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Substructures and Polypeptides of Visna Virus

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The protein of Visna virus, disrupted by 8 M guanidine hydrochloride and heating, was resolved into 10 polypeptides by agarose gel column chromatography in 6 M guanidine hydrochloride. Two of the peaks contained glycopolypeptides. Nonidet-disrupted virions were resolved into two fractions by potassium tartrate gradient centrifugation, with densities of 1.08 and 1.24 g/ml, respectively. About 70% of the viral DNA polymerase directed by added template was released into the light fraction, in which very little endogenous enzyme activity was detected. Also released into the light fraction were all of the glycopolypeptides, 50% of the viral RNA, and a part of each of the other viral protein components. The data indicate that extensive degradation of subviral structures occurred, even under mild conditions for virion disruption. The 1.24-g/ml fraction was composed of 50% of the viral RNA, most of the endogenous DNA polymerase activity (80%), and a major internal polypeptide (GuHCl6) with an estimated mol wt of 28,000. Two other polypeptides were also consistently detected in the heavy fraction, but they constituted less than 25% of the ribonucleoprotein complex, compared with 75% for GuHCl6.

Visna virus is the causative agent of a neurological disease of sheep. The virions contain a 60 to 70*S* RNA (6, 20, 25) and a DNA polymerase directed by RNA as well as DNA (13, 24, 34, 35). The high-molecular-weight RNA and the DNA polymerase were found first in oncogenic RNA viruses (oncornaviruses; 2, 10, 31, 32, 37). Mountcastle et al. (27) and Haase and Baringer (19) reported that visna virus contains 11 to 15 polypeptides, as analyzed by polyacrylamide gel electrophoresis. Three of these polypeptides are glycoproteins which are localized on the surface of the virion. Nowinski et al. (28) showed that the protein of the progressive pneumonia virus could be resolved into five polypeptides by agarose gel filtration in 6 M guanidine hydrochloride (GuHCl). In general, the proteins of the slow viruses found by these investigators are similar to the oncornaviral proteins which have been extensively studied by a number of laboratories (3, 7, 9, 11, 15, 21, 29, 30). It has been reported recently that the Visna viral RNA contains a long, homogeneous stretch of poly(A) (17) similar to the poly(A) tract of the oncornaviral RNA (16, 18, 22, 33). Thus, the biochemical properties of Visna virus are very similar to those of oncornaviruses. In addition, Visna virus and oncornaviruses are also similar with regard to a number of other properties (36, 38). However, Visna virus has not yet been shown to be oncogenic in its natural host. Further, Visna virus causes marked cytopathic effect in tissue culture, whereas the oncornaviruses do not usually show such an effect. These differences suggest that Visna virus represents a separate group of RNA viruses. Although the general protein composition of Visna virus and progressive pneumonia virus has been studied recently (19, 27, 28), some of the major protein components have not yet been localized in the virion. It was, therefore, of interest to study the location of the major internal proteins and their serological properties. In initiating this study, we selected agarose gel filtration in 6 M GuHCl as the primary method to separate the polypeptides because of its high resolving power and its good recovery of antigenic activity of the viral proteins from the column, as demonstrated by Fleissner (15) and Nowinski et al. (29). This paper reports the separation of viral subunits and the location of some of the proteins in these subunits. The activity of DNA polymerase in the protein fractions was also investigated.

MATERIALS AND METHODS

Chemicals and reagents. The chemicals were obtained from the suppliers indicated below: ^{14}C- or ^{3}H-labeled L-amino acid mixtures and ^{14}C- or ^{3}H-labeled D-glucosamine, Omnifluor, Aquasol, and Triton X-100 from New England Nuclear, Boston, Mass.; ^{14}C-labeled amino acids derived from the hydrolysis of

algael-protein from Amersham/Searle, Arlington, Ill.; GuHCl, [^{3}H]thymidine 5'-triphosphate, L-[^{14}C]leucine and [^{3}H]uridine from Schwarz/Mann, Orangeburg, N.Y.; Bio-Gel A-5M (200 to 400 mesh) from Bio-Rad Laboratories, Rockville Center, N.Y.; 2-mercaptoethanol from Eastman Organic Chemicals, Rochester, N.Y.; the unlabeled deoxyribonucleoside 5'-triphosphates and dithiothreitol from Sigma Chemicals, St. Louis, Mo.; blue dextran, aldolase (mol wt 158,000), ovalbumin (mol wt 45,000), chymotrypsinogen A (mol wt 25,000), and ribonuclease A (mol wt 13,700) from Pharmacia Fine Chemicals, Piscataway, N.J.; bovine serum albumin (mol wt 69,000) from Nutritional Biochemicals Corp., Cleveland, Ohio; membrane filter (B6, 25 mm) from Schleicher & Schuell, Keane, N.H.; Nonidet P-40 was a gift from Shell Chemical Co; oligo(dT)-rA from Collaborative Research Inc., Waltham, Mass.; and type E fiber glass filter (25 mm) from Gelman Instrument Co., Ann Arbor, Mich.

