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Polypeptides of Mumps Virus

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Mumps virus was propagated in the extra-embryonic fluids of embryonated chicken eggs and was labeled by coinjection of radioactively labeled amino acids. The virus was purified by density gradient centrifugation, and its polypeptides were analyzed by polyacrylamide gel electrophoresis. The virus was found to be composed of six polypeptides, ranging in size from 40,000 to 64,000 daltons. Viral proteins 1 and 3 were the glycoproteins of the virons. When the virus particle was treated with nonionic detergents, a small fraction of these glycoproteins could be released into the supernatant. After treatment with nonionic detergents in high salt and alkaline conditions, more of the surface glycoproteins were removed. This treatment also released the smallest viral polypeptide from the virion. The glycoproteins were separated using an affinity chromatographic column of agarose-fetuin. The heavier glycoprotein, viral protein 1, was found to contain both the neuraminidase and hemagglutinating activity. The two glycoproteins were tested for their ability to react in complement-fixing tests with mumps antisera. Only the heavier glycoprotein reacted with antisera possessing both anti-S and anti-V activity. Neither glycoprotein reacted with antisera specific for the S antigen. Thus, it was concluded that this glycoprotein corresponds to the classical V antigen of mumps virus.

The etiologic agent of mumps, or epidemic parotitis, was first identified in 1935 as a filterable agent by Johnson and Goodpasture (16). By 1960 the virus had been shown to belong to the paramyxovirus group on the basis of its morphology, the sensitivity of its infectivity to ether, its ability to agglutinate certain erythrocytes, the presence of an enzyme neuraminidase on the virus, its ability to induce erythrocyte hemolysis and to induce cell fusion in vitro, and the serological cross-reaction between mumps virus and other members of the paramyxovirus group, such as Newcastle disease virus (NDV), Sendai virus, simian virus 5 (SV5), and measles virus (7).

Preliminary serological analysis of the mumps virus particle revealed the presence of at least two antigens (13)—a viral-associated, or V, antigen which remained associated with the virus particle after centrifugation of homogenates of virus-infected cells, and a soluble, or S antigen, which also was associated to some degree with the virus particle, but which also remained in the supernatant after centifugation of similar cell homogenates. During the course of mumps virus infections, antibody to the S antigen appeared first, whereas anti-V antibodies appeared only later (13).

Further biochemical investigations into the structure and replication of mumps virus were hampered by the comparative inefficiency of mumps virus replication in vitro (7). East and Kingsbury (10) obtained initial results on the nucleic acid composition and replicative mechanism of mumps virus replication in chicken embryo lung cells. They demonstrated that the virion contained a 50 to 55*S* single-stranded RNA as the parental genome; they also demonstrated the accumulation of subgenomic fragments of RNA complementary to the parental RNA molecules. Bernard and Northrop (5) recently demonstrated the existence of an RNA-dependent RNA polymerase in chicken egg-grown mumps virus. These findings were in agreement with results from work with more thoroughly studied paramyxoviruses such as NDV and SV5 (17). This paper presents the preliminary results of further biochemical analyses of mumps virus, and it was presented by S.C.J. in partial fulfillment of the requirements for the Doctor of Philosopy degree in the graduate College, University of Illinois, 1974.

MATERIALS AND METHODS

Buffers. TSV buffer contained 0.01 M Tris-hydrochloride (pH 7.4), 0.1 M NaCl, and 0.001 M EDTA.

BKE buffer contained 0.1 M boric acid Tris buffer (pH 8.5), 0.002 M EDTA, and 0.1 M KCl. VBD (veronal-buffered diluent) was prepared as described in Lennette and Schmidt (20).

Chemicals and isotopes. Bovine serum albumin, urease, chymotrypinogen, ovalbumin, ^{3}H-labeled glucosamine (5 to 10 Ci/mmol), and ^{3}H-labeled leucine (30 to 50 Ci/mmol) were purchased from Schwartz-Mann (Orangeburg, N.J.). Sodium dodecyl sulfate was purchased from the Matheson, Coleman, and Bell Co., Norwood, Ohio. Pronase and human glycoprotein #6 were purchased from Calbiochem, Los Angeles, Calif. Triton N-101 was purchased from the Sigma Chemical Co., St. Louis, Mo. Acrylamide, *N,N*-methylene bisacrylamide, *N,N,N',N''*-tetramethylethylene diamine, 2-mercaptoethanol, and ammonium persulfate were purchased from the Bio-Rad Laboratories, Richmond, Calif. Cyanogen bromide-activated Sepharose-4B was obtained from Pharmacia Fine Chemicals, Piscataway, N.J. Lyophilized complement was purchased from the Cordis Company, Miami, Fla. Fetuin and trypsin were obtained from the Grand Island Biological Co., Grand Island, N.Y. Antisera specific for mumps virus containing both anti-S and anti-V activity obtained from guinea pigs were purchased from Microbiological Associates, Bethesda, Md.

Virus. The Jo Ann strain of mumps virus was utilized in these experiments. This strain was an isolate of the Jones strain (4, 28) which was serially passed in embryonated chicken eggs. The chicken eggs used for virus propagation were avian leukosis-free and were purchased from Spafas, Norwich, Conn.

Virus production in ovo. Seven-day-old leukosis-free embryonated chicken eggs were inoculated intra-allantoically with $10^{4.0}$ to $10^{5.0}$ mean egg infectious doses in 0.2 ml. The eggs were incubated in a humid atmosphere at 36 C for 6 days and then cooled at 4 C overnight. The amniotic and allantoic fluids were collected the next days and large debris was removed by centrifugation at 1,500 $\times$ *g* for 20 min at 4 C.

