PH-dependent Fusion Induced by Vesicular Stomatitis Virus Glycoprotein Reconstituted into Phospholipid Vesicles

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Purified G-protein from vesicular stomatitis virus was reconstituted into egg phosphatidylcholine vesicles by detergent dialysis of octyl glucoside. A homogeneous population of reconstituted vesicles could be obtained, provided the protein to lipid ratio was high (about 0.3 mol % protein) and the detergent removal was slow. The reconstituted vesicles were assayed for fusion activity using electron microscopy and fluorescence energy transfer. The fusion activity mediated by the viral envelope protein was dependent upon pH, temperature, and target membrane lipid composition. Incubation of reconstituted vesicles at low pH with small unilamellar vesicles containing negatively charged lipids resulted in the appearance of large conate structures, as shown by electron microscopy using negative stain. This process did not cause leakage of a vesicle-encapsulated aqueous marker. The rate of fusion was PH-dependent with a pK of about 4 and the apparent energy of activation for the fusion was 16 ± 1 kcal/mol. G-protein-mediated fusion showed a large preference for target membranes which contain phosphatidylserine or phosphatidic acid. Inclusion of 36% cholesterol in any of the lipid compositions had no effect on the rate of fusion. These reconstituted vesicles provide a system to study the mechanism of PH-dependent fusion induced by a viral spike protein.

Enveloped viruses often contain surface glycoproteins which participate in the attachment and penetration processes of viral infection (1–5). VSV is a rhabdovirus containing one glycosylated protein, G-protein, which is vital for viral infectivity. Removal of G-protein by trypsinization (6–8) or reacting VSV with anti-G-protein antibodies (9) results in a marked decrease in viral infectivity. One of the functions of the G-protein is to facilitate attachment of the virus to the cell surface. Although the precise cellular attachment site for VSV is unknown, the finding that VSV attachment is unaffected by protease treatment of the cell surface (6–8) but markedly inhibited by exogenous phosphatidylserine (10) suggests that this phospholipid may have an important role in the binding of VSV G-protein to cells. VSV associates with "coated pit" regions of the plasma membrane (11–13) and is internalized into the cells via an endocytic pathway morphologically identical with that of receptor-bound ligands (13, 14). Upon endocytosis, the virus is transferred into cytoplasmic vacuoles termed "endosomes" or "receptosomes" (15, 16), which are rapidly acidified (17). This pH change apparently initiates VSV fusion with the endosome membrane and release of the viral nucleocapsid into the cell cytoplasm. Evidence exists that acidic pH initiates the second function of G-protein, that of a membrane fusogen: low pH can induce direct VSV cell membrane fusion as well as cell-cell fusion of VSV-infected cells (18).

The purpose of this study was to construct an in vitro system for analyzing the role of G-protein in membrane fusion. The first step was to reconstitute the G-protein in phospholipid vesicles. Petri and Wagner (19) have shown that VSV G-protein can be reconstituted in egg phosphatidylcholine vesicles by detergent dialysis using octyl glucoside. The insertion and orientation of G-protein in these reconstituted vesicles is the same as in intact virions (19). Similar reconstituted vesicles can inhibit VSV infectivity (21).

In this study we found that a homogeneous population of reconstituted vesicles (called virosomes (22)) could be obtained by dialysis of octyl glucoside, provided the protein/lipid ratio was high (about 0.3 mol % protein) and the detergent removal was slow. Incubating virosomes with target vesicles containing negatively charged lipids and lowering the pH resulted in massive fusion as shown by electron microscopy. We examined the mixing of membrane lipid resulting from membrane fusion by monitoring the efficiency of resonant energy transfer between two fluorescent lipid probes (23) incorporated in the same virospme membrane.

