JOURNAL OF VIROLOGY, Nov. 1972, p. 925-932

Vol. 10, No. 5
Printed in U.S.A.

Formation of Sindbis Virus Proteins: Identification of a Precursor for One of the Envelope Proteins

SONDRA SCHLESINGER AND MILTON J. SCHLESINGER

Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

Received for publication 24 July 1972

Exposure of Sindbis virus-infected chicken embryo cells to a short pulse of radioactive amino acids revealed the formation of primarily three proteins: the nucleocapsid (C) of the virus, one of the viral envelope proteins (E1), and a glycoprotein that did not appear in the virion. This third protein (PE2) has now been identified as a precursor of the other viral envelope protein (E2) on the basis of two observations: (i) the simultaneous disappearance of radioactive PE2 and appearance of labeled E2 in pulse-chase experiments, and (ii) the identity of ^{14}C-arginine tryptic peptides in fingerprints of the two proteins. The nucleocapsid was the most heavily labeled protein in the cell and appeared in the virus during the short pulse. The two ^{14}C-labeled envelope proteins, although having different kinetics of labeling in the cell, appeared simultaneously in the virus only after the chase. Addition of pactamycin, a drug inhibiting initiation of protein synthesis, preferentially inhibited the formation of capsid protein Assuming that Sindbis virus proteins are formed initially as a single polypeptide, our studies locate the nucleocapsid at the amino-terminal end of the polypeptide chain.

Sindbis virus is a relatively simple enveloped virus. The nucleocapsid contains a single strand of ribonucleic acid (RNA) (molecular weight about 4×10^6 [3]) and a single protein (16). A lipid bilayer is believed to separate the nucleocapsid from the outer envelope protein (4). We recently described the resolution of two glycoproteins in the envelope (14).

Radioactive, virus-specific proteins synthesized during viral replication are easily identifiable in the cell because infection by Sindbis inhibits host cell protein synthesis (15). There is increasing evidence that the proteins of the virion are initially synthesized as a large polypeptide which is subsequently cleaved to the smaller proteins found in the virion. Although large-molecular-weight precursor proteins are not found during normal replication of Sindbis in chicken embryo fibroblasts, a large-molecular-weight protein associated with viral infection has been identified in BHK cells (15). The accumulation of an even larger-molecular-weight protein occurred in chicken embryo fibroblasts infected with certain temperature-sensitive Sindbis mutants at a nonpermissive temperature (13, 15). Similar results were obtained when infected cells were exposed to an inhibitor of protease action (9).

When we labeled Sindbis-infected chicken embryo fibroblasts with ^{14}C-amino acids for 10 min, we found that three proteins in the cell contained most of the radioactivity (14). Two of them corresponded to two of the three proteins of the virion: the nucleocapsid (C) and one of the envelope proteins (E1). Essentially no isotope was found in the region of the other virus envelope protein (E2); instead, a radioactive protein slightly larger than E1 and not present in the virion was resolved. The most reasonable model to explain this pattern of short isotopic labeling was that the unidentified virus protein was a precursor of E2 and that its conversion to E2 was relatively slow. In the present report we present evidence to support this model.

MATERIALS AND METHODS

Virus and tissue culture. Wild-type Sindbis virus was obtained from B. Burge (Massachusetts Institute of Technology). For the preparation of primary chicken embryo fibroblasts and the titration of virus, we followed the techniques of Pfefferkorn and Hunter (10). Eagle medium contained 3% fetal calf serum.

Labeling of virus proteins for the pulse-chase experiment. Four monolayers of chicken embryo fibroblasts in 60-mm tissue culture dishes were infected with Sindbis at a multiplicity of 30. After 1 hr of adsorption, 8 ml of Eagle medium lacking amino acids was added to each dish. Five hours later, the medium was removed from each monolayer and replaced with 3 ml of the same medium containing 40 μCi of a mixture

of ^{14}C-labeled amino acids. After 20 min at 37 C, the radioactive medium was removed. One milliliter of 2% sodium dodecyl sulfate (SDS) was immediately added to one dish to remove the monolayer. These cells and the radioactive medium represented the zero time point of the chase. Three milliliters of medium containing a fourfold excess of the standard amount of amino acids was added to each of the remaining three monolayers, and incubation at 37 C was continued. At 20, 40, and 60 min, one of the dishes was removed, the medium was saved for virus purification, and the monolayer was harvested with 2% SDS as described above.

Labeling of virus proteins with ^{14}C-arginine. Infection was similar to that described for the pulse-chase experiment except that the medium contained amino acids. Before addition of ^{14}C-arginine, the monolayer was washed two times with Eagle medium lacking arginine. Three milliliters of this medium containing 50 μCi of ^{14}C-arginine was then added to the monolayer, which was incubated at 37 C for 6 hr. After removing the radioactive medium, the monolayer was collected with 1 ml of 2% SDS.

