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Application of Fluorescence Photobleaching Recovery To Assess Complex Formation between the Two Envelope Proteins of Sendai Virus in Membranes of Fused Human Erythrocytes†

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ABSTRACT: Fusion of human erythrocytes by Sendai virions is accompanied by lateral mobilization of the viral envelope proteins (F, the fusion protein, and HN, the hemagglutinin/neuraminidase protein) in the target cell membrane; the dynamic parameters characterizing the lateral diffusion of F and HN in the fused cell membrane are identical [Henis, Y. I., & Gutman, O. (1987) *Biochemistry 26*, 812-819; Aroeti, B., & Henis, Y. I. (1988) *Biochemistry 27*, 5654-5661]. This identity raised the possibility that F and HN diffuse together in the cell membrane in mutual heterocomplexes. In order to investigate the possible formation of F-HN complexes in the target cell membrane, which could be important for the fusion process mediated by the viral envelope proteins, we combined fluorescence photobleaching recovery (FPR) measurements of the lateral mobility of the viral glycoproteins with antibody-mediated cross-linking of F or HN. After fusion, one viral glycoprotein type was immobilized by cross-linking with highly specific bivalent polyclonal IgG. The other glycoprotein type was labeled with fluorescence monovalent Fab' fragments that do not induce cross-linking, and its mobility was measured by FPR. Neither the mobile fraction nor the lateral diffusion coefficient of the Fab'-labeled viral glycoproteins was affected by immobilization of the second viral envelope protein, demonstrating that F and HN diffuse independently in the target cell membrane and are not associated in mutual complexes.

Membrane fusion events are involved in a variety of physiological and pathological processes (Poste & Pasternak, 1978; White et al., 1983; Blumenthal, 1987). The penetration of enveloped viruses into animal cells involves a fusion event, either between the viral envelope and the cellular plasma membrane (as in the case of paramyxoviruses) or between the envelope of lysosomally trapped virions and the lysosomal membrane following adsorptive endocytosis (White et al., 1983). Virally mediated fusion is the best characterized system of biological membrane fusion, and its identification with specific viral envelope proteins (Poste & Pasternak, 1978; Hsu et al., 1979; White et al., 1983; Florkiewicz & Rose, 1984)

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makes it an attractive system for studies on the fusion mechanism.

Among the paramyxoviruses, which fuse with cell membranes at neutral pH values, the fusogenic activities of Sendai virus were studied most extensively. The envelope of these virions contains two glycoproteins: the hemagglutinin/neuraminidase (HN)[1] and the fusion (F) proteins. Until recently, it was accepted that the HN protein mediates the binding of the virions to cellular receptors while the F protein promotes virus–cell and cell–cell fusion (Poste & Pasternak, 1978; Choppin & Scheid, 1980). However, recent findings from several laboratories suggested that the HN protein may also play an active role in the fusion event (Ozawa et al., 1979; Miura et al., 1982; Gitman & Loyter, 1984). The mechanism by which the viral envelope proteins induce fusion is yet unknown, although hydrophobic interactions between the fusogenic viral glycoproteins and target membranes were proposed to be involved (Gething et al., 1978; Richardson et al., 1980; Hsu et al., 1981; White et al., 1983).

We have recently employed fluorescence photobleaching recovery (FPR) to demonstrate that the envelope proteins of Sendai virions (F and HN) become laterally mobile in the membrane of human erythrocytes following fusion (Henis et al., 1985). This lateral mobilization in the target cell membrane appears to be involved in the mechanism of cell–cell fusion, since under conditions that selectively inhibit cell–cell fusion but not virus–cell fusion, a strict correlation was found between the level of laterally mobile viral glycoproteins in the cell membrane and the extent of cell–cell fusion (Henis & Gutman, 1987; Aroeti & Henis, 1988). An important observation in these studies was that in all cases, the dynamic parameters characterizing the lateral diffusion of F and HN in the target cell membrane (D, the lateral diffusion coefficient, and R_f, the mobile fraction) were similar (Henis et al., 1985; Henis & Gutman, 1987; Aroeti & Henis, 1988). This similarity raises the possibility that F and HN diffuse together in the cell membrane in mutual heterocomplexes. If formed, such complexes could have a role in the induction of membrane fusion; participation of HN in the formation of an active complex together with F could provide an explanation for the suggestion that not only F but also HN is required in Sendai virus mediated fusion (Miura et al., 1982; Gitman & Loyter, 1984; Citovsky et al., 1986).