EXPERIMENTAL PROCEDURES

Materials—Solutions of phosphatidylserine (bovine brain), egg L-α phosphatidylcholine, phosphatidic acid, N-NBD-PE, and N-Rho-PE in chloroform (Avanti Biochemicals, Birmingham, AL) were kept in sealed glass ampules at −70 °C in the dark until used. Trypsin was obtained from Sigma (type III) and was used at a final concentration of 50 µg/ml. Octyl glucoside was obtained from Calbiochem-Behring. Dialysis tubing (Spectra-2, 10 mm) was purchased from Spectrum Medical Industries (Los Angeles, CA). Purification of virus and G-protein—Large quantities of VSV (strain Indiana) were obtained by infecting 850-cm² roller bottles of Vero cells with 1 plaque-forming unit of VSV per cell. The culteres were kept at 37 °C overnight and the virus was harvested and purified by rate-velocity and equilibrium centrifugation of sucrose gradients (24). Viral proteins were radioactively labeled by the addition of 5 µCi/ml of [3H]leucine to the infection medium. G-protein was ex-
tracted from the purified virus with 30 mM octyl glucoside and purified free of phospholipid according to Petri and Wagner (19) by sedimentation into a 15–30% sucrose gradient containing 60 mM octyl glucoside, 0.5 M NaCl, and 50 mM Tris, pH 7.6 (19). The pooled protein fractions from the gradient contained only G protein as determined by polyacrylamide gel electrophoresis; the recovery of G protein from the sucrose gradient was roughly 70%. Aliquots of G protein were stored at -70°C.

Anti-G-protein Antibodies—Antibodies against VSV (strain Indiana) G protein were prepared as described previously (20). Purified G protein was injected into rabbits according to the method of Miller et al. (21). Five hundred µg of G protein was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia) and used to affinity purify the anti-G antibodies.

Reconstitution of G-protein into Lipid Vesicles (Virosomes)—A mixture of 250 µg of egg phosphatidylcholine, 2.5 µg of N,NBD-PE, and 2.5 µg of N-Rh-PE in chloroform was dried under a stream of argon and lyophilized for 4 h to remove any residual solvent. The lipid was resuspended in a 300-µl solution containing G protein (250 µg/ml), 60 mM octyl glucoside, 0.5 M NaCl, and 50 mM Tris, pH 7.6. This suspension was dia lyzed at 4°C in Spectra-2 dialysis tubing against 3 volumes of buffer (145 mM NaCl, 10 mM Hepes, pH 7.4). After 3 days, the volume ratio was increased gradually to 10. The change of volume schedule was either two times a day for 4 days or three times a day for 3 days.

Preparation of Phospholipid Vesicles—Phospholipids, dried from chloroform solution and lyophilized, were suspended in NaCl/Hepes buffer (2–4 mg of lipid/ml, final). Small unilamellar vesicles were formed by sonication in an argon atmosphere at a stirring rate of 15 min in a W-375 probe sonicator (Heat Systems-Ultrasonics). These vesicles had an average area of 5.05 × 10⁻² µm² as measured using a Hewlett-Packard digitizer and computer. Large vesicles, used in the electron microscopy studies, were formed by drying lipids from a chloroform/methanol solution in a rotary evaporator, then slowly hydrating in NaCl/Hepes pH 7.4 buffer and, gently vortexing.

