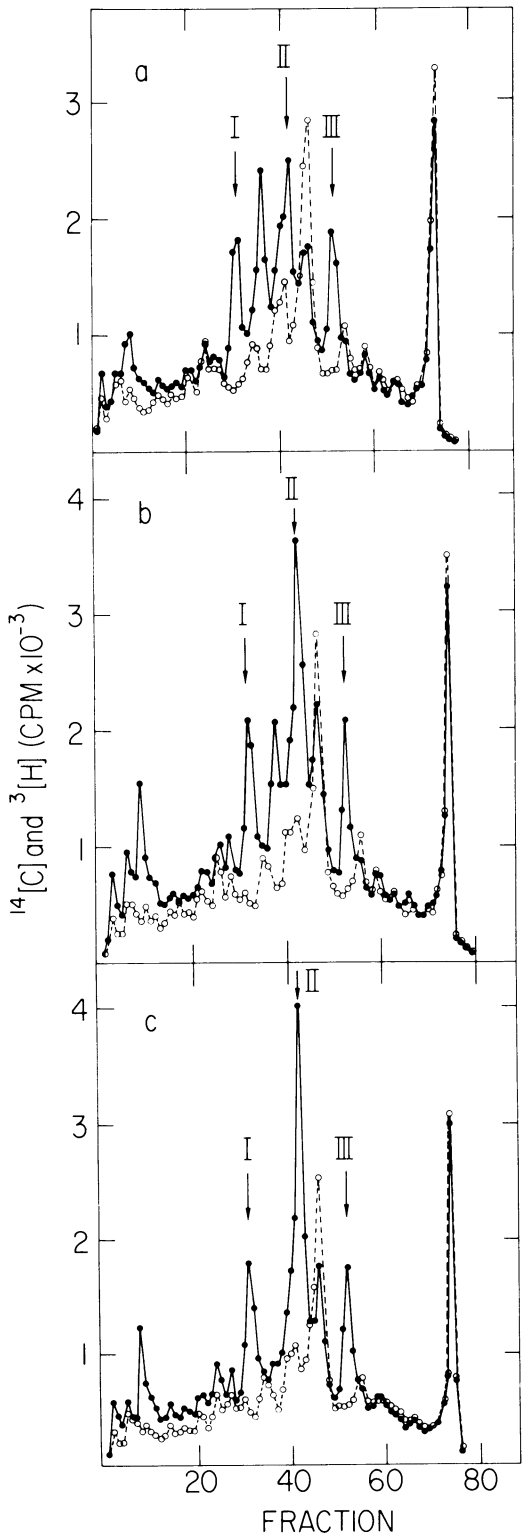


FIG. 1. *Electrophoresis of a mixture of proteins from uninfected chick cells labeled for 5 h with ^{14}C-isoleucine with proteins from uninfected chick cells labeled for 10 min with ^{3}H-isoleucine. a, Cells labeled with ^{3}H-isoleucine were processed for electrophoresis immediately after the 10-min pulse incubation (no chase). b, Medium containing ^{3}H-isoleucine was removed at the end of the 10-min pulse incubation and fresh medium 199 IC containing 200 μg of unlabeled isoleucine per ml was added for a further 30-min chase incubation. Cells were then processed as in (a). Symbols: ○, ^{14}C; ●, ^{3}H. Between 80 and 160 μg of protein was applied to each gel. Arrows I, II, and III are the predicted positions for the three major polypeptides in purified egg-grown NDV virions. In these and subsequent electrophoretic patterns, migration is towards the right (anode).*



vary in their relative amounts depending upon the length of the chase period after the pulse-label incubation (Fig. 2a, b, c). Increasing the chase period decreases the amount of radioactivity in peak PII (so named because it is a precursor to a class II polypeptide) and increases the amount of radioactivity in peak II. The data in Fig. 2a, b, and c are treated in Fig. 3a, b, and c, respectively. The ^{3}H radioactivity in NDV-infected cells is corrected for the contribution by host protein synthesis. Fig. 3a, b, and c show the above-mentioned relationships more clearly and also reveal that the two ^{3}H excess peaks, I and III, do not alter significantly in absolute amounts during chase periods of increasing times. An additional ^{3}H excess peak of high molecular weight is occasionally found near the top of the gel. This peak is not seen when the dialysis step is omitted and may represent aggregates of the various viral polypeptides.