Labeling of chicken embryo fibroblasts in the presence of pactamycin. Incorporation of ^{14}C-amino acids into protein. Monolayers of primary chicken embryo cells were prepared on five round cover slips placed inside 60-mm tissue culture dishes. Six hours postinfection, the complete Eagle medium was removed and the cells were washed with amino acid-free medium. Three milliliters of the amino acid-free medium was then added to the dishes. Pactamycin was added to all the dishes except for the control and, 2 min later, 10 μCi of a mixture of ^{14}C-amino acids was added to each dish. At subsequent times, a cover slip was removed from each dish, dipped briefly into medium containing a fourfold excess of amino acids, and then into four successive solutions of 10% trichloroacetic acid. The cover slips were thoroughly dried, broken, and placed into scintillation vials for counting.

Formation of virus proteins. Six and one-half hours postinfection, monolayers of chicken embryo cells, in amino acid-free medium as above, were exposed to 10^{-6} M or 3×10^{-7} M pactamycin. Two minutes later, 100 μCi of uniformly labeled ^{14}C-amino acids was added to each dish as well as to a third control culture with no pactamycin. After an additional 8 min, the medium was removed and the cells were dislodged from the dish with 1 ml of 2% SDS.

Purification of virus. Two milliliters of medium from infected cells was layered onto 16 ml of a 15 to 30% sucrose gradient containing 0.1 M NaCl, 0.05 M tris-(hydroxymethyl)aminomethane (Tris) (*p*H 7.5), 0.001 M ethylenediaminetetraacetic acid, and 0.1% fetal calf serum. The samples were centrifuged at 27,000 rev/min for 3 hr in an SW27 rotor at 4 C. Approximately 40 fractions were collected from each tube by using an Isco density gradient fractionator (model 640). Samples from each tube were precipitated with 10% trichloroacetic acid. The precipitated protein was collected on Millipore filters, dried, and counted in a Packard scintillation counter. Tubes containing the radioactive virus were pooled.

Preparation of proteins for acrylamide gel electrophoresis. Dialyzed, lyophilized samples of intact virus, purified by sucrose gradient centrifugation, were suspended in 2% SDS containing 10 mM Tris, *p*H 7.4. To 50-μliter samples were added 2 μliters of 2-mercaptoethanol, and the solutions were heated for 20 min at 80 C. Prior to loading onto the slab gels, 10 μliters of 60% sucrose was added.

The viscous solution of virus-infected cellular material suspended in 2% SDS was forced through a 26-gauge, 3.5-inch (8.9 cm) needle several times to shear deoxyribonucleic acid DNA. To reduce and alkylate the proteins, samples (0.5 ml) were diluted with an equal volume of 1 M Tris, *p*H 9.0, containing 20 μliters of 2-mercaptoethanol. After 4 hr at 37 C, 55 mg of iodoacetamide was added, and the sample was incubated an additional hour. Prior to electrophoresis, samples were dialyzed against 5 mM Tris (*p*H 7.4), 0.1% SDS, and 15% sucrose.

Acrylamide gel electrophoresis. For analysis of the pulse-chase and pactamycin experiments, samples (10 to 50 μliters) of reduced-alkylated proteins derived from infected cells or of reduced-heated proteins from virus were loaded onto thin, vertical slab gels devised by Reid and Bieleski (11). The lower gels were composed of 10% acrylamide in 0.2 M Tris (*p*H 8.8) and 0.1% SDS; the upper spacer gels contained 5% acrylamide in .075 M Tris (*p*H 6.8), and 0.1% SDS as described by Laemmli (7). Electrophoresis was at constant voltage, either at 50 v for 8 hr or at 30 v for 16 hr. The gel was transferred to Whatman 3 MM paper, heat-dried while subjected to vacuum, and exposed to Kodak No-Screen X-ray film. Exposure time varied from 3 to 9 days.

Separation of ^{14}C-arginine proteins was achieved with cylindrical acrylamide gels containing the same ingredients used for the slab gels. Reduced-alkylated proteins were subjected to electrophoresis on 10 identical gels at 100 v for 9 hr. The gels were removed from the glass tubes, frozen at −70 C, and sliced into discs about 1-mm thick. Protein was eluted by incubating the discs in 0.5 ml of 0.1% SDS at 30 C for 16 hr. Radioactive protein was pooled and lyophilized.

Quantitative evaluation of autoradiograms. Strips of autoradiograms were scanned in a Gilford Spectrophotometer equipped with a linear transport device. The instrument was set at 600 nm, and the film densities were recorded as peaks of absorbancy. Exposure times of the film and the amount of label applied were adjusted to insure that film densities were linearly related to the amount of ^{14}C in the particular protein bands. The areas under the peaks of absorbance were calculated, and the relative amount of ^{14}C-protein was assigned to each of the virus-specific proteins on the basis of the total amount of radioactivity in the particular sample applied to the gel.

