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Mutants of Sindbis Virus

III. Host Polypeptides Present in Purified HR and *ts*103 Virus Particles

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The amounts of host-encoded protein present in purified Sindbis virions of both the HR strain and of a mutant (*ts*103) which makes multicored particles were examined. Cells were labeled with [^{35}S]methionine before infection and with [^{3}H]methionine postinfection. Virions were purified by velocity sedimentation and isopycnic banding, and their polypeptides were examined by polyacrylamide gels in a sodium dodecyl sulfate-containing discontinuous buffer system. Host prelabeled material was found principally in a small number of discrete polypeptides in HR virions, which contained as little as 0.2% host-encoded protein. Virus-sized particles of mutant *ts*103 contained significantly more host material, and multiploid particles from *ts*103 infection contained up to 12% host prelabeled protein.

Sindbis virus and other alphaviruses are the simplest enveloped viruses (20). The icosahedral nucleocapsid of the virus, which contains several hundred molecules of a single protein species surrounding the viral RNA, is assembled in the cytoplasm of the infected cell. Upon diffusion to the cell surface, it interacts directly with the internal regions of two virus-encoded glycoproteins, E1 and PE2, present in the plasma membrane, leading to virus budding. During maturation a proteolytic cleavage of PE2 to E2 and E3 occurs, and the mature virion thus contains only three or four protein species, depending upon whether E3 is retained in the virion. Other enveloped viruses such as rhabdoviruses, orthomyxoviruses, and paramyxoviruses mature by related mechanisms, but they contain a more complex nucleocapsid and also contain, in addition to the external glycoproteins, a matrix protein. This matrix protein is intimately associated with the inner (cytoplasmic) surface of the bilayer and appears to interact with both the capsid and the glycoproteins. In all cases the major polypeptide components of the virion are virus encoded (8, 14), whereas the lipid constituents are largely host specific, with little selection by the virus (3, 9, 13, 15).

Early experiments on virus maturation suggested that host components were excluded from sites of virus budding (8, 14). Subsequent experiments showed that the virus-specific envelope polypeptides are free to diffuse laterally in the host cell membranes and form patches of modified membrane by interaction with internal proteins or nucleocapsids (20). It was then tacitly assumed that virions contained only virus-specific polypeptides and that residual host material found in a given virus preparation was the result of insufficient purification (see review by Lenard and Compans [11]). Recently, however, there has been renewed interest in the possibility that certain cellular polypeptides form integral membrane components of some enveloped viruses. Therefore, Sindbis virions were examined for the presence of host polypeptides which had been synthesized before infection. The amount and type of cellular material found in the HR strain and *ts*103 virions were compared. *ts*103 is a mutant of Sindbis virus which forms aberrant particles containing multiple nucleocapsids within one envelope (19).

MATERIALS AND METHODS

Cell and virus. Preparation of monolayers of chicken embryo fibroblasts has been described previously (16). Virus stocks used were the HR strain of Sindbis virus (2) and the mutant *ts*103 (19).

Labeling conditions. Roller bottles (750 cm^2) of subconfluent chicken embryo fibroblasts were washed for several hours with Eagle minimal essential medium (6) containing Earles salts, 2% dialyzed fetal calf serum, and 1/3 to 1/5 the normal concentration of essential amino acids. Then [^{35}S]methionine (460 to 500 Ci/mmol; Amersham/Searle) was added to 4 to 4.2 mCi/roller bottle (depending on the experiment) in 150 ml of Eagle medium containing 1/3× of all essential amino acids except methionine (which was at 0.017 mM or 1/6×) and 3% dialyzed fetal calf serum. In this medium, the monolayers reached confluency in 48 h, as opposed to 24 h in medium containing normal amino acid concentrations.

At 3 h before infection, the ^{35}S-containing medium was replaced with Eagle medium containing unlabeled methionine at 0.2 mM (2×) and 2% dialyzed fetal calf

serum. Bottles were infected with 10 ml of phosphate-buffered saline (5) containing 1% dialyzed fetal calf serum, 1 μg of actinomycin D per ml, and sufficient HR or *ts*103 virus to give a multiplicity of 5 to 10 PFU/cell. Mock-infected rollers received 10 ml of diluent without virus.