The present work was aimed to investigate the possible formation of F–HN complexes. To explore this point, we combined FPR measurements of the lateral mobility of F and HN with antibody cross-linking experiments. After fusion, one of the viral glycoproteins (e.g., F) was immobilized by cross-linking with specific bivalent IgG; the other glycoprotein (e.g., HN) was labeled by fluorescent monovalent Fab′ fragments that do not induce cross-linking, and its mobility was measured by FPR. The results of these experiments demonstrate that F and HN diffuse independently in the target cell membrane and are not associated in mutual complexes.

Materials and Methods

Virus. Sendai virus (Z strain) was grown in the allantoic sac of 10–11-day-old embryonated chicken eggs, harvested 48 h after injection, and purified by established procedures (Peretz et al., 1974; Hsu et al., 1982; Henis et al., 1985). The virus was resuspended in 160 mM NaCl/20 mM Tricine, pH 7.4 (solution A), and stored at −70 °C. Viral protein concentration was determined by a modified Lowry procedure (Markwell et al., 1978). Viral hemagglutinating activity (around 13 000 HAU/mg of viral protein) was measured in hemagglutinating units (HAU) as described (Peretz et al., 1974).

Reconstituted Sendai Virus Envelopes. RSVE were prepared as described in detail previously (Vainstein et al., 1984; Henis & Gutman, 1987), by solubilization of the virions with Triton X-100, removal of insoluble material by centrifugation, and removal of the detergent by direct addition of SM-2 Biobeads into the solution containing detergent, viral envelope lipids, and the viral glycoproteins F and HN. The resulting envelopes, which contain F and HN at a ratio similar to that of the native virions (Vainstein et al., 1984), were collected by centrifugation and suspended in solution A. The RSVE typically showed 3–4-fold higher HAU per milligram of protein than native virions, since they are enriched with the envelope proteins; for this reason, the amounts of RSVE and Sendai virions were calibrated by using HAU rather than the amount of viral protein (Henis & Gutman, 1987).

Interactions of Sendai Virions and RSVE with Human Erythrocytes. Fresh human blood (group O, Rh-positive) was obtained from a blood bank and stored with sodium citrate up to 7 days at 4 °C. Prior to use, erythrocytes were washed with solution A, diluted to 2% (v/v) with the same buffer, and incubated (4 °C, 15 min) with 400 HAU/mL Sendai virions or RSVE. The viral particles were earlier subjected to mild sonication and filtrated through a 0.45-μm Acrodisc filter (Gelman, Ann Arbor, MI) to eliminate large viral aggregates (Henis et al., 1985). After being washed twice with solution A, the cells were suspended in 1 mL of solution A and incubated 30 min at 37 °C to induce fusion. The degree of cell–cell fusion was evaluated by determining the percentage of fused cells among the total cell population, employing phase-contrast microscopy (Henis et al., 1985; Peretz et al., 1974).

Antibodies and Fab′ Fragments against Viral Envelope Proteins. Anti-F and anti-HN antibodies were raised in rabbits as described by us earlier (Henis et al., 1985), by intracutaneous injections of vesicles containing either F or HN proteins. These vesicles were prepared by a modification (Nussbaum et al., 1984) of the method of Nakanishi et al. (1982). Monovalent Fab′ fragments labeled with TMR–isothiocyanate were prepared from the IgG fractions as described (Henis et al., 1985; Henis & Gutman, 1987), following standard labeling procedures (Brandtzaeg, 1973). The TMR–Fab′ fragments were not contaminated by Fab'_2 or IgG, as demonstrated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under nonreducing conditions. The antibodies did not cross-react with erythrocyte membrane components, as demonstrated by their failure to bind to erythrocytes which were not exposed to Sendai virions (Henis et al., 1985). Moreover, they were specific to the viral glycoprotein originally incorporated into each type of vesicle used for the immunization (see Results).