Electron Microscopy Studies—A 10-µl drop of each preparation (vesicles alone, liposomes alone, and a combination of the two) all diluted in the plane of the newly enlarged membrane. The increase in mean distance between donor and acceptor causes a reduction in energy transfer efficiency, resulting in an increase of the intensity of NBD fluorescence. NBD fluorescence was measured using a MPF 44B spectrofluorimeter (Perkin-Elmer) at 473 nm excitation and 535 nm emission with a 515 nm high pass filter to reduce light scattering. Virosome- Vesicle Fusion—The kinetics of pH-dependent fusion was determined by measuring energy transfer between two fluorescent lipid probes, N,NBD-PE and N-Rh-PE, incorporated into the same virosome during reconstitution. The details of this method, which has been described previously, have been described elsewhere (23, 25–28). Briefly, at appropriate surface densities of the fluorescent probes (1% each with respect to lipids), efficient energy transfer between donor (NBD) and the acceptor (rhodamine) is observed. Thus, the fluorescence emission of NBD is quenched by energy transfer to rhodamine and, concomitantly, rhodamine emission is “sensitized” by excitation of NBD. Upon fusion of virosomes with unlabeled target vesicles, the fluorescently labeled lipids are diluted in the plane of the newly enlarged membrane. The increase in mean distance between donor and acceptor causes a reduction in energy transfer efficiency, resulting in an increase of the intensity of NBD fluorescence. NBD fluorescence was measured using a MPF 44B spectrofluorimeter (Perkin-Elmer) at 473 nm excitation and 535 nm emission with a 515 nm high pass filter to reduce light scattering. Virosomes containing 1 µg of G protein, 4 µg of egg phosphatidylcholine, and 0.04 µg of each fluorescent lipid were injected into a fluorimeter cuvette containing 2.5 ml of buffer (145 mM NaCl, 10 mM Hepes, pH 7.4) and target vesicle (200 µg of lipid). The background intensity, due to light scattering and residual fluorescence, was recorded. Then, the medium was acidified by addition of HCl (10 µl, 1 N) to pH 3.3 or as indicated. The increase in NBD fluorescence was measured with time and recorded on a strip chart recorder.

Determination of Fusion Rate—Estimated fusion rates were calculated as the reciprocal of the time from mixing of vesicles and virosomes until the fluorescence signal reached a given value. Usually, the results are expressed in terms of t½, the time in which the fluorescence change has reached half the maximum value. At a given virosome and target lipid concentration, this parameter provided a quantitative comparison of fusion rates under different conditions (pH, lipid composition, temperature).

Results
Preparation of VSV G-protein Reconstituted Vesicles (Virosomes)—Reconstitution of purified G-protein into lipid vesicles was performed by mixing purified G-protein with dried lipids as described under "Experimental Procedures" and dialyzing out the detergent. The reconstituted preparation was evaluated by two criteria: (a) homogeneity of vesicle size and G-protein distribution and (b) fusogenic activity at low pH. The first criterion was emphasized because a homogeneous population is required for a valid interpretation and analysis of fusion data.

Preliminary attempts to prepare virosomes by dialysis against large volumes (>1000:1) of buffer produced populations of proteolipid vesicles which were inhomogeneous, as judged from negative staining electron microscopy. Vesicle sizes ranged from 20 nm to more than 200 nm and the distribution of G-proteins was not uniform; there were vesicles containing no protein while other vesicles contained dense patches of G-protein. We therefore developed a protocol of slow dialysis against small volumes with about 10 changes of buffer. The rationale for this method is that the slow removal of octyl glucoside allows the glucose aldehyde to form a dense lipid-protein micelles to occur before the structures close to form vesicles upon the final elimination of octyl glucoside (29–32). Slow dialysis produced vesicles with a fairly uniform size distribution, but the distribution of G-protein in the vesicles was dependent upon the starting G-protein to lipid ratio. At low G-protein to lipid ratio, there was a subpopulation of vesicles containing large amounts of spikes, while other vesicles lacked spikes observable by negative staining (data not shown). This was similar to the behavior found by Petri and Wagner (19) in the reconstitution of the VSV G-protein and by Rivnay and Metzger (32) in the reconstitution of IgE receptor. It seems to indicate that the proteolipid vesicles are formed with a defined ratio of G-protein to phospholipid, while the excess lipid forms protein-free vesicles. Homogeneous preparations were obtained when the G-protein to lipid ratio was similar to that of the native virus envelope (about 1 mol %). The virosomes obtained are shown in Fig. 1A. The vesicles had a fairly uniform size and a homogeneous distribution of G-protein in their membranes. The average area of a virosome was 5.74 × 10⁻³ µm² as measured using a Hewlett-Packard digitizer and computer.

