JOURNAL OF VIROLOGY, June 1973, p. 1013-1016

Vol. 11, No. 6
Printed in U.S.A.

NOTES

Large-Molecular-Weight Precursors of Sindbis Virus Proteins

MILTON J. SCHLESINGER AND SONDRA SCHLESINGER
Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

Received for publication 22 January 1973

Infection of chicken embryo fibroblasts with a temperature-sensitive mutant of Sindbis virus at the nonpermissive temperature leads to the accumulation of a large-molecular-weight protein. We have shown that this protein contains ^{14}C-arginine tryptic peptides present in the three virion proteins. We have also found that a slightly smaller protein which is detected in Sindbis-infected BHK cells contains the ^{14}C-arginine tryptic peptides of the two envelope proteins but not those of the capsid protein. Pulse-chase experiments indicate that the Sindbis virus protein in BHK cells is cleaved to the envelope proteins.

Sindbis virions contain three proteins: two glycoproteins (E1 and E2) located in the envelope and an internal protein that is part of the nucleocapsid (4, 9). There may be as many as 10 additional virus-specific proteins in Sindbis virus-infected cells; some of these are believed to be large-molecular-weight precursors of the virion proteins (8). We recently concluded that one of the virus-specific cellular proteins (PE2) is a direct precursor of the E2 glycoprotein on the basis of pulse-chase experiments and an analysis of the fingerprints of tryptic peptides (5). It has been suggested that the proteins of Sindbis virus originate as a single, large polypeptide that is cleaved to the virion proteins in a manner analogous to that described for picornaviral proteins (1, 2, 10). Evidence to support this idea came from the observation that chicken embryo fibroblasts (CEF) infected with a particular class of temperature-sensitive mutants at a nonpermissive temperature accumulated a large-molecular-weight protein at the expense of the viral proteins (3, 8). Furthermore, another potential precursor was detected in Sindbis virus-infected BHK cells (8). The latter protein is much larger than any of the individual virion proteins but is smaller than the protein associated with the temperature-sensitive mutant.

Our approach to determining the relationship of these larger proteins to the proteins of the virion was essentially the same as the one we used in establishing the precursor role of PE2 (5). As a first step, we prepared ^{14}C-arginine-labeled virus-specific proteins from infected cells and purified them by acrylamide gel electrophoresis. Figure 1 shows the distribution of radioactivity obtained in CEF infected with the temperature-sensitive mutant (ts2) of Sindbis at 40 C. Almost all of the isotope was located in a single, large-molecular-weight protein. The protein pattern observed with BHK cells labeled with ^{14}C-arginine for 30 min at 5 h after infection is depicted in Fig. 2. The protein marked B1 is the one associated with infection of BHK cells. From several gels such as the one shown in Fig. 2, we isolated B1, the capsid protein, and a mixture of PE2 plus E1. The latter two proteins are often not well resolved on cylindrical gels, but are clearly distinct on autoradiograms of slab gels (Fig. 4).

The proteins were digested with trypsin, and the peptides were analyzed by paper electrophoresis and paper chromatography as described previously (5). Autoradiograms of these fingerprints are presented in Fig. 3. Panel A is that of the largest polypeptide formed by the ts2 mutant; eight of the arginine peptides are in positions identical to those found in the fingerprint of the capsid protein (panel C), and the others can be assigned to tryptic peptides found in the glycoproteins PE2 and E1 noted in panel D. Panel B is obtained from the large-molecular-weight protein isolated from BHK-infected

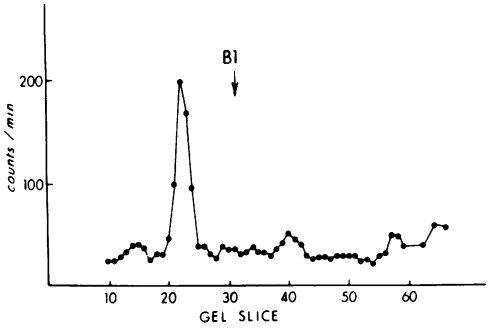

FIG. 1. *Pattern of ^{14}C-arginine-labeled protein synthesized in chicken embryo fibroblasts infected with the ts2 mutant of Sindbis virus at 40 C. CEF were infected with ts2 at a multiplicity of 50 PFU/cell at 40 C; 5 h later the cells were labeled with 50 μCi of ^{14}C-arginine (336 mCi/mmol) for 2 h. The methods for removing cells from the dish, for reduction and alkylation of the protein and for acrylamide gel electrophoresis were described previously (5). The gel was 7.5% acrylamide, and the arrow indicates the mobility of B1 run in a parallel gel.*