Purification of mumps virus. After removal of cellular debris from the egg fluids, the virus was pelleted by centrifugation in an SW27 rotor (Beckman Instrument Co., Palo Alto, Calif.) at 25,000 rpm for 1 h at 4 C. The pelleted virus was resuspended in approximately 1/25 the original volume in 10% (wt/wt) sucrose in TSV, and the large aggregates of virus were easily disrupted by homogenization with several strokes in a Dounce homogenizer. The virus suspension was then layered onto a discontinuous zone gradient of 50% (wt/wt) sucrose in TSV overlaid by 25% (wt/wt) sucrose in TSV. This material was centrifuged in a Beckman SW41 rotor at 40,000 rpm for 1 h at 4 C. The virus formed a dense layer on top of the 50% sucrose layer, and it could easily be removed with a Pasteur pipette. Afterwards the virus was dialyzed against 500 volumes of TSV at 4 C for 16 h. This virus was then layered onto a linear 10 to 50% (wt/wt) sucrose in TSV gradient which had been preformed by layering in succession 1.8 ml of 60, 50, 40, 30, 20, and 10% (wt/wt) sucrose in TSV solutions and allowing this zone gradient to diffuse overnight at 4 C. This gradient, after the virus had been added, was centrifuged in an SW41 rotor at 40,000 rpm for 150 min at 4 C, and the virus was collected after fractionation with an ISCO model D fractionator (Instrumentation Specialty Co., Lincoln, Neb.) with continuous monitoring at 254 nm. Virus concentration was assayed by the microtiter hemagglutination method of Sever (27). Viral protein determinations were done by the method of Lowry et al. (21).

Production of radioactive virus in embryonated chicken eggs. Approximately 36 h after inoculation, eggs were recandled to ascertain continued viability, and they were then injected with either 100 μCi of [^{3}H]leucine or 100 μCi of [^{3}H]glucosamine. The eggs were reincubated at 36 C, the fluids were harvested 5 days later, and the radioactive virus was collected and purified as described above.

The gradients were fractionated into 1-ml fractions by the same procedure as described previously, and from each fraction 5 μl was assayed for acid-insoluble radioactivity by the addition of 0.1 mg of bovine serum albumin and trichloroacetic acid to a final concentration of 5% (wt/vol). After incubating the fractions for 15 min at 4 C, the precipitates were collected on cellulose nitrate filters (Scheicher and Schuell, Keene, N.H.) and washed with 5% trichloroacetic acid. The filters were dried and counted in a liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.) using a toluene-based scintillation fluid containing 0.6% (wt/vol) Omnifluor (New England Nuclear, Boston, Mass.).

Preparation of samples for polyacrylamide gel electrophoresis. Purified mumps virus was prepared for and assayed on polyacrylamide gels by methods previously described (22). Viral proteins which remained in solution and which could not be pelleted by centrifugation were dialyzed against 0.01 M sodium phosphate buffer (pH 6.0) for 16 h at 4 C. Five volumes of cold absolute alcohol were added to the dialysate and the samples were incubated at -20 C for at least 12 h. The precipitated proteins were then centrifuged in the Beckman J 7.5 rotor at 5,000 rpm for 30 min at 4 C. The pelleted protein precipitates were dried in vacuo and resuspended in the same electrophoresis buffer as described above. This procedure was found to be an excellent method to separate the proteins from the nonionic detergents, since dialysis and exclusion chromatography on Sepharose 2B were found to be unsatisfactory for this prupose.

The gels were then subjected to electrophoresis at 5 mA/gel for 14 h at room temperature. Gels which were to be examined for radioactivity were cut into approximately 1-mm slices with a gel slicer from Diversified Scientific Instruments, San Leandro, Calif., and each fragment was dispensed into a scintillation vial (Beckman Instrument Co.). To each vial was added 10 ml of toluene containing 0.6% (wt/vol) Omnifluor and 0.3% (vol/vol) Protosol (New England Nuclear, Boston, Mass.). The gel slices were incubated at 60 C for at least 12 h, allowed to cool, and counted in a Packard liquid scintillation counter.

Unlabeled proteins which were used as markers for molecular weight determinations included urease,

bovine serum albumin, ovalbumin, and chymotrypsinogen (subunit molecular weight 83,000, 68,000, 46,000, and 25,000, respectively). After electrophoresis the gels were stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.) by a procedure outlined by Maizel (22). Gels containing viral protein which were to be analyzed for glycoprotein were stained by the periodic-acid Schiff method of Fairbanks et al. (11). These latter gels were scanned on a spectrophotometer (Beckman Acta V) possessing an appropriate adapter for gel use.

Treatment of mumps virus with detergents. Purified mumps virus was dialyzed against 500 volumes of one of four buffers: (i) TSV, (ii) TSV with 2.0 M KCl, (iii) 0.02 M sodium bicarbonate (pH 10.0), or (iv) 0.02 M sodium bicarbonate (pH 10.0), containing 2.0 M KCl. The virus was dialyzed for 16 h at 4 C. Afterwards the dialysates were allowed to warm to room temperature and Triton N-101 was added to a final concentration of 2% (vol/vol). The viral suspensions remained at room temperature for 30 min and were then centrifuged in the Beckman 40.2 rotor at 40,000 rpm for 30 min at 4 C. The supernatants were removed and the pellets were resuspended in an equal volume of TSV. The supernatants were dialyzed against 500 volumes of TSV for 16 h at 4 C. After dialysis the supernatants and pellets were assayed for hemagglutinating activity as described previously. Neuraminidase activity was assayed only after supernatant and pellet preparations had been dialyzed against 500 volumes of 0.02 M sodium phosphate buffer (pH 6.0). The supernatant and pellet fractions were prepared for polyacrylamide gel analysis by the alcohol precipitation method described previously; this proved an excellent method to remove the nonionic detergent from the specimen prior to electrophoresis.