At 1 h postinfection the inoculum was removed and replaced with 40 ml of low-salt medium (containing NaCl at 0.065 M in place of the normal concentration of 0.115 M) per roller bottle with 1/3× essential amino acids except methionine (which was at 1× or 2×) and 1 μg of actinomycin D per ml. After 1 h this was replaced with medium containing [^{3}H]methionine (5 to 10 mCi/bottle), 0.065 M NaCl, 1/3× essential amino acids except methionine, 3% dialyzed fetal calf serum, and a total concentration of methionine of 1/2× to 2× depending upon the experiment. ([*Methyl*-^{3}H]methionine [5.5 Ci/mmol] was purchased from Amersham/Searle, lyophilized to remove mercaptoethanol, dissolved in the labeling medium, and sterilized by filtration.) Bottles were incubated at 30°C for 16 to 17 h after HR virus infection and 20 to 21 h after *ts*103 infection. The low-salt radioactive medium was removed, and the virus was harvested by incubating the monolayers with a small volume of medium containing 0.16 M NaCl (16).

Purification of virus. The entire virus harvest from one roller bottle (~7 ml) was layered onto 32-ml gradients of 15 to 30% sucrose in 0.2 M NaCl–0.05 M Tris, pH 7.6–0.001 M EDTA–0.3% fetal calf serum and sedimented in an SW27 rotor for 90 min at 27,000 rpm and 4°C. Fractions were collected by pumping, and a small portion of each one was assayed for ^{35}S and ^{3}H. Fractions containing virion-sized *ts*103, multiploid *ts*103 sedimenting 1.7× as rapidly, and 2.0× multiploids were pooled separately. Comparable regions of the sucrose gradients containing HR and the harvest from a mock-infection roller bottle were also pooled.

Pooled fractions were layered directly onto 11-ml preparative isopycnic gradients containing 22 to 44% sucrose, 0.2 M NaCl, 0.05 M Tris (pH 7.6), 0.001 M EDTA, and 0.3% fetal calf serum in 90% D_2O. Centrifugation was for 12 h or more in an SW40 rotor at 32,000 rpm and 4°C. A small amount of each of the pools was also centrifuged in a parallel analytical isopycnic gradient with ^{32}P-labeled HR virions as a marker. In either case the gradients were collected by pumping, and a small amount of each fraction was counted in a three-channel Beckman liquid scintillation spectrometer. Correction of the counts for channel overlap was made by computer.

For quantitation of host polypeptides, pooled fractions from the preparative isopycnic gradients were diluted with 5 volumes of 0.2 M NaCl–0.05 M Tris, pH 7.6–0.001 M EDTA and further purified by a second cycle of sucrose gradient velocity sedimentation followed by isopycnic separation.

Polyacrylamide gel electrophoresis. Gels containing 10% acrylamide, 0.26% bisacrylamide in 0.19 M Tris-chloride buffer (pH 8.6), and 0.1% sodium dodecyl sulfate (SDS) were prepared as described by Laemmli (10). Stacking gels contained 5% acrylamide, 0.067% bisacrylamide, 0.063 M Tris (pH 6.8), and 0.1% SDS. Samples were first dialyzed against buffer containing 0.05 M Tris-chloride, pH 6.8, 1% SDS, 10% glycerol, and 1 to 2% β-mercaptoethanol and subjected to electrophoresis until the bromophenol blue front was approximately 0.5 cm from the bottom of the gel. Gels were frozen and sliced on a Mickle gel slicer, and the fractions were counted in a scintillation cocktail containing toluene, Liquifluor, and NCS.

Gels were calibrated for molecular weight by using a series of standard proteins. Electrophoresis conditions were the same as described above, except that, in the case of the unlabeled markers, the gels were stained overnight with 0.05% Coomassie brilliant blue–25% isopropyl alcohol–10% glacial acetic acid and destained with several changes of water–methanol–glacial acetic acid at 66:33:10. Gels were scanned for absorbance at 580 nm with a gel scanning attachment for an ISCO model UA-5 Absorbance Monitor (Instrumentation Specialties Co.). Standards used in addition to the three virion proteins from purified HR virus were phosphorylase A (molecular weight, 94,000 [94K]), bovine serum albumin (66K), pyruvate kinase (57K), heavy chain of gamma globulin (50K), actin (43K), D-amino acid oxidase (37K), and soybean trypsin inhibitor (21K).

We have found that small changes in pH significantly influence the rate of migration of the virion polypeptides relative to the bromophenol blue marker but have little effect on the migration of the Sindbis polypeptides relative to one another. For this reason all measurements of migration rates were made relative to the migration rate of Sindbis capsid protein.

Laemmli slab gels containing an exponential gradient of acrylamide from 8 to 20% were used to better display the lower-molecular-weight components. Standard proteins used to calibrate this gel system were insulin (two chains of molecular weights 2.3K and 3.4K), α-bungarotoxin (8K), cytochrome *c* (11.7K), β-lactoglobin (18.4K), and soybean trypsin inhibitor (21K).