Cell cultures and media. The choroid plexus was removed from a sheep brain, minced with scissors, suspended in 3 ml of growth medium (25), and put into two 30-ml Falcon plastic flasks. After 2 to 3 days, the fluid was carefully removed, and 5 ml of growth medium was added to each flask. When confluent outgrowth had formed, the cultures were trypsinized and subcultured as previously described (25).

Standard buffers. TNE buffer (pH 7.4) contained 0.01 M Tris-hydrochloride, 0.1 M NaCl, and 1.0 mM EDTA; buffer A contained 0.05 M Tris-hydrochloride (pH 8.5), 8 M GuHCl, 2% 2-mercaptoethanol, and 0.01 M EDTA; buffer B (pH 6.5) contained 0.02 M sodium phosphate, 6 M GuHCl, and 0.01 M dithiothreitol; and buffer C contained 0.01 M Tris-hydrochloride (pH 7.9), 1.0 mM EDTA, and 0.02 M dithiothreitol.

Assay of DNA polymerase. The activity of DNA polymerase was assayed according to our previously published method (24), except that Nonidet was omitted and 0.02 M dithiothreitol was substituted for 2-mercaptoethanol.

Preparation of radioactive virus. The sheep choroid plexus cells grown in 250-ml Falcon plastic flasks were inoculated with Visna virus K796 with a multiplicity of about one infectious dose per cell. Forty milliliters of the maintenance medium (25) containing 5 μCi of tritiated L-amino acid mixture per ml and 0.5 μCi of D-[^{14}C]glucosamine per ml (224 mCi/mmol), or 0.5 μCi of ^{14}C-labeled L-amino acid mixture per ml (54 mCi per matom of carbon) and 5 μCi of D-[6-^{3}H]glucosamine (3.6 Ci/mmol), or 0.5 μCi of L-[^{14}C]leucine per ml (312 mCi/mmol) and 5 μCi of [5-^{3}H]uridine per ml (28 Ci/mmol) were added to each flask. The culture was incubated at 37 C for 3 to 4 days until the marked cytopathic effect was observed. The radioactive virus was harvested from the infectious fluid and used as protein source.

The unlabeled virus was similarly prepared as described for the labeled virus.

Purification of virus. The Visna virus was concentrated by zinc acetate precipitation and was purified by banding twice in a potassium tartrate gradient in TNE buffer as described previously (23, 25).

Gel filtration of Visna viral protein in GuHCl. The agarose gel (Bio-Gel A-5M) was packed in a column by the method of Nowinski et al. (29). The flow rate was set at 1 ml/h by differential pressure according to Fish et al. (14). The purified virus was suspended in 1 ml of buffer A and boiled for 3 min. To the heated viral suspension, crystal sucrose and blue dextran were added to 10 and 0.6% (wt/wt), respectively. The solution was mixed by pipetting and was layered onto the column underneath the eluting buffer. The column was eluted with buffer B, and 1-ml fractions were collected. Each fraction was weighed to calculate the ratio of elution volume to void volume, as described by Fish et al. (14). To each fraction, 200 μg of bovine serum albumin (0.1 ml) and 1 ml of 10% trichloroacetic acid were added to precipitate the radioactive polypeptides. The mixture was incubated in ice water for at least 10 min and filtered through a membrane or a fiber glass filter which was presoaked in 5% trichloroacetic acid. The acid-precipitable radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrophotometer in 10 ml of toluene containing Omnifluor and Triton X-100.

Preparation of subunits from Visna virus. The method of Coffin and Temin (8) was modified for this study. The purified radioactive virus was suspended in 1 ml of buffer C containing 50 μg of bentonite and 0.5% Nonidet P-40. The viral particles were disrupted by pipetting gently, and the mixture was incubated at 0 to 4 C for 90 s. The solution was centrifuged on a 10 to 50% potassium tartrate gradient in buffer C for 3 h in a Beckman SW27 rotor at 27,000 rpm (95,000 $\times$ g). Centrifugation of the disrupted viral suspension for up to 10 h did not significantly change the densities of the subviral structures. The gradient was fractionated from the top in 1-ml portions with an ISCO gradient fractionator, and a 0.1-ml sample was taken from each fraction for radioactivity measurement. Gradient fractions in each radioactive peak were pooled, and the proteins were further analyzed by gel filtration.

The subunits of unlabeled virus were similarly prepared as above. For the assay of DNA polymerase activity in each gradient fraction, the potassium tartrate was removed by dialysis of each fraction against TNE buffer overnight.

Protein determination. The method of Lowry et al. (26) was employed by using bovine serum albumin as standard.

