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Analysis of Structural Polypeptides of Purified Human Cytomegalovirus

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Human cytomegalovirus strain C87 was purified by the following procedures. (i) Extracellular virus was concentrated by centrifugation at 100,000 × *g* for 90 min and passed through a Bio-Rad Bio-Gel A-15m column. Most of the virus was recovered in the void volume. (ii) After two consecutive isopycnic potassium tartrate gradient centrifugations (20 to 50%), coinciding peaks of plaque titer, protein, and radioactivity were found at a density of from 1.20 to 1.21 g/cm^3. To characterize the structural polypeptides of human cytomegalovirus and to establish relative purification criteria, virus was purified from two mixtures: (i) [^{35}S]methionine-labeled extracellular virus mixed with an equal volume of unlabeled normal culture fluid; (ii) unlabeled extracellular virus mixed with an equal volume of [^{35}S]methionine-labeled normal culture fluid. The extent of purification, as judged by the ratio of cellular to viral radioactivity, was 39-fold; i.e., about 2.5% of the protein in the purified virus preparation could be accounted for by host protein contamination. Electrophoresis of purified [^{35}S]methionine-labeled virus on a polyacrylamide gel slab showed that there were at least 33 viral structural polypeptides (VPs), and their molecular weights ranged from 11,000 to 290,000. Autoradiograms obtained from electropherograms of purified [^{14}C]glucosamine-labeled virus showed six bands. Four of these were so broad that several VPs corresponded to each of the glycosylated bands. When heavy (two fractions close to 1.21 g/cm^3) and light (two fractions close to 1.20 g/cm^3) fractions of the PFU peak from the second potassium tartrate gradient were analyzed separately, the number of polypeptides observed was the same, but the relative amounts of some polypeptides differed. The major polypeptide, VP17, was found in greater amounts in the heavy fraction (35%) than in the light fraction (22%). The amount of DNA as a percentage of the weight of protein was 2% for the light fraction and 1% for the heavy fraction.

The very low virus yields and the narrow host range of human cytomegalovirus (HCMV) have mitigated against precise enumeration and characterization of the structural polypeptides of HCMV. Recently, Sarov and Abady (7) demonstrated 23 structural polypeptides in purified HCMV, using polyacrylamide gel slab electrophoresis. This number is less than that reported for other members of the herpesvirus group, such as Epstein-Barr virus (2) and herpes simplex virus (3). Using Bio-Rad Bio-Gel A-15m column chromatography, followed by a series of two potassium tartrate gradient centrifugations, Kim et al. (5) obtained highly purified murine cytomegalovirus. The starting material was extracellular virus found in the culture fluid of infected cells. The final viral preparation was purified at least 70-fold. Analysis of structural polypeptides of purified HCMV by electrophoresis on a 5 to 20% polyacrylamide gradient gel slab demonstrated at least 33 structural polypeptides. Purified HCMV strain C87 was obtained by similar methods, and the purity of this virus was determined. In addition, we report on the number, molecular weight, and relative amounts of each glycosylated and nonglycosylated viral polypeptide (VP), and the DNA content of purified virus preparations.

MATERIALS AND METHODS

Cells. Human fetal tonsil cells, a diploid cell strain, were obtained from Bertina Wentworth. The growth medium was Eagle basal medium containing 10% fetal calf serum with 100 U of penicillin and 100 μg of streptomycin per ml.

Virus. The C87 strain of HCMV was obtained from Matilda Benyesh-Melnick. The virus was concentrated 50-fold by pelletizing in an A-147 angle rotor at 27,000 rpm for 1 h in an International ultracentrifuge, model B-60, and suspending the resulting pellet in 1/50 of the original volume of medium.

Chemicals and isotopes. Components for poly-

acrylamide gels were obtained from Canalco Co., Rockville, Md. Sodium dodecyl sulfate (SDS) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. D-[1-^{14}C]glucosamine-hydrochloride (57.5 mCi/mmol) was purchased from New England Nuclear Corp., Boston, Mass. [^{35}S]methionine (186 Ci/mmol) was obtained from Amersham/Searle Corp., Arlington Heights, Ill. RNA polymerase was purchased from Boehringer Mannheim Corp., New York, N.Y., and the other standard proteins were obtained from Pharmacia Fine Chemicals, Inc., Uppsala, Sweden. *N,N'*-diallyltartardiamide (DATD) was purchased from Aldrich Chemical Co., San Leandro, Calif.

Plaque titration. The plaque titration procedure was the same as that described by Wentworth et al. (9).