Immune precipitation. As a final method of purification of certain virus fractions, the material was precipitated by a two-stage immune precipitation, in which rabbit anti-Sindbis immunoglobulin G (IgG) and goat anti-rabbit IgG were used. The anti-Sindbis IgG fraction was a gift of C. Birdwell and was absorbed three times on monolayers of uninfected chicken cells before use. Goat anti-rabbit IgG was obtained as a lyophilized powder from Calbiochem. Fractions from the isopycnic gradients were precipitated at 4°C with anti-Sindbis serum for 4 h, followed by incubation overnight at 4°C with goat anti-rabbit serum. Precipitates were collected by centrifugation at 27,000 × *g* for 20 min and dissolved in the same buffer as used for dialysis of gel samples. Bromophenol blue was added, and the samples were boiled for 2 min. Electrophoresis, slicing, and counting were as described above.

RESULTS

Amount of prelabeled protein in HR and *ts*103. Several prelabeling experiments were performed, with some changes in procedural detail, especially in the specific activities of methionine used; with each experiment an attempt was made to improve the efficiency of the chase. Several preliminary experiments were done in

which the purification consisted of only a single cycle of velocity and isopycnic centrifugation. In these experiments, the amount of host material in 1× *ts*103 virions varied between 0.5 and 2.8%, depending on the preparation. A final experiment with a two-cycle purification reduced the amount of host material to an apparent minimum and yielded the quantitative data shown in Table 1.

Chicken embryo fibroblasts were labeled for 2 days with [^{35}S]methionine at a specific activity of 1,700 mCi/mmol, infected with either *ts*103 or HR or mock infected, and labeled postinfection in low-salt medium containing [*methyl*-^{3}H]methionine at 1,150 mCi/mmol; virus was harvested in normal salt. The entire virus harvest from each infection was sedimented in sucrose velocity gradients, and materials sedimenting at 1× (the major virus peak), 1.7× (containing multiploid particles sedimenting 1.7× as fast as HR virions), and 2.0× were pooled for further analysis.

Each of the pooled fractions was layered onto both analytical and preparative isopycnic gradients. Figure 1 shows profiles obtained with preparative isopycnic gradients of 1× (virion-sized) and 1.7× material for HR, *ts*103, and mock infections. The gradients from virus-infected material contained well-defined peaks of ^{3}H label, which was coincident with the ^{32}P marker HR virus on the parallel analytical gradients. The mock-infected monolayer showed no such peak and illustrated that most of the ^{35}S label associated with the virus from the infected cells was specific to virus infection. The location of the virus region shown on this gradient was determined by reference to the ^{32}P marker HR on the analytical gradient. In all three samples from the 1.7× region there was a second peak (band 2; usually split into two poorly resolved peaks) near the density of 1.19 g/ml (at 5°C), midway between the sample overlay and the Sindbis peak at 1.21 g/ml. From the density we postulate that this fraction contained membrane fragments which sedimented at greater than 400S.

The virus peaks from such isopycnic gradients were diluted and resedimented in velocity sucrose gradients, and the appropriate fractions were again selected and rebanded on isopycnic gradients. Selected isopycnic gradients from such a two-cycle purification are shown in Fig. 2. On these gradients, little or no radioactivity, either ^{35}S prelabel or ^{3}H postlabel, was found outside the virus peak. In the case of the 1× *ts*103 the two halves of the final isopycnic gradient were pooled separately (1× *ts*103 "dense" and 1× *ts*103 "light"). In the case of 1.7× *ts*103, the virus band was split according to density after the first isopycnic gradient (see below).

Figure 3 shows the result of the second-cycle velocity gradient of the 1.7× HR. The majority of the ^{3}H label (>60%) sedimented at 1×, with only 25% of the material resedimenting at 1.7×. This is characteristic of HR preparations; multiploid particles are produced in very small amounts and are often obscured by reversible aggregates of 1× particles sedimenting in the same region. (The 1.7× particles from the second cycle all sediment at 1.7×, however.) It is readily apparent from this figure that there was much more ^{35}S associated with the multiploid particles than with the 1× virions (also see below). The 1.7× particles and the 1× particles from this velocity gradient were pooled separately and sedimented to equilibrium on isopycnic gra-