pH-dependent Association of Virosomes and Vesicles—Incubating virosomes with small unilamellar PS:PC vesicles and lowering the pH caused massive vesicle-vesicle association as indicated by changes in light scattering (not shown) and in sedimentation rate of vesicles as shown by Table I. Small unilamellar vesicles labeled with [¹⁴C]dipalmitoylphosphatidylcholine were incubated with virosomes containing [³H]G-protein and centrifuged at 120,000 × g, for 15 min. When the incubation was done at pH 8.0, less than 10% of the [¹⁴C]label were pelleted. However, at pH 3.0, over 90% of the vesicles were pelleted. There was no pelleting of vesicles in the absence of virosomes at either pH value. Under these centrifugation conditions, about 55% of the [³H]-virosomes were pelleted by themselves at pH 7.4 either with or without added vesicles and at pH 3.0 without added vesicles (see Table 1), whereas at pH 3.0 about 97% of the [³H] label were pelleted (together with the [¹⁴C]-vesicles). This indicates that the G proteins are released into solution until the fluorescence signal reached a given value. Usually, the results are expressed in terms of t½, the time in which the fluorescence change has reached half the maximum value. At a given virosome and target lipid concentration, this parameter provided a quantitative comparison of fusion rates under different conditions (pH, lipid composition, temperature).

By weight, VSV is 20% in lipid (33) and 19% in G protein (34). Assuming molecular weights of 1,000,000 for lipid and protein, respectively, we calculate that the protein/lipid ratio is 1.5 mol %.
Fig. 1. Electron micrographs of negatively stained vesicles, virosomes, and fusion products. Virosomes and small unilamellar vesicles were prepared as described under "Experimental Procedures." Electron microscopy was performed after negative staining with Na phosphotungstate (pH 7.4). The micrographs were at ×89,000 magnification. A, purified virosomes (40 μg/ml of protein, 150 μg/ml of lipid). B, small unilamellar vesicles (PS:PC = 1:1, 2 mg/ml of lipid). C, virosomes (150 μg/ml of lipid) incubated at pH 3.3 with PS:PC (1:1) vesicles (2 mg/ml) forming cochleate structures. Note the "closed bag" appearance at the arrow.

protein was part of an enlarged virosome-vesicle structure. The virome surface could only accommodate 3-4% of the total amount of small unilamellar vesicles initially present. Therefore, those large structures probably represent fused rather than aggregated structures. We used ultrastructural examinations to study this process in more detail.

*Electron Microscopy Studies of Virosome-Vesicle Fusion*—Virosomes (Fig. 1A) were incubated with small unilamellar PS/PC (1:1) vesicles (Fig. 1B). Lowering the pH to 3.3 resulted in the appearance of large, irregularly sized, cochleate structures (Fig. 1C). The layers in the structures appear as closed flattened bags (see arrow in Fig. 1C) rather than as open sheets. Cochleate structures have been observed to result from Ca^{2+}-induced fusion of negatively charged vesicles (35).
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FIG. 2. Electron micrographs of a large unilamellar vesicle fusing with a virosome. Virosomes and large unilamellar vesicles PS:PC (1:1) prepared as described under “Experimental Procedures” were incubated at pH 7. The vesicles were stained with Na phosphotungstate and the magnification was ×115,000. A, large vesicle near two small virosomes decorated by G-protein on their surfaces. B, virosome touching the surface of the large vesicle. C, fusion of the large vesicle with a fringed virosome, showing a continuous “neck” between the two.

TABLE I

pH dependence of the association between virosomes and PS/PC small unilamellar vesicles

| pH | Virosomes alone | Vesicles alone | Virosomes and vesicles |
|----|----------------|---------------|-----------------------|
|     | [H]G-protein | 14C | [H] | 14C |
| 7.4 | 0.53 | <0.02 | 0.55 | <0.02 |
| 3.0 | 0.56 | 0.05 | 0.97 | 0.84 |

There was no fusion of virosomes alone incubated at pH 3.3 (data not shown). In order to follow the process of fusion more closely, we incubated virosomes with large vesicles which were clearly distinguishable from virosomes (Fig. 2). Lowering the pH resulted in massive fusion, with little resolution of the structures formed. Incubating the virosomes with target vesicles at neutral pH, however, led to fusion at a very slow rate (see below). Fig. 2, A–C shows the fusion steps between virosome and large vesicle. In Fig. 2A, they are separate, in Fig. 2B adherent, and in Fig. 2C the virosome and target vesicle appear to be in the early fused state.

