JOURNAL OF VIROLOGY, May 1973, p. 810-813

Vol. 11, No. 5
Printed in U.S.A.

Proteins Specified by Herpes Simplex Virus

IX. Contiguity of Host and Viral Proteins in the Plasma Membrane of Infected Cells

JOCHEN W. HEINE AND BERNARD ROIZMAN
Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

Received for publication 24 January 1973

Artificial mixtures of plasma membrane vesicles produced by microcavitation from infected and uninfected cells band at the same density on isopycnic centrifugation in sucrose density gradient. However, after reaction with antiviral antibody, the density of the infected cell plasma membrane vesicles increases, and the infected and uninfected cell membranes are quantitatively separable on isopycnic centrifugation. Plasma membrane vesicles prepared from cells doubly labeled before and after infection with radioactive amino acids and reacted with antibody banded at a high density. Polyacrylamide gel electropherograms show that the vesicles reacted with antibody consist of both host- and virus-specific membrane proteins. Microcavitation does not disrupt viral envelopes since infectivity is not affected by this procedure. We conclude that viral and cellular proteins in the plasma membrane preparations are contiguous.

In a preceding paper of this series (3), we reported that purified plasma membrane preparations from human epidermoid carcinoma no. 2 (HEp-2) cells infected with herpes simplex virus 1 (HSV-1) contained viral glycoproteins similar to those present in the purified virion (Fig. 1). In that series of experiments, the plasma membrane was prepared by microcavitation of cells. The purification of the plasma membrane vesicles involved extensive washing of the vesicles with buffers of different ionic strengths to release entrapped soluble proteins (4). The purification was monitored by enzyme markers specific for plasma membrane (7) and fucose reported to be preferentially incorporated into plasma membranes (1). Based on polyacrylamide gel electropherograms, we concluded that viral glycoproteins constitute approximately 10% of the proteins in the plasma membrane preparation. In this paper we report that the viral glycoproteins contained in plasma membranes are contiguous with host membrane proteins.

The design of these experiments was based on observations reported earlier (5) that the buoyant density of smooth membranes from infected and uninfected cells is identical. However, upon reaction with antiviral antibody, the density of the infected cell membrane increases, and the uninfected and infected cell membranes are readily separated by isopycnic centrifugation in a sucrose density gradient. In the present studies, purified plasma membranes were prepared as previously described (3) from (i) uninfected HEp-2 cells labeled with ^{3}H-amino acids (3 μCi/ml, Schwarz/Mann, Orangeburg, N.Y.) for

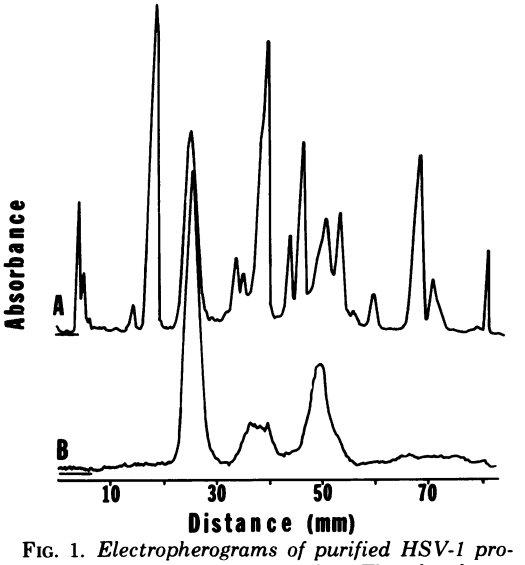

FIG. 1. *Electropherograms of purified HSV-1 proteins labeled with ^{14}C-glucosamine. The absorbance tracing of Coomassie brilliant blue-stained acrylamide gel (A) shows the profile of the viral structural proteins, whereas the absorbance tracing of the autoradiogram (B) prepared from the same gel shows the profile of viral structural glycoproteins. The procedures for labeling and purifying HSV-1 and preparation for acrylamide gel electrophoresis are described in reference 6.*