Preparation of agarose-fetuin for affinity chromatography. The agarose-fetuin gel was prepared by the method outlined by Becht and Rott (3). Sepharose 4-B, which had been activated by cyanogen bromide, was allowed to swell for 1 h in 0.1 M boric acid-NaOH buffer (pH 9.0), 0.1 M NaCl. Freeze-dried fetuin (500 mg) was then dissolved in 25 ml of the above buffer and added to an equal volume of the packed agarose beads. The fetuin was coupled to the resin under gentle stirring for 48 h at 4 C; afterwards it was stirred for 2 h at room temperature in 0.1 M Tris-glycine buffer (pH 8.2). The agarose-fetuin was washed successively with BKE, BKE with 3.0 M KCl, with more BKE, and finally it was equilibrated with BKE containing 1.0% Triton N-101.

Preparation of the virus for affinity chromatography. Purified mumps virus was dialyzed against 500 volumes of 0.02 M sodium bicarbonate buffer-2.0 M KCl (pH 10.0). Detergent was added as described previously, and after a similar 30-min incubation period the undisrupted virus was removed by centrifugation. The supernatant was dialyzed against 500 volumes of BKE containing 1.0% Triton N-101 overnight at 4 C. During this dialysis a precipitate formed which was removed by centrifugation in the 40.2 rotor at 10,000 rpm for 15 min at 4 C.

The supernatant was layered onto a column of the agarose-fetuin. The column was then washed with BKE containing 1.0% Triton N-101 at a temperature of 4 C; 1-ml fractions were collected and tested for radioactivity by diluting 100 μl of the sample with 500 μl of water and 10 ml of a scintillation cocktail A 70 from RPI, Elk Grove Village, Ill. After no further radioactivity could be detected in the effluent, the column was transferred to a 37 C incubator and eluted with prewarmed BKE containing 1.5% M KCl. Fractions (1 ml) were collected again until no additional absorbed radioactive material could be eluted from the column. The fractions containing the radioactivity were pooled and dialyzed against 0.01 M sodium phosphate buffer (pH 6.0), so as to lower the high salt concentration prior to preparation for acrylamide gels or biological activity assays as described previously.

Serological tests. Hemagglutination, hemagglutination-inhibition, and complement fixation tests were performed by the microtiter methods outlined by Sever (27). The presence of the nonionic detergent in the samples caused erythrocyte lysis, so this was removed by the alcohol precipitation of proteins. The samples were resuspended or dialyzed against VBD.

Neuraminidase assays. Free neuraminic acid was measured by the thiobarbiturate procedure of Aminoff (2). Human glycoprotein fraction #6 was used as a substrate; the use of commercial fetuin was unsatisfactory due to the presence of free neuraminic acid in certain lots of the preparation, whereas the use of the purified substrate, neuraminlactone, was restricted for reasons of expense. Each assay contained 100 μl of the particular sample in 0.01 M sodium phosphate (pH 6.0), 100 μl of 0.1 M sodium acetate buffer (pH 5.5) with 0.002 M $CaCl_2$, and 100 μl of substrate solution consisting of the glycoprotein solution of 10 μg/ml in 0.01 M sodium phosphate buffer (pH 6.0). The reaction mixtures were incubated at 37 C, and the reactions were terminated by the addition of 100 μl of 9.0 M phosphoric acid. This mixture was allowed to sit at room temperature for 20 min, after which time 1 ml of 10% (wt/wt) sodium arsenite was added. The samples were shaken until the reddish brown color disappeared, and then 3 ml of 0.6% thiobarbituric acid in 10% (wt/wt) sodium sulfite solution was added. The reaction mixtures were incubated at 100 C for 15 min. After cooling, 4 ml of cyclohexanone was added and the mixtures were shaken thoroughly. The red chromophore formed by the combination of the *N*-acetyl neuraminic acid and the thiobarbituric acid was extracted into the organic phase; after centrifugation this layer was removed by pipette and the absorbency was measured at 549 nm on a Zeiss PMQII spectrophotometer (Carl Zeiss, Oberkochen, W. Germany).

RESULTS

Purification of mumps virus. After the egg fluids had been harvested, the virus was concentrated by pelleting in SW27 rotors as described. The pellets of viral material were easily resuspended with a Dounce homogenizer; no delete-

rious effect on viral infectivity was noted. After further purification on discontinuous zone gradients, the virus was centrifuged on linear 10 to 60% sucrose gradients. A typical gradient profile is shown in Fig. 1. The radioactive label corresponds well with the pattern of absorbancy at 254 nm. Previous work has shown that allantoic and amniotic fluid from uninfected eggs contains no material which sediments in this region of the sucrose gradient (15).

Polyacrylamide gel electrophoresis of mumps virus. Mumps virus was labeled in ovo with ^{3}H-labeled leucine. The virus was purified and examined on polyacrylamide gels as described in Materials and Methods. Figure 2 shows the electropherogram of the polypeptides of the mumps virus particle. There are six peaks of radioactively labeled mateial, and the size range of these polypeptides was from 40,000 to 64,000 daltons. The pattern of radioactivity was identical whether the virions were labeled with radioactive leucine or with an amino acid mixture. Table 1 shows the estimated molecular weights of the viral polypeptides.