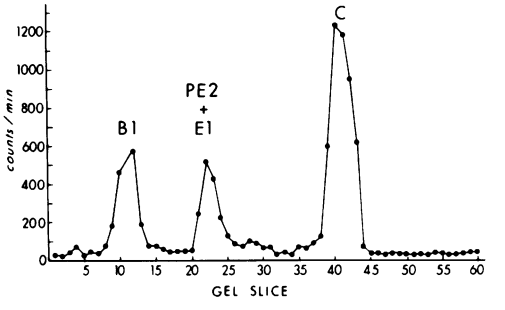

FIG. 2. *Pattern of labeled protein synthesized in Sindbis virus-infected BHK cells. The cells were labeled with ^{14}C-arginine for 30 min, 5 h after infection. After reduction and alkylation, the protein from the infected cell was subjected to electrophoresis on 10% acrylamide gels.*

cells, and it contains the arginine-peptides found in both PE2 and E1. Thus, the data clearly establish that the largest-molecular-weight polypeptide contains peptides identical to those found in all of the virion proteins. The slightly smaller protein B1 is missing those peptides associated with the capsid protein.

The conclusion that the protein B1 found in Sindbis virus-infected BHK cells is actually an intermediate in the formation of the viral envelope proteins was substantiated by its metabolic instability in a pulse-chase experiment. This protein was clearly discernible after a 20-min pulse of ^{14}C-amino acids (Fig. 4). When the radioactivity was chased with an excess of unlabeled amino acids for 20 or 40 min, the amount of isotope in B1 decreased substantially. The relative amount of isotope in each protein band was quantitated by densitometer tracings of the autoradiogram (Table 1). Seventy-five percent of the radioactivity was lost from B1 after 20 min of chase. Almost half of this can be accounted for by an increase in the radioactivity of PE2, E1, and E2. Another protein, which migrates in the gels between B1 and PE2 (Fig. 4), increases during the 20-min chase in a manner that is proportional to the loss of isotope from B1. After 40 min of chase, this protein accounts for about half the loss of isotope from B1, and thus it may be an intermediate in the pathway from B1 to PE2 and E1. However, we do not have peptide maps to confirm this. After a 40-min chase, significant amounts of E1 and E2 appear in virions (5), and this can account for the loss of radioactive material from cellular PE2, E1, and E2 that is observed in the gels of Fig. 4. Because of the latter and the appearance of a possible additional precursor, we have not attempted to account for the complete loss of radioactive B1 during the chase. The extensive labeling of the capsid protein and PE2 and the complete absence of radioactive E2 after the 20-min pulse were results similar to those obtained previously with CEF. Thus, in BHK as in chicken cells, the conversion of PE2 to E2 is a slow step in viral maturation.

There is evidence that the large protein accumulating in cells infected with the ts2 mutant at the nonpermissive temperature may not be a normal intermediate in the formation of Sindbis virion proteins. According to the studies of Scheele and Pfefferkorn, this protein does not degrade to the virion proteins when the temperature is lowered (3). Our previous study had indicated that the capsid protein is at the amino terminal end of the large polypeptide and that it is cleaved and released before the synthesis of the entire polypeptide is completed (5).

The molecular weight of this large protein is greater than 100,000 (8) and is large enough to accomodate all of the virion proteins. The estimated molecular weights of PE2, E1, and the capsid protein are 60,000, 53,000, and 30,000, respectively (8). Their total molecular weight accounts for about one-third of the potential genetic information in Sindbis RNA (mol wt 4.3×10^6 [6]). No large polypeptide corresponding to the entire genome of Sindbis virus has been detected; however, Simmons and Strauss (6, 7) recently isolated a major species of RNA in Sindbis virus-infected cells that had a molecular weight of 1.6×10^6. This RNA corresponds closely to the size required for the