Fluorescence Photobleaching Recovery. Lateral diffusion coefficients (D) and mobile fractions (R_f) of the viral glycoproteins in the cell membrane were measured by FPR (Koppel et al., 1976; Axelrod et al., 1976) using a previously described apparatus (Henis & Gutman, 1983). The bleaching conditions in the FPR studies were shown not to alter the lateral mobilities measured (Wolf et al., 1980; Koppel & Sheetz, 1981). Following incubation with the virions, the ghosts formed by the viral hemolytic activity were attached to glass coverslips precoated with polylysine [10-min incubation of the coverslip

[1] Abbreviations: D, lateral diffusion coefficient; F, fusion protein; FPR, fluorescence photobleaching recovery; HAU, hemagglutinating unit(s); HN, hemagglutinin/neuraminidase protein; R_f, mobile fraction; RSVE, reconstituted Sendai virus envelope(s); TMR, tetramethylrhodamine; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine.

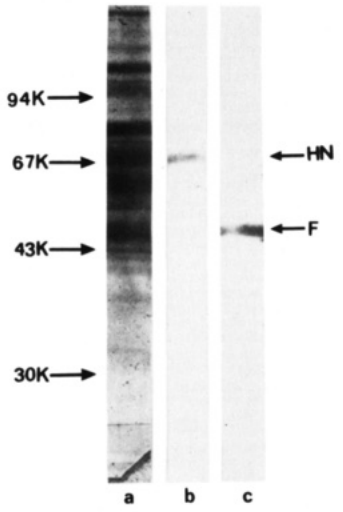

FIGURE 1: Immunoblot assay of IgGs raised against Sendai virus envelope glycoproteins. The gel (10% polyacrylamide) was loaded with 100 μg (lane a) or 5 μg (lanes b and c) of protein of Sendai virions. After the gel was run in SDS–PAGE and electroblotted [according to Towbin et al. (1979)], nitrocellulose paper strips representing the various lanes were cut. Lane a was stained for proteins with amidoblack, while the other two lanes were incubated with 5 μg/mL anti-HN IgG (b) or 5 μg/mL anti-F IgG (c) under the conditions described by Towbin et al. (1979). The bands labeled by the antibodies were visualized by a peroxidase-conjugated second antibody (goat IgG directed against rabbit IgG). The numbers on the left are molecular weights determined by running marker proteins under the same conditions. Similar results were obtained for TMR–Fab′ fragments prepared from the above IgG preparations.

with 5 μg/mL poly(L-lysine) in 20 mM phosphate buffer, pH 8]. The cells were incubated with whole IgG (30 min, 22 °C, in solution A containing 0.2% bovine serum albumin) directed against F or HN, using IgG concentrations sufficient to induce immobilization by cross-linking but low enough to enable adequate labeling of the remaining antigenic determinants by the fluorescent Fab′ fragments for FPR experiments (see Results). After being washed 3 times, the cells were labeled with anti-F or anti-HN TMR-Fab′ fragments in the same buffer (100 μg/mL, 30 min, 22 °C). The coverslips were wet-mounted in solution A supplemented with 0.2% bovine serum albumin and taken for the FPR experiments.

The monitoring laser beam (529.5 nm, 0.5 μW, argon ion laser) was focused through the microscope to a Gaussian radius of 0.93 μm with an 100× oil-immersion objective. A brief pulse (5 mW, 20 ms) bleached 50–70% of the fluorescence in the illuminated region. The time course of fluorescence recovery was followed by the attenuated monitoring beam. D and R_f were determined from the fluorescence recovery curves (Axelrod et al., 1976) by nonlinear regression analysis (Petersen et al., 1984). Incomplete recovery was interpreted to represent fluorophores which are immobile on the experimental time scale ($D \leq 5 \times 10^{-12}$ cm^2/s).