Preparation of fingerprints of ^{14}C-arginine-labeled tryptic peptides. The separated, lyophilized ^{14}C-proteins, eluted from the cylindrical acrylamide gels, were resuspended in one-tenth their original volume in 0.2 M NH_4HCO_3. Bovine gamma globulin (0.5 mg) was added, and the tubes were chilled on ice. To remove the SDS, nine volumes of cold acetone (−20 C) were added rapidly, and the solution was maintained at 0 C for 2 hr. The precipitated protein was

separated by centrifugation, and the supernatant fractions were carefully decanted. Recovery of ^{14}C-labeled protein was 85%. The pelleted protein was suspended in 1 ml of 0.1 M NH_4HCO_3, and 50 μg of TPCK-trypsin (Worthington) was added. Proteolysis was carried out at 37 C for 4 hr, and the samples were lyophilized. The tryptic peptides were suspended in 0.2 ml of H_2O, and 0.1 ml was applied to Whatman 3 MM paper. Electrophoresis was performed at 3.5 kv for 1 hr at 140 ma in Varsol-cooled tanks. The paper was dried, and chromatography was carried out in a closed chamber with butanol-acetic acid-water (2:0.5:2.5) for 20 hr at 23 C. Chromatograms were air-dried and exposed to Kodak No-Screen X-ray film.

Material. ^{14}C-amino acids and ^{14}C-glucosamine were purchased from New England Nuclear Corp.; ^{14}C-arginine was obtained from Amersham Searle. Pactamycin was a gift from D. Rekosh (Massachusetts Institute of Technology).

RESULTS

Kinetics of formation of virus proteins. The first indication that the unidentified virus-specific protein which accumulated in the cell but did not appear in virions was a direct precursor of the envelope protein, E2, came from experiments in which we followed the flow of radioactive amino acids into the virus-specific proteins of the infected cell as well as into the structural proteins of the virion. Infected monolayers of chicken embryo fibroblasts were exposed to a pulse of ^{14}C-amino acids for 20 min, and the radioactivity was then chased by the addition of an excess of unlabeled amino acids (details are described in Materials and Methods). The cells and medium were collected immediately after the pulse and at several 20-min intervals during the chase. Proteins were separated by electrophoresis on polyacrylamide slab gels, and the distribution of radioactivity among the virus-specific proteins was analyzed from autoradiograms of the dried gels (Fig. 1). After the 20-min exposure to ^{14}C-amino acids, the radioactivity in the cell was distributed primarily among three proteins: the nucleocapsid (C), E1, and the putative precursor to E2. During the chase period, the ^{14}C-label in the nucleocapsid and E1 declined gradually in the cells. Between 20 and 40 min, a much faster decrease was observed in the third protein, and