To determine which of the polypeptides were glycoproteins, mumps virus was labeled with [^{3}H]glucosamine and purified by the usual methods. Figure 3A demonstrates the pattern of radioactivity; two peaks are evident, and by comparison to the pattern of radioactivity of viral polypeptides labeled with [^{3}H]leucine, it is seen that viral proteins 1 and 3 correspond to the peaks containing the glycoprotein label. The large amount of radioactivity at the top of the gel was thought to represent aggregation of labeled virus. Gels of unlabeled mumps virus were stained for glycoproteins. Figure 3B shows the graph of the densitometer tracing of a representative gel; by comparison of Fig. 3A and B it can be seen that the viral proteins 1 and 3 are the only glycoproteins of the virus. The peak of absorbancy of fractions 14 and 15 (Fig. 3B) was thought to be due to artifactual aggregates.

Detergent treatment of mumps virus. From

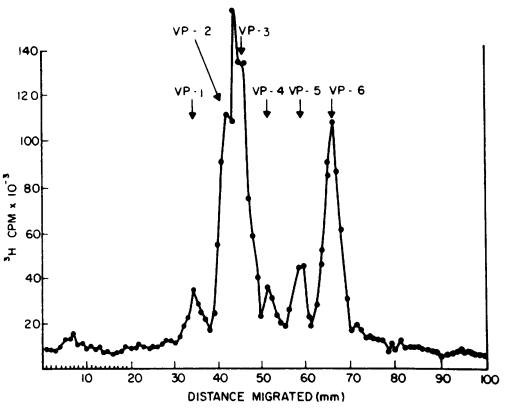


FIG. 2. *Polyacrylamide gel electrophoresis of mumps virus labeled with [^{3}H]leucine. Mumps virus was labeled with [^{3}H]leucine, purified, and analyzed on acrylamide gels. The gels were cut and analyzed for radioactivity as described in Materials and Methods.*

TABLE 1. *Molecular weights of polypeptides of mumps virus*[a]

Viral polypeptide	Mol wt
VP-1	66,000
VP-2	58,000
VP-3	56,000
VP-4	48,000
VP-5	44,000
VP-6	40,000

[a] Four proteins with polypeptide chains of known molecular weight (urease, 83,000; bovine serum albumin 68,000; ovalbumin, 48,000; and chymotrypsinogen, 25,000) were subjected to electrophoresis on individual polyacrylamide gels in a manner similar to that described for the viral proteins (see Fig. 2). The distance each marker protein migrated was plotted against the logarithm of the molecular weight and the migration of the six polypeptides of the mumps virion was then used to calculate the approximate molecular weight.

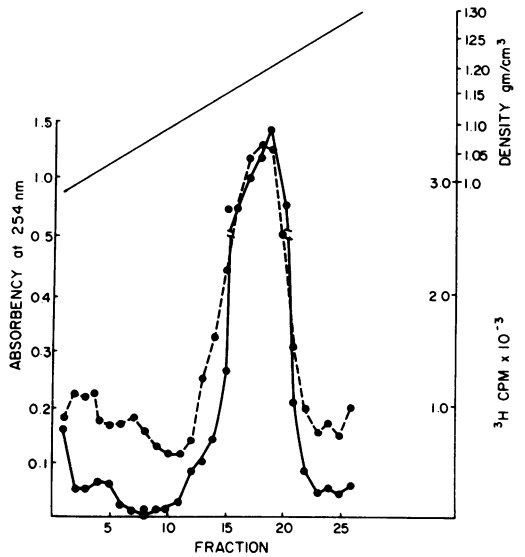


FIG. 1. *Isopycnic density gradient centrifugation of radioactively labeled mumps virus. Isopycnic sucrose density gradient of [^{3}H]leucine-labeled mumps virus. Symbols: —●—, absorbancy at 254 nm; - -●- -, ^{3}H radioactivity in counts per minute per 50 μl; ——, density. The density determinations were determined on an Abbe-3L refractometer (Bausch and Lomb, Inc., Skokie, Ill.).*

previous work (8, 24, 25) it is known that the treatment of paramyxoviruses such as NDV and SV5 with nonionic detergents results in the release of surface glycoproteins from the viral envelope. It was assumed that such agents would have a similar effect on the glycoproteins of mumps virus, and Triton N-101 was selected to effect virus disruption. Table 2 presents the results of Triton N-101 treatment on mumps virus under varying conditions of ionic strength and pH. The elevated salt concentration was chosen on the basis of the work of Scheid et al. (24, 25), who demonstrated that paramyxoviruses were disrupted more completely in the presence of high salt. An alkaline solution (pH 10.0, 0.02 M sodium bicarbonate) was assessed on the basis of results from both Webster and Darlington (30) and Hosaka (14), who presented evidence that paramyxoviruses were more thoroughly disrupted by detergent at high pH. The effectiveness of detergent disruption at each of the conditions was determined by several methods. From the work of Laver (18) it was known that the persistence or presence of hemagglutinating activity in a preparation of viral surface glycoproteins was an indication that the viral surface proteins had not been completely disrupted. If the glycoproteins were not completely solubilized into individual units, then there were probably still mixed aggregates of glycoproteins in solution and further attempts to separate the individual proteins would not be successful. Table 2 shows that there was no residual hemagglutinating activity in the supernatants after detergent treatment.