The observed fusion process was studied quantitatively by examining the mixing of virosome and target liposome membrane lipids (which results from fusion) by resonance energy transfer between two lipid probes incorporated into the virosome.

Kinetics of Virosome-Vesicle Fusion.—Quantitation of virosome-vesicle fusion was performed by continuously monitoring the efficiency of energy transfer between two fluorescent lipid probes incorporated into virosomes: N-NBD-PE (donor) and N-Rho-PE (acceptor) (see “Experimental Procedures”). The fluorescently labeled virosomes were then mixed with various amounts of PC:PS (4:1) vesicles and the intensity of NBD fluorescence was measured. The recordings of NBD fluorescence intensity with time are shown in Fig. 3.

After addition of HCl to adjust the pH to 3.3, the fluorescence intensity increased, indicating that the fluorescent labels had been diluted by fusing with unlabeled liposomes. Both the rate of increase and the total change in fluorescence was dependent on the amount of target lipid present. The inset shows the fluorescence intensity change after 1 h when most of the fusion reaction has gone to completion. As a quantitative measure of fusion rates, we used in subsequent experiments the parameter $t_{1/2}$, the time needed to attain the half-maximum fluorescence change (see “Experimental Proce-
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Under the experimental conditions described in Fig. 3, the half-maximum fluorescence change corresponded to the dilution of virosome lipids with about 40 μg of target lipid, reducing the probe surface density from 1% to 0.3%.

**pH Dependence of Virosome-Vesicle Fusion**—Fig. 4 shows the rates of fusion between virosomes and PC/PS (1:1) vesicles incubated together at various pH values. There was no significant change in fusion rate when the pH was lowered to pH 5.5. However, there was a marked increase as the pH was lowered further. From Fig. 4, we deduce that the apparent pK for G-protein-mediated fusion is lower than pH 4. A similar pH profile was noted with PC/PA (1:1) vesicles (not shown). Fig. 5 shows that the G-protein was not inactivated by exposure to low pH: adding HCl to a final pH of 3.3 initiated fusion and titration back to pH 7.0 by NaOH stopped the fusion reaction. Subsequent additions of HCl and NaOH caused the fusion to start again or to stop, respectively.

**Dependence of Fusion on Lipid Composition**—Fig. 6 shows the fusion rate as a function of PS content in mixed PC:PS target vesicles. Addition of up to 10% PS had little effect on the rate of fusion. Further increases caused a progressive increase in fusion rate. For target vesicles containing 50% PS and 50% PC, the rate of virosome fusion was found to be 22–25-fold increased relative to the background level of fusion with pure PC vesicles. Table II shows the fusion rates for vesicles with different lipid compositions at a given ratio of phospholipid to PC. Fusion rate was enhanced 120-fold by substituting 50% of the phosphatidylincholine in the vesicle membranes with phosphatidic acid and 25-fold with 50% phosphatidylyserine. Phosphatidylinositol appeared to enhance the rate slightly; replacement with 50% phosphatidylethanolamine had no effect. Inclusion of 36% cholesterol in any of these vesicle compositions also had no effect.

**Fusion Is Dependent upon G-protein**—Several experiments were designed to verify that the fusion of virosomes to vesicles was dependent upon the presence of functional G-protein. (a) Elimination of G-protein in the reconstitution protocol re-

Fig. 4. **pH dependence of fusion.** Fluorescently labeled virosomes were injected into a fluorimeter cuvette containing PC:PS vesicles (200 μg of lipids) in 2.5 ml of buffer (145 mM NaCl, 10 mM Hepes) set to the indicated pH value. Fusion was followed fluorometrically and the rate of fusion was estimated from the half-time for the fluorescence signal change (see "Experimental Procedures"). The inset shows raw data of fluorescence records obtained in a different set of measurements.