Two ^{3}H-deficient troughs are also discerned. One lies between peaks II and III, and the other lies at the position of the tracking dye. These troughs indicate that the synthesis of certain host proteins is either depressed or that their turnover rates are increased after infection by NDV.

Kinetics of polypeptide PII disappearance and appearance of a class II polypeptide. The concomitant decrease in peak PII and increase in peak II, together with the identity of total excess counts computed for polypeptides PII plus II for the three chase incubation periods, 0, 10, and 30 min (Table 1), suggest a precursor role for PII, the product being a class II polypeptide. The molecular weight assignments in Table 1, calculated by using the calibration curve in Fig. 4, are only apparent, since the contribution of glycosylated moieties to electrophoretic mobilities of polypeptides in SDS gels is unknown. These estimates are in fair agreement with the data of other workers (4, 5, 15) for NDV virion polypeptides. Discrepancies may

FIG. 2. *Electrophoresis of a mixture of proteins from uninfected chick cells labeled for 5 h with ^{14}C-isoleucine with proteins from NDV-infected chick cells labeled for 10 min with ^{3}H-isoleucine 5.5 h after virus adsorption. a, Cells were not subjected to a chase incubation. b, Cells were subjected to a chase incubation for 10 min in medium 199 IC containing 200 μg of unlabeled isoleucine per ml. c, Chase incubation period was increased to 30 min. Symbols: ○, ^{14}C; ●, ^{3}H. Between 80 and 160 μg of protein per gel was applied. Arrows I, II, and III are the predicted positions for the three major polypeptides in purified NDV virions.*