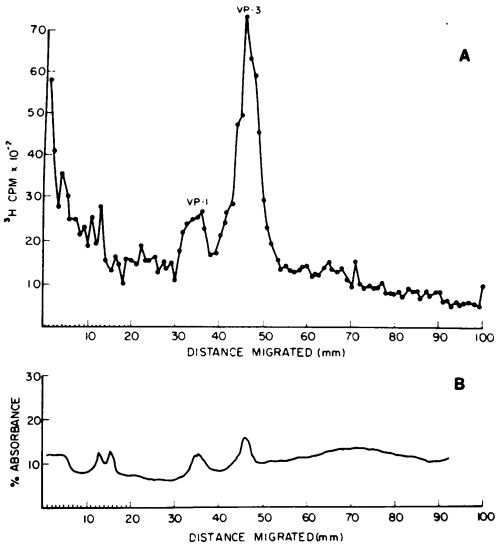


FIG. 3. *Polyacrylamide gel electrophoresis and densitometer tracing of acrylamide gels of mumps virus. A, Mumps virus was labeled with [^{3}H]glucosamine, purified, and analyzed on acrylamide gels as described. B, Unlabeled mumps virus was subjected to electrophoresis on acrylamide gels and the gels were then stained with periodic acid-Schiff's technique. After staining, the gels were scanned for absorbancy at 540 nm on a Beckman Acta V spectrophotometer. The gels were scanned with a full scale of 1.000, a slit width of 0.2 mm, and a scan speed of 6.0 cm/min.*

Neuraminidase assays were done for two reasons. The first was to guarantee that the biological activity of the viral glycoproteins was not destroyed by the detergent treatment. Treatment of paramyxoviruses with certain detergents, such as sodium dodecyl sulfate, results in complete and irreversible loss of biological activity. Thus the presence of neuraminadase activity in the supernatant preparations (Table 2) is evidence that the Triton N-101 did not result in complete loss of biological activity. In addition, it indicates the relative effectiveness

TABLE 2. *Comparison of the relative efficiency of disruption of mumps virus by Triton N-101 under various conditions*[a]

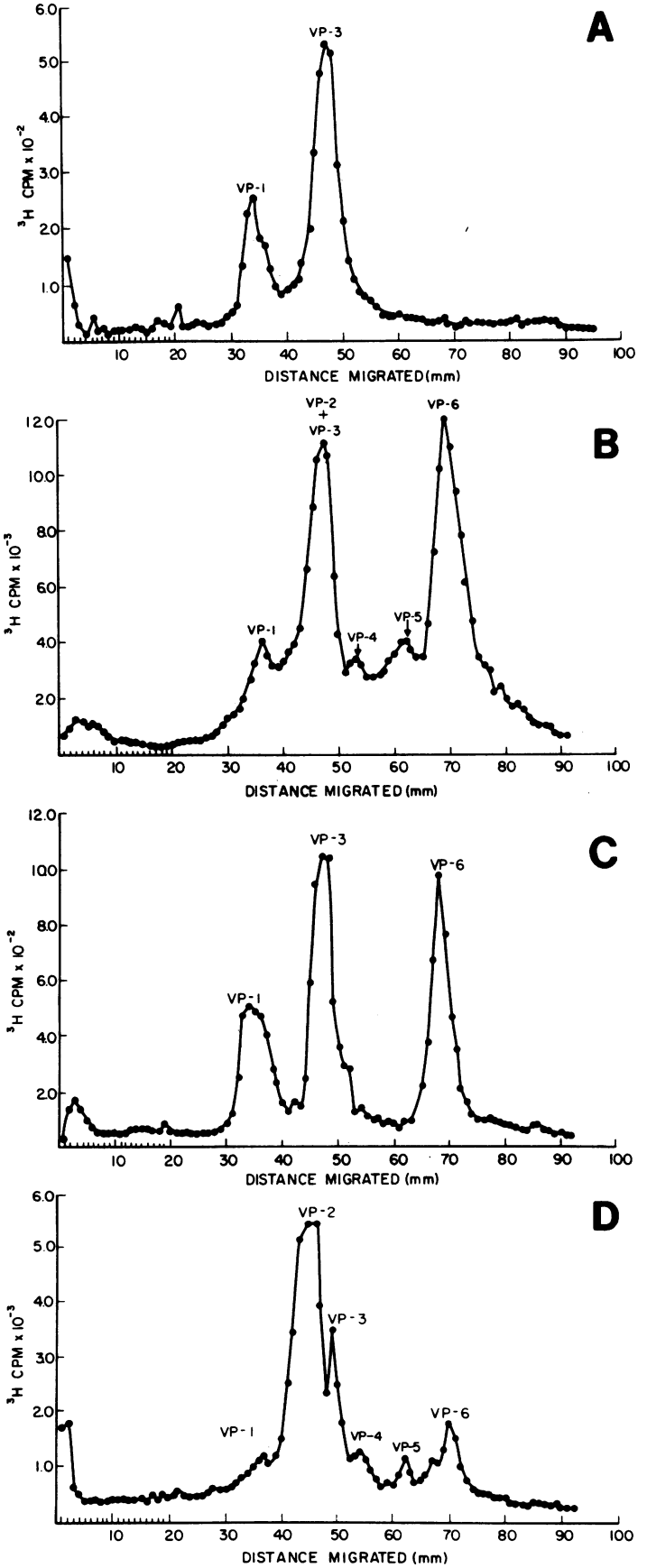
Procedure	Supernatant			Pellet		
	HA	Neuraminidase[b]	Counts/min	HA	Neuraminidase[b]	Counts/min
Control	<2	0	50	256	12.0	3,000
2% TN-101[c]	<4	1.9	900	64	ND[d]	2,500
2% TN-101, 2.0 M KCl	<4	2.2	1,000	ND	ND	2,200
2%TN-101, 0.02 M bicarb (pH 10.0)	<4	2.1	1,200	ND	ND	2,000
2% TN-101 with 0.02 M bicarb (pH 10.0), 2.0 M KCl	<4	4.2	1,600	ND	ND	1,800

[a] Purified mumps virus was treated with detergent in the various procedures described. A small amount of virus labeled with [^{3}H]leucine was added to give a total final radioactivity of approximately 4,000 counts/min. The viral preparations were centrifuged as before and the supernatants and pellets were assayed for hemagglutination, neuraminidase, and radioactivity as described in the text.