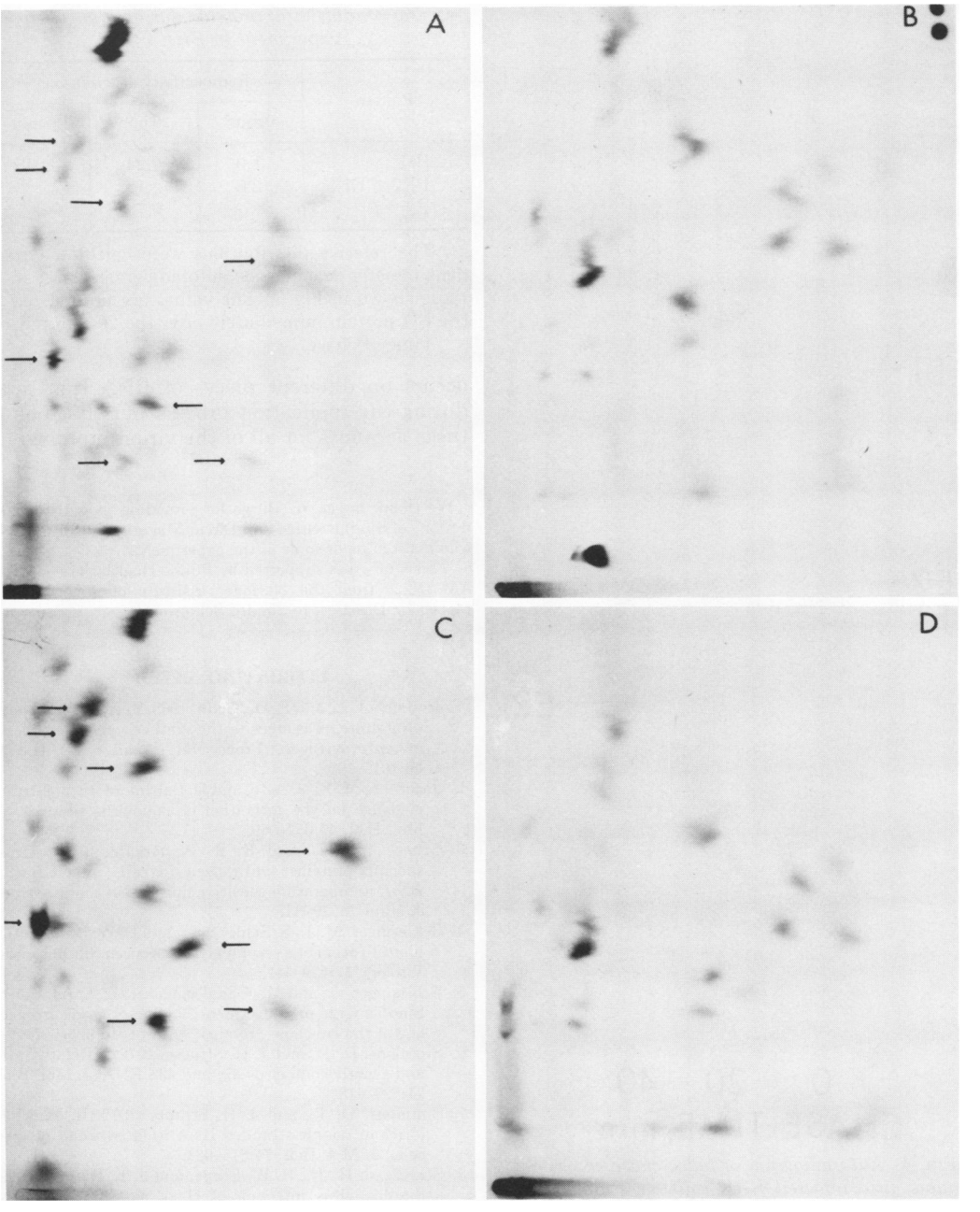

FIG. 3. *Autoradiograms of tryptic-peptide fingerprints. Proteins labeled with ^{14}C-arginine were purified on sodium dodecyl sulfate-polyacrylamide gels (refer to Fig. 1 and 2) and digested with trypsin. In the photograph, the origin is at the lower left corner of each panel; electrophoresis in pH 3.5 buffer was upwards, and decending chromatography in butanol-acetic acid-water (2:0.5:2.5) was to the right; approximately 100,000 counts/min were applied to the paper. Panel A, the ts2 protein; panel B, the B1 protein from BHK cells; panel C, the capsid; panel D, proteins PE2 and E1. The arrows in A and C indicate peptides with identical positions. Some of the peptides noted in C are not clearly resolved in A from those found in B and D.*