TABLE 1. *Quantitation of host polypeptides in Sindbis virus*

Sample[a]	% Host polypeptides[b]	Total [^{3}H]methionine radioactivity (cpm)	% as E1 + E2 + C	Total [^{35}S]methionine radioactivity[c] (cpm)	% as E1 + E2 + C
1× HR	0.24	385,600	94.3	9,690	68.0
1× HR from 1.7× HR pool	0.56	28,700	89.4	1,010	49.7
1.7× HR	4.01	5,300	77.0	770	24.1
1× *ts*103 dense	0.58	110,100	88.8	3,950	49.1
1× *ts*103 light	1.14	30,170	84.6	1,560	33.5
1.7× *ts*103 intermediate density	3.80	9,050	81.5	1,320	24.5
1.7× *ts*103 light	12.31	2,650	75.5	1,070	18.2

[a] Preparation and purification of these samples is described in the text.

[b] Host polypeptides are computed as follows: % host polypeptides = (counts of ^{35}S not in E1 + E2 + C/counts of ^{3}H in E1 + E2 + C) × (specific activity of ^{3}H in counts per minute per mole/specific activity of ^{35}S in counts per minute per mole). This formula expresses the ^{35}S present corrected for the amount reutilized in virion proteins but uncorrected for any other turnover and normalized to the total amount of virion polypeptide on the gel. It gives a mass ratio if one assumes that the average methionine content of the host polypeptides is similar to that of the virion polypeptides.

[c] ^{35}S counts have been corrected for decay.

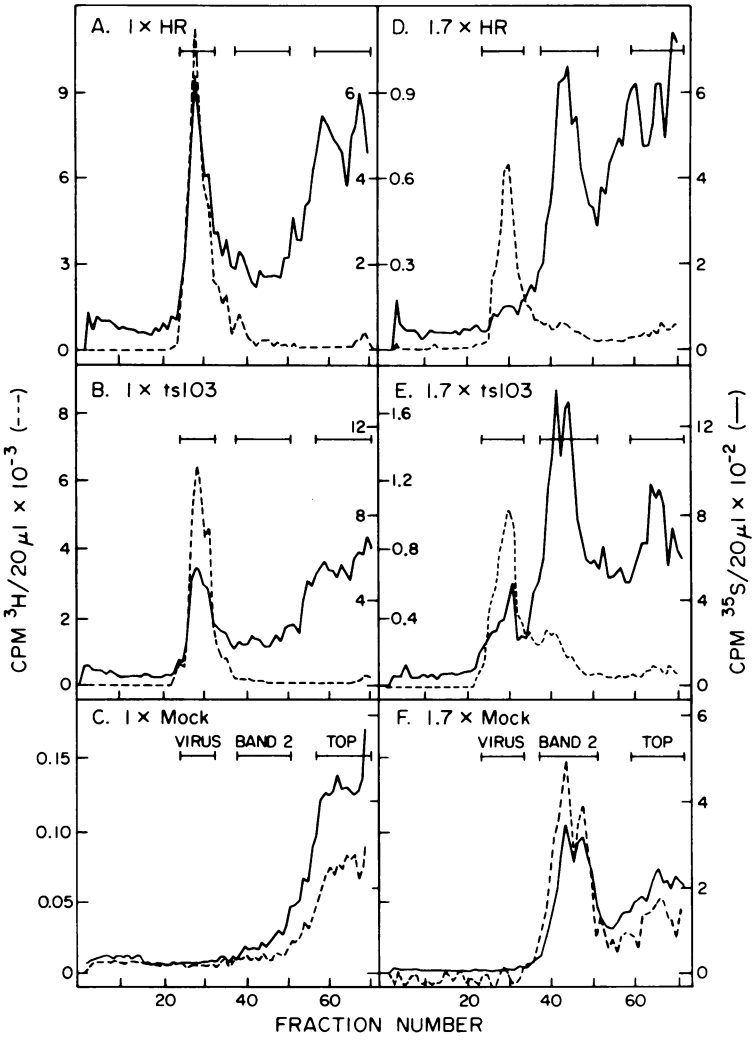

FIG. 1. *Preparative isopycnic gradients of 1× and 1.7× regions from HR, ts103, and mock infections. Samples (20 µl) of each fraction were counted in Aquasol to determine the distribution of prelabel and postlabel. (A) 1× HR; (B) 1× ts103; (C) 1× mock; (D) 1.7× HR; (E) 1.7× ts103; (F) 1.7× mock.* (——) *[^{35}S]-methionine prelabel;* (---) *[^{3}H]methionine postlabel. Sedimentation was from right to left.*

dients. They are referred to below as 1× HR from 1.7× pool and 1.7× HR, respectively.