Infection of cells and incorporation of radioactive isotopes. Fetal tonsil cell monolayers, prepared in 32-ounce (ca. 1-liter) Brockway bottles, were used for infection. After the growth medium was removed from each bottle, virus was added at a multiplicity of infection of 5 PFUs/cell, and absorption was continued for 2 h at 37°C. At the end of the absorption period, unabsorbed virus was removed and 60 ml of complete medium was added to each bottle. After 7 days, the culture fluid was used as a source of virus.

[^{35}S]methionine-labeled virus was prepared in the following way. After fetal tonsil cells were infected at multiplicity of infection of 5 PFUs/cell, the infected cells were maintained in medium containing 10% fetal calf serum plus [^{35}S]methionine at a concentration of 1 mCi/ml. After 7 days, extracellular virus was harvested and purified.

[^{14}C]glucosamine-labeled virus was prepared by growing infected cells in Eagle basal medium containing 10% fetal calf serum, 10% of the normal concentration of glucose, and 1 mCi of D-[1-^{14}C]glucosamine-hydrochloride per ml. Extracellular virus was harvested and used for purification.

Virus purification. The method described by Kim et al. (5) for purification of murine cytomegalovirus was used, except for the following. Before the determination of VPs, the virus peak from the second tartrate gradient centrifugation was divided into two portions, a "heavy" and a "light" portion. Of the four fractions from the peak area, the two with lower density were pooled and are referred to as the light fraction, and the two with heavier density were pooled and are referred to as the heavy fraction. The heavy and light fractions were dialyzed overnight against two changes, 4 liters each, of phosphate-buffered saline (PBS).

As a control, the extracellular fluid from mock-infected cultures was processed in the same manner.

To determine the extent of purification, the data for heavy and light fractions were pooled.

Polyacrylamide gel electrophoresis, autoradiography, and densitometry. Electrophoresis in a high-resolution, 5 to 20% gradient polyacrylamide gel slab, in the presence of 0.1% SDS, was done as described by Kim et al. (5). For some experiments DATD was used in place of methylene bisacrylamide (MBA) as the cross-linking agent. In these gels, 1.5 g of DATD was substituted for 1 g of MBA as described by Heine et al. (3). Labeled proteins (^{14}C and ^{35}S) were prepared for electrophoresis by the addition of 2% SDS and 5% mercaptoethanol. The sample was boiled for 2 min and subjected to electrophoresis for 18 h at 6 mA/gel slab. The gel slab was 14 cm wide, 12 cm high, and 1.5 mm thick. At the end of electrophoresis, the gel slabs were fixed, stained with Coomassie brilliant blue (CBB) as described by Weber and Osborn (8), and dried on filter paper. The dried gel was placed in contact with Cronex X-ray film (E. I. duPont de Nemours & Co., Inc., Wilmington, Del.) and kept in a Kodak X-ray exposure holder under high pressure for the exposure interval. The film was developed with Kodak liquid X-ray developer and fixed with Kodak rapid fixer. Densitometer tracings of autoradiographs were done by using a soft laser scanning densitometer, model SL-508 (Biomed Instruments, Inc., Chicago, Ill.).

Molecular weight determination. The method described by Weber and Osborn (8) was used to determine the approximate weights of viral proteins by gel electrophoresis.

Determination of protein and DNA. The protein determination method of Lowry et al. (6) was used to estimate the amount of protein. The amount of DNA was assayed by the method of Burton (1), with calf thymus DNA as a standard.

RESULTS

Purification of HCMV. To assess the degree of purity after purification of HCMV from extracellular fluid, the following experiments were carried out. One liter of [^{35}S]methionine-labeled extracellular virus from day 7 postinfection was mixed with an equal volume of unlabeled normal cell protein found in mock-infected cell culture fluid. This mixture will be referred to as ^{35}S-virus + cell protein. In another experiment, 1 liter of [^{35}S]methionine-labeled extracellular fluid from mock-infected cells maintained in medium for 7 days was mixed with an equal volume of unlabeled extracellular virus. This mixture will be referred to as ^{35}S-cell protein + virus. To eliminate any cellular debris, the mixtures were centrifuged on a Sorval GSA rotor at 3,000 rpm for 20 min. Each mixture was then centrifuged at 33,000 rpm for 90 min using a Beckman L42 rotor. After centrifugation, about 1% of the radioactivity and 0.66% of the protein were recovered in the pellet of the ^{35}S-virus + cell protein preparation, whereas 0.42% of radioactivity and 0.67% of the protein were recovered from the ^{35}S-cell protein + virus preparation.