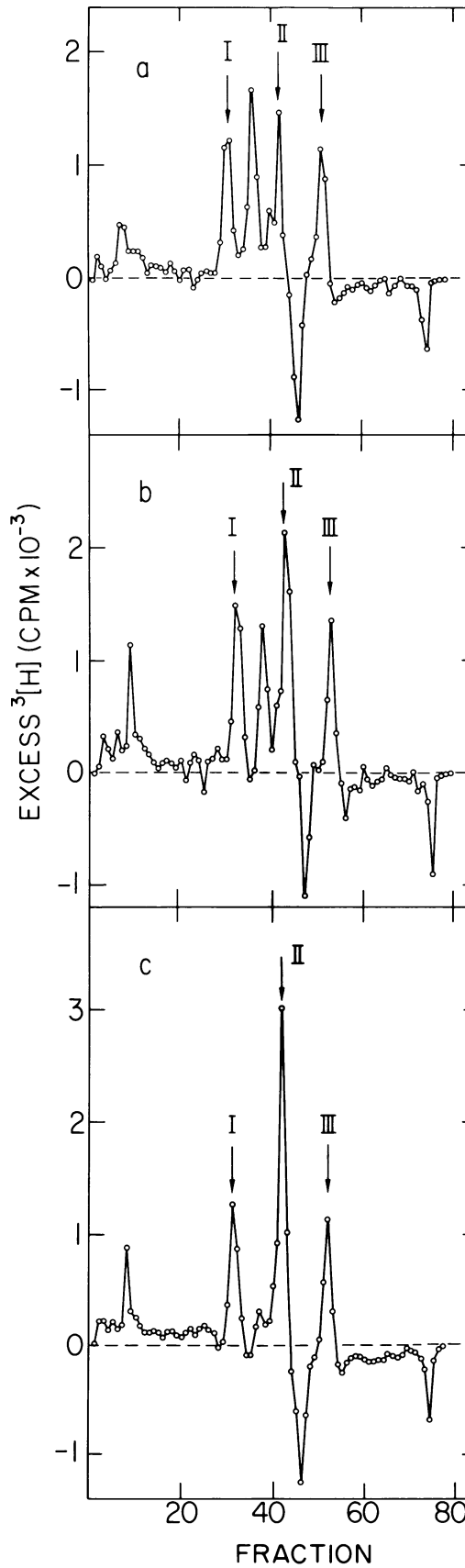


FIG. 3. *Difference profiles computed from the data in Fig. 2. The ^{14}C counts were normalized to the ^{3}H counts by multiplying by the $^{3}H/^{14}C$ ratio in the nonvariable ratio regions of the gel pattern and subtracting this normalized value from the ^{3}H counts in all fractions. Both positive and negative values are shown. Difference profiles (a), (b), and (c) were obtained from the dual label patterns depicted in Fig. 2a (10-min pulse), 2b (10-min pulse, 10-min chase), and 2c (10-min pulse, 30-min chase), respectively. Arrows I, II, and III are the predicted positions for the three major polypeptides in purified NDV virions.*

TABLE 1. *Molecular weights and kinetic data for NDV-induced polypeptides*

Polypeptide peak	Estimated mol wt × 10^{-3}	10-min pulse		10-min pulse, 10-min chase		10-min pulse, 30-min chase	
		Excess ^3H counts in peak (%)	Molar ratio[a]	Excess ^3H counts in peak (%)	Molar ratio[a]	Excess ^3H counts in peak (%)	Molar ratio[a]
I	78.4	23.0	1.0	21.4	1.0	20.4	1.0
PII	64.8	26.4 } 45.9	2.6	17.4 } 47.1	3.0	5.3 } 45.3	3.2
II	53.5	19.5 }		29.7 }		40.0 }	
III	37.6	19.6	1.8	15.5	1.5	16.2	1.7

[a] Minimum molar component I expressed as unity.

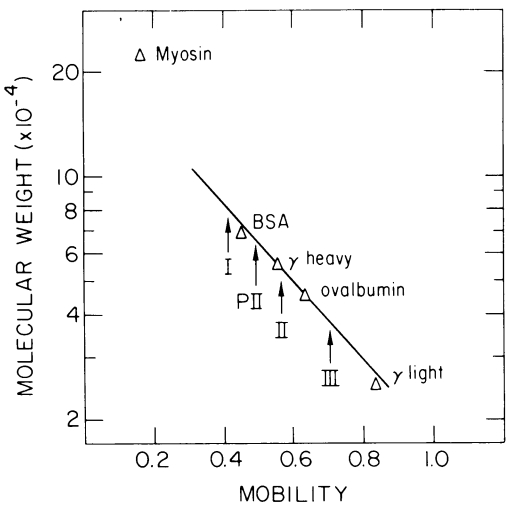


FIG. 4. *Molecular weight calibration curve. Arrows I, II, and III are positions of the three major polypeptides from purified egg-grown virions. Arrow PII is the position of polypeptide PII. Assumed mol wts are: myosin, 220,000; bovine serum albumin (BSA), 68,000; heavy chain of gamma globulin (γ heavy), 50,000; ovalbumin, 45,000; light chain of gamma globulin (γ light), 25,000. Mobility is relative to the tracking dye, bromophenol blue.*

reflect NDV strain or host differences, or the molecular weight values assumed for the standards for calibrating SDS gels.

Appearance of virion proteins on Coomassie Blue-stained acrylamide gels. Figure 5 compares the Coomassie Blue-stained gel patterns of SDS-disrupted egg-grown (i) virions, (ii) nucleocapsids, and (iii) nucleocapsid-depleted virions, and identifies the protein with electrophoretic mobility equal to that of the protein in region II as nucleocapsid protein. In addition, very little protein having the same electrophoretic mobility as nucleocapsid protein is observed in the nucleocapsid-depleted extract of these virions, and no protein band corresponding to PII is observed in purified egg-grown virions.

Labeling pattern of isolated nucleocapsid protein. If the product of polypeptide PII cleavage is indeed nucleocapsid protein and not one of the minor proteins having the same electrophoretic mobility as nucleocapsid protein, then the amount of radioactive label in pulse-labeled purified nucleocapsids should increase during a chase incubation period without radioactive amino acids.