The 1.7× *ts*103 particles show considerable density heterogeneity on isopycnic gradients (19). Therefore, after the first isopycnic centrifugation, three regions of the virus band were pooled: the dense side, the central (intermediate) region, and the light side. Each of these was diluted and resedimented separately on 15 to 30% sucrose gradients, where virtually all the radioactivity resedimented at 1.7×. The 1.7× material from the three second-cycle velocity gradients were pooled separately and rebanded on three isopycnic gradients, where each formed a single band. These preparations are referred to as 1.7× *ts*103 dense, 1.7× *ts*103 intermediate, and 1.7× *ts*103 light, respectively.

Selected fractions from these gradients were analyzed by acrylamide gel electrophoresis for radiolabel in virion and host polypeptides. Representative gels are shown in Fig. 4, and the results of one such experiment are summarized in Table 1. Several conclusions can be drawn from these data. The first is that the amount of host polypeptide found in HR virions was strikingly small, as little as 0.24%. The amount of prelabel in 1× *ts*103 was severalfold higher, on the other hand, and depended on the density of the particle. Multiploid particles, whether HR or *ts*103, contained more host material; for ex-

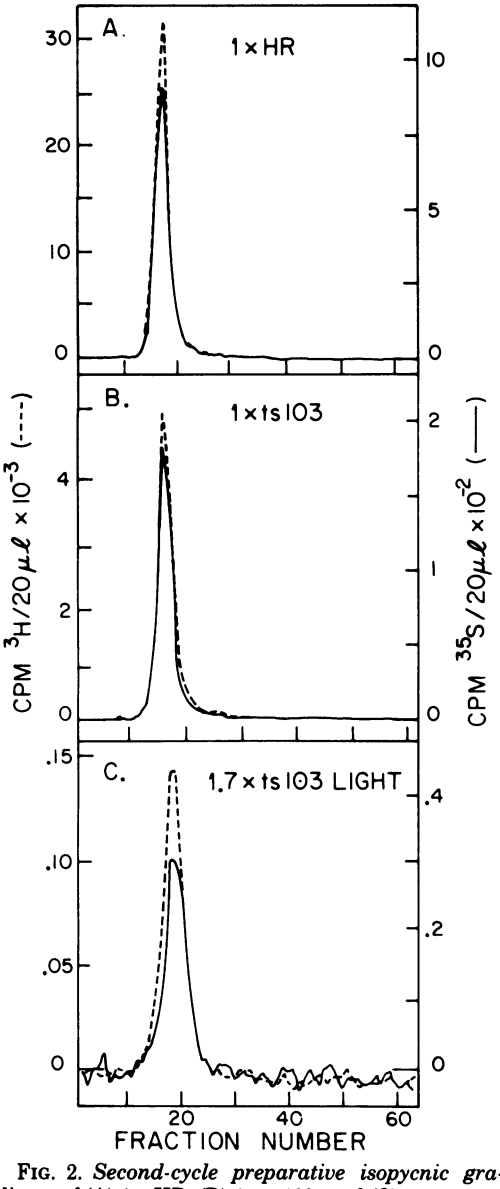

FIG. 2. *Second-cycle preparative isopycnic gradients of (A) 1× HR, (B) 1× ts103, and (C) 1.7× ts 103 light. The origin of the samples is described in the text.* (——) *[^{35}S]methionine prelabel;* (- - -) *[^{3}H]methionine postlabel.*

ample, the light 1.7× *ts*103 contained up to 12% host protein, 10 to 20 times as much as was found in 1× *ts*103. Despite these differences, earlier experiments have shown that these multiploids are fully infectious. The 1.7× HR are also naturally occurring multiploids, but have not been as well characterized (19). Note that 1.7× HR were made in very small amounts and contained levels of prelabeled material comparable to that found in 1.7× *ts*103 of intermediate density. These results suggest that abnormalities in budding lead to the inclusion of more host material in the virions produced.