RESULTS

DNA polymerase activity in subunits of Visna virus. Purified, unlabeled Visna virus (2.5 mg of protein) was treated with Nonidet P-40 and centrifuged on a tartrate gradient as described above. The profiles of endogenous and exogenous activities of the viral DNA polymerase are shown in Fig. 1. About 70% of the enzyme activity directed by the homopolymer oligo(dT)-rA was released from the virions and was located on the top of the gradient at a

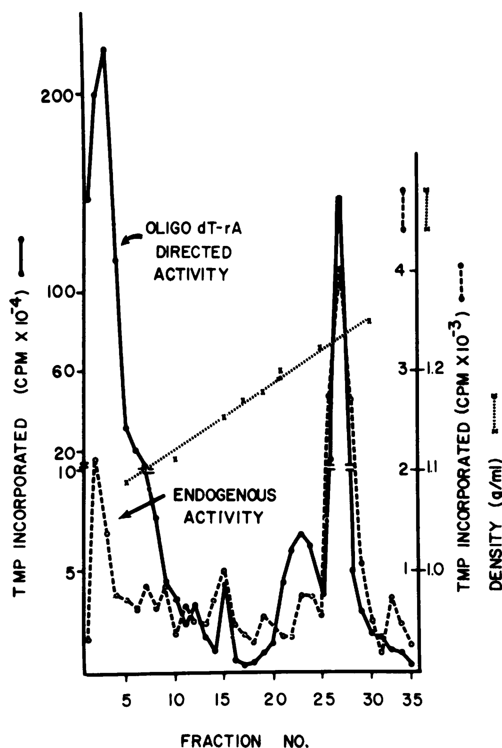

FIG. 1. *DNA polymerase activity in subunits of Visna virus. Purified, unlabeled visna virus was disrupted by Nonidet and centrifuged on a 10 to 50% potassium tartrate gradient. The gradient was fractionated from the top (left) to the bottom (right). After dialyzing for 12 h, a 0.1-ml sample of each fraction (1 ml) was assayed for DNA polymerase activity with [0.5 μg of oligo(dT)-rA, exogenous activity] and without added template (endogenous activity). The specific radioactivity of [^{3}H]thymidine 5'-triphosphate was 6,000 counts per min per pmol. The density was measured by weighing 0.1-ml samples from randomly selected fractions. Note that the scale break does not apply to endogenous reaction.*

density of 1.08 g/ml. Most of the remaining 30% exogenous activity was banded sharply at a density of 1.23 g/ml; on the other hand, more than 80% of the endogenous activity was detected in the 1.23-g/ml band. A small activity peak was also observed at a density of 1.21 g/ml.

Fractionation of subunits of Visna virus. Visna virus labeled with [^{3}H]uridine and [^{14}C]leucine was treated with Nonidet, and the radioactivity profiles of the disrupted virus in a potassium tartrate gradient is shown in Fig. 2. It is seen that the intact virus was banded at a density of 1.16 g/ml (Fig. 2A), whereas the two isotopes were resolved into low (1.08 g/ml) and high (1.24 g/ml) density fractions after treatment of the doubly labeled virus with the nonionic detergent. The [^{3}H]uridine label was found in almost equal amounts in the two fractions, whereas 89% of the [^{14}C]leucine was found in the light fraction and the remaining 11% banded in the heavy fraction (Fig. 2B). The recovery of ^{3}H and ^{14}C was 89 and 92%, respectively, from the two fractions. The data obtained from this experiment, and from the endogenous DNA polymerase in 1.23-g/ml fractions described above, strongly indicate that the 1.24-g/ml fraction contained a ribonucleoprotein.