Prior to potassium tartrate gradient centrifugation, pellets were homogenized with a Dounce homogenizer and passed through a Bio-Rad Bio-Gel A-15m column to remove any contaminating cellular protein with molecular weights less than 15×10^6. The column was loaded with 7 ml of the material and 5-ml fractions were collected. The peaks of radioactivity, PFUs, and absorbance at 280 nm were found in

the void volume. This step provided about twofold purification, as determined by comparing radioactivity and the amount of protein present (Table 1). Total PFUs recovered in the void volume was 3.0×10^8. Judging by PFUs, most of the virus was recovered in two fractions in the void volume. These were layered on a 20 to 50% linear potassium tartrate gradient, which was centrifuged at 25,000 rpm for 18 h at 4°C in a Beckman SW27 rotor. A visible band was seen at a density between 1.20 and 1.21 g/cm^3. One-milliliter fractions were collected from the bottom of the tube, and the density and PFUs of each fraction were determined. The visible band contained the peak of PFUs. The fractions at the density region of 1.20 to 1.21 g/cm^3 were pooled and dialyzed against 4 liters of PBS for 2 h before being layered on a second 20 to 50% tartrate gradient. After the second gradient centrifugation at 25,000 rpm for 18 h, 1-ml fractions were again collected from the bottom of the tube, and the PFUs, radioactivity, absorbancy of 280 nm, and density were determined. Based on the determination of radioactivity and PFUs, the peak from the second gradient centrifugation showed a twofold increase in purity compared with the peak from the first gradient.

The density profile for ^{35}S-virus + cell protein after the second gradient showed coinciding peaks of radioactivity, PFUs, and protein concentration (Fig. 1). The second tartrate gradient profile of the ^{35}S-cell protein + virus preparation is similar to that seen in gradient profiles of ^{35}S-virus + cell protein preparations, except that the amount of radioactivity is reduced markedly (Fig. 2). The relative purity of the virus preparations achieved at each step in the purification procedure is summarized in Table 1. The final ratio of viral to cellular radioactivity was 39. This means that approximately 2.5% of the total radioactivity in purified virus represents cellular material. The total infectivity decreased from 1.4×10^9 to 9.6×10^6 PFUs.

Fractions 16 and 17 from Fig. 1 were pooled, as were fractions 18 and 19 of Fig. 1, fractions 16 and 17 of Fig. 2, and fractions 18 and 19 of Fig. 2. These pooled fractions were dialyzed against two changes of PBS. Pooled fractions 16 and 17

TABLE 1. *Purification of HCMV: recovery of PFUs, virus-specific label, and protein*

Determination	Mixture of ^{35}S-labeled virus protein with unlabeled cell protein					Mixture of ^{35}S-labeled cell protein with unlabeled virus protein				Ratio: cpm ^{35}S-virus + cell protein to ^{35}S-cell protein + virus
	Total PFU	cpm × 10^{-3}	Fraction recovered	Protein (mg)	Fraction Recovered	cpm × 10^{-3}	Fraction recovered	Protein (mg)	Fraction recovered	
3,000-rpm[a] supernatant	1.4×10^9	274,120	(1.0000)[b]	2,425	(1.0000)	289,712	(1.0000)	2,541	(1.0000)	0.946
33,000-rpm[c] pellet	1.0×10^9	2,774.4	0.0100	16.1	0.0066	1,226.4	0.0042	17.1	0.00070	2.262
Bio-Gel[d] virus	3.0×10^8	1,466.1	0.0053	8.2	0.0034	138.6	0.00048	8.8	0.00350	10.578
Peak fractions[e] from second tartrate gradient (fractions 16 through 19)	9.6×10^6	396	0.0013	1.8	0.0007	10.6	0.00004	2.4	0.00094	37.358

[a] Mixtures of both [^{35}S]methionine-labeled virus with unlabeled cell protein and ^{35}S-labeled cell protein with unlabeled virus were centrifuged at 3,000 rpm in a Sorval GSA rotor for 20 min. The supernatant obtained from it was termed the 3,000-rpm supernatant.

[b] Total radioactivity in extracellular fluid was arbitrarily set at 1.00.

[c] The 3,000-rpm supernatant wes centrifuged in a Beckman L42 rotor at 33,000 rpm for 1 h. The pellet was homogenized with a Dounce homogenizer and centrifuged at 2,000 rpm in a Sorval GLC-1 centrifuge for 5 min. The fluid removed from the pelleted debris was termed the 33,000-rpm pellet.

[d] The 33,000-rpm pellet was placed on a Bio-Rad Bio-Gel A-15m column prepared in PBS. Fractions (5 ml) were collected at 4°C. A peak of PFUs, counts per minute, and absorbance at 280 nm was recovered in the void volume (fractions 11 and 12), and this was referred to as the Bio-Gel virus.