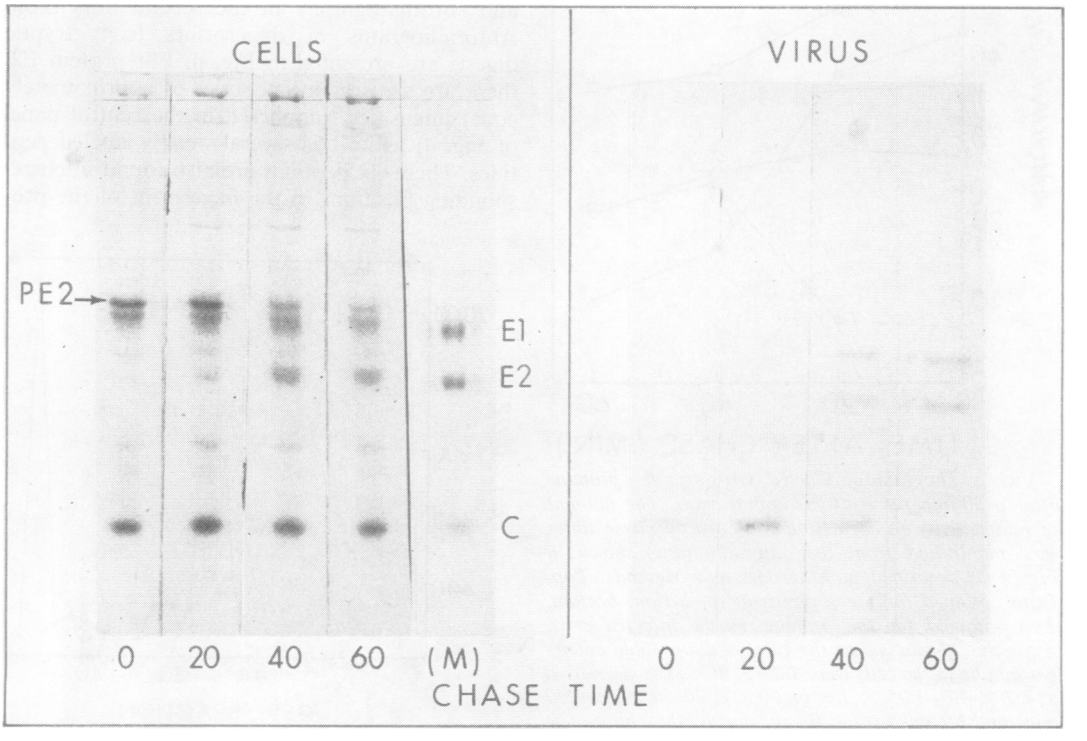

FIG. 1. *Autoradiograms of ^{14}C-labeled viral proteins at several periods after a 20-min pulse of radioactive amino acids. The details of the pulse-chase experiment, slab gel electrophoresis, and autoradiography are described in Materials and Methods. A marker (M) of purified virus is included in the gel of cell extracts. PE2 refers to the putative precursor of E2.*

this was accompanied by an increase in the amount of ^{14}C-label in E2. The appearance and disappearance of these proteins are analyzed in Fig. 2. These kinetics are consistent with the proposed precursor-product relationship.

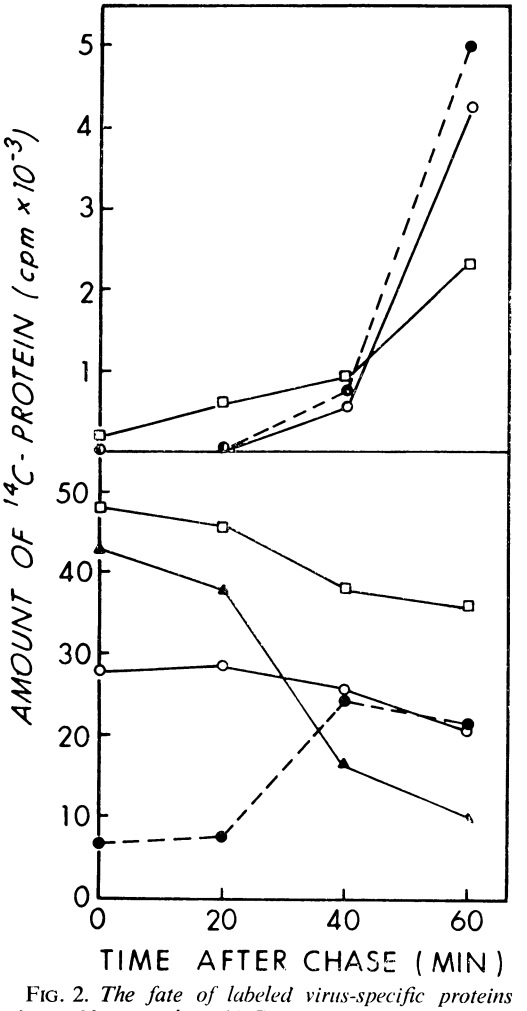

FIG. 2. *The fate of labeled virus-specific proteins after a 20-min pulse of ^{14}C-amino acids. The amount of radioactivity in each protein at several chase times was quantitated from the autoradiograms shown in Fig. 1 as described in Materials and Methods. Top, Data obtained for the proteins in the virion; bottom, data obtained for the proteins in the infected cells. After the 20-min pulse, the total acid-insoluble counts per minute in the cells were 1.37 × 10^5. They decreased to 1.2 × 10^5, 1.05 × 10^5, and 0.9 × 10^5 at 20 min, 40 min, and 60 min, respectively, during the chase. The total acid-insoluble counts per minute recovered in the virus after purification by sucrose gradient centrifugation were 234 after the 20-min pulse. They increased to 600, 2,330, and 8,950, respectively, at the 20-, 40-, and 60-min chase times. Capsid protein, □——□; E1, ○——○; E2, ●- - -●; the putative precursor to E2, ▲——▲.*

The pulse-chase experiment also revealed that, at the conclusion of the pulse, the nucleocapsid was the most heavily labeled protein in the cell and was the only labeled protein detectable in the virions. There was substantial label in E1 in the cells compared to E2 at the end of the pulse and after 20 min of chase, yet labeled E1 did not appear in the virions at these times. The appearance of E1 in the virion occurred simultaneously with E2 at 40 min, and both proteins contained essentially the same amount of radioactivity (Fig. 2).

