Solubilization of Envelopes of HVJ (Sendai virus) with Alkali-Emasol Treatment and Reassembly of Envelope Particles with Removal of the Detergent

KAZUFUMI SHIMIZU,¹ YASHUHIRO HOSAKA, AND YOHKO K. SHIMIZU

Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, Suita City, Osaka, Japan

Received for publication 5 January 1972

The envelopes of HVJ (Sendai virus) virions were solubilized with alkali-Emasol treatment. The solubilized envelope subunit(s) associated with hemagglutination-inhibiting antibody blocking, neuraminidase, and low hemagglutinating (HA) activities had a sedimentation coefficient of 8.8S. Envelope fragment-like structures were assembled from the solubilized subunits after Emasol was removed by gel filtration. These reassembled envelope particles with HA activity had cell-fusion activity as well as hemolytic activity. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the reassembled particles revealed that they mainly consisted of two kinds of polypeptides.

The envelopes of paramyxoviruses have neuraminidase, hemagglutinating (HA), hemolytic, and cell-fusion activities; the relationship of these activities to envelope structure is very interesting. Isolation of the envelope components and reconstitution of envelopes from them are useful methods for the investigation of the subunit or molecular structure of the envelope. Hosaka (8) isolated the nucleocapsids of HVJ (Sendai virus) from disrupted envelopes with the alkali-Emasol (Tween 20) treatment. We then set out to study the envelope subunits solubilized by the same treatment and further properties of envelope fragment-like structures reassembled after removal of the detergent.

By this method (4, 21, 24) or sodium dodecyl sulfate (SDS) treatment (11, 23), envelope subunits with biological activities of influenza virus (11, 23, 24) and ribonucleic acid (RNA) tumor viruses (4, 21) have been prepared and studied. However, reassembly of envelope-like particles from them were not reported for these viruses, although polymers of several spikes were found (11, 24).

The present paper describes properties of the solubilized envelope subunits and of the reassembled envelope particles of parainfluenza virus HVJ.

MATERIALS AND METHODS

Virus. HVJ, strain Z, was grown in chorioallantoic membranes of 10-day-old chick embryos and purified

by differential centrifugations and sedimentations through a sucrose gradient as previously described (9).

Alkali-Emasol treatment. The virions were disrupted in 0.02 M bicarbonate buffer (pH 10) with 0.25 to 1.0% Emasol 1130 (or Tween 20; Kao-Atlas Co., Ltd.) at 20°C for 1 hr as previously described (8). Nucleocapsids and partially disrupted virions were removed by a centrifugation at 40,000 rev/min for 1 hr in a Beckman no. 40 rotor.

Gel filtration. A sample (2 ml) was applied to the column of BioGel A-15m or Sephadex G-200. The effluents were collected in 3-ml fractions and assayed for biological activities and protein content. The void volume of the column was determined with blue dextran as a marker. Stokes radius of the test material was estimated by using catalase [Stokes radius of 5.2 nm (1)] as a standard protein.

Zonal rate centrifugation. This was carried out in 5-ml linear sucrose gradients [5 to 20% sucrose in 0.02 M bicarbonate buffer, pH 10, containing 0.5% Emasol or in phosphate-buffered saline (PBS; 0.0025 M, pH 7.0)]. Sedimentation coefficients were estimated by the method of Martin and Ames (15) with catalase (11S) and rabbit gamma globulin (7S) as reference proteins.

HA titration. HA activity was assayed by Salk's pattern method (19).

Hemagglutination-inhibiting antibody blocking (HI blocking) test. Hemagglutinin antigens were titrated by the antibody blocking test for HA by the method of Webster and Darlington (24).

Neuraminidase assay. A sample (0.05 ml) was mixed with 0.05 ml of 0.2 M sodium acetate buffer (pH 5.0), 0.2 ml of fetuin (10 mg/ml) was added as substrate, and the mixture was incubated at 37°C for 6 min. Then liberated *N*-acetylneuraminic acid was assayed by the 2-thiobarbituric acid method (2, 22).

¹ Present address: Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan.

Assay of hemolytic activity. A sample (0.2 ml) was mixed with 1.8 ml of 1.5% chicken erythrocytes in PBS, and the mixture was incubated at 37 C for 1 hr. Hemolytic activity was expressed as the optical density of the liberated hemoglobin at 540 nm.