RESULTS

Antibody-Mediated Immobilization of the Viral Glycoproteins. In order to investigate whether the viral envelope proteins F and HN form mutual heterocomplexes in the cell membrane, we employed a combination of lateral mobility measurements and antibody-mediated cross-linking of the viral glycoproteins with anti-viral antibodies.

The specificity of the anti-F and anti-HN IgG preparations toward the viral glycoproteins against which they were raised was estimated by subjecting Sendai virions to SDS–PAGE followed by immunoblotting (Figure 1). This experiment demonstrated that each antibody labeled only the viral glycoprotein against which it was raised and no cross-reactivity could be detected.

Table I: Effect of Treatment with Anti-F or Anti-HN IgG on the Lateral Mobility of the Two Viral Glycoproteins in the Membrane of Erythrocytes Fused with Sendai Virions[a]

cross-linking IgG	TMR–Fab′-labeled glycoprotein	R_f	D (cm^2/s × 10^{10})
none	F	0.53 ± 0.03	2.5 ± 0.2
anti-F	F	NR[b]	≤0.05
anti-F	HN	0.45 ± 0.05	3.0 ± 0.3
none	HN	0.52 ± 0.03	2.5 ± 0.2
anti-HN	HN	NR[b]	≤0.05
anti-HN	F	0.47 ± 0.04	2.4 ± 0.2

[a] Erythrocytes (2% v/v) were fused with Sendai virions (400 HAU/mL) in solution A, by incubation at 4 °C followed by 37 °C. The ghosts formed by the viral hemolytic activity were attached to polylysine-coated coverslips and incubated (30 min, 22 °C) with either 5 μg/mL anti-F IgG or 10 μg/mL anti-HN IgG as described under Materials and Methods. These antibodies are referred to as the "cross-linking IgG". The cells were washed and labeled with anti-F or anti-HN TMR–Fab′ fragments (100 μg/mL). The FPR measurements were conducted in solution A containing 0.2% bovine serum albumin at 22 °C. The results are the mean ± SE of 50–60 measurements in each case. Student's t test indicated that the changes in the dynamic parameters of each viral envelope protein (F or HN) upon cross-linking of the other were not statistically significant ($p > 0.1$ for the effects on the R_f values, $p > 0.25$ for the effect of anti-HN IgG on D of the F proteins, and $p > 0.05$ for the effect of anti-F IgG on D of the HN proteins). This is contrasted with the high significance ($p < 0.00005$) of the effects of the cross-linking IgG on either R_f or D of the viral glycoproteins against which they were directed. [b] NR represents no recovery (complete immobility) on the time scale of the FPR experiment ($D \leq 5 \times 10^{-12}$ cm^2/s).

In order to explore whether F and HN are complexed together in the cell membrane, the effects of immobilizing one viral glycoprotein (e.g., F) on the lateral mobility of the other (HN) were examined. After fusion with native Sendai virions, the cells were incubated with anti-F or anti-HN IgG to specifically cross-link one type of the viral envelope proteins. The cells were washed and labeled with either anti-F or anti-HN TMR–Fab′ fragments, and FPR measurements were performed to determine the lateral mobility of each viral glycoprotein (for details, see Materials and Methods). The IgG concentrations employed for cross-linking were the lowest that induced complete immobilization of the viral glycoproteins against which they were directed. This enabled adequate labeling of the remaining antigenic determinants by TMR–Fab′ fragments, allowing determination of the lateral mobility of the viral glycoprotein that was cross-linked by IgG, and ensuring that the level of cross-reactivity of the IgG with the other viral glycoprotein will be kept to the minimum. Thus, 10 μg/mL anti-HN IgG was sufficient to induce complete lateral immobilization of the HN proteins in the erythrocyte membrane, and 5 μg/mL anti-F IgG induced an analogous effect on the F proteins (Table I). Binding measurements on erythrocytes fused by the virus, using TMR-labeled IgG followed by quantitative measurement of the fluorescence on the surface of the cells employing the FPR instrumentation under nonbleaching conditions, indicated that the incubation with 10 μg/mL anti-HN IgG saturated 30% of the total antibody binding sites on the surface of the fused cells and incubation with 5 μg/mL anti-F IgG resulted in the occupation of 20–22% of the sites available for this antibody.