Peptide maps of virus proteins. Definitive evidence for the relationship between the envelope protein E2 and its putative precursor was obtained from an analysis of the tryptic-peptide fingerprints of the two proteins. ^{14}C-arginine-labeled virus proteins were prepared from monolayers of infected chicken embryo fibroblasts exposed to ^{14}C-arginine from 6 to 12 hr postinfection. Virus-specific proteins were separated on SDS-polyacrylamide gels (Fig. 3) and digested with trypsin after the removal of SDS (refer to Materials and Methods). Fingerprints were prepared by electrophoresis at *p*H 3.5 in one direction and chromatography in the second dimension. Autoradiograms of fingerprints from tryptic digests are presented in Fig. 4. For protein E2, there are six arginine peptides of approximately equal intensities (numbered in the central panel of Fig. 4) as well as several weakly labeled peptides. These six peptides are also found in corresponding positions in the fingerprint of the pro-

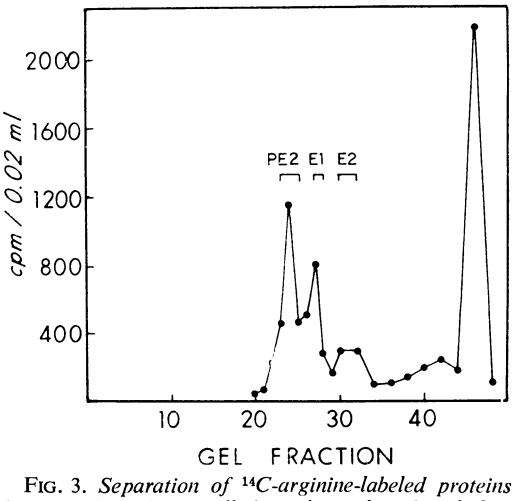

FIG. 3. *Separation of ^{14}C-arginine-labeled proteins from Sindbis-infected cells by polyacrylamide gel electrophoresis. A 0.1-ml sample containing 2.6 × 10^5 counts/min was applied to the gel. Electrophoresis, gel slicing, and elution of the proteins are described in Materials and Methods. PE2 refers to the proposed precursor to E2.*

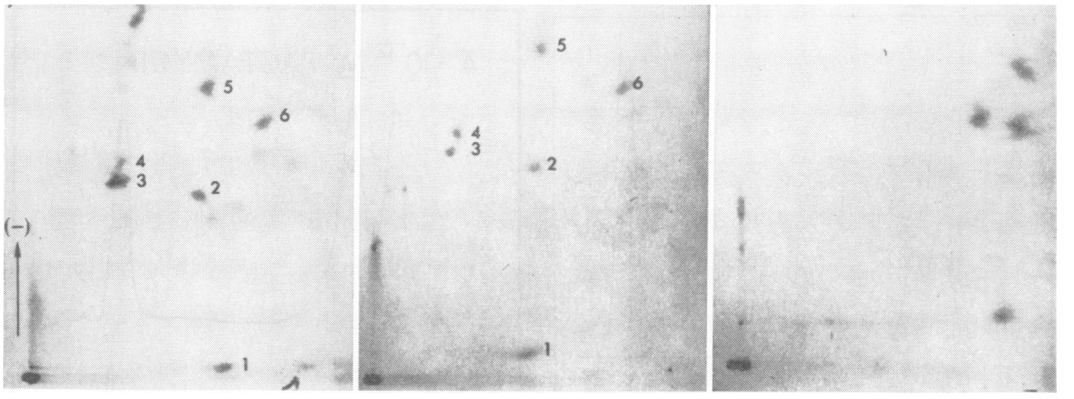

FIG. 4. *Autoradiograms of tryptic-peptide fingerprints. ^{14}C-arginine-labeled proteins were purified on SDS-polyacrylamide gels prior to digestion with trypsin. The fingerprint of protein E1 is on the right, E2 in the center, and the proposed precursor on the left. About 60,000 counts/min were applied to the paper.*

posed precursor (Fig. 4, left) and contain approximately equivalent amounts of arginine except for peptide 3, which was found in another fingerprint to consist of an additional peptide present in the precursor but absent in E2. Several other weaker spots are visible in the fingerprint of the precursor, and some of them correspond to those weakly labeled peptides in E2, possibly indicative of partial tryptic cleavages. Four of the weak spots in the precursor fingerprint correlate with the four strong peptides detected in the fingerprint of E1. The most likely explanation for this is the presence of some E1 protein in the pool of precursor protein eluted from the acrylamide gels (refer to Fig. 3). The patterns presented in Fig. 4 were obtained by performing electrophoresis prior to chromatography, and essentially the same patterns were found in fingerprints prepared by carrying out the chromatography first, followed by electrophoresis.

In addition to establishing partial identity in the primary structures of E2 and the presumptive precursor, these fingerprints also show that the two virus envelope proteins, E1 and E2, have distinctly different arginine peptides. The four distinct peptides of E1 all move very close to the solvent front in the chromatographic system, suggesting that they are quite hydrophobic in composition. This observation may prove significant in considering how E1 and E2 interact with the lipid envelope of the virion and possibly with each other.