Gel electrophoresis patterns of ^3H-isoleucine pulse-labeled and pulsed-chased isolated nucleocapsids are shown in Fig. 6a and b, respectively. First, an approximately 2.5-fold increase in radioactivity in peak II after a 30-min chase compared with no chase is observed, in fair agreement with the 2-fold increase shown in Table 1. Moreover no significant radioactivity in the region predicted for polypeptide PII is observed in the nucleocapsid material derived from cells not subjected to a chase incubation. This implies that PII is not transiently associated with nucleocapsid and later cleaved to polypeptide II. However, the possibility that polypeptide PII is associated with a pronucleocapsid having a lower sedimentation coefficient than mature nucleocapsid is not excluded.

DISCUSSION

Preliminary experiments involving labeling of NDV-infected chick cells with radioactive amino acids for 2-h periods and analysis of labeled proteins by SDS-PAGE indicated the presence of a small quantity of a polypeptide in addition to the three polypeptides observed in NDV L. Kansas virions.

An earlier study reported the appearance of a nonvirion polypeptide in cells infected by the L. Kansas, La Sota, or F strains of NDV labeled for 2 h with radioactive amino acids (12). In these three strains of NDV, the novel polypeptide occupied the same region of the gel pattern as polypeptide PII of this study. Other workers have reported a fourth protein, 1a (lying between polypeptides I and II of our study), in

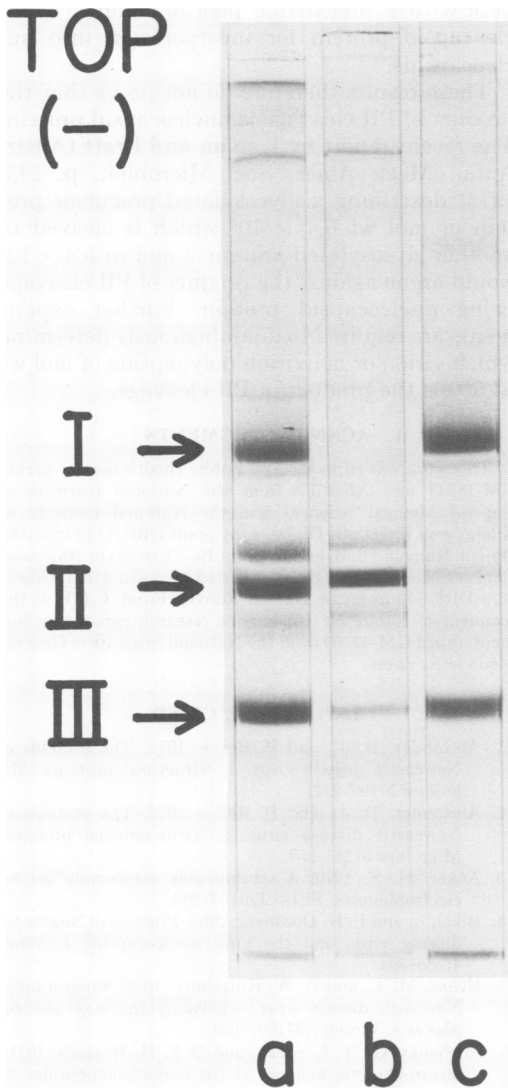


FIG. 5. *Appearance of polypeptides from fractionated and unfractionated virions on SDS polyacrylamide gels stained with Coomassie Blue. a, Unfractionated virions; b, nucleocapsid fraction; c, nucleocapsid depleted fraction. Purified egg-grown virions were disrupted in Triton X-100, sodium deoxycholate buffer, and nucleocapsids sedimented through a discontinuous cesium chloride gradient as described in Materials and Methods. Approximately 10 μg of protein was applied to each gel. Arrows I, II, and III indicate the three major virion polypeptides.*

virions isolated from chick monolayers infected with the N strain of NDV (18). Resolution on acrylamide gels was poor, however, and it remains to be seen if polypeptide Ia and PII are the same. A complex pattern of virus-induced polypeptides, depending upon the NDV strain used and the time after virus infection, was revealed in another recent study (2). Again 2-h labeling periods were used, and no precursor-product relationship between viral-induced proteins was discerned.