Effect of Immobilization of F or HN on the Lateral Mobility of Each Other. Immobilization of one of the viral glycoproteins (e.g., F) in the cell membrane by specific cross-linking with IgG can have one of several effects on the lateral mobility of the other viral envelope protein (HN). If

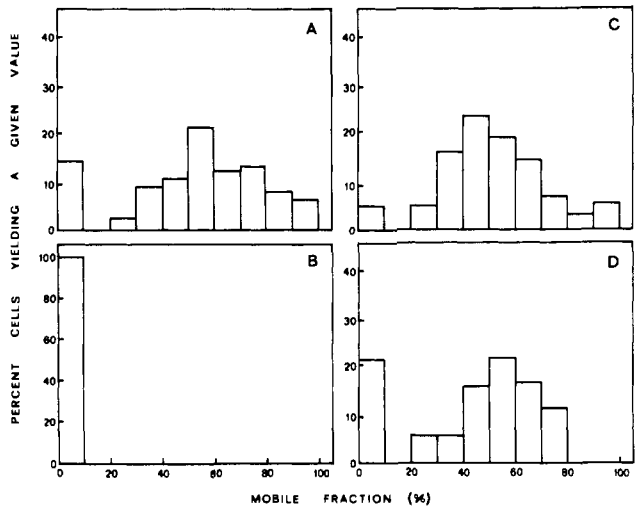

FIGURE 2: Effect of cross-linking with anti-F IgG on the distribution of the R_f values of F and HN on erythrocytes fused with Sendi virions. The cells were fused, cross-linked (or mock-incubated in solution A) with 5 μg/mL anti-F IgG, and labeled with TMR–Fab′ directed against F or HN as described in Table I. A total of 50–60 cells were measured in each case. (A) R_f of the F proteins on fused cells which were not exposed to anti-F IgG. (B) Mobile fractions of the F proteins after cross-linking with anti-F IgG. (C) R_f of HN proteins on fused cells which were not exposed to anti-F IgG. (D) R_f of HN proteins after cross-linking with anti-F IgG.

the two types of proteins do not reside in the same complex, the lateral mobility of HN will be unaltered. If they form mutual complexes whose lifetime is long relative to the characteristic lateral diffusion time, t_D ($t_D = w^2/4D$, where w is the Gaussian radius of the laser beam on the cell surface), the mobile fraction of HN is expected to drop due to the association of HN molecules with immobile cross-linked F molecules for the entire duration of the lateral diffusion measurement (Elson & Reidler, 1980; Petersen et al., 1984). In case the complex formed has a lifetime which is short relative to t_D, each HN molecule will undergo many association–dissociation cycles with immobile F molecules during the observed fluorescence recovery time, a phenomenon which would lead to a reduction in the effective diffusion coefficient measured for HN (Elson & Reidler, 1980; Petersen et al., 1984).

In view of the above, it was important to determine the effects of immobilization of F or HN on both the R_f and the D values of the other, TMR–Fab′-labeled glycoprotein. The results (Table I, Figures 2 and 3) demonstrate that incubation of erythrocytes fused by Sendai virions with anti-F IgG had no effect on either R_f or D measured for HN, under conditions that induce complete lateral immobilization of the F proteins in the cell membrane. Similarly, immobilization of HN had no effect on either R_f or D exhibited by the F protein in the same preparation (Table I). Moreover, the distribution of the R_f and D values of the HN proteins was not affected by treatment of the virus-fused cells with anti-F IgG; the distributions of both dynamic parameters were not significantly different from those observed on fused cells which were not incubated with the IgG (Figures 2 and 3). Similar results (data not shown) were obtained for the distributions of R_f and D of the F proteins following treatment of cells fused by Sendai virus with anti-HN IgG; namely, immobilization of HN did not affect significantly the distribution of either the R_f or the D value measured for the F proteins. These results indicate that F and HN diffuse independently in the membrane of erythrocytes fused by Sendai virions and are not associated in mutual complexes, either with long or with short lifetimes