[e] The Bio-Gel virus was placed on a tartrate gradient (20 to 50%) and centrifuged at 25,000 rpm for 18 h. Fractions 16 and 17 of the PFU peak were pooled, as were fractions 18 and 19, and these were designated heavy and light fractions, respectively. For calculations of purity, the data for the heavy and light fractions were pooled.

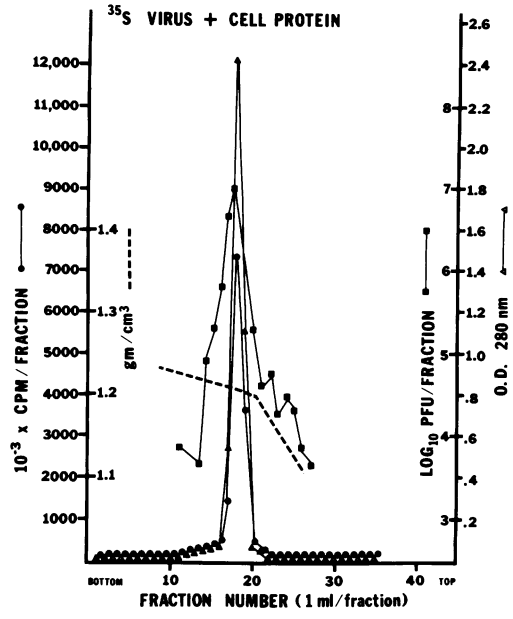

FIG. 1. *Second potassium tartrate gradient (20 to 50%) centrifugation ^{35}S-virus + cell protein. The fractions containing the coincident PFU and radioactivity peaks from the first tartrate gradient were pooled and dialyzed for 2 h against PBS. Then the material was layered on a second tartrate gradient and centrifuged at 25,000 rpm for 18 h in a Beckman SW27 rotor. One-milliliter fractions were collected from the bottom of the tube, and absorbancy of 280 nm, radioactivity, PFUs, and density were determined.*

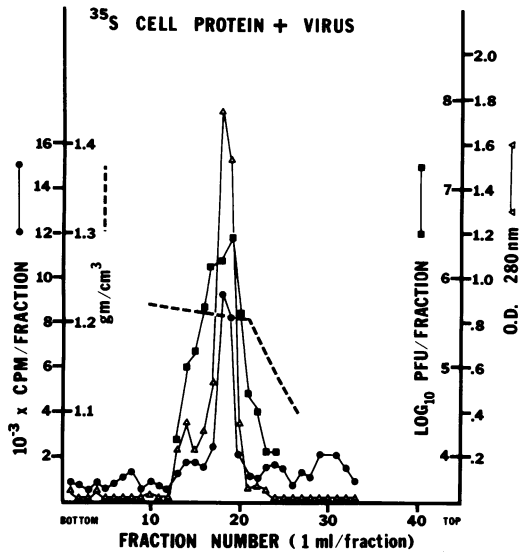

FIG. 2. *Second potassium tartrate gradient (20 to 50%) centrifugation of ^{35}S-cell protein + virus. The methods used were the same as those described for Fig. 1.*

will be referred to as the heavy fraction, and pooled fractions 18 and 19 will be referred to as the light fraction. These preparations were used for analysis of structural polypeptides of HCMV using gradient polyacrylamide gel slab electrophoresis.

Electrophoresis of purified HCMV preparations in 5 to 20% polyacrylamide gradient gel slabs. Purified preparations were solubilized with 2% SDS and subjected to electrophoresis in the 5 to 20% polyacrylamide gradient gel slab containing 0.1% SDS, as described in Materials and Methods. A photograph of the CBB-stained gel slab and the autoradiograph developed from it is shown in Fig. 3. Slot A contained standard proteins with molecular weights ranging from 160,000 to 13,370. Slot B contained 100 μg of heavy fractions of purified ^{35}S-cell protein + virus preparation. Slot C contained 100 μg of heavy fractions of purified ^{35}S-virus + cell protein preparations. The CBB-stained gel slots containing these preparations showed at least 36 bands. Autoradiograms seen in slots b and c correspond to stained gel slots B and C. Since the autoradiograms seen in slot b were prepared from [^{35}S]methionine-labeled normal protein + unlabeled virus, any bands seen in this slot are due to labeled normal cell protein. There are three faint bands in slot b (two of which are difficult to see in photographic reproductions) that were consistently found in both [^{35}S]methionine-labeled normal protein and [^{35}S]methionine-labeled virus preparations. These are assumed to be normal cellular proteins and are labeled CP. Depending on the virus preparations, we could occasionally count one more band, labeled 1A, that is slightly heavier than VP1. Since we do not see this band consistently, we have not counted this as a VP.