[b] Neuraminidase activity was expressed as the absorbancy at 549 nm.

[c] TN-101, Triton N-101.

[d] ND, Not done.


A
VP-3
VP-1
^{3}H CPM x 10^{-2}
DISTANCE MIGRATED(mm)
B
VP-2
+
VP-3
VP-6
VP-1
VP-4
VP-5
^{3}H CPM x 10^{-3}
DISTANCE MIGRATED (mm)
C
VP-3
VP-6
VP-1
^{3}H CPM x 10^{-2}
DISTANCE MIGRATED (mm)
D
VP-2
VP-3
VP-1
VP-4
VP-5
VP-6
^{3}H CPM x 10^{-3}
DISTANCE MIGRATED (mm)

of the detergent treatments; it was assumed that the more neuraminidase in the supernatant, the more glycoproteins had been removed from the viral envelope. Thus, treatment of mumps virus with detergent at both high salt and a high pH results in more released neuraminidase activity from the viral particle (Table 2).

This assumption was supported by further experiments in which a small amount of radioactively labeled virus was added to the viral preparations prior to detergent treatment. Table 2 shows that relatively more of the viral proteins were released into the supernatant when the virus was treated with detergent at high ionic strength and at high pH.

Polyacrylamide gel electrophoresis of the supernatants and pellet fractions of detergent-treated mumps virus. Figure 4A-D shows the electrophoretic patterns of the supernatants and pellets of mumps virus after treatment with Triton N-101 under varying conditions. Treatment of the virus with the detergent released the two glycoproteins from the virus, but only a relatively small percentage of the surface glycoproteins was solubilized, and after centrifugation the viral pellet is seen to contain most of the surface protein (Fig. 4A and B). When the virus was treated with detergent at high salt concentration at a high pH, there was a considerable increase in the proportion of viral surface proteins which had been released (Fig. 4C and D). In addition, the low-molecular-weight polypeptide viral protein 6 (VP-6) had also been solubilized. Nevertheless, despite the improved release of viral surface glycoproteins under high salt and alkaline conditions, there was still significant retention of surface proteins on the viral envelope. The work of Scheid et al. (24, 25) demonstrated that all the surface glycoproteins of SV5 and NDV were removed under similar conditions. Numerous attempts to further improve the yield of viral surface proteins, such as with concentrations of detergent up to 20% (vol/vol) and temperatures to 37 C, were unsuccessful with mumps virus.

Separation of viral glycoproteins. The surface proteins of NDV and SV5 were successfully separated by sucrose density gradient centrifugation, as demonstrated by the work of Scheid (24, 25). Numerous attempts to reproduce this excellent separation for the surface glycoproteins of mumps virus by comparable centrifugation techniques were never successful. Therefore, the affinity chromatography procedure of Becht and Rott (3) was attempted. Virus was disrupted with detergent in high salt and at alkaline pH; after centrifugation to remove the undisrupted virus, the supernatant was dialyzed against BKE buffer and chromatographed on the agarose-fetuin system as described previously. Figure 5 illustrates the chromatographic separation of this supernatant

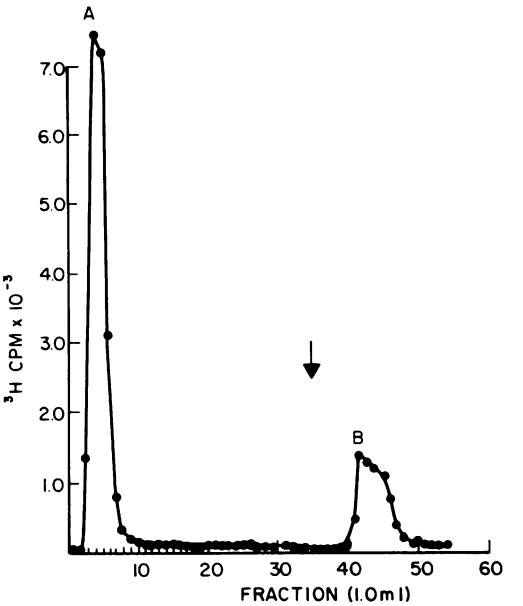


FIG. 5. *Agarose-fetuin chromatography of the viral glycoproteins. The mumps virus labeled with [^{3}H]leucine was treated with detergent and a high salt concentration at high pH to yield a supernatant fraction containing the two glycoproteins and the low-molecular-weight membrane protein. This supernatant was was dialyzed against BKE; a precipitate was removed by low-speed centrifugation and the final supernatant was applied to the affinity chromatography resin. The column was then washed with BKE at 4 C and 1-ml fractions were collected. After several hours, the column was transferred to 37 C and eluted with BKE containing 1.5 M KCl prewarmed at 37 C. This point is designated by the arrow. Additional 1-ml fractions were collected until no further radioactive material could be eluted.*

FIG. 4. *Electrophoresis of supernatants and pellets of mumps virus treated with Triton N-101 under various conditions. A and B, Mumps virus was labeled in ovo, purified, and treated with 2% Triton N-101 in TSV buffer. The virus preparation was centrifuged and the supernatant and pellet fractions were separated and analyzed on polyacrylamide gel electrophoresis. C and D, Another sample of purified, labeled mumps virus was treated with 2% Triton N-101 in high salt and bicarbonate concentration. After centrifugation the supernatant and pellet were analyzed on acrylamide gels.*