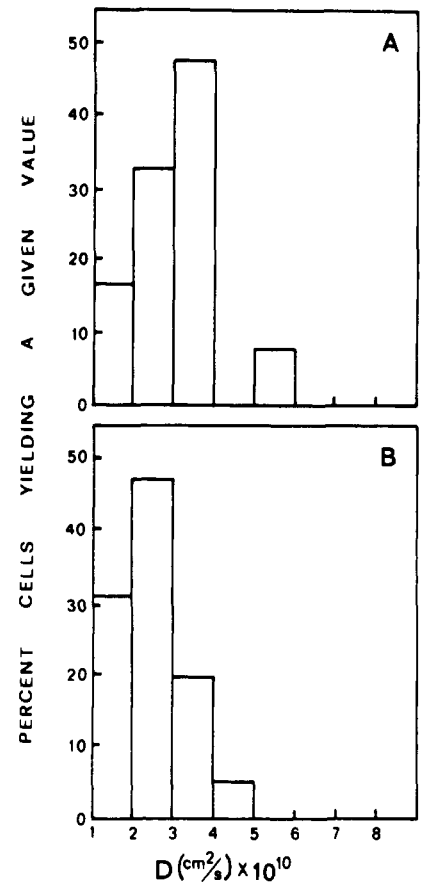

FIGURE 3: Effect of cross-linking with anti-F IgG on the distribution of the D values of the HN proteins on erythrocytes fused with Sendai virions. Experiments were performed as in Figure 2. A total of 50–60 cells were measured in each case. Similar results were obtained for the F proteins after cross-linking the HN proteins on the fused cells with 10 μg/mL anti-HN IgG. (A) Measurements on fused cells which were not exposed to anti-F IgG. (B) Measurements on fused cells incubated with 5 μg/mL anti-F IgG.

relative to the characteristic diffusion time.

Analogous experiments were performed on human erythrocytes fused with RSVE rather than with native Sendai virions. The RSVE, which are lipid envelopes containing F and HN, are as active as native virions in envelope–cell fusion but display a much lower cell–cell fusion activity (Henis & Gutman, 1987). These experiments (Table II) yielded results along the same lines found for erythrocytes fused with native virions, the only difference being that the initial R_f values for F and HN (on fused cells not treated with anti-F or anti-HN IgG) were significantly lower than on cells fused with native virions, as observed by us earlier (Henis & Gutman, 1987). Thus, immobilization of F by antibodies directed against it did not affect the R_f and D values measured for HN, and vice versa (Table II).

Discussion

The purpose of the present experiments was to explore the existence of complexes containing both F and HN in the target cell membrane. This question was investigated by immobilizing the lateral motion of one glycoprotein type on the cell surface by cross-linking with specific IgG, and following the effect of this immobilization on the lateral mobility of the other viral envelope protein. The results of these experiments, depicted in Table I and in Figures 2 and 3, clearly demonstrate that IgG-mediated cross-linking of the F proteins had no effect on the lateral mobility of HN, and vice versa. As discussed in detail under Results [see Elson and Reidler (1980) and

Table II: Effect of Treatment with Anti-F or Anti-HN IgG on the Lateral Mobility of F and HN on Human Erythrocytes Fused with RSVE[a]

cross-linking IgG	TMR-Fab'-labeled glycoprotein	R_f	D ($cm^2/s \times 10^{10}$)
none	F	0.28 ± 0.02	3.3 ± 0.2
anti-F	F	NR[b]	≤0.05
anti-F	HN	0.24 ± 0.03	3.1 ± 0.3
none	HN	0.29 ± 0.04	3.0 ± 0.2
anti-HN	HN	NR[b]	≤0.05
anti-HN	F	0.28 ± 0.03	3.4 ± 0.3