Excluding these 3 cellular protein bands and band 1A, there were 33 labeled VPs seen in purified ^{35}S-virus + cell protein (slots C and c), and they represent the structural polypeptides of HCMV, strain C87. These VPs were labeled 1 through 33, as shown in Fig. 3.

Autoradiograms and CBB-stained gels prepared from light fractions of purified virus preparations showed that they contained the same number of VPs seen in heavy fractions of virus preparations.

Determination of molecular weights and relative amounts of VPs. Seven standard proteins (RNA polymerase, subunits of 160,000, 150,000, 90,000, and 40,000 daltons; bovine serum albumin, 68,000 daltons; chymotrypsinogen, 23,240 daltons; and cytochrome *c*, 13,370 daltons) were subjected to electrophoresis in the gel slab with the purified virus proteins (Fig. 3,

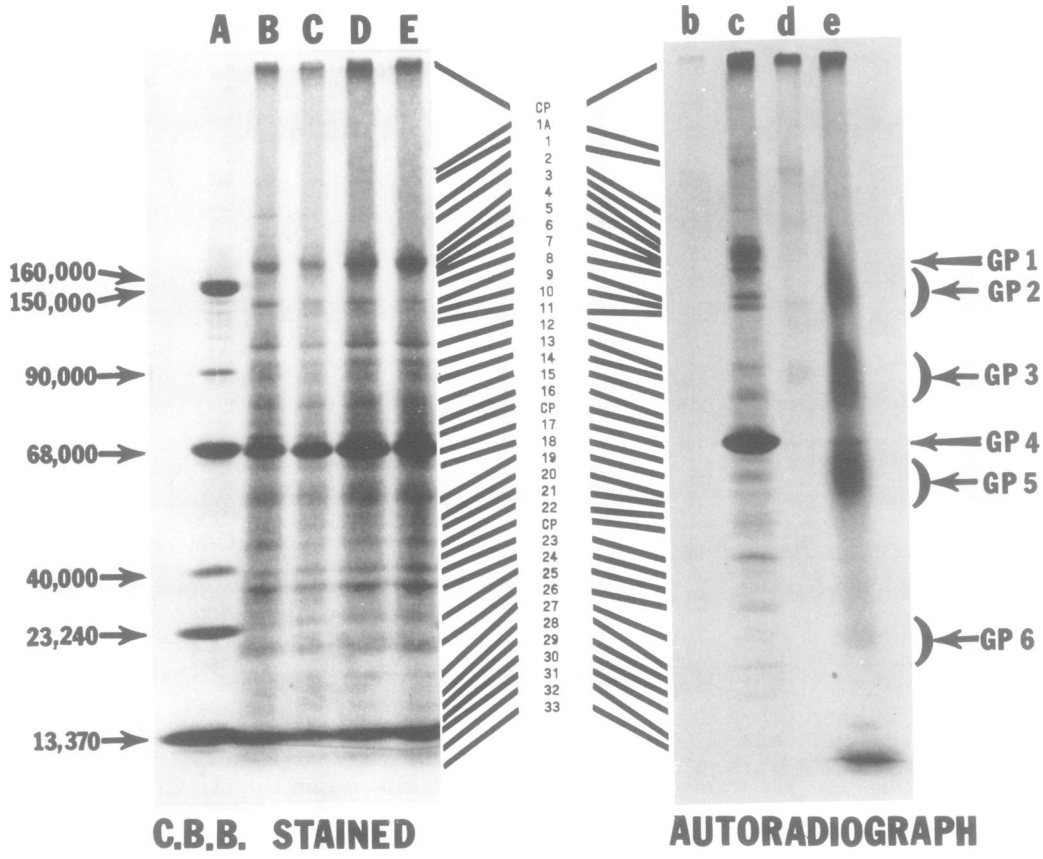

FIG. 3. *Polyacrylamide gradient (5 to 20%) gel slab electropherograms of proteins of purified ^{35}S-virus + cell protein, ^{35}S-cell protein + virus, and [^{14}C]glucosamine-labeled cell protein + virus. CBB-stained gel (left side) and autoradiograph (right side) prepared from the same gel slab are shown. Slot A contains standard proteins. All of the slots except A received 100 μg of protein. Slots B and b, Heavy fraction of ^{35}S-cell protein + virus; slots C and c, heavy fraction of ^{35}S-virus + cell protein; slot D and d, [^{14}C]glucosamine-labeled cell protein + virus; slots E and e, [^{14}C]glucosamine-labeled virus + cell protein. The six glycosylated polypeptides detected by autoradiography are labeled GP1 through GP6.*

slot A). The molecular weights of VP1 through VP33 were determined by comparing the mobilities of these standard proteins (Fig. 4). The molecular weights of VPs ranged from 290,000 to 11,000 (Table 2). The sum of the molecular weights of 33 VPs was $2,998 \times 10^3$. The molecular weights of VPs greater than 160,000 may not represent the correct value, since we estimated them on the assumption that linearity exists beyond 160,000 daltons in the 5 to 20% polyacrylamide gradient gel slab system.