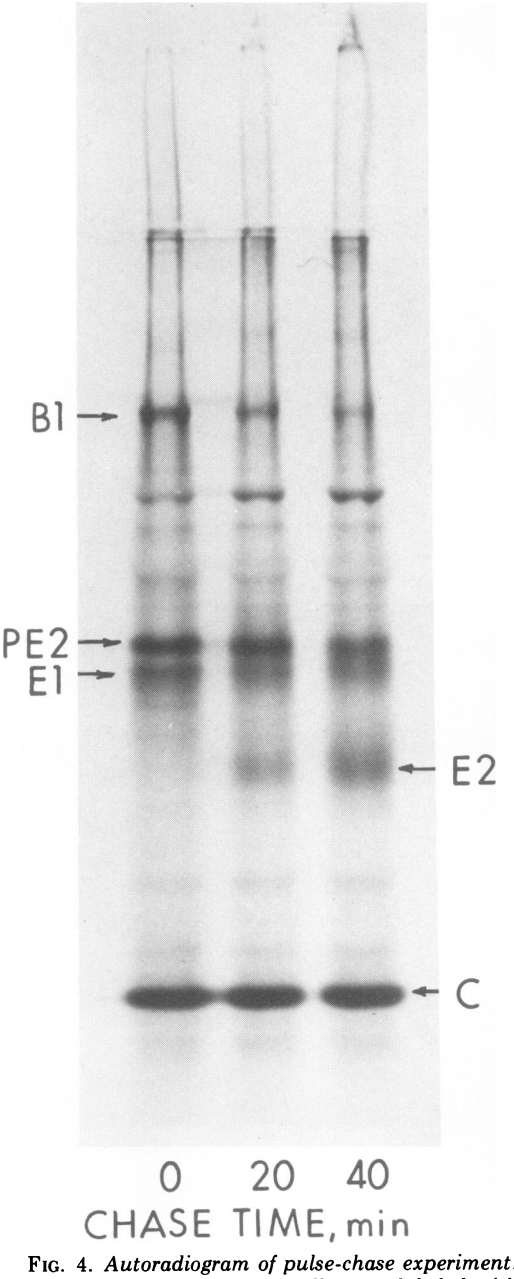

FIG. 4. *Autoradiogram of pulse-chase experiment. Sindbis virus-infected BHK cells were labeled with ^{14}C-amino acids for 20 min, 6 h after infection, and then chased with unlabeled amino acids. The details of the pulse-chase experiment, slab gel electrophoresis and autoradiography have been described (5).*

translation of the large-molecular-weight protein analyzed here. Thus, it appears that translation of the various Sindbis viral proteins occurs on different pieces of RNA that arise during the replication process but that one of these accounts for all of the virion proteins.

TABLE 1. *The relative distribution of radioactivity in several Sindbis viral proteins during a pulse-chase experiment in BHK cells*

Protein	Radioactivity distribution[a]		
	0 min[b]	20 min	40 min
B-1	1.0	0.24	0.048
PE2 + E1	2.6	2.1	1.1
E2		0.7	0.9

[a] The relative distributions were calculated from densitometer tracings of autoradiograms similar to those shown in Fig. 4. The values are normalized to the B-1 protein immediately after the 20-min pulse.
[b] Time of chase.

We thank Boyce W. Burge for providing us with the ts2 mutant of Sindbis virus, and David Magarik and Erno Duda who assisted us in some of the experiments.

This study was supported by Public Health Service grant AM 09226 from the National Institute of Arthritis and Metabolic Diseases, to Sondra Schlesinger.

LITERATURE CITED

1. Holland, J. J., and E. D. Kiehn. 1968. Specific cleavage of viral proteins as steps in the synthesis and maturation of enteroviruses. Proc. Nat. Acad. Sci. U.S.A. **60**:1015-1022.
2. Jacobson, M. F., J. Asso, and D. Baltimore. 1970. Further evidence on the formation of poliovirus proteins. J. Mol. Biol. **49**:657-669.
3. Scheele, C. M., and E. R. Pfefferkorn. 1970. Virus-specific proteins synthesized in cells infected with *RNA*$^+$ temperature-sensitive mutants of Sindbis virus. J. Virol. **5**:329-337.
4. Schlesinger, M. J., S. Schlesinger, and B. W. Burge. 1972. Identification of a second glycoprotein in Sindbis virus. Virology **47**:539-541.
5. Schlesinger, S., and M. J. Schlesinger. 1972. Formation of Sindbis virus proteins: identification of a precursor for one of the envelope proteins. J. Virol. **10**:925-932.
6. Simmons, D. T., and J. H. Strauss. 1972. I. Relative size and genetic content of 26S and 49S RNA. J. Mol. Biol. **71**:599-613.
7. Simmons, D. T., and J. H. Strauss. 1972. II. Multiple forms of double-stranded RNA isolated from infected cells. J. Mol. Biol. **71**:615-631.
8. Strauss, J. H. Jr., B. W. Burge, and J. E. Darnell. 1969. Sindbis virus infection of chick and hamster cells: synthesis of virus-specific proteins. Virology **37**:367-376.
9. Strauss, J. H., Jr., B. W. Burge, E. R. Pfefferkorn, and J. E. Darnell, Jr. 1968. Identification of the membrane protein and "core" protein of Sindbis virus. Proc. Nat. Acad. Sci. U.S.A. **59**:533-537.
10. Summers, D. F., and J. V. Maizel, Jr. 1968. Evidence for large precursor proteins in poliovirus synthesis. Proc. Nat. Acad. Sci. U.S.A. **59**:966-971.