Assay of cell-fusion activity. A sample (0.75 ml) was diluted with an equal volume of Eagle minimal essential medium (MEM) containing 4% calf serum, and inoculated to KB monolayer cells (kindly supplied by M. Takahashi, Osaka Univ.) in a Leighton tube. These monolayer cells were incubated at 37 C for 3 hr (7) and stained with hematoxylin-eosin. Cell fusion was estimated by the formation of multinuclear cells.

Protein measurement. Protein was measured by the method of Lowry et al. (12) with bovine serum albumin as the standard.

SDS-polyacrylamide gel electrophoresis. Proteins were dissociated by first heating the sample for 1 min at 100 C in the presence of 1% SDS and 1% 2-mercaptoethanol and then subjecting it to electrophoresis in a 7.5% acrylamide gel by the method of Maizel (13, 14).

Electron microscopy. Sample materials were observed by negative-staining with 1% uranyl acetate in an electron microscope (Hitachi HU 11 DS type).

RESULTS

Solubilization of HVJ envelopes with alkali-Emasol treatment. HVJ virions were treated with Emasol and then centrifuged as described above. The resulting supernatant fluid possessed about 40% of the original HI blocking titer, neuraminidase activities, and proteins, but only 7% of the original HA titer.

HVJ virions negatively stained with uranyl acetate were covered with spikes (10 nm length) which had a knob (6 nm diameter) with a central hole (2 nm diameter) at their tips (Fig. 1a). In the supernatant fraction, there were particles with single or a few spikes similar to those seen on the surface of the virion, and granular particles of 5 to 14 nm in diameter (Fig. 1b). This supernatant fluid was used as the fraction of the solubilized envelopes.

Sedimentation and gel-filtration patterns of the solubilized envelopes. Figure 2 shows the sedimentation pattern of the solubilized envelopes on a sucrose gradient containing Emasol. HI blocking and neuraminidase activities as well as proteins sedimented together at 5 to 11S.

The solubilized envelope subunits were subjected to gel filtration on a BioGel A 15-m column equilibrated with bicarbonate buffer containing Emasol. HI blocking and neuraminidase subunits were eluted together from fraction 26 to fraction 36 having a peak at fraction 31 (Fig. 3). Their Stokes radii were calculated to be 9.4 nm (6.2 to 13 nm) according to Ackers table (1) with catalase as a standard protein.

Kinds of polypeptides in the solubilized fraction. Polypeptides in the solubilized fraction were analyzed by SDS-polyacrylamide gel electrophoresis. They had two main peaks (Fig. 4a; see legend). One of them coincided with P2 (molecular weight = 51,000 daltons) and the other did with P4 (molecular weight = 67,000

FIG. 1. Electron micrographs (a) of HVJ virions, and (b) of the solubilized envelope preparation obtained with 0.5% Emasol treatment. Negative-staining by 1% uranyl acetate. (s) Particle with spikes, (g) granular particle.

FIG. 2. Sedimentation of the solubilized envelopes. An amount (0.5 ml) of the solubilized fraction obtained with 0.5% Emasol treatment was layered onto a sucrose gradient containing Emasol and centrifuged at 50,000 rev/min for 4 hr at 4°C in a Beckman SW50 rotor. Viral activities and proteins were measured after dialysis against PBS overnight.

daltons) of the virion glycoproteins (Fig. 4c; Shimizu and Hosaka, *manuscript in preparation*). Only small amounts of the nucleocapsid proteins (P3; molecular weight = 60,000 daltons) (references 3 and 16; Shimizu and Hosaka, *manuscript in preparation*) and inner proteins (P1; molecular weight = 35,000 daltons) (Shimizu and Hosaka, *manuscript in preparation*) were observed in this fraction.

Sedimentation, gel filtration, and electrophoresis patterns of the solubilized envelopes prepared by 0.25, 0.5, and 1% Emasol treatments did not significantly differ from one another, although when less than 0.5 mg/10,000 HA units of the virions was used, solubilized viral activities and proteins were greatly decreased.