Radioactivity profiles of intact and Nonidet-

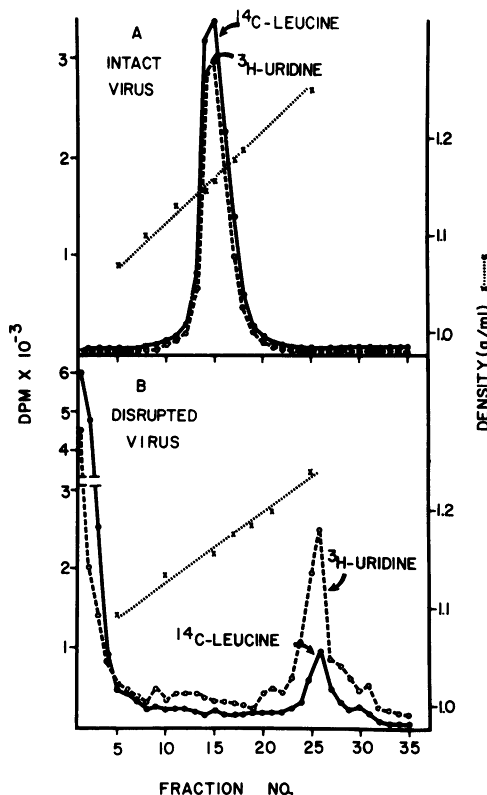

FIG. 2. *Potassium tartrate gradient centrifugation of intact and disrupted visna virus. Visna virus was labeled with [5-^{3}H]uridine and L-[^{14}C]leucine and centrifuged on a potassium tartrate gradient. A solution (0.1 ml) from each fraction was used for the measurement of radioactivity. The radioactivity profiles from a second banding of the intact virus are shown in (A). Fractions (13 to 17) in the viral band were pooled, and the virus was pelleted by centrifugation at 95,000 × g for 1 h. The particles were disrupted and centrifuged on tartrate gradient. The profiles of acid-precipitable radioactivity of each fraction (1 ml) are shown in (B). The method of fractionation and measurement of density were the same as those described in Fig. 1.*

disrupted Visna virus, which was labeled with [³H]amino acids and [¹⁴C]glucosamine, is shown in Fig. 3. It is seen that a single peak of the two labels was located at a density of 1.15 to 1.16 g/ml (Fig. 3A) in the gradient containing the untreated virus. In contrast, after the detergent treatment, the [³H]amino acids label was resolved into two fractions; one was located on the top of the gradient (1.08 g/ml), accounting for 86% of the ³H detected in the gradient, and the other (14%) was banded at the heavy subunit position. Ninety-five percent of the initial ³H label in the intact virus was recovered from the two fractions. The ³H radioactivity in the 1.24-g/ml fraction was estimated to constitute 12% of the intact viral protein. It is also seen that almost all the [¹⁴C]glucosamine label (97%) was recovered from the top 5 ml of the gradient. The heavy fraction (1.24 g/ml) was contaminated with about 2% of [¹⁴C]glucosamine radioactivity.

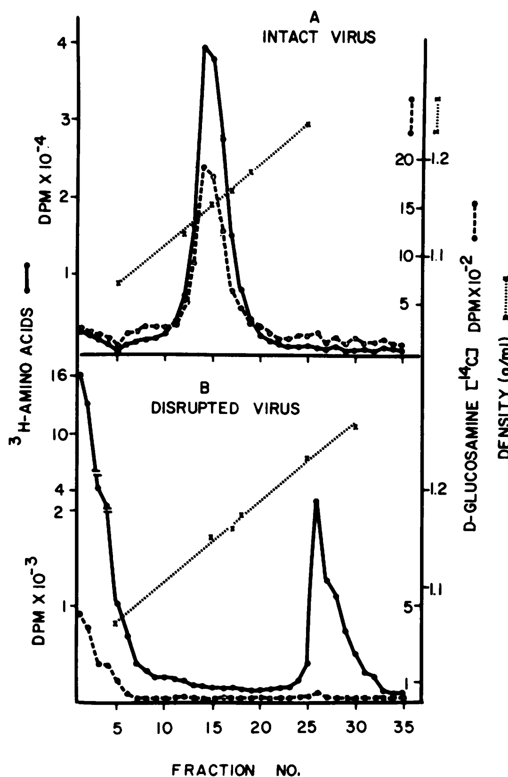

FIG. 3. *Potassium tartrate gradient centrifugation of intact and disrupted visna virus. Visna virus (6 × 10¹¹ mean tissue culture infected doses) was labeled with [³H]amino acids and D-[1-¹⁴C]glucosamine. The procedures of gradient centrifugation of intact (A) and disrupted (B) virions were the same as those described in Fig. 2. A sample (0.1 ml) from each fraction (1 ml) was used for the measurement of radioactivity.*

Separation of polypeptides of Visna virus by agarose gel column chromatography in GuHCl. Visna virus was labeled with a mixture of [³H]amino acids and with D-[¹⁴C]glucosamine and was treated with 8 M GuHCl as described above. The viral protein preparation was chromatographed on an agarose gel column. The acid-precipitable radioactivity profiles are illustrated in Fig. 4. For the purpose of convenient identification, an arabic numeral prefixed with GuHCl will be assigned to each peak of polypeptide eluted from the agarose column. It is seen that the Visna proteins were clearly resolved into 10 peaks by the gel filtration. Two glycopolypeptides were located in GuHCl 1 and 3. GuHCl 3 and 4 were not well separated, but the two polypeptides could be clearly distinguished by the ratio of ¹⁴C to ³H. GuHCl 5 through 10 are free of glucosamine label and account for more than 60% of the total viral