12 h at 37 C in a medium consisting of 10% dialyzed calf serum, vitamins, and amino acids in concentrations of 1× and 0.25×, respectively, of those recommended by Eagle (2); (ii) HEp-2 cells infected with a multiplicity of 15 PFU of the F prototype of HSV-1 per cell, and then labeled with ^{14}C-amino acids (0.3 μCi/ml, Schwarz/Mann, Orangeburg, N. Y.) between 4 and 18 h postinfection in a medium consisting of 1% dialyzed calf serum, vitamins, and amino acids in concentrations of 1× and 0.1×, respectively, of those recommended by Eagle (2); and (iii) HEp-2 cells labeled with ^{3}H-amino acids for 12 h in a medium consisting of 10% dialyzed calf serum, vitamins, and amino acids in concentrations of 1× and 0.25×, respectively, and then incubated for 4 h in medium containing twice the recommended concentration of unlabeled amino acids, infected, and lastly, labeled with ^{14}C-amino acids between 4 and 18 h postinfection, as described above. Samples of the three batches of plasma membranes in amounts of 400 μg in 1 mM Tris buffer (pH 7.4) containing 0.25 M sucrose, and 0.15 M NaCl were chilled for 15 min in an ice bath, and then each was mixed with 0.1 ml of rabbit anti-type 1 HSV-infected rabbit kidney cell serum (see legend, to Fig. 2). After continuous stirring for 1 h in an ice bath, the suspensions containing the ^{3}H-labeled uninfected cell membranes and the ^{14}C-labeled infected cell membranes were pooled. The mixture of infected and uninfected cell membranes, as well as the suspension of membranes labeled with ^{3}H-amino acids before infection and with ^{14}C-amino acids after infection, were layered on 10-ml sucrose density gradients (25–60% [wt/wt] made up in Tris saline buffer, pH 7.4) and centrifuged in a Beckman SW41 rotor for 22 h at 26,000 rpm and 4C. Examination of the tubes after centrifugation revealed one band in the tube containing doubly labeled membranes and two bands in the tube containing the artificial mixture of ^{3}H- and ^{14}C-labeled membranes reacted with antiviral serum. The gradients were then collected in fractions containing four drops each. Samples of the various fractions were removed for determination of the refractive index and radioactivity, as indicated in the legend to Fig. 2. The distribution of the radioactivity (Fig. 2, panel 1) indicates that the artificial membrane mixture of infected and uninfected cells exposed to antiviral serum formed two distinct bands at densities of 1.161 g/cm^3 (37% [wt/wt] sucrose) and 1.122 g/cm^3 (29% [wt/wt] sucrose), respectively. On the other hand, the membranes labeled before and after infection and reacted with antiserum (Fig. 2, panel 2) formed a major band corresponding to the density of 1.161 g/cm^3 and a minor band comprising 1% of the total ^{3}H activity at the density of 1.122 g/cm^3. Plasma membranes of infected and uninfected cells not exposed to the antiserum have the same density (1.122 g/cm^3), i.e., the density reported for plasma membrane of uninfected cells (7). In subsequent steps in this experi-