The relative amount of each VP in light and heavy fractions was estimated by measuring the amounts of [^{35}S]methionine in each band. The intensity of each VP band in the autoradiogram was determined, using a soft laser scanning densitometer. Figure 5A and B represent the relative absorbancy profiles of the autoradiograms prepared from light and heavy fractions of purified [^{35}S]methionine-labeled viral preparations, respectively. The amount in each polypeptide band is expressed as a percentage of the total amount of structural polypeptides. The relative amount of each VP is shown in Table 2. Although there are small differences in the relative amounts of some VPs found in the light and heavy fractions, the most striking difference was the consistent presence of a much greater amount of VP17 in the heavy virus fraction (35.2%) than in the light virus fraction (22.2%).

Determination of viral DNA content in light and heavy fractions of purified HCMV preparations. Sarov and Abady (7) reported that the cytomegalovirus dense bodies, which contain very little DNA, banded at a higher-density region (1.21 g/cm^3) than enveloped HCMV (1.20 g/cm^3) upon sucrose gradient centrifugation. Since a certain amount of dense bodies might be present in the heavier fraction of the virus

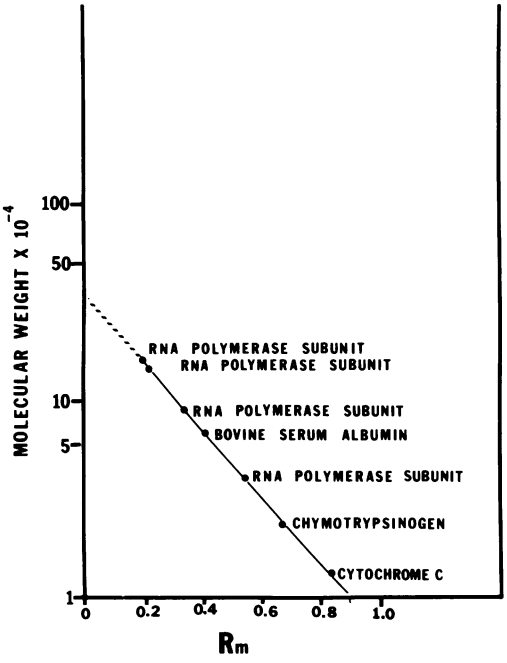

FIG. 4. *Plot of the molecular weights (log_{10}) of standard proteins versus their mobilities on a 5 to 20% polyacrylamide gradient gel slab containing 0.1% SDS. The molecular weights of the HCMV polypeptides were calculated from the data for standard proteins shown in this figure. Since the largest standard protein marker was of molecular weight 160,000, the determination of molecular weights of VPs greater than that was estimated on the assumption that linearity exists above that size (dotted line).*

peak in a tartrate gradient, the relative amounts of DNA present in the heavy and light fractions were determined.

To estimate the percent viral DNA content, the amount of protein and DNA were determined as described in Materials and Methods. The DNA content of the light fraction was 2% of the total protein, whereas the heavy fraction contained only 1% DNA (Table 3).

Glycopeptides of HCMV. To determine how many of the 33 VPs are glycosylated polypeptides, [^{14}C]glucosamine-labeled virus was purified by the same method used for the purification of [^{35}S]methionine-labeled virus. The purified [^{14}C]glucosamine-labeled virus preparation was subjected to electrophoresis and compared with that of purified virus labeled with [^{35}S]methionine. The CBB-stained gel and an autoradiogram prepared from it are shown in Fig. 3. Slot D contained 100 μg of purified [^{14}C]glucosamine-labeled cell protein + virus, and slot d is an autoradiogram developed from slot D. Slot E contained 100 μg of purified [^{14}C]glucosamine-labeled virus + cell protein, and slot e is an autoradiogram developed from slot E. Slots D and E both showed 36 bands by the CBB stain. By autoradiography, at least six glycosylated peptides are seen in slot e. Four of the six glycosylated polypeptide bands were so broad that they corresponded to more than one VP band, making it impossible to assign a specific VP number. None of the six glycosylated polypeptide bands seen in the la-

TABLE 2. *Distribution of [^{35}S]methionine and [^{14}C]glucosamine in structural polypeptides present in HCMV*