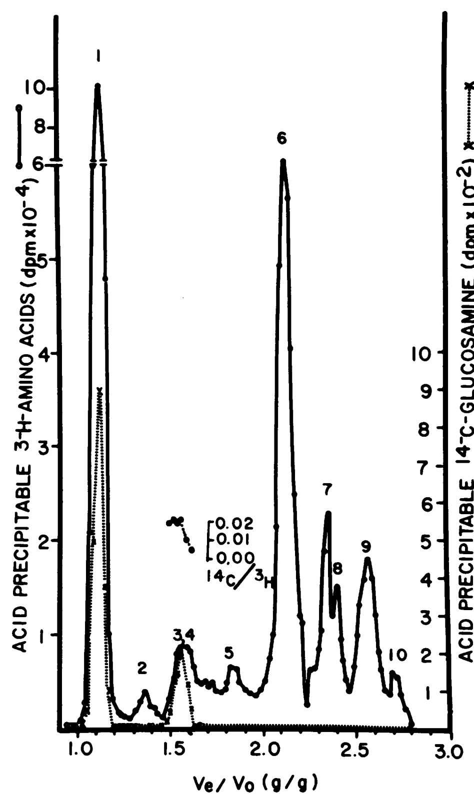

FIG. 4. *Agarose gel filtration of the protein of visna virus. The purified [³H]amino acids and D-[¹⁴C]glucosamine-labeled visna virus was disrupted with buffer A, and the dissociated proteins were chromatographed on an agarose gel column.*

protein. It was also estimated that GuHCl 6 constituted about 30% of the Visna protein.

The polypeptide composition of each of the two fractions obtained from Nonidet-disrupted virus on tartrate gradient was analyzed by gel filtration in 6 M GuHCl. A sample of each fraction was dialyzed against deionized water for 18 h and was dried by lyophilization. The powder was dissolved in 1 ml of buffer A and boiled for 3 min. The solution was chromatographed on an agarose gel column. The results are illustrated in Fig. 5. It is seen that the light fraction contains seven major polypeptides (Fig. 5A). Two of the polypeptides are glycopolypeptides. GuHCl 1 was greatly reduced in radioactivity and not as well separated from GuHCl 2 as compared with the peak in Fig. 4. The possible effects of dialysis and lyophilization on these results will be discussed later. Whether or not GuHCl 2 was present in the light fraction was difficult to determine in this experiment because of the overlapping of GuHCl 1 into the GuHCl 2 area. However, in another experiment (see Fig. 8A and B), GuHCl 2 was not detectable in the light density fractions; on the other hand, GuHCl 2 was consistently present in the heavy fraction (Fig. 5B and 8C). GuHCl 3 and 4 are clearly separated in Fig. 5A. Almost all of GuHCl 7 and 8 were located in the light fraction, whereas GuHCl 6 appeared in both light and heavy density fractions (Fig. 5A and B). The results showed that 66% of GuHCl 6 was found in the 1.08-g/ml fraction and 34% was found in the 1.24-g/ml fraction. It was further calculated from the data, as shown in Fig. 5B, that GuHCl 6 accounted for about 75% of the protein in the heavy fraction. The remaining 25% consisted of other nonglycosylated polypeptides of high molecular weights and of GuHCl 9. Since the heavy fraction contained about 12% of the viral protein (Fig. 3), and 75% of the protein content of this subunit was contributed by GuHCl 6 (Fig. 5B), it was calculated that GuHCl 6 in the heavy fraction accounted for 9% of the viral protein. In addition, two times this quantity of GuHCl 6 was found in the light fraction (Fig. 5A). Thus, the total amount of GuHCl 6 found in both fractions represents 27% of the viral protein. This figure (27%) agrees well with that (30%) found in the complete viral protein preparation (Fig. 4). However, it should be pointed out that the amount of GuHCl 6 estimated from the data presented in Fig. 3 and 5B was based on the assumption that the recovery of this internal polypeptide was proportional to the recoveries of other polypeptides found in the heavy fraction. [^{14}C]glucosamine radioactivity was not detectable in any of the eluates from the gel column loaded with the heavy fraction. Except for GuHCl 1, whose molecular weight exceeds 100,000, the molecular weights of the polypeptides were estimated according to Fish et al. (14). They are shown in Table 1.

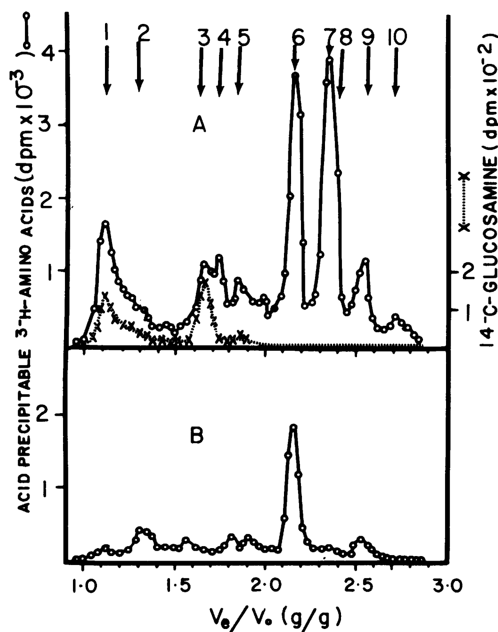

FIG. 5. *Column chromatography of viral subunits. (A) Fractions 1 to 8 from the tartrate gradient shown in Fig. 3B were pooled, dried, and denatured. The solution was chromatographed on an agarose gel column. The fractionation and processing of acid-precipitable polypeptides were described in the Methods. (B) Fractions 25 to 30 of tartrate gradient shown in Fig. 3B were pooled and chromatographed as described in (A).* D-[^{14}C]*glucosamine label was not detectable and is not shown.*