![Fig. 4](http://www.jbc.org/)

**Fig. 5. Activation of fusion by low pH.** Virosomes (15 μg of lipid, 4 μg of protein) and vesicles (PC/PS, 1:1, 200 μg) were mixed with 2.5 ml of buffer (145 mM NaCl, 10 mM Hepes, pH 7.4). Fusion was measured by following the change in NBD fluorescence as described under "Experimental Procedures." Before the addition of HCl, the fluorescence intensity remained constant at the background level. With the additions of HCl (pH 3.3 final), fusion commences and additions of NaOH to neutralize the pH causes fusion to stop. The dashed line shows for comparison a time course of fusion after a single addition of HCl.

![Fig. 5](http://www.jbc.org/)

**Table II**

| Phospholipid composition | Fluorescence signal half-time |
|-------------------------|------------------------------|
|                         | Cholesterol                  | +Cholesterol                 |
| PS:PC                   | 30 s                         | 30 s                         |
| PA:PC                   | 5 s                          | N/D*                         |
| PI:PC                   | 6 min                        | N/D*                         |
| PE:PC                   | >15 min                      | >15 min                      |
| PC                      | 10 min                       | 10 min                       |

*N/D, not done.*
resulted in formation of PC vesicles which did not fuse with PS/PC vesicles at any pH (data not shown). In fact, if the initial protein/lipid ratio was too low or the initial detergent removal too fast, the resulting virosomes were fusion-incompetent. (b) When fusion-competent virosomes were mildly treated with trypsin (50 μg/ml, 30 min, room temperature), the rate of fusion was slowed down to about 70% of the original value (data not shown). (c) Virosomes treated with a 1:50 dilution of anti-G-protein anti-serum (20) (see "Experimental Procedures") were inhibited by more than 90% in their fusion ability (Fig. 7).

Temperature Dependence—The fusion of PC/PS (1:1) vesicles with virosomes was also studied at various temperatures. An Arrhenius plot of the dependence of fusion rate on temperature (Fig. 8) showed a single linear slope between 0 and 30°C. This indicates that there was no change in membrane structure in that temperature range that affects fusion. The calculated energy of activation was 15 ± 1 kcal/mol.


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Retention of Vesicle Contents during Fusion.—To test whether the fusion process was "leaky," we prepared target vesicles (PS:PC, 1:1) containing self-quenching concentrations of lucifer yellow. The method to monitor release of contents was similar to that used for vesicles containing carboxyfluorescein (36, 37). Upon release of the contents, the dye is diluted into a larger volume and consequently an increase in fluorescence is measured due to relief of self-quenching. We used lucifer yellow for these experiments since this compound did not leak spontaneously upon lowering the pH. Incubating virosomes with those lucifer yellow-containing vesicles under the same conditions as shown in Fig. 3 resulted in no increased fluorescence (data not shown). This indicated that virosones-vesicle fusion was non-leaky.

**DISCUSSION**

The results of the present study indicate that VSV G-protein can be functionally reconstituted into lipid vesicles. Although reconstitution of G-protein into lipid vesicles had been reported previously (19, 21), no functional (fusion) studies were performed. In order to obtain a functional reconstitution product, appropriate reconstitution conditions and techniques must be chosen. The underlying processes which lead to the formation of a desired reconstitution product are not well understood. However, a number of detailed reconstitution studies published recently (29–32) have guided us in these experiments. Jackson and Litman (30) have shown that in a micellar mixture of phospholipid, octyl glucoside, and membrane protein, the octyl glucoside-phospholipid micelles are less stable than the protein-lipid-detergent micelles. Therefore, lowering the octyl glucoside concentration rapidly by dialysis against a large volume of buffer results in the formation of protein-depleted phospholipid vesicles. As the detergent concentration is further decreased, the micelles containing protein become unstable and reconstituted vesicles are formed. Petri and Wagner (38) in fact have shown that G-protein can partition spontaneously from glycoprotein micelles into preformed sonicated vesicles. This model for asymmetric membrane reconstitution had also been proposed by Helenius et al. (31). However, we found in this study that virosomes reconstituted in this fashion (i.e. fast dialysis) were not fusogenic.