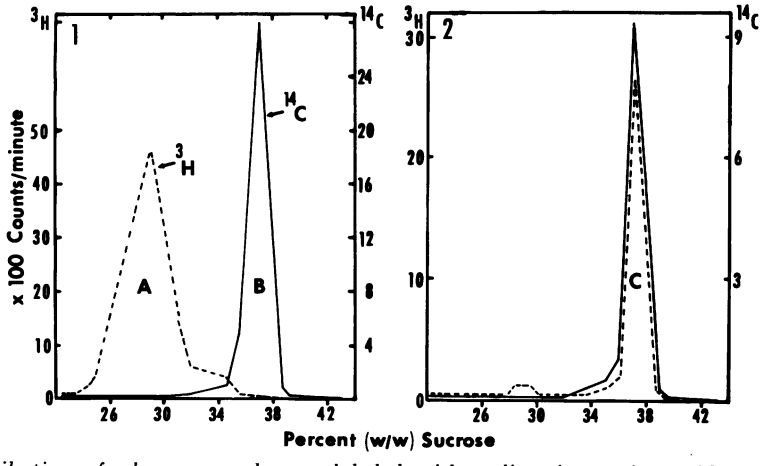

FIG. 2. *Distribution of plasma membranes labeled with radioactive amino acids and reacted with anti-HSV-1 serum after centrifugation in sucrose density gradients. The serum was prepared by immunization of rabbits with virus produced in rabbit kidney cells (8) and was the kind gift of Douglas Watson. The radioactivity was determined by drying 25 μliters of each fraction onto glass fiber disks (Whatman) and placing the disks into vials containing 3 ml of a toluene-based scintillation fluid for counting in a Packard scintillation counter. Panel 1, artificial mixture of plasma membrane vesicles from uninfected HEp-2 cells labeled with ^{3}H-amino acid and from HSV-1-infected HEp-2 cells labeled with ^{14}C-amino acids. Panel 2, plasma membrane vesicles from HSV-1-infected HEp-2 cells labeled with ^{3}H-amino acids before infection and with ^{14}C-amino acids after infection.*

ment, the fractions from the two gradients containing the radioactive bands were pooled, diluted with Tris saline buffer, and centrifuged at 120,000 × *g* for 60 min. The sedimented membranes were solubilized and subjected to electrophoresis on polyacrylamide gels, as described elsewhere (3). After the electrophoresis the gels were sliced into 1-mm slices and the radioactivity in each slice was determined as previously described (6). The electrophoretic profiles (Fig. 3) show that the bands obtained for the artificial mixture of ^{3}H- and ^{14}C-labeled membranes contained, respectively, host and viral membrane proteins similar to those present in the virion (Fig. 1) and reported previously (3). The major band obtained by centrifugation of the membranes labeled before and after infection contained ^{3}H label in host proteins and ^{14}C label in viral proteins. The observation that, in the doubly labeled membrane preparation, 99% of the host membrane proteins cosedimented with viral membrane proteins indicates that the host and membrane proteins are contiguous on the same membrane vesicle.

A rough estimate of the number of antigenic sites reactive with viral antiserum and present on the surface of the plasma membrane vesicles can be calculated from the increase in density after reaction with antibody. Thus, the average vesicle diameter determined from measurements of several hundred vesicles in the electron microscope (Fig. 4) was 1.60×10^{-4} mm. Based on the density of unreacted vesicles (1.122 g/cm^3) and assuming that the vesicles are spherical, the average weight of the vesicles was calculated to be 2.42×10^{-15} g. The average weight of vesicles reacted with antibody was 2.50×10^{-15} g. On the basis of the average weight (2.7×10^{-19} g) of an antibody molecule (immunoglobulin G), we calculated that the average number of antibody molecules binding to the vesicles is about 300.

Additional evidence that membrane vesicles containing viral-specific membrane proteins do not originate from virions de-enveloped during the fractionation of the cells is based on the following observation. Two samples with equal numbers of 16-h infected cells were disrupted, one by microcavitation, and the other in a Dounce homogenizer. The total infectivity of the disrupted samples in terms of plaque-forming units was identical. Since naked capsids are not infectious, we conclude that microcavitation does not strip the envelope.

We conclude from the experiments reported here that viral proteins present in the plasma membrane are contiguous with host proteins and that the presence of the viral proteins in the plasma membrane preparations is not a consequence of manipulation of the infected cell constituents during the fractionation and purification of the plasma membrane.