Host polypeptides in purified virus particles and in other fractions. Figure 4 shows three of the profiles from which the data in Table 1 were obtained and illustrates that purified virions from HR and *ts*103 differ in composition of prelabeled material. In these preparations, a small number of discrete species of prelabeled host polypeptides was identified. The same host polypeptides appeared reproducibly in all prelabeled preparations examined and have been numbered in order of decreasing molecular weight. 1× HR virions (Fig. 4A) contain polpeptides called 1 (molecular weight, 68K), 3 (41K), 7 (22K), 8 (21K), and F (polypeptides of <20K which migrated with the solvent front). (For reference, the apparent molecular weights of E1, E2, and C in this system are 54K, 48K, and 32K, respectively.) Prelabel ^{35}S was also found in E1, E2, and C, presumably due to inefficiencies in the chase or from breakdown and reutilization of the label after infection, but there was very little ^{35}S in other regions of the gel. 1× *ts*103 (Fig. 4B) contained the host polypeptides found in 1× HR as well as peaks called

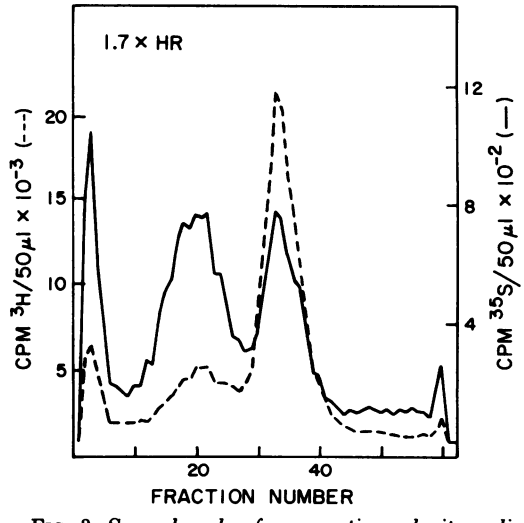

FIG. 3. *Second cycle of preparative velocity sedimentation of 1.7× HR. 1.7× HR material was purified by velocity sedimentation followed by isopycnic centrifugation, and the virus band was diluted and centrifuged on a 15 to 30% sucrose gradient. Portions of each sample were counted for ^{35}S and ^{3}H. Samples 14 through 23 were pooled, banded on an isopycnic gradient, and designated 1.7× HR. Samples 30 through 36 were treated similarly and designated 1× HR from 1.7× HR pool.* (——) *[^{35}S]methionine prelabel;* (- - -) *[^{3}H]methionine postlabel. Sedimentation was from right to left.*

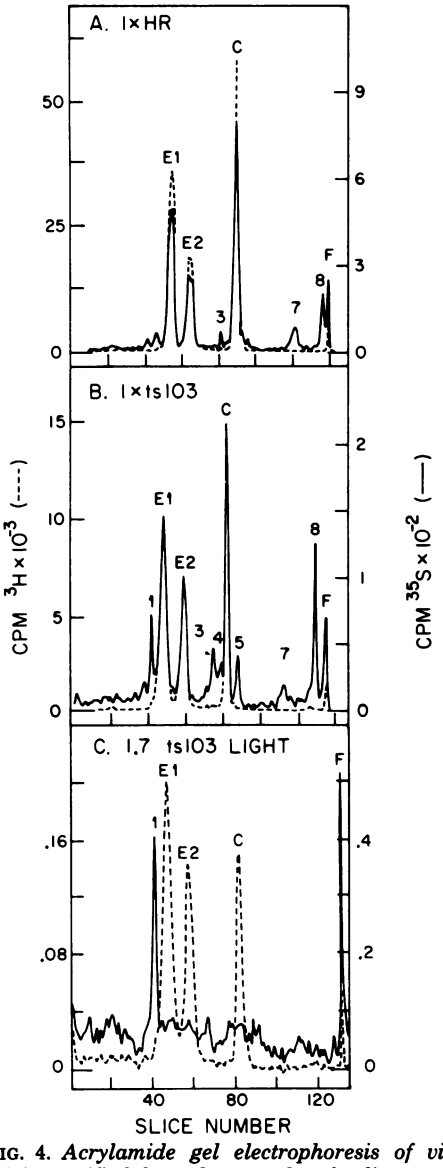

FIG. 4. *Acrylamide gel electrophoresis of virus particles purified through two cycles of sedimentation followed by isopycnic density banding. Electrophoresis was from left to right; F comigrated with the bromophenol blue marker. (A) 1× HR. Same preparation as in Table 1, line 1. (B) 1× ts103, dense side of virus peak. Same preparation as in Table 1, line 4. (C) 1.7× ts103, light side of virus peak. Same preparation as in Table 1, line 7.* (——) *[^{35}S]methionine prelabel;* (- - -) *[^{3}H]methionine postlabel.*

4 (33K) and 5 (29K). All of the host peaks were more prominent in the *ts*103 material, and in addition there was more of a background smear of ^{35}S throughout the gel. The light 1.7× *ts*103 particles (Fig. 4C) contained ^{35}S primarily in peptides 1 and F, plus a general smear. In Fig. 4, the ^{3}H postlabel was found only in the Sindbis polypeptides and in the small material migrating at the front.