Reassembly of the solubilized envelope subunits. The preparation of the solubilized envelope subunits from 0.25% Emasol treatment was subjected to gel filtration on the BioGel A15-m column equilibrated with PBS. HI blocking and neuraminidase activities were eluted together having two peaks (Fig. 5a). The first peak occurred slightly after the void volume, separated from Emasol, and had high HA and hemolytic activities. The second peak partially overlapped with the Emasol peak (Fig. 5c). The leading portion of the second peak, not overlapping with the Emasol one, had a considerable HA titer and hemolytic activity, but its trailing portion, overlapping with the Emasol one, had a negligible HA titer and hemolytic activity.

Comparing this pattern with that on the Emasol

FIG. 3. Gel filtration of the solubilized envelopes through BioGel A-15m column (25 by 280 mm) in the presence of Emasol. An amount (2 ml) of the sample obtained with 0.25% Emasol treatment was subjected to gel filtration on a column equilibrated with 0.02 M bicarbonate buffer containing 0.05% Emasol. The void volume of this column was equivalent to fraction 15. Catalase as a reference protein was eluted in a peak at fraction 38.

column (Fig. 3), it was clear that on the PBS column about one-half of the charged materials was separated from Emasol and eluted considerably faster than on the Emasol column. The advancing of elution and the increase of HA titer suggested that the solubilized subunits aggregated together to form large structures immediately after being separated from Emasol. On this assumption their elution volumes should decrease according to both the dimensions of aggregates and the times at which aggregates were formed in the column. Therefore, an increase of Emasol concentration in the sample should cause delay of their aggregation and then of their elution. This assumption was confirmed by the following experiments.

When the solubilized envelopes from 0.5% Emasol treatment were subjected to gel filtration on the same PBS column (Fig. 5b), the first peak moved backward, but the second peak was at the same position as that in Fig. 5a (0.25% Emasol treatment). Moreover, when the sample from 1% Emasol treatment was applied, the first peak diminished to a shoulder of the second peak. This dependency of the elution patterns on the Emasol concentration supported the assumption that the envelope subunits, which were solubilized with the alkali-Emasol treatment, aggregated together consecutively to form large particles in the gel column according to their separation from Emasol.

Morphology of the reassembled particles.

FIG. 4. SDS-polyacrylamide gel electrophoresis (a) of the solubilized envelopes obtained with 0.25% Emasol treatment, (b) of the reassembled preparation (fraction 22 of Fig. 7b), and (c) of HVJ virions. An amount (0.1 ml) of dissociated proteins (see Materials and Methods) was applied to the gel, and electrophoresis was carried out at 5 ma in a 7.5% diacrylate-cross-linked polyacrylamide gel column (5.5 by 60 mm) containing 0.1 M sodium phosphate (pH 7.2) and 0.1% SDS until bromphenol blue marker migrated about 55 mm (for

Figure 6a shows the morphology of the first peak of Fig. 5b. There were many particles with several spikes. Similar particles were observed in the first peak of Fig. 5a.

Figure 6b shows the morphology of the HA and hemolytic peak at the leading portion of the second peak (just before the Emasol peak as seen in Fig. 5a). Very large membranous particles with many spikes are seen, and their morphological feature is similar to that of the viral envelopes. When these large particles were again gel-filtered, they were eluted at the void volume. The unusual occurrence of these particles in the later tubes in the first gel filtration could be explained by their formation after the gel filtration.

The morphology of particles in the trailing parts of the second peak, overlapping with the Emasol peak, was like that of the solubilized envelope fraction. These findings indicated that the presence of Emasol prevented the aggregation of the solubilized subunits, which would be necessary for expression of HA and hemolytic activities.

Emasol could not be removed by dialysis through cellulose tubing [Visking and Neffex 100 (Union Carbide Co.) tubings were tested]. After 3 days of dialysis of the solubilized envelope fraction, the HA titer did not increase, and envelope fragment-like particles were not observed.

Reassembly of 8.8S-envelope subunits. To find out whether the envelope-like particles were reassembled only from the 8.8S subunits, the 8.8S fraction of the solubilized envelopes after centrifugation on a sucrose gradient containing Emasol was subjected to gel filtration on the PBS column. The elution pattern was like that of the unfractionated preparations, and envelope fragment-like particles were observed too in the effluent fractions having high HA and hemolytic activities (Fig. 6c).

Removal of Emasol from the solubilized envelope fraction by Sephadex G-200 gel filtration. By means of gel filtration on a BioGel A-15m column, only half of the charged subunits were separated from Emasol, and they, forming aggregates, distributed widely as seen in Fig. 5. Therefore, Sephadex G-200 was used for the more effective and more concentrated preparation of the reassembled envelopes.