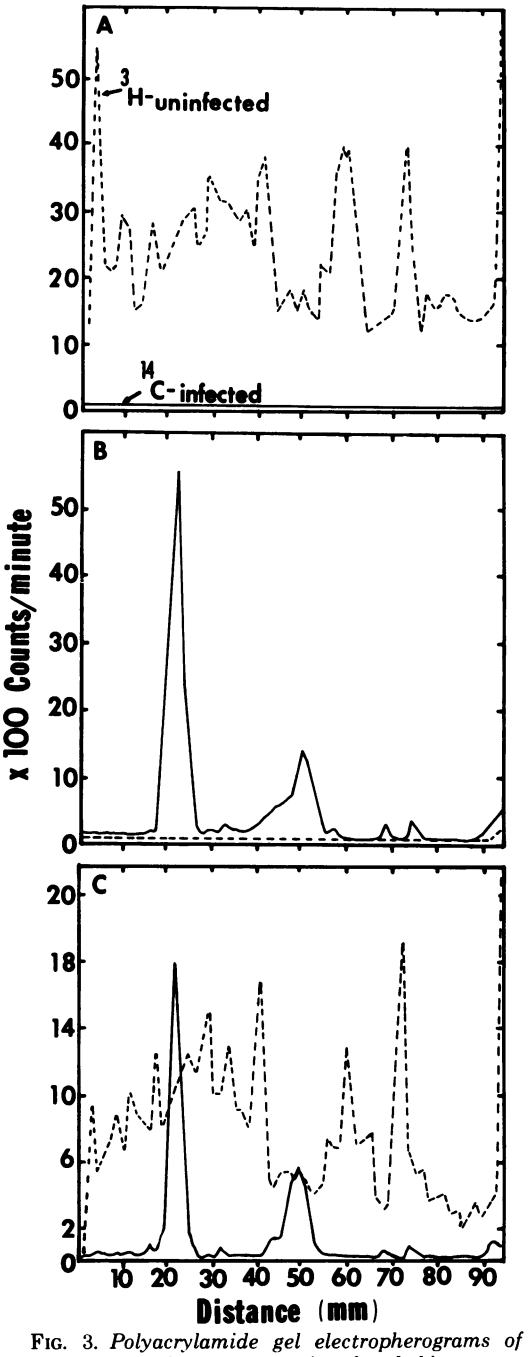

FIG. 3. *Polyacrylamide gel electropherograms of the plasma membrane preparations banded in sucrose density gradients shown in Fig. 2. The designations A, B, and C refer to the bands A and B of Fig. 2, panel 1, and the band C in Fig. 2, panel 2.*

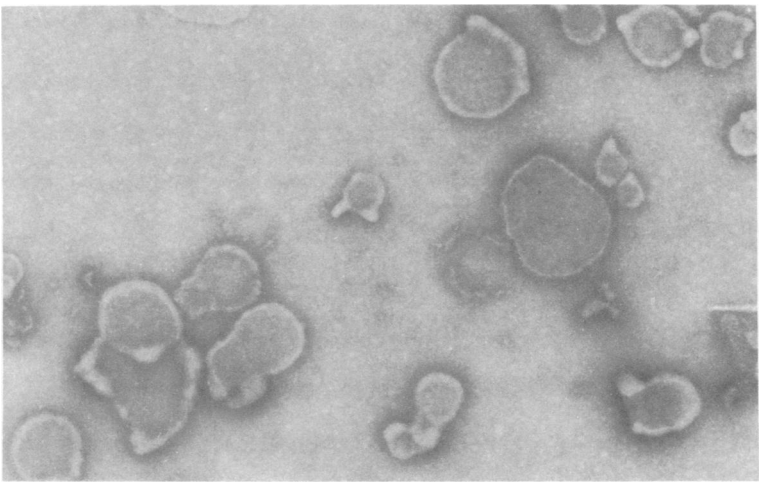

FIG. 4. *Electron micrograph of purified plasma membrane vesicles. Plasma membrane vesicles in 1 mM Tris at pH 7.4 containing 0.25 M sucrose were stained with 2% phosphotungstic acid, and electron micrographs were taken with an AEI-EM6B electron microscope. Final magnification is* $\times$ *45,000.*

We thank Douglas Watson for the gift of the rabbit serum prepared against HSV-1 produced in rabbit kidney cells.

These studies were aided by grants from the Public Health Service, National Institutes of Health (CA 08494), the American Cancer Society (VC-103H), an American Cancer Society Institutional grant (IN 416), and the National Science Foundation (GB27356).

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