Peptide designation[a]	Mol wt × 10^{-3} [b]	Glycosylated[c] peptides	Percent total [^{35}S]methionine incorporation	
			Light fraction	Heavy fraction
CP				
1	290		4.4	6.7
2	220		2.2	1.0
3	210		1.1	1.9
4	195		2.8	3.8
5	185		2.2	4.8
6	175	+ GP1	1.7	1.9
7	165	+ GP1	3.3	5.3
8	155		1.1	1.4
9	140	+ GP2	7.1	1.4
10	135	+ GP2	1.1	1.0
11	130	+ GP2	2.2	2.9
12	105	+ GP3	3.3	1.0
13	98	+ GP3	1.1	1.4
14	90	+ GP3	1.7	1.9
15	84	+ GP3	3.9	5.7
16	78		2.2	1.0
CP	70			
17	66	+ GP4	22.2	35.2
18	52	+ GP5	5.0	3.3
19	50	+ GP5	2.8	1.4
20	48	+ GP5	3.9	1.9
21	44	+ GP5	3.9	1.4
22	42		5.6	1.9
CP	40			
23	38		2.2	4.8
24	35		1.7	1.9
25	30		1.1	0.7
26	28		2.8	1.0
27	22	+ GP6	1.1	0.5
28	19		1.7	0.8
29	17		0.6	0.3
30	15		1.7	0.6
31	14		0.6	0.4
32	12		0.6	0.3
33	11		1.1	0.5
Totals				
33	2,998,000		100.0	100.0

[a] Structural polypeptides were labeled 1 through 33, and host cell polypeptides consistently found with the purified virus preparations are labeled CP.

[b] Molecular weights were calculated to the nearest 1,000.

[c] The glycopeptides corresponded to one or more VPs, as shown by brackets.

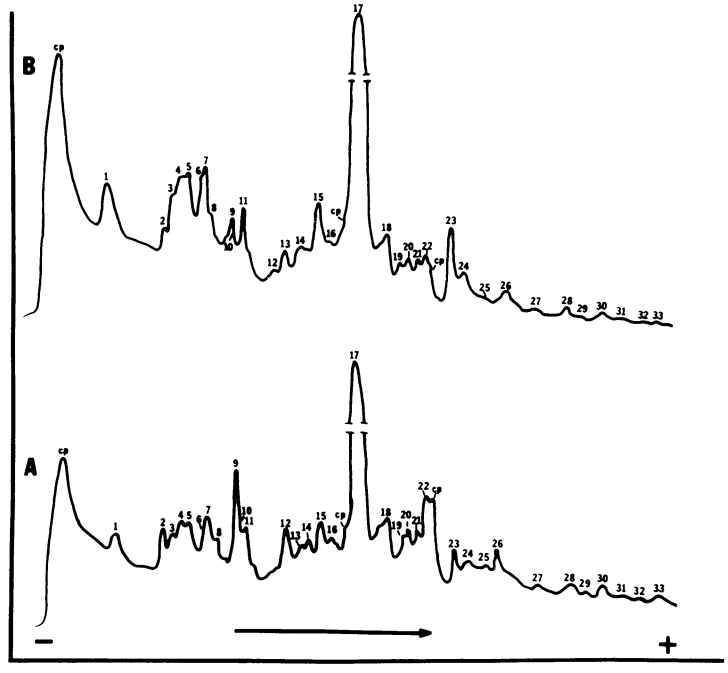

FIG. 5. *Absorbance profile of autoradiogram of polyacrylamide gradient gel slab containing electrophoretically resolved polypeptides from purified ^{35}S-virus + cell protein preparations. Densitometer tracings of polypeptides from light (A) and heavy (B) fractions of PFU peaks were done with a soft laser densitometer.*

TABLE 3. *Concentration of protein and DNA in light and heavy fractions of HCMV*

Fraction[a]	Protein (μg/ml)	DNA (μg/ml)	DNA content (% of total protein)
Light	120	2.4	2
Heavy	250	2.5	1

[a] Fractions were derived from the PFU peak of tartrate gradient centrifugation of HCMV.

beled virus preparation was present in the control (slot d). There were several faint bands seen in the control, but they did not correspond to any VP bands seen in slot c. The glycosylated polypeptide bands shown in Fig. 3, slot e were arbitrarily labeled GP1 through GP6, so that these can be conveniently compared with [^{35}S]methionine-labeled VP bands. Each glycosylated protein corresponded to one or more VPs as follows: GP1 to VP6 and VP7; GP2 to VP9, VP10, and VP11; GP3 to VP12, VP13, VP14, and VP15; GP4 to VP17; GP5 to VP18, VP19, VP20, and VP21; GP6 to VP27. Therefore, except for GP4 and GP6, precise designation of other glycosylated peptides is not possible at present. Since it has been reported that DATD cross-linked gels yield better resolution than gels cross-linked with MBA when both glycosylated and nonglycosylated polypeptides are present (3), both the purified [^{14}C]-glucosamine-labeled and [^{35}S]methionine-labeled virus preparations were compared by electrophoresis in 5 to 20% polyacrylamide gel slabs prepared by using DATD as a cross-linking agent. The results were identical to those seen in gels cross-linked with MBA. We consistently observed a band in autoradiograms beyond the smallest VP band, and this may be due to labeled glycoproteins that were subsequently degraded.