The solubilized envelope subunits eluted at the

about 6 hr). The stained gels were scanned by a densitometer (Gilford, linear transport) at 570 nm. The proteins of HVJ virions were separated into five main peaks which were designated as P1, P2, P3, P4, and P5 in order of their mobilities. P2 and P4 are glycoproteins. P3 is nucleocapsid protein, and P1 is inner protein (Shimizu and Hosaka, manuscript in preparation).

FIG. 5. Gel filtration of the solubilized envelopes through BioGel A-15m in the absence of Emasol. An amount (2 ml) of the sample prepared (a) with 0.25% and (b) with 0.5% Emasol treatment was subjected to gel filtration on a column equilibrated with PBS. Emasol was eluted in a peak at fraction 40 through the same column. The void volume was equivalent to fraction 15, and catalase was eluted in a peak at fraction 40.

void volume from the Sephadex G-200 column containing Emasol because of their smaller molecular weight exclusion limit (Fig. 7a). Gel filtration on this column without detergent separated almost all of the charge subunits from Emasol (Emasol was eluted as a broad peak at fraction 32) and formed a sharper peak at the void volume; the HA activity was greatly increased, and the hemolytic activity was restored (Fig. 7b). The morphology of this peak fraction was similar to that of the reassembled particles formed through the gel filtration on the BioGel A-15m column.

These findings show that reassembly of the solubilized subunits also occurred through Sephadex G-200 gel filtration. Thus, this gel

filtration proved to be suitable for preparative procedures.

Sedimentation patterns of the reassembled envelope particles. The viral activities of the reassembled envelope fractions sedimented at much greater rate than those of the solubilized one on a sucrose gradient (Fig. 8). This finding confirmed reassembly of the solubilized subunits through gel filtration.

The reassembled particles distributed widely after the centrifugation on the sucrose gradients (Fig. 8a and b), showing that they were heterogeneous in size or shape. Separation of the peaks of neuraminidase, HA, and hemolytic activities is clearly seen in Fig. 8b. This may be caused by differences in the optimal size or shape of ag-

FIG. 6. Morphology of the reassembled envelope particles. (a) Fraction 23 of Fig. 5b; (b) fraction 31 of Fig. 5b; (c) the reassembled particles from 8.8S subunits (in the fraction corresponding to fraction 31 of Fig. 5b).

FIG. 7. Gel filtration of the solubilized envelopes through Sephadex G-200 column (25 by 360 mm) (a) in the presence and (b) in the absence of Emasol. An amount (2 ml) of the sample prepared with 0.25% Emasol treatment was applied to the column equilibrated (a) with 0.02 M bicarbonate buffer (pH 10) containing 0.05% Emasol and (b) with PBS. The void volume was equivalent to fraction 22. Emasol was eluted in a peak at fraction 32. Catalase also eluted at fraction 32.

gregates for HA and hemolytic activities and seemed to be consistent with the results from sonically disrupted envelopes by Hosaka (7).

Cell fusion activity of the reassembled envelope particles. Cell fusion activity of the reassembled envelope particles was examined on KB monolayer cells. As shown in Fig. 9, they induced many multinuclear cells after 3 hr of 37°C incubation. Thus, the reassembled envelope particles had cell-fusion activity in addition to HA and hemolytic activity.

Polypeptides of the reassembled envelope particles. SDS-polyacrylamide gel electrophoretic pattern of the reassembled particles had two main peaks at the positions corresponding to those of HVJ glycoprotein P2 and P4 and a small sharp peak between the origin and the P4 peak (Fig. 4b). This pattern was similar to that of the solubilized fraction (Fig. 4a), but nucleocapsid proteins (P3) and inner proteins (P1) were proportionately lower than in the solubilized fraction.

Alkali-Emasol treatment of the reassembled envelope particles. When the reassembled particles

FIG. 8. Sedimentation of the reassembled envelope particles. An amount (0.5 ml) of (a) fraction 31 of Fig. 5b and (b) fraction 22 of Fig. 7b was layered onto the sucrose gradient in PBS. For comparison, (c) 0.5 ml of the solubilized fraction was layered onto the sucrose gradient containing Emasol. Centrifugation was at 35,000 rev/min for 1 hr at 4°C in a Beckman SW39 rotor.

were subjected to alkali-Emasol treatment, their envelope-like structures disappeared, their HA titer was greatly decreased, and their sedimentation pattern was similar to that of the solubilized subunits. The solubilization of HVJ envelopes by alkali-Emasol treatment and the reassembly of the envelope-like particles with the removal of Emasol seemed to be reversible.