Evidence of the presence of GuHCl 6 in a substructure associated with the low-density fraction. The possibility of a pelletable substructure associated with the low-density fraction was investigated. The [^{3}H]amino acids and [^{14}C]glucosamine-labeled virus was treated with Nonidet for 90 s, and the mixture was centrifuged at 105,000 × *g* for 1 h. The supernatant (soluble fraction) and the pellet (insoluble fraction), which was suspended in 2 ml of TNE buffer by repeated pipetting until homogenized, were then separately centrifuged on 10 to 50% potassium tartrate gradient at 95,000 × *g* for 3 h (Fig. 6). It is seen that all the [^{3}H]amino acids and [^{14}C]glucosamine labels in the soluble fraction remained on the top of the gradient (Fig. 6A) having a density of 1.05 g/ml. In a similar

TABLE 1. *Molecular weights of the polypeptides of visna virus*

GUHCl[a]	Mol wt
1	
2	110,000
3	75,000
4	70,000
5	44,000
6	28,000
7	17,000
8	15,500
9	11,500
10	9,000

[a] Molecular weights were determined by the method of Fish et al. (14). The procedure of plotting the V_e/V_o (wt/wt) versus the logarithm of molecular weight was employed to obtain a standard curve. V_e, Elution volume (grams) of samples; V_o, elution volume (grams) of blue dextran. The standard proteins were bovine serum albumin (69,000), ovalbumin (45,000), chymotrypsinogen A (25,000), and ribonuclease A (13,700).

that the light fraction (Fig. 8A) derived from the preparation from unlabeled virus, it was found that 70% of the original oligo(dT)-rA-directed activity of DNA polymerase was detected in this fraction. The endogenous enzyme activity was not detectable. In contrast, the insoluble fraction was resolved into two fractions having densities of 1.08 and 1.21 g/ml, respectively. No detectable [^{14}C]glucosamine label was observed in either fraction of the insoluble material. More than 90% of the exogenous DNA polymerase activity originally present in the insoluble fraction was located in the 1.08-g/ml band. The endogenous enzyme activity in either band was very low.

The possible presence of viral RNA in the insoluble 1.08-g/ml subunit was investigated by labeling Visna virus with [^{3}H]uridine. The preparation of the insoluble fraction and the gradient centrifugation were carried out as described above. The radioactivity profile of the gradient is shown in Fig. 7. It is seen that the [^{3}H]uridine label was resolved into two major peaks with a distribution of 20 and 80%, respectively, in the 1.08-g/ml and 1.24-g/ml subunits. The proportion of the labeled RNA in the soluble and insoluble fractions were 48 and 52%, respectively. Thus, it was calculated that the light insoluble subunit contained about 10% of the original viral RNA. A small radioactive peak was also detected at a density of 1.20 g/ml. This minor peak was also frequently observed in other experiments (Fig. 1 and 2).

Analyses of the polypeptide composition in each fraction of the tartrate gradient were carried out by gel filtration (Fig. 8) It is seen soluble protein contained the two glycopolypeptides, as well as GuHCl 5, 7, and 8. GuHCl 9 was about equally distributed in the soluble and insoluble fractions (Fig. 8A and C), whereas GuHCl 10 was found only in the soluble fraction. GuHCl 6 was completely absent from the soluble protein preparation. The light fraction of the insoluble protein preparation contained GuHCl 6, 7, 8, and a third peak that was not identified (Fig. 8B). In the heavy fraction (Fig. 8C), GuHCl 6 accounted for about 70% of the protein. The remaining 30% was located mainly in GuHCl 9. The data indicate that GuHCl 6 found in the light-density fraction (Fig. 3 and 5A) was associated with a substructure pelletable at 105,000 × *g*.