Presence of carbohydrate in the precursor. Both envelope proteins of Sindbis contain carbohydrate residues (14). To establish whether the precursor of E2 also is a glycoprotein, ^{14}C-glucosamine was added to cells 6 hr postinfection for varying lengths of time. The distribution of isotope among the virus-specific proteins clearly revealed the presence of glucosamine in the precursor (Fig. 5). The labeling pattern shown in Fig. 5 was obtained from cells that had been exposed to isotope for 6 hr, but label was also detected in the precursor after 30 min. The relative amounts of ^{14}C-glucosamine and ^{14}C-arginine in the precursor and E2 were constant, suggesting that the conversion to E2 did not entail further glucosylation. It is possible, however, that the two proteins differ in other carbohydrate residues.

The effect of pactamycin on the synthesis of virus proteins. Additional information about the formation of Sindbis viral proteins has come from studies using pactamycin, a drug that preferentially inhibits the initiation of protein synthesis (2, 8). Several investigators have taken advantage of this fact to order the proteins of poliovirus which are synthesized initially as one long polypeptide chain (5, 6, 17). Assuming one site for initiation of protein synthesis, the incorporation of radioactive amino acids into those proteins located at the amino-terminal end of the large polypeptide should be decreased to the greatest extent when pactamycin is present. In fact, differential inhibition of the formation of poliovirus-specific proteins was observed after the addition of pactamycin (18, 19).

Sindbis virus proteins are postulated to be synthesized initially as a single large polypeptide (13, 15). Thus, studies with pactamycin should also permit an ordering of these virus proteins. The effect of pactamycin on the initiation of protein synthesis would be most apparent at concentrations of the drug that do not completely inhibit protein synthesis. Pactamycin at 10^{-5} M almost completely inhibited the incorporation of ^{14}C-amino acids into protein in Sindbis-infected chicken embryo cells; at 10^{-7} M it was almost without effect (Fig. 6). To determine if pacta-

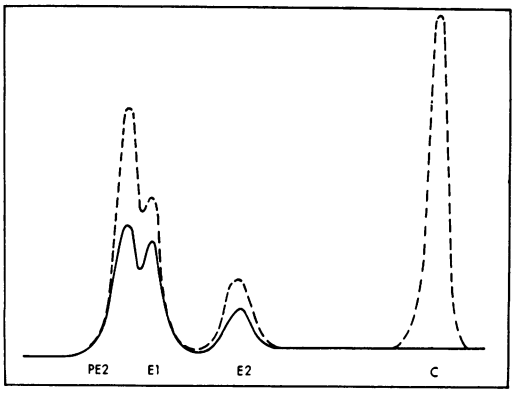

FIG. 5. *Densitometer tracings of an autoradiogram of ^{14}C-glucosamine-labeled virus proteins separated by SDS-polyacrylamide slab gel electrophoresis. The procedure for labeling infected cells with glucosamine (50 μCi) was identical to that described for ^{14}C-arginine. The graph shows the data obtained from a 6-hr labeling period with ^{14}C-glucosamine (——) and with ^{14}C-arginine (----).*

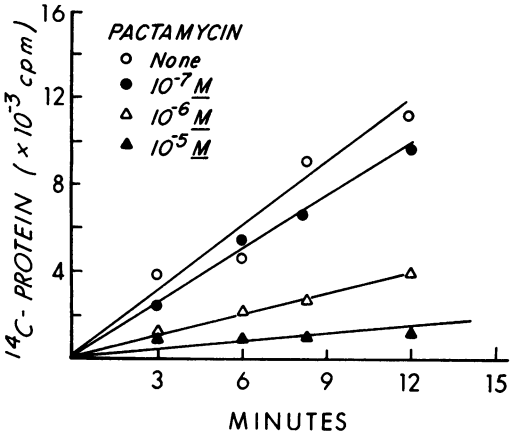

FIG. 6. *The effect of pactamycin on protein synthesis in Sindbis-infected chicken embryo cells.*