Selected fractions from first-cycle isopycnic gradients (such as those shown in Fig. 1) were also examined as a check on virus purification and to determine the distribution of polypeptides throughout the gradients. Figure 5 shows gel profiles of the band 2 material from mock-infected and *ts*103-infected preparations. One predominant peak (6, 25K to 26K) was found in addition to F. Polypeptide 6 (as well as F) was labeled with both ^{35}S and ^{3}H in both cases, showing that it is a species which turns over rapidly, is synthesized after actinomycin treatment of the cells, and is independent of virus infection. Band 2 from *ts*103 infection contained the Sindbis polypeptides in addition to 6 and F. The absence of polypeptide 6 from the various purified virion preparations in Fig. 4 demonstrates that the virus preparations were not detectably contaminated with band 2 material. The origin of band 2 is unclear, although its isopycnic density of 1.18 to 1.19 indicates that it contains membrane fragments. Whatever its origin, it is curious that it contains such a limited distribution of polypeptides.

It should also be pointed out that host polypeptides 1, 5, and 7 could not be detected in any fraction examined from uninfected cells (whether the virus region, band 2, or the soluble material at the top of the isopycnic gradient). These polypeptides appear to be specifically associated with virus particles in some way.

The low-molecular-weight material migrating with the solvent front on these gels (F) has been shown to be composed of polypeptides. It is nondialyzable, precipitable in hot trichloroacetic acid, and insoluble in chloroform-methanol. When F was displayed on 8 to 20% Laemmli slab

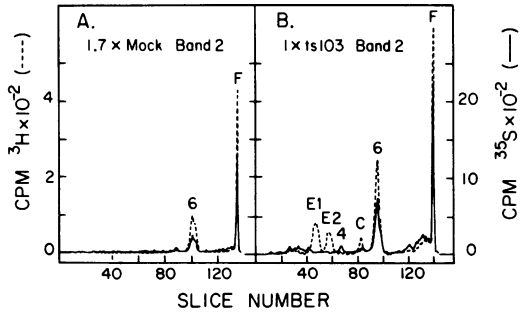

FIG. 5. *Polyacrylamide gel electrophoresis of band 2 fractions from isopycnic gradients. F comigrated with the bromophenol blue marker. (A) 1.7× mock, band 2 (pool of fractions 41, 42, 43 from gradient in Fig. 1F). (B) 1.7× ts103, band 2 (pool of fractions 37, 38, 39 from gradient in Fig. 1E).* (——) *[^{35}S]methionine prelabel;* (- - -) *[^{3}H]methionine postlabel.*

gels with appropriate low-molecular-weight markers, both the prelabel and postlabel showed a small number of discrete bands, between two and four depending on the sample, with molecular weights between 10K and 20K. F was present in the most highly purified virus preparations examined (Fig. 4A and B) and was not removed by immunoprecipitation with anti-Sindbis antibody, implying that some or all of these small polypeptides may be integral components of the virion. On the other hand, it is possible that these low-molecular-weight components are in part generated by proteolysis occurring during purification.

DISCUSSION

Host polypeptides in purified Sindbis virions have been examined to determine the absolute level of host protein present in virus particles, to ascertain the nature and degree of heterogeneity of host protein found in virions, and to determine whether the amount or type of host material depends upon the efficiency of budding. First of all, the host material found in HR virions was a very small percentage of the protein of the Sindbis virion, although it is always difficult to know whether residual host label in virions is due to true virion components or to residual nonvirion contaminants. Pfefferkorn and Clifford (14) placed an upper limit of 5% host material in Sindbis virus, but improved techniques and the availability of higher specific activity labels have reduced this limit considerably. The present results indicate that during a standard infection with the virus, the amount of host protein found in purified virions was as low as 0.2 to 0.5% of the total protein, which translates to only two to four host polypeptides per particle. What proportion of the protein of the infected cell plasmalemma is represented by E1 and PE2 is unknown, but it is probably less than 50%, considering the length of the infection and the relative rates of protein synthesis in infected and uninfected cells. Thus, an area of membrane which contains more than 400 host proteins and less than 400 virus-specific molecules at the onset of budding produces a mature virion containing 800 virus polypeptides and not more than 2 to 4 host polypeptides in its envelope. This rather remarkable selectivity in an essentially fluid system is probably provided by the interaction between the nucleocapsid and the transmembranous portions of the glycoproteins (20).