DISCUSSION

The protein of Visna virus labeled with

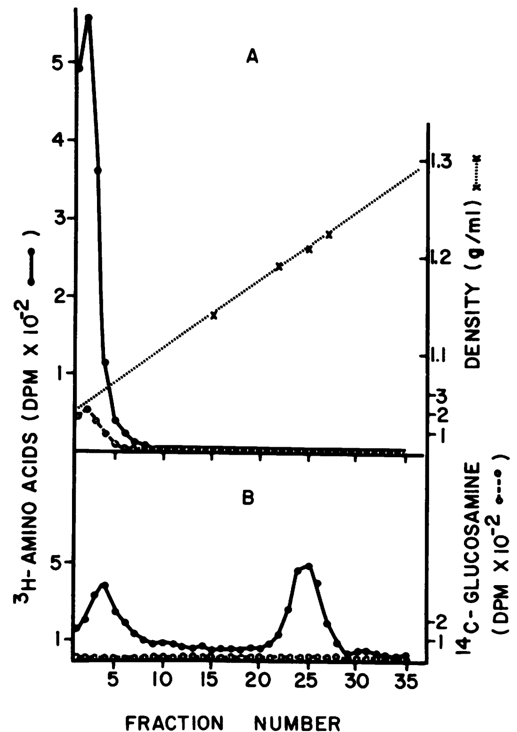

FIG. 6. *Potassium tartrate gradient centrifugation of soluble and insoluble fractions of visna viral subunits. Visna virus labeled with [^{3}H]amino acid and* D-[^{14}C]*glucosamine was disrupted with Nonidet. The disrupted virions were centrifuged at 105,000 × g for 1 h. The supernatant (soluble fraction) and the pellet (insoluble fraction) were subjected to equilibrium centrifugation separately on a potassium tartrate gradient (10 to 50%). The fractionation and sampling were the same as those described in Fig. 3. (A), soluble fraction; (B), insoluble fraction.*

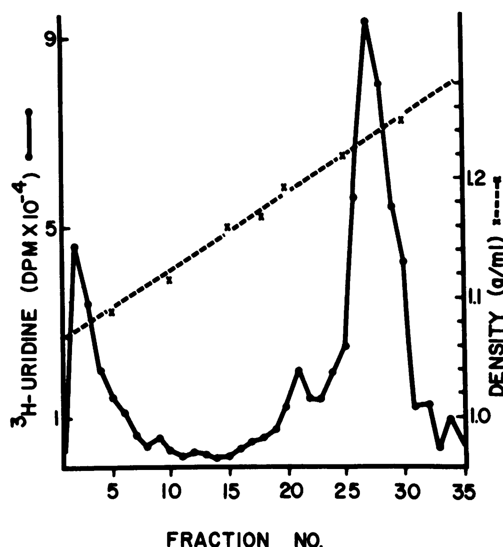

FIG. 7. *Potassium tartrate gradient centrifugation of insoluble fraction from visna virus. Visna virus was labeled with [5-³H]uridine (8 Ci/mmol). The disruption of the virus, the preparation of the insoluble fraction, and the gradient centrifugation were the same as those described in Fig. 6. After fractionation, 0.1 ml of yeast RNA (0.1 mg) and 1 ml of 10% trichloroacetic acid containing 0.1 M sodium pyrophosphate were added to each fraction (1 ml). The acid-precipitable radioactivity was measured as described.*

distinct radioactive peaks by column chromatography of the labeled viral protein on an agarose gel in 6 M GuHCl. Two of these polypeptides are glycopolypeptides. The glycopolypeptide GuHCl 1 could be resolved into two glycosylated components with molecular weights higher than that of glycopolypeptide GuHCl 3 by polyacrylamide-sodium dodecyl sulfate-gel electrophoresis (unpublished data). In general, these results are in agreement with those of Mountcastle et al. (27) and of Haase and Baringer (19).

There was a great loss in glycopolypeptide GuHCl 1 (Fig. 4 and 5) after the fractionation of the subviral structures. The loss did not occur in the tartrate gradient centrifugation because almost all of the [¹⁴C]glucosamine was recovered (Fig. 3). It is possible that the glycopolypeptide was lost during dialysis and lyophilization, due to its high affinity to the walls of dialyzing bags and to glass. A loss of viral DNA polymerase to a dialyzing tube has been reported by Tronick et al. (39). Heating of the lyophilized light fraction might result in partial disaggregation or irreversible denaturation of GuHCl 1. This effect could cause the appearance of a heterogeneous peak, as shown in Fig. 5A. This explanation is substantiated by the result shown in Fig. 8A, since heating was omitted in that experiment.

The treatment of Visna virus with Nonidet P-40 resulted in separation of the viral components into two major fractions demonstrable by density gradient centrifugation. The data indicate that the 1.24-g/ml subunit was a ribonucleoprotein (Fig. 1 and 2), which was predominantly composed of GuHCl 6 with an estimated mol wt of 28,000. Our results strongly indicate that GuHCl 6 is located in the innermost layer of the viral particle. The light fraction contained all the components of the viral protein (Fig. 5A). The high content of GuHCl 6 (66%) in the light fraction compared with only 34% in the ribonucleoprotein subunit indicated extensive breakdown of the internal substructure, even under very mild conditions for viral disruption. We estimated that GuHCl 6 constituted 30% of the total viral protein. Since GuHCl 6 accounted for only about 75% of the protein in the heavy fraction, it follows that the protein of the