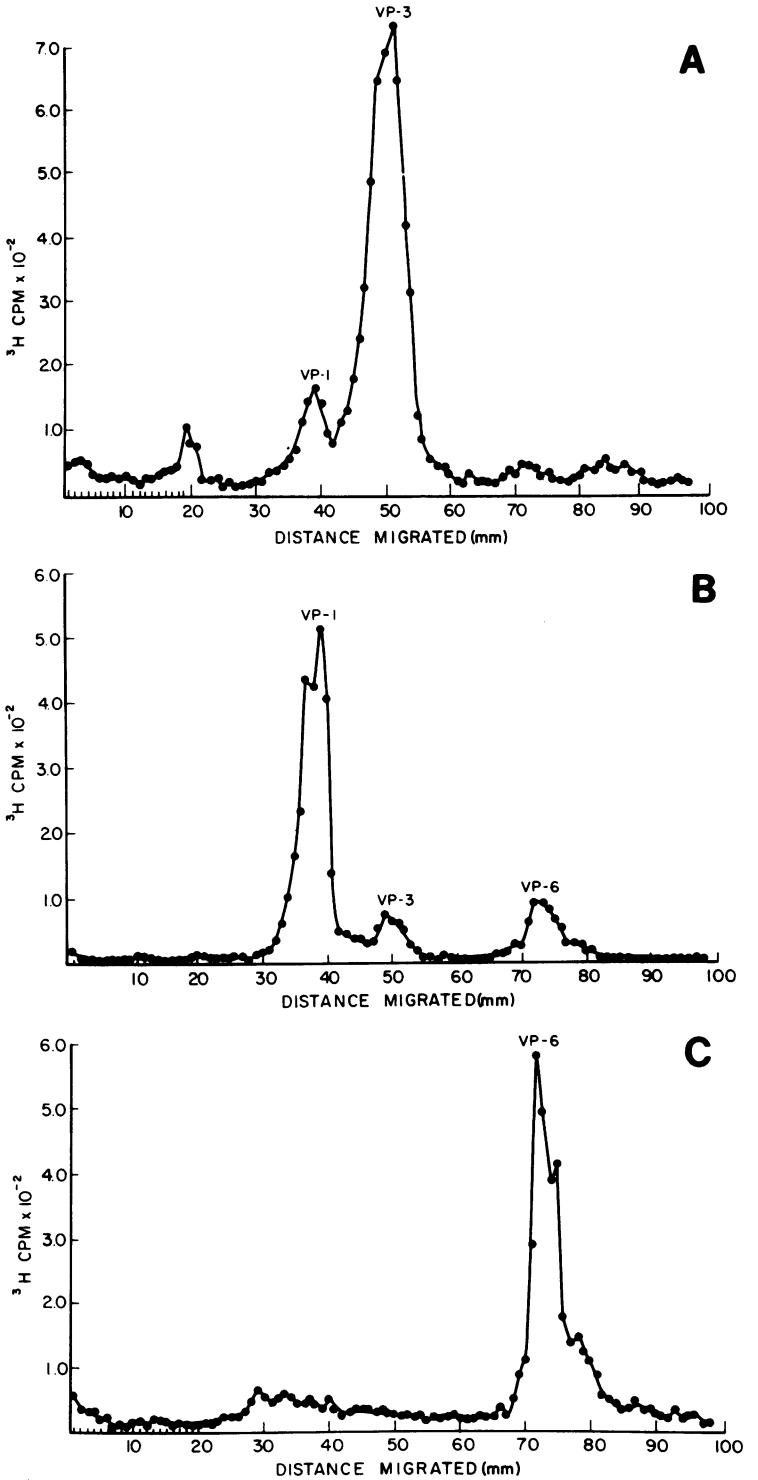


FIG. 6. *Polyacrylamide gel electrophoresis of the fractions of the affinity chromatography. The two peaks of radioactivity from the affinity chromatography column, as shown in Fig. 5, were dialyzed to remove salt, alcohol precipitated, and analyzed by acrylamide gels. A, Material from fractions 3 to 8 of Fig. 5; B, material from fractions 40 to 46; C, precipitate which formed during the dialysis of the viral proteins against BKE, prior to affinity chromatography, and which was removed by centrifugation, was analyzed on acrylamide gels.*

fraction, and Fig. 6A and B demonstrate the electrophoretic analysis on the material eluting in the two peaks. The precipitate which formed during the dialysis against BKE buffer was also analyzed on polyacrylamide gel electrophoresis. This precipitate is comprised of only the low-molecular-weight polypeptide VP-6 (Fig. 6C). The material which was not adsorbed to the fetuin during the initial chromatography consisted primarily of the smaller glycoprotein, VP-3, and the material which eluted only in the high salt concentration at 37 C was mainly VP-1. Table 3 presents the hemagglutinating activity, the neuraminidase activity, radioactivity, and protein concentrations of the two preparations. It is evident that the VP-1 contains both the hemagglutinating activity and the neuraminidase activity; the low background activities found in the second fraction, the one containing primarily VP-3, are explained by the minimal contamination of this fraction with VP-1.

Serological investigations. The two glycoproteins which had been separated by the agarose-fetuin procedure were dialyzed to remove the high salt, alcohol-precipitated to remove the detergent, and then resuspended in VBD. Protein determinations were performed to equalize the amount of antigen used. Table 4 contains the results of complement fixation tests between several different antisera and the two purified glycoproteins. Serum specific for virus nucleocapsid (S. Jensik, manuscript in preparation) showed no reactivity to either glycoprotein. The hyperimmune guinea pig serum, which contained both anti-S and anti-V activity, reacted only to glycoprotein VP-1. Since VP-1 displayed no S antigenic activity, it is therefore the site of the classical V antigen described by Henle (13).