One important feature which was necessary for successful reconstitution of G-protein was the slow removal of octyl glucoside. We performed an initial 1:3 dilution of the detergent followed by a number of changes of the dialyzing solution gradually decreasing the concentration of octyl glucoside in the dialysate over a period of 3–4 days. Since the initial concentration of octyl glucoside was 60 mM, the initial dilution yields 20 mM, which is about the critical micellar concentration of octyl glucoside (39). The protein to lipid ratio was also important for proper reconstitution. The ratio of G-protein to phospholipid in the virosomes was about equal to that in the original VSV membrane, suggesting that protein-protein interactions have an important role in functional reconstitution.

In this study, we demonstrated pH-dependent, non-leaky fusion of the virosomes with negatively charged lipid vesicles. Fig. 1C shows massive fusion of small vesicles into large vesicles. Those large vesicles appeared as cochleate structures. Papahadjopoulos et al. (35) have observed similar structures resulting from Ca2+-induced fusion of negatively charged vesicles, interpreting them to be large sheets folded spirally to form cylinders. Since we did not observe leakage of contents,
we believe that the structures are not sheets but flattened bags which role up into spirals. The electron micrographs also show closed structures at the ends of the swells (Fig. 1C). The exact nature of the fusion product needs to be more closely examined by freeze-cleavage and thin section electron microscopy.

The fusion of virosomes with negatively charged lipids was pH-dependent with a pK around 4.0. This pattern differs from that found by White et al. (18) for pH dependence of VSV-induced cell fusion (pK around 6.0). In those experiments, fusion of virus with cells is induced by lowering the pH of the medium for 30–60 s. The cells subsequently fuse into giant polykaryons. The difference in pH profile might be due to the fact that we work with a reconstituted system. Moreover, White et al. (18) consider extent of fusion. An interesting feature of the fusion reaction was that it could be turned on and off by lowering and raising the pH (Fig. 5). Although the fused particles (the product of the reaction) were irreversible structures in that they did not fall apart upon raising the pH, the activation of fusion was reversible. This indicates that the G-protein was not denatured by exposure to the low pH.

G-protein-mediated fusion showed a large preference toward target membranes containing lipids which are negatively charged at neutral pH or uncharged at the pH where fusion occurs (Table II). Although phosphatidylethanolamine facilitates Ca²⁺-induced fusion of phospholipid vesicles (40), consistent with the greater ability to remove the water of hydration from its head group, it did not enhance G-protein-mediated fusion. Probably this was due to the positive charge on the head group at the low pH. The greater fusion capacity of PA over PS and PI (Table II) is consistent with data on Ca²⁺-induced fusion of vesicles containing those head groups (41) and on the relative hydration energies of those head groups. Table II also shows that cholesterol was not required for fusion. This is in contrast with the absolute requirement for cholesterol in the target membrane for Semliki forest virus fusion (42). On the other hand, influenza virus does not require cholesterol in its target membrane (43, 44). The specificity for fusion of the negatively charged lipids in the target membrane contrasted with the data on binding of lipids to the virus, which shows specificity for PS over all other negatively charged lipids (10). However, binding experiments were done at neutral pH. At low pH, the G-protein appears to bind (and fuse) to lipids which are uncharged at that pH.

Several mechanisms can explain the pH-dependent activation step. One is a pH-dependent conformational change in the protein leading to the exposure of a “fusion peptide” as in the case of influenza virus (45). Other possibilities include the titration of amino acid residues on the protein and/or head groups on the target phospholipid resulting in association of virosome and target membranes. The pK of about 4 is consistent with the titration of carboxylic groups of glutamic and aspartic acids. We hope to follow possible structural changes in the G-protein with pH and correlate this with its fusogenic activity.

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