The experiments described in this study involved brief pulse-labeling periods and demonstrate the existence of a precursor-product relationship between the nonvirion polypeptide PII and a class II polypeptide. The rapid rate of disappearance of precursor peak PII and appearance of product peak II explains why such a

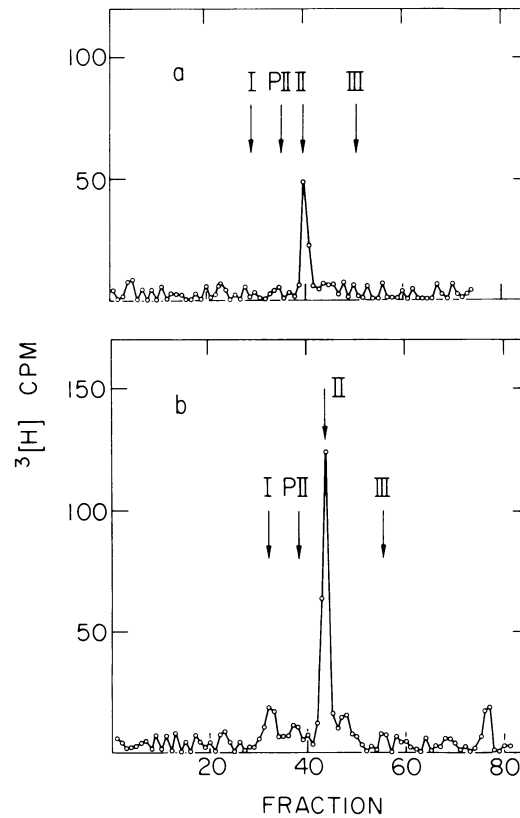


FIG. 6. *Electrophoresis of nucleocapsid material isolated from NDV-infected chick cells 10-min pulse labeled with ^{3}H-isoleucine 5.5 h after completion of virus adsorption. a, Cells were not subjected to a chase incubation. b, Cells were subjected to a 30-min chase incubation in medium 199 IC containing 200 μg of unlabeled isoleucine per ml prior to extracting nucleocapsid material. The material applied to each gel (approximately 7 μg of protein) was derived from an equivalent amount of radioactivity incorporated into total cellular material (60,000 counts/min). Arrows I, II, and III are the predicted positions for the three major polypeptides of purified virions; PII is the predicted position for the class II polypeptide precursor.*

relationship was not seen in studies which employed a 2-h labeling period.

The technique of dual labeling used here to distinguish between proteins synthesized in infected versus uninfected cells is valid if any effect of NDV upon host protein synthesis is nonspecific. That this is not always the case is shown by the existence of ^{3}H-deficient troughs after SDS-PAGE of proteins from cells infected with NDV and pulse labeled with ^{3}H-isoleucine (Fig. 3a, b, c). Viral-induced proteins in the trough regions could remain undetected. Furthermore, regions without troughs or peaks could conceivably contain a viral-induced peak masked by an equivalent trough arising from selective inhibition of synthesis of host proteins. Despite these reservations, it is seen that the three major virion polypeptide classes are clearly distinguished by this method. In addition, a nonvirion polypeptide PII is detected in cells labeled for a short period with radioactive amino acids.

The susceptibility of mature nucleocapsid protein to proteolytic cleavage with trypsin has been demonstrated for several paramyxoviruses, including NDV (14). Cells infected with NDV and subsequently removed from dishes by trypsin treatment (but not by disodium EDTA alone) show discrete degradation of the isolated nucleocapsid protein (14). The conversion of polypeptide PII to a class II polypeptide occurs in the absence of trypsin. (Trypsin was used at an earlier stage in the preparation of chick primary and chick secondary cultures, but extensive washing of trypsinized cells prior to seeding for secondary cultures had no effect on the polypeptide PII to II conversion.) In addition, no further conversion of the class II polypeptide to a trypsin-degraded nucleocapsid form was detected in the experiments reported here. The conversion of PII to II is likely to involve a proteolytic cleavage step mediated by either a host or a viral-induced enzyme.