DISCUSSION

Paramyxoviruses were initially classfied as a different subgroup of the influenza viruses or orthomyxoviruses on the basis of morphological, immunological, genetic, and biochemical differences (1, 6, 7, 29). In studies with NDV, SV5, Sendai, and measles virus (12, 17, 23), the paramyxoviruses have all been found to be remarkably similar. The viruses are pleomorphic in shape, range in size from 150 to 300 nm, and are covered with a lipid envelope which has surface projections. The viruses all possess a nucleocapsid composed of 50 to 55S RNA encased in a protein coil constructed from subunits containing only a single polypetide chain. The base ratios of the paramyxoviruses are similar and are characterized by a relatively high uridine content (6). All of the viruses studied have an RNA-dependent RNA polymerase (5, 17). The polypeptide composition of the paramyxoviruses, as analyzed by polyacrylamide gel electrophoresis, is reasonably similar. There are five to six polypeptides (23): a large glycoprotein of molecular weight 65,000 to 74,000, a nucleocapsid protein of molecular weight 55,000 to 60,000, a second glycoprotein of

TABLE 4. *Serological properties of the two mumps virus glycoproteins*[a]

Antigen preparation	Serum	Dilution of antigen fixing 2 units of complement at a 1:10 dilution of serum
VP-3	Hamster anti-S	<1:10
VP-1	Hamster anti-S	<1:10
Purified nucleocapsid	Hamster anti-S	1:40
VP-3	Hyperimmune guinea pig serum	<1:10
VP-1	Hyperimmune guinea pig serum	1:80

[a] The two fractions from the affinity chromatography, corresponding to the viral glycoproteins VP-1 and VP-3, were tested for their ability to react in complement fixation tests with various sera. Purified nucleocapsid from mumps virus was prepared from eggs infected with mumps virus by a method similar to that described for SV5 by Compans and Choppin (9; see also S. Jensik, manuscript in preparation).

TABLE 3. *Comparison of biological activities of the two fractions obtained from the affinity chromatography*[a]

Fraction from affinity chromatography	Protein (μg)	Counts/min	HA	Neuraminidase (OD_{549})[b]
A (VP-3)	40	5,000	2	0.16
B (VP-1)	10	1,000	64	1.85

[a] Fractions 3 to 8 (peak A) and 40 to 48 (peak B) were collected and pooled, and 100-ml volumes were assayed for hemagglutination activity, radioactivity, neuraminidase activity and protein concentration by methods previously described.

[b] OD_{549}, Optical density measured at 549 nm.

molecular weight 52,000 to 56,000, and a low-molecular-weight polypeptide of molecular weight 38,000 to 40,000. There are also two minor polypeptides present. The heavier glycoprotein has always been the one associated with the hemagglutination and neuraminidase activity (24, 25). The smallest polypeptide is believed to be structural protein in the membrane envelope of the virion, because it is a major viral polypeptide and because it can be removed from the virus particle by digestion with detergent and high salt (24). The function of the smaller glycoprotein and the minor viral polypeptides is not known.

The data presented in this work demonstrate that mumps virus has a polypeptide composition similar to the other paramyxoviruses. Gel electrophoresis of the proteins of the whole virion revealed the presence of six polypeptides; the range of molecular weights and distribution were similar to other paramyxoviruses studied. There were only two glycoproteins, and the heavier one was the site of the hemagglutinating and neuraminidase activity. The degree of separation between VP-2 and VP-3 was never complete. Nevertheless, the smaller glycoprotein was repeatedly found to migrate faster than the other component of the large band of radioactivity between fractions 40 and 50 (see Fig. 2, 4A, B, C, and D). Double-label experiments with ^{14}C-amino acid-labeled mumps virus could not be done because of the inadequacy of in ovo incorporation of ^{14}C-amino acids into mumps virus and because of inefficiency in labeling mumps virus satisfactorily with any radioactively labeled amino acids in tissue culture systems.

The association of the neuraminidase and hemagglutinating activity with the same polypeptide in mumps virus and other paramyxoviruses is in contrast to the results with influenza virus, in which these two biological activities were found to be associated with separate polypeptides (19, 26). The biological function of the smaller paramyxovirus glycoprotein, VP-3, has not yet been fully elucidated, although it has been suggested that this polypeptide contains the hemolytic and cell fusion properties of the paramyxovirus (12). Preliminary experiments to demonstrate hemolytic activity for the mumps virus VP-3 have been unsuccessful in this laboratory. Other workers have done similar experiments with the purified glycoproteins of measles virus (12) and it was found that the purified glycoproteins alone did not exhibit any hemolytic activity. However, with the addition of a lipid component, such as phosphatidylethanolamine, cell fusion and hemolytic activities were restored; these biological activities could not be attributed to a specific glycoprotein, however.

Table 4 demonstrates that only VP-1 reacted in complement fixation tests with a hyperimmune guinea pig serum containing anti-V and anti-S activity. Since the use of hamster serum specific for the S antigen demonstrated that the VP-1 did not contain any S antigen as well as the site of hemagglutination-inhibition activity of immune sera. It cannot yet be stated with certainty that this glycoprotein is the site against which the infectivity-neutralization activity of immune serum is also directed. Further experiments are being conducted to resolve this and other aspects regarding the antigenic and biochemical properties of the mumps virus proteins.

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