In comparing HR virus with *ts*103, it was found that 1× *ts*103 contained more prelabel material than did the comparable HR fraction. In addition to the discrete species of polypeptides seen in HR particles, *ts*103 particles also contained more of a smear of many polypeptides, suggesting a nonspecific inclusion of host material. Larger *ts*103 virion particles, the multiploids which contain four to eight nucleocapsids in a single envelope, contained significantly more host polypeptides than did the 1× virions with a single core, and the smear of background polypeptides was quite pronounced. Furthermore, the *ts*103 multiploids of lowest density contained up to 12% ^{35}S prelabel; these particles have a higher proportion of envelope to core, in other words, loosely fitting membranes. It is also of note that multiploids of HR contain more host protein than does the standard HR virion; this is readily apparent in Fig. 3 and Table 1.

There are three possible origins for the host material which was found associated with virus. The first is that the ^{35}S material consists of residual contaminants which are adsorbed onto the surface of the virions. Such a mechanism would not explain the differences seen between 1× HR and 1.7× HR or between 1× HR and 1× *ts*103, which should have very similar surface properties for adsorption. The second possibility is that the ^{35}S-labeled protein is due to intrinsic contamination, i.e. to material which copurifies with particular virus classes. This seems highly unlikely because of the absence of comparable prelabeled material in mock-infected preparations and because of the disparate levels of host polypeptides present in the various virus subclasses. I personally favor the third alternative, i.e., that some of the prelabeled material (above the 0.2 to 0.5% level which may be a measure of the purity of the preparations) represents integral host membrane components which are inserted in the envelope of the virion.

All of the results support the general model for Sindbis maturation previously presented (20) as well as our explanation of the defect in the mutant *ts*103 (19). The *ts*103 defect is probably in the nucleocapsid protein; we have shown that the majority of nucleocapsids synthesized early in infection are aberrant in their sedimentation behavior, but that most of these defective cores do not bud and are not recovered in released virions. We have postulated that all of the manifestations of the *ts*103 mutation can be explained if the *ts*103 nucleocapsid can only interact weakly with the glycoproteins in the cell surface. Whereas *ts*103 very seldom buds singly and usually buds as multiploid particles, it is easy to see how host material could be included adventitiously.

The most highly purified preparations of HR virions contain the equivalent of only two to five molecules of host polypeptides per virion, and these are present as several polypeptide species.

In contrast to this, actin has been found to be a regular component of both paramyxoviruses, such as Sendai virus (17), and the avian, monkey, and murine RNA tumor viruses (21). Comprising up to 8% of the protein mass of the Rous-associated virus-2 virion, this large amount of a cellular protein incorporated is thought to result from a budding mechanism which requires the juxtaposition of microfilaments to the emerging virion. Actin, which should migrate at the position of host polypeptide 3, could be present in only insignificant quantities in Sindbis virions. In addition, there have been numerous reports which suggest an association of polypeptides of the major histocompatibility complex with enveloped virus particles. On the one hand, H-2 antigens have been reported to be integral components of vesicular stomatitis virions grown in mouse L-cells (7). On the other hand, studies with several viruses, including lymphocytic choriomeningitis virus and Rauscher leukemia virus, have shown that sensitized cytotoxic T-lymphocytes are most efficient in killing virus-infected cells when the target and the killer cells share the same haplotype of the H-2 histocompatibility locus, suggesting that the cell surface antigen recognized might be a hybrid of viral and cellular components (4, 18). Sindbis virions have not been tested directly for such antigens, but they could be present only in very small quantities in these preparations.

There have also been numerous reports of host cell enzymatic activities associated with purified viruses, but whether these represent polypeptides necessary for production of infectious virions, adventitious components which are engulfed or included in the envelope at the time of budding, or stubborn contaminants which copurify with virions is unclear (1). It has been suggested that the host-encoded ribosomes which are found in the arenaviruses belong to the second category and are not required for virus replication (12).

Finally, there is supporting qualitative evidence indicating that alphaviruses are much more selective in their envelope components than other virus groups. A recent series of phenotypic mixing experiments has shown that it is possible to produce pseudotypes containing either vesicular stomatitis virus or avian tumor virus genomes with Sindbis envelope glycoproteins. However, no particles could be detected containing Sindbis nucleocapsids and heterologous viral antigens (22). This implies that the interaction of the alphavirus nucleocapsid with its envelope is highly specific, much more so than the interaction between glycoproteins and the matrix proteins of rhabdoviruses or oncornaviruses. This may explain the almost complete exclusion of host polypeptides from alphavirus virions which has been observed, in contrast to the situation with other enveloped viruses discussed above.

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