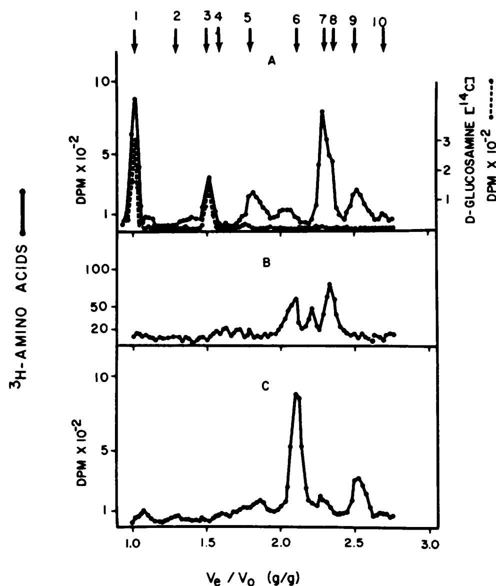

FIG. 8. *Agarose column chromatography of visna viral subunits from soluble and insoluble fractions. The procedures described in Fig. 5 were employed in these experiments except that heating was omitted. (A) Elution profiles of agarose column chromatography of the soluble protein collected from fractions 1 to 5 of the tartrate gradient shown in Fig. 6A; (B) insoluble proteins collected from fractions 1 to 6 of the tartrate gradient shown in Fig. 6B; and (C) insoluble proteins collected from fractions 22 to 27 of the tartrate gradient shown in Fig. 6B.*

[^{3}H]amino acids has been resolved into 10 ribonucleoprotein subunit makes up more than 30% of the total viral protein. Our data regarding the proportion of the nucleoprotein in the viral particle are consistent with the results of Coffin and Temin (8) who have prepared a ribonucleoprotein from Rous sarcoma virus and showed a similar protein proportion of this substructure in the virion.

A core structure sedimented at 1.25 g/ml was isolated from avian myeloblastosis virus (AMV), by Bolognesi et al. (4), by treating the virus with a combination of Nonidet P-40 and ether. In an earlier report, this showed that a polypeptide with a mol wt of 28,000 was predominantly associated with the AMV core. However, with a different method of core preparation, three major polypeptides, namely, p27, p15, and p12, were found in the AMV core as reported recently by Bolognesi et al. (5). They showed that p27, which corresponds to the 28,000-mol-wt polypeptide in their original core preparation, and p12 are equally represented in the AMV core. Upon further treatment of the AMV core, p27 was released from the subviral structure and located at a density of 1.10 g/ml, whereas p12 (mol wt 12,000) was found in the resultant ribonucleoprotein banded at 1.34 g/ml. A similar small polypeptide (mol wt from 11,000 to 14,000) was also found by other workers (9, 30) in ribonucleoprotein (density from 1.27 to 1.34 g/ml) from Rous sarcoma virus. The reported data indicate that the ribonucleoprotein from the oncornaviruses contains primarily a polypeptide having a mol wt of around 12,000. Based on the density and the polypeptide composition, it appears that the Visna ribonucleoprotein reported in this paper is similar to the AMV core as reported by Bolognesi et al. (4). However, our attempt to visualize a core-like structure under electron microscope has been unsuccessful.

In this study, evidence has been presented that GuHCl 6 released into the light-density fraction can be pelleted as a ribonucleoprotein. It has been reported by a number of investigators (1, 4, 8, 10) that the RNA of the oncornaviral core was susceptible to digestion by a ribonuclease. Since GuHCl 6 is the dominant component of the Visna ribonucleoprotein, it is possible that a subribonucleopeptide containing this internal polypeptide was released from the major subunit as a result of the action of a ribonuclease on the structure. This subribonucleopeptide can be in an extended form or loosely coiled and its density, therefore, lower.

The endogenous DNA polymerase activity was largely present in the GuHCl 6-associated ribonucleoprotein band (1.24 g/ml) obtained upon direct fractionation of the disrupted virus on equilibrium gradient centrifugation (Fig. 1). However, when the insoluble viral substructures were pelleted, resuspended in TNE buffer, and then subjected to equilibrium gradient centrifugation as described in Fig. 6, the exogenous DNA polymerase activity was shifted from the 1.24-g/ml band to the light fraction. Little endogenous activity was detected in either fraction. These results indicate that the residual enzyme can be readily released from the nucleoprotein complex, and they suggest that the DNA polymerase is not situated at the center of the virus. The GuHCl 7 and 8 were consistently located only in the light protein fraction. The data suggest that GuHCl 7 and 8 probably attaches to a light lipid-containing substructure which is a component of the viral envelope.

The serological properties of the Visna internal proteins are not yet known. Whether any of the Visna polypeptides has antigenic determinants in common with oncornavirus polypeptides is of great interest. Study on this aspect will shed more light on the relationship between Visna and oncogenic viruses.

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