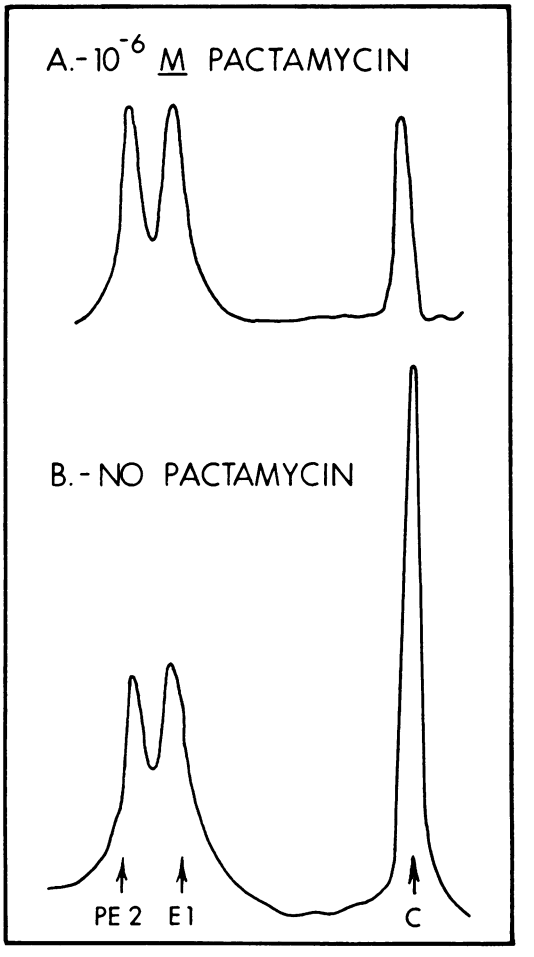

FIG. 7. *Densitometer tracings of an autoradiogram of virus-specific proteins separated by SDS-polyacrylamide slab gel electrophoresis. The relative distribution of radioactivity in cells exposed to 10^{-6} M pactamycin is compared to an uninhibited culture. The results obtained with 3×10^{-7} M pactamycin were similar to those shown for 10^{-6} M (refer to Table 1). The experimental details and methods for quantitating the data are described in Materials and Methods.*

mycin preferentially inhibited any of the intracellular virus-specific proteins, we exposed infected cells to the drug at 10^{-6} M and 3×10^{-7} M. Radioactive amino acids were added 2 min later, and the monolayers were collected after an additional 8 min (see Materials and Methods for details). Samples from the SDS-treated cell extracts were subjected to electrophoresis on acrylamide gel slabs. Densitometer tracings of the autoradiograms obtained from the control (no pactamycin) and from cells exposed to 10^{-6} M pactamycin are shown in Fig. 7. A summary of the data is reported in Table 1. At these concentrations of pactamycin, the relative amount of radioactivity in the capsid protein was reduced 50%, but there was no decrease in the relative amounts of E1 or the precursor to E2. E2 itself was not detected in this short exposure to radioactive amino acids.

DISCUSSION

The peptide maps obtained for the virus-specific proteins E1, E2, and the putative precursor to E2 demonstrate a remarkable similarity between the latter two and the uniqueness of E1. These results, together with the pattern of forma-

TABLE 1. *Effect of pactamycin on the relative distribution of radioactivity in the three major virus-specific proteins in Sindbis-infected chicken embryo fibroblasts*

Pactamycin (M × 10^{-6})	Protein[a]		
	Capsid	E1	PE2[b]
0	1.0	.71	.68
0.3	0.55	.72	.70
1.0	0.46	.74	.71

[a] The relative distributions were calculated from densitometer tracings of autoradiograms of acrylamide gels as described in Materials and Methods. Values were normalized to the capsid in the absence of pactamycin.

[b] PE2 refers to the precursor of E2.

tion and disappearance of these proteins in the infected cell, offer convincing evidence that E2 is formed directly from the precursor PE2. The chemical basis for the conversion has not been established. E2 may be formed from PE2 by proteolytic cleavage, but the conversion might also entail changes in carbohydrate composition. PE2 is the only intermediate that accumulates in appreciable quantities during the normal processing of Sindbis virion proteins in chicken embryo fibroblasts. A polypeptide with an estimated molecular weight close to 130,000 has been detected in chicken embryo fibroblasts infected with a temperature-sensitive mutant of Sindbis at nonpermissive temperature (13, 15). This particular protein accumulated at the expense of the envelope and capsid proteins, suggesting that all of the viral proteins originate as a single polypeptide.

The estimated molecular weights of the nucleocapsid, E1, and PE2 are 30,000, 53,000, and 60,000, respectively (15). After a 20-min pulse of ^{14}C-amino acids, the nucleocapsid was the most heavily labeled protein in the cell. It was also the first protein to become radioactive in the virion. Preferential labeling of the nucleocapsid in the virion after a short pulse of isotope had also been observed by Scheele and Pfefferkorn (12). To account for these results, the capsid protein would have to be synthesized at a much faster rate than the other virus proteins. This conclusion seemed incompatible with the hypothesis that all of the virus proteins are synthesized initially as a single large polypeptide. The two ideas are consonant, however, if we assume that (i) the capsid is derived from the NH_2-terminal end of the polypeptide and (ii) the capsid is cleaved from the growing polypeptide chain and its synthesis is reinitiated while the polyribosomes are still translating the remaining RNA. Although there is no evidence for the second assumption, our studies with pactamycin provide support for the first. Pactamycin preferentially inhibits the initiation of protein synthesis, and the nucleocapsid was the only protein affected by treating infected cells with the drug. This effect would be expected if the capsid were located in that region of the polypeptide where synthesis begins. The results with pactamycin, however, do not by themselves prove that the Sindbis virus proteins are synthesized initially as a single polypeptide. For if the capsid were translated from a separate, smaller messenger RNA as a unique polypeptide, it would still have been the protein most affected by pactamycin because it is the smallest.