DISCUSSION

The alkali-Emasol treatment of HVJ partially disrupts their envelopes but does not disintegrate their nucleocapsids (8). Envelope subunits with biological activities of influenza and Newcastle disease viruses (24) and RNA tumor viruses (4, 21) are solubilized with this treatment.

In the case of HVJ, total HI blocking and neuraminidase activities were not decreased with this treatment, and about 40% remained in the supernatant fraction after centrifugation at 40,000 rev/min for 1 hr. Since this supernatant fluid contained a lot of envelope proteins but a small amount of nucleocapsid and inner proteins, as shown by SDS-polyacrylamide gel electrophoresis (Fig. 4a), envelope components were specifically solubilized with this treatment.

FIG. 9. Cell fusion caused by the reassembled envelope particles. (a) Treated KB monolayer cells (sample used; fraction 23 of Fig. 7b). (b) Control KB monolayer cells (see Materials and Methods).

The solubilized envelope subunits had a sedimentation coefficient of 8.8S (5 to 11S; Fig. 2) and a Stokes radius of 9.4 nm (6.2 to 13 nm; Fig. 3). Their particle weight was calculated to be 360,000 daltons (140,000 to 630,000 daltons) based on their sedimentation coefficient and Stokes radius but ignoring specific volume differences between the envelope subunits and standard protein catalase, whereas the estimated molecular weights of their constituent proteins P2 and P4 were 51,000 and 67,000 daltons, respectively. Therefore, they were considered to be not single molecules but aggregates of several molecules of P2 or P4, or both.

The solubilized proteins, P2 and P4, are glycoproteins. The carbohydrate chains have much less mobility than their glycoproteins in SDS-polyacrylamide gel electrophoresis (3, 4), and the molecular weight of fetuin (a glycoprotein; molecular weight = 43,000 to 50,000 daltons) (5, 20) was estimated to be 60,000 daltons by electrophoresis (Shimizu, *unpublished data*). It

seems that the molecular weights of glycoproteins are estimated to be greater than the true values by the electrophoresis. Further investigations of correlation between the molecular weights of glycoproteins and their behavior on electrophoresis are necessary for the determination of true molecular weights of P2 and P4.

When the solubilized envelope subunits were separated from Emasol through gel filtration, they self-assembled to form envelope-fragment particles (Fig. 6). Dialysis of the solubilized fraction with Visking or Neflex 100 tubing was unsuccessful for removal of the detergent and reassembly of the envelope-like particles, although the theoretical molecular weight of Emasol (polyoxyethylene sorbitan monolaurate, $C_{58}O_{26}H_{114}$) is 1,226 daltons. Emasol had the same elution volume at its peak as did catalase (molecular weight 230,000 to 250,000 daltons) on the gel filtrations (Fig. 5 and 7). This fact suggested that it formed large micelles in PSB and explained the above contradiction.

The reassembled particles had not only HI blocking and neuraminidase activities, but also HA, hemolytic, and cell-fusion activities. This is taken as direct evidence that these viral activities are associated with the viral envelopes. The resemblance of the reassembled and virion envelopes in morphology and activities lead to a view that the primary structure of the virion envelopes also consists of the two glycoproteins (P2 and P4 in Fig. 4c), although the assignment of the envelope activities to the component proteins was not accomplished.

The reassembled envelope particles gave a cell-fusion factor free from viral genomes. They will be useful for homokaryocyte, heterokaryocyte, and hybrid-cell formation, instead of inactivated HVJ virions by ultraviolet irradiation (6, 17, 18 25) or β -propiolactone (10).

ACKNOWLEDGMENTS

We thank K. Fukai for his interest and encouragement and J. Tomizawa (Faculty of Science, Osaka University) and Y. Okada (Department of Preventive Medicine, Osaka University) for their advice and criticism, N. Iwa for his excellent technical assistance in staining cells, and H. Shinagawa and K. Yagi for reviewing the manuscript.