A delay in incorporation of pulse-labeled radioactive protein into mature viral nucleocapsid was recently reported for the Beaudette strain of NDV (8). A delay of 30 min before maximal incorporation of pulse-labeled protein into nucleocapsids is in good agreement with the kinetic data of polypeptide PII conversion to a class II polypeptide (Table 1). This delay in incorporation of pulse-labeled radioactive protein into nucleocapsid may be a consequence of PII cleavage to nucleocapsid protein. Alternatively, the nucleocapsid protein labeled during the pulse incubation period may have to compete with a preexisting pool of unlabeled nucleocapsid protein for incorporation into nucleocapsids.

These results therefore do not prove that the product of PII cleavage is nucleocapsid protein. The recent report by Kaplan and Bratt (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 243, 1973) describing a glycosylated precursor protein of mol wt 6.4×10^4 which is cleaved to another glycosylated protein of mol wt 5.4×10^4 would argue against the product of PII cleavage being nucleocapsid protein. Further experiments are required to unambiguously determine which virion or nonvirion polypeptide of mol wt 53,500 is the product of PII cleavage.

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LITERATURE CITED

1. Alexander, D. J., and P. Reeve. 1972. The proteins of Newcastle disease virus. 1. Structural proteins. Microbios **5**:199-212.
2. Alexander, D. J., and P. Reeve. 1972. The proteins of Newcastle disease virus. 2. Virus-induced proteins. Microbios **5**:247-257.
3. Anker, H. S. 1970. A solubilizable acrylamide gel for electrophoresis. FEBS Lett. **7**:293.
4. Bikel, I., and P. H. Duesberg. 1969. Proteins of Newcastle disease virus and the viral nucleocapsid. J. Virol. **4**:388-394.
5. Evans, M. J., and D. W. Kingsbury. 1969. Separation of Newcastle disease virus by polyacrylamide gel electrophoresis. Virology **37**:597-604.
6. Fairbanks, G., T. L. Steck, and D. F. H. Wallach. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10**:2606-2617.
7. Haslam, E. A., I. M. Cheyne, and D. O. White. 1969. The structural proteins of Newcastle disease virus. Virology **39**:118-129.
8. Kaverin, N. V. 1972. Delay in the incorporation of protein into virus nucleocapsid in Newcastle disease virus infected cells. J. Gen. Virol. **17**:337-341.
9. Kiehn, E. D., and J. J. Holland. 1968. Multiple components of mammalian cell membranes. Proc. Nat. Acad. Sci. U.S.A. **61**:1370-1377.
10. Kingsbury, D. W., and R. W. Darlington. 1968. Isolation and properties of Newcastle disease virus nucleocapsid. J. Virol. **2**:248-255.
11. Laemmli, U. K. 1971. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
12. Lomniczi, B., A. Meager, and D. C. Burke. 1971. Virus RNA and protein synthesis in cells infected with different strains of Newcastle disease virus. J. Gen. Virol. **13**:111-120.

13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
14. Mountcastle, W. E., R. W. Compans, L. A. Caliguiri, and P. W. Choppin. 1970. Nucleocapsid protein subunits of simian virus 5, Newcastle disease virus, and Sendai virus. J. Virol. **6**:677-684.
15. Mountcastle, W. E., R. W. Compans, and P. W. Choppin. 1971. Proteins and glycoproteins of paramyxoviruses: a comparison of simian virus 5, Newcastle disease virus, and Sendai virus. J. Virol. **7**:47-52.
16. Scheid, A., and P. W. Choppin. 1973. Isolation and purification of the envelope proteins of Newcastle disease virus. J. Virol. **11**:263-271.
17. Schimke, R. T., and D. Doyle. 1970. Control of enzyme levels in animal tissues. Ann. Rev. Biochem. **39**:929-976.
18. Shapiro, S. C., and M. S. Bratt. 1971. Proteins of four biologically distinct strains of Newcastle disease virus. Proc. Soc. Exp. Biol. Med. **136**:834-838.