[a] Human erythrocytes were fused with RSVE as described under Materials and Methods. The ghosts formed by the hemolytic activity of the RSVE were incubated with anti-F IgG (5 μg/mL) or anti-HN IgG (10 μg/mL) and labeld with TMR-Fab' fragments (anti-F or anti-HN) as in Table I. The results shown are the mean ± SE of 50–60 FPR measurements in each case. As demonstrated in Figures 2 and 3 for native Sendai virions, immobilization of one viral glycoprotein had no effect on the distributions of the R_f and D values of the other on erythrocytes fused with RSVE (data not shown). Student's t test indicated that the changes in the dynamic parameters of each viral envelope protein upon cross-linking of the other were not statistically significant ($p > 0.25$ for the effect of anti-HN IgG on the R_f of the F proteins, and $p > 0.1$ for the effect of anti-F IgG on the R_f of the HN proteins; $p > 0.25$ for the equivalent effects on the D values). This is contrasted with the high significance ($p < 0.005$) of the effects of the cross-linking IgG on either R_f or D of the viral glycoproteins against which they were directed. [b] NR represents no recovery (complete immobility) on the time scale of the FPR experiment ($D \leq 5 \times 10^{-12}$ cm^2/s).

Petersen et al. (1984)], the insensitivity of the R_f values of one viral glycoprotein type to lateral immobilization of the other suggests the lack of stable F–HN complexes (complexes that do not dissociate on the time scale of the FPR experiments). Similarly, the fact that the diffusion coefficients of, e.g., HN do not decrease following lateral immobilization of F demonstrates the absence of F–HN complexes whose lifetime is short relative to the characteristic diffusion time (Elson & Reidler, 1980; Petersen et al., 1984). These conclusions apply to the population of laterally mobile viral glycoproteins (those comprising the mobile fraction) and do not rule out the possibility that F–HN complexes may exist among the immobile viral glycoproteins. However, this possibility is unlikely in view of the fact that chemical cross-linking experiments could not detect any F–HN complexes even in the membranes of native Sendai virions (Markwell & Fox, 1980; Sechoy et al., 1987). Another possibility that should be mentioned is that the cross-linking antibodies somehow interfere with F–HN complexes and induce their dissociation; however, this possibility is highly unlikely, especially in view of the fact that the effect would have to be induced by both the anti-F and anti-HN antibody preparations, and under conditions where only a small fraction of the antibody binding sites is occupied.

The insensitivity of the dynamic parameters of one viral glycoprotein to lateral immobilization of the other by cross-linking with specific IgG also demonstrates the lack of cross-reactivity between the anti-HN and anti-F IgG at the concentration range employed for lateral immobilization of either viral envelope protein in the FPR studies (Table I, Figures 2 and 3), as suggested also by the immunoblotting data (Figure 1). In as much as such cross-reactivity exists, it must therefore be below the levels that affect the lateral diffusion measurements reported here.

The small size of the viral particles relative to the spot illuminated by the laser beam does not allow measurement of the lateral motion of the viral glycoproteins in the viral envelope itself. Therefore, we could not examine whether F and HN are associated in mutual heterocomplexes in the viral envelope before fusion. However, as stated above, studies based on the use of chemical cross-linkers (Markwell & Fox, 1980; Sechoy et al., 1984) demonstrated only homooligomers of the envelope glycoproteins in native virions. The existence of such homooligomers also in the membranes of the fused cells is possible, and would increase the sensitivity of the viral glycoproteins to cross-linking by the IgG directed against them. Such a phenomenon could be one of the reasons for the ability of low IgG concentrations (resulting in the occupancy of 20–30% of the available binding sites) to immobilize F or HN in the target cell membrane.