LITERATURE CITED

1. Ackers, G. K. 1964. Molecular exclusion and restricted diffusion processes in molecular-sieve chromatography. *Biochemistry* 3:723-730.
2. Aminoff, D. 1961. Methods for the quantitative estimation of N-acetylneuraminic acid and their application to hydrolysates of sialomucoids. *Biochem. J.* 62:384-392.
3. Content, J., and P. H. Duesberg. 1970. Electrophoretic distribution of the proteins and glycoproteins of influenza virus and Sendai virus. *J. Virol.* 6:707-716.
4. Duesberg, P. H., G. S. Martin, and P. K. Vogt. 1970. Glycoprotein components of avian and murine RNA tumor viruses. *Virology* 37:597-604.

5. Green, W. A., and C. M. Kay. 1963. The influence of buffer composition on the hydrodynamic behavior of fetuin. *Arch. Biochem. Biophys.* **102**:359-366.
6. Harris, H., and J. F. Watkins. 1965. Hybrid cells derived from mouse and man: artificial heterokaryons of mammalian cells from different species. *Nature (London)* **205**:640-646.
7. Hosaka, Y. 1970. Biological activities of sonically treated Sendai virus. *J. Gen. Virol.* **8**:43-54.
8. Hosaka, Y. 1968. Isolation and structure of the nucleocapsid of HVJ. *Virology* **35**:445-457.
9. Hosaka, Y., H. Kitano, and S. Ikeguchi. 1966. Studies on the pleomorphism of HVJ virions. *Virology* **29**:205-221.
10. Knowles, B. B., F. C. Jensen, Z. Steplewski, and H. Koprowski. 1968. Rescue of infectious SV 40 after fusion between different SV 40-transformed cells. *Proc. Nat. Acad. Sci. U.S.A.* **61**:42-45.
11. Laver, W. G., and R. C. Valentine. 1969. Morphology of the isolated hemagglutinin and neuraminidase subunits of influenza virus. *Virology* **38**:105-119.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
13. Maizel, J. V. 1966. Acrylamide-gel electrophorograms by mechanical fractionation: radioactive adenovirus proteins. *Science* **151**:988-990.
14. Maizel, J. V., D. O. White, and M. D. Scharff. 1968. The polypeptides of adenovirus. *Virology* **36**:115-125.
15. Martin, R. G., and B. N. Ames. 1961. A method for determination the sedimentation behavior of enzymes: application to protein mixture. *J. Biol. Chem.* **236**:1372-1379.
16. Mountcastle, W. E., R. W. Compans, L. A. Caligiuri, and P. W. Choppin. 1970. Nucleocapsid protein subunits of simian virus 5, Newcastle disease virus, and Sendai virus. *J. Virol.* **6**:677-684.
17. Okada, Y. 1961. Multinucleated giant cell formation by fusion of two different strains of cells. *Biken J.* **4**:145-146.
18. Okada, Y., and F. Murayama. 1965. Multinucleated giant cell formation by fusion between cells of two different strains. *Exp. Cell Res.* **40**:154-158.
19. Salk, J. E. 1944. A simplified procedure for titrating hemagglutinating capacity of influenza virus and the corresponding antibody. *J. Immunol.* **49**:87-98.
20. Spiro, R. G. 1960. Studies on fetuin, a glycoprotein of fetal serum. I. Isolation, chemical composition, and physicochemical properties. *J. Biol. Chem.* **235**:2860-2869.
21. Tozawa, H., H. Bauer, T. Graf, and H. Gelderblom. 1970. Strain-specific antigen of the avian leukosis sarcoma virus group. I. Isolation and immunological characterization. *Virology* **40**:530-539.
22. Warren, L. 1959. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.* **234**:1971-1975.
23. Webster, R. G. 1970. Estimation of the molecular weights of the polypeptide chains from the isolated hemagglutinin and neuraminidase subunits of influenza viruses. *Virology* **40**:643-654.
24. Webster, R. G., and R. W. Darlington. 1969. Disruption of myxoviruses with Tween 20 and isolation of biologically active hemagglutinin and neuraminidase subunits. *J. Virol.* **4**:182-187.
25. Yerganian, G., and M. B. Nell. 1966. Hybridization of DWARE hamster cells by UV-inactivated Sendai virus. *Proc. Nat. Acad. Sci. U.S.A.* **55**:1066-1073.