DISCUSSION

The purpose of the present study was to find a suitable procedure to obtain purified HCMV so that VPs could be characterized. The procedure we have established for the purification of murine cytomegalovirus was found to be a very effective method for obtaining highly purified HCMV preparations. The purification procedures used only extracellular virus as the starting material. At 7 days postinfection, about half of the total infectious virus is found in extracellular fluid. Since extracellular fluid contains less contaminating protein than intracellular preparations, it is advantageous for use as the starting material for purification. The maximum amount of contaminating radioactively labeled cellular protein material present

in the virus peak after the second tartrate gradient centrifugation was about 2.5% of the total labeled proteins. In the autoradiogram prepared from the gel containing virus purified from ^{35}S-cell protein + virus mixtures, only three labeled host cell protein bands were seen, and they were constantly present in virus purified from ^{35}S-virus + cell protein mixtures.

The primary purpose of using a Bio-Rad Bio-Gel A15m column was to eliminate any soluble serum components and cell proteins smaller than 15×10^6 daltons. By using virus recovered in the void volume, the possibility of soluble protein coaggregation with virus during gradient centrifugation was reduced. About 30% of the PFUs was recovered in the void volume. The loss may be due to inactivation of infectious virus during column chromatography.

In the present study, purified HCMV (strain C87) was found to contain at least 33 structural polypeptides with molecular weights ranging from 11,000 to 290,000. Sarov and Abady (7) observed 23 structural polypeptides in purified HCMV (strain AD169), with the molecular weight ranging from 24,000 to 171,000. It is not clear whether this discrepancy is related to strain differences or to differences in resolving power of the gel systems used. In preliminary work, we found that VPs with molecular weights of less than 20,000 are not detected in the standard 10% polyacrylamide gel system that was used by Sarov and Abady (7). Thus, the difference in gel systems could account for at least six of the additional VPs observed in this study. The number of polypeptides found in this study is comparable to the findings for several other herpes-group viruses (2, 3).

Autoradiographic analysis revealed that peptides present in both heavy and light portions of the PFU peak contained all 33 VPs. However, there were minor differences in the relative amounts of some VPs and a marked difference in the relative amount of VP17. The heavy fraction contained 35.2% VP17, whereas the light fraction contained only 22.2%. The DNA content of the light portion of the PFU peak was 2% of the weight of protein, whereas the heavy fraction contained only 1%. Sarov and Abady (7) reported that a polypeptide with a molecular weight of 67,000, comparable to VP17, constituted a great portion (42%) of the polypeptides of the cytomegalovirus dense bodies. These workers also reported that the dense bodies contain comparatively little DNA. In the present study, the heavy fraction of the PFU peak, when compared to the light fraction, contained large amounts of VP17 and smaller amounts of DNA. Therefore, these data suggest that the heavy portion of the PFU peak contains some cytomegalovirus dense bodies plus fully or partially de-enveloped HCMV, which tends to band at density higher than the complete virus. A thorough analysis of cytomegalovirus dense bodies will be done, using electron microscope monitoring of fractions from this and other purification methods.

VP17 is the most abundant VP. It is glycosylated and has a molecular weight of 66,000. It is interesting that in a previous study (K. S. Kim et al., manuscript in preparation), a glycosylated protein with a similar molecular weight was found in complement-fixing antigen preparations obtained from five strains of HCMV, including C87 and AD169.

The sum of molecular weights of the 33 VPs was 2,998,000. Since the molecular weight of HCMV DNA was reported to be 100×10^6 (4), 62% of the viral DNA is involved in making VPs. This estimate is calculated on the assumption that viral mRNA is transcribed asymmetrically.

Six glycosylated polypeptides were detected by electrophoresis of [^{14}C]glucosamine-labeled virus. Four of six bands were so broad that more than one VP corresponded to the bands. Electrophoresis of [^{14}C]glucosamine-labeled purified virus preparation in a gradient gel slab cross-linked with DATD did not resolve these glycosylated bands any better than gels cross-linked with MBA.

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