The experiments performed using RSVE instead of native virions (Table II) yielded analogous results, demonstrating the lack of F–HN complexes among the mobile viral glycoproteins also in this case. This conclusion does not apply to the viral glycoproteins that remain immobile following fusion with RSVE, and our results do not eliminate the possible existence of complexes containing both F and HN in this population. In fact, the significantly lower R_f values obtained for the viral envelope proteins on erythrocytes fused by RSVE as compared with erythrocytes fused by native virions (Tables I and II) could be due to an increased level of nonspecific aggregation of the viral glycoproteins, preventing them from moving laterally in the cell membrane following fusion. Such nonspecific aggregation could be the outcome of partial denaturation of viral envelope proteins in the course of solubilizing the virions and reconstituting the viral envelopes, as suggested by Metsikko et al. (1986) for the analogous preparation of virosomes (reconstituted envelopes) from vesicular stomatitis virus.

Recent data suggested that the HN protein may also have an active role in Sendai virus mediated fusion (Ozawa et al., 1979; Miura et al., 1982; Nakanishi et al., 1982; Gitman & Loyter, 1984; Citovsky et al., 1986). The demonstration that F and HN diffuse independently in the target cell membrane, together with the failure to detect F–HN complexes in native Sendai virions by chemical cross-linking (Markwell & Fox, 1980; Sechoy et al., 1987), indicates that the role of HN in the fusion process does not rely on the formation of active complexes together with F proteins. In view of the strict correlation between the level of laterally mobile Sendai virus glycoproteins in the cell membrane and the extent of cell–cell fusion (Henis & Gutman, 1987; Aroeti & Henis, 1988), and the independent motion of F and HN in the target cell membrane, it will be interesting to explore whether lateral motion of both envelope proteins or only one is required for cell–cell fusion. This subject is currently under investigation in our laboratory.

Registry No. Neuraminidase, 9001-67-6.

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Functional Reconstitution of the Glycine Receptor†

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ABSTRACT: The functional reconstitution of the chloride channel coupled glycine receptor is described. Glycine receptors were purified from the cholate extract of rat spinal cord membranes by affinity chromatography and incorporated into phospholipid vesicles by the addition of phosphatidylcholine and removal of detergent by gel filtration. The reconstituted vesicles showed the same polypeptide composition as the purified receptor (proteins of M_r 48 000 and 58 000). The pharmacological characteristics of the glycine receptor were also preserved in the proteoliposomes, as demonstrated by the displacement of [^{3}H]strychnine binding by several glycinergic ligands and by photoaffinity labeling experiments. In order to observe functional responses (i.e., specific agonist-induced anion translocation), we have developed an assay based on the fluorescence quenching of an anion-sensitive entrapped probe, SPQ [6-methoxy-*N*-(3-sulfopropyl)quinolinium]. Reconstituted vesicles were loaded with the fluorescent probe during a freeze–thaw–sonication cycle in the presence of added liposomes containing cholesterol. In such a reconstituted system, glycine receptor agonists are able to increase the rate of anion influx into the vesicles. The action of agonists is blocked by the simultaneous presence of strychnine or other glycine antagonists. Our results show that the purified 48 000- and 58 000-dalton polypeptides reconstituted into phospholipid vesicles can bind ligands and promote specific ion translocation in a way similar to the glycine receptor in its native environment.

Glycine is a major inhibitory neutrotransmitter in the spinal cord and other regions of the central nervous system. The interaction of this amino acid with the glycine receptor (GlyR)[1] increases the chloride conductance of the postsynaptic membrane, thus producing a hyperpolarization (Krnjevic, 1974; Aprison & Daly, 1978). Glycine-mediated inhibitory actions are selectively antagonized by the alkaloid strychnine. The latter compound has been utilized in pharmacological, biochemical, and localization studies of the glycine receptor

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[1] Abbreviations: FTS vesicles, reconstituted vesicles subjected to a freeze–thaw–sonication cycle; GlyR, glycine receptor; SPQ, fluorescent probe 6-methoxy-*N*-(3-sulfopropyl)quinolinium.

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