The kinetics of appearance of radioactive E1 and E2 in the virion were unexpected. Even though there was considerably more radioactivity in E1 than in E2 in the cell at the conclusion of the pulse and after the first 20 min of chase, no ^{14}C-labeled E1 was detected in the virion at these times. This, together with the observation that E1 and E2 appear in the virion with equal amounts of isotope, suggests that labeled E2 must accompany labeled E1 into the extracellular virion. A possible model to explain these findings is that, subsequent to their cleavage from a large precursor polypeptide, E1 and PE2 remain in the cell as a stable complex. As such, E1 alone would be unavailable for virion maturation, and only after PE2 was converted to E2 would these proteins become part of the virion. Formation of E2 must be essential for virus production because PE2 has not been found in purified virions. Assuming that the complex between E1 and E2 was also stable, these two polypeptides would appear in the virion simultaneously. Experiments are presently in progress to determine the extent to which these two envelope proteins occur as a complex.

Sindbis virus proteins have been located in the plasma membranes of infected cells (1). Our data indicate that a substantial amount of radioactive protein in these membranes is PE2. Thus, the conversion of PE2 to E2 must occur at the plasma membrane of infected cells and may be an important step in the process by which the virion buds from the cell.

ACKNOWLEDGMENTS

We thank Boyce W. Burge for introducing us to Sindbis virus, David Rekosh for suggesting the pactamycin experiment, and Stephen Halford for helpful discussions.

The work was supported in part by Public Health Service Health Sciences Advancement Award no. 5 SO4 FR06115.

LITERATURE CITED

1. Bose, H. R., and M. A. Brundige. 1972. Selective association of Sindbis virion proteins with different membrane fractions of infected cells. J. Virol. 9:785–791.

2. Cohen, L. B., A. E. Herner, and I. H. Goldberg. 1969. Inhibition by pactamycin of the initiation of protein synthesis. Binding of N-acetylphenylalanyl transfer ribonucleic acid and polyuridylic acid to ribosomes. Biochemistry 8:1312–1326.
3. Dobos, P., and P. Faulkner. 1970. Molecular weight of Sindbis virus ribonucleic acid as measured by polyacrylamide gel electrophoresis. J. Virol. 6:145–147.
4. Harrison, S. C., A. David, J. Jumblatt, and J. E. Darnell. 1971. Lipid and protein organization in Sindbis virus. J. Mol. Biol. 60:523–528.
5. 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.
6. Jacobson, M. F., J. Asso, and D. Baltimore. 1970. Further evidence on the formation of poliovirus proteins. J. Mol. Biol. 49:657–669.
7. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.
8. Macdonald, J. S., and I. H. Goldberg. 1970. An effect of pactamycin on the initiation of protein synthesis in reticulocytes. Biochem. Biophys. Res. Commun. 41:1–8.
9. Pfefferkorn, E. R., and M. K. Boyle. 1972. Selective inhibition of the synthesis of Sindbis virion proteins by an inhibitor of chymotrypsin. J. Virol. 9:187–188.
10. Pfefferkorn, E. R., and H. S. Hunter. 1963. Purification and partial chemical analysis of Sindbis virus. Virology 20:433–445.
11. Reid, M. S., and R. L. Bieleski. 1968. A simple apparatus for vertical flat-sheet polyacrylamide gel electrophoresis. Anal. Biochem. 22:374–381.
12. Scheele, C. M., and E. R. Pfefferkorn. 1969. Kinetics of incorporation of structural proteins into Sindbis virions. J. Virol. 3:369–375.
13. 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.
14. Schlesinger, M. J., S. Schlesinger, and B. W. Burge. 1972. Identification of a second glycoprotein in Sindbis virus. Virology 47:539–541.
15. 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.
16. 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.
17. 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.
18. Summers, D. F., and J. V. Maizel, Jr. 1971. Determination of the gene sequence of poliovirus with pactamycin. Proc. Nat. Acad. Sci. U.S.A. 68:2852–2856.
19. Tabor, R., D. Rekosh, and D. Baltimore. 1971. Effect of pactamycin on synthesis of poliovirus proteins: a method for genetic mapping. J. Virol. 8:395–401.