specific antibody, such as anti-FLAG. This is shown by quantification of the amount of europium released from the bound liposomes after addition of the DELFIA enhancement solution containing detergent.

Concluding Remarks

The one-step purification of proteins appears efficient and incorporation of the two proteins into liposomes seems quantitative, as the pellets, after ultracentrifugation, contain both fluorescent label and RV proteins. Coexpression of the two proteins in insect cells should enable formation of heterodimeric complexes, which would broaden the use of this type of RV-specific virosome, entrapping a lanthanide chelate as a label. In addition to the potential use of this kind of particle in diagnostics, this strategy may also enable studies aimed at solving problems related to basic biology.

Acknowledgment

The authors acknowledge the support of the European Union (Contract BIO-2CT94-3069).

[26] Receptor-Activated Binding of Viral Fusion Proteins to Target Membranes

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Introduction

All known viral membrane fusion proteins are type I integral membrane proteins, and each contains a fusion peptide within its ectodomain. A special feature of viral fusion proteins is that they reside on the virion surface as metastable entities. For the three viral fusion proteins whose metastable structures have been probed by high-resolution X-ray crystallography or electron microscopy—the influenza hemagglutinin (HA),[1] the E glycoprotein of tick-borne encephalitis virus (TBE),[2] and the E1 glycoprotein of Semliki Forest virus (SFV)[3]—the fusion peptide is buried

[1] P. A. Bullough, F. M. Hughson, J. J. Skehel, and D. C. Wiley, *Nature* **371,** 37 (1994).
[2] F. A. Rey, F. X. Heinz, C. Mandl, C. Kunz, and S. C. Harrison, *Nature* **375,** 291 (1995).

0076-6879/03 $35.00

within the ectodomain of the protein. Consequently, the ectodomains of these proteins are fully water soluble when released from their transmembrane domains.

Viral fusion proteins have been divided into two classes.[4] The influenza HA is the prototype of class I viral fusion proteins, which employ coiled-coil motifs for fusion.[1,5] The TBE E and SFV E1 glycoproteins are class II viral fusion proteins that employ alternate motifs for fusion.[4] Nonetheless, all three glycoproteins undergo dramatic conformational changes at low pH, a condition that these viruses encounter in cellular endosomes after receptor-mediated endocytosis (Table I). Although the specifics of the conformational changes differ between class I and class II viral fusion proteins, the major consequence of the low pH-induced conformational changes is the same: the formerly water-soluble protein ectodomains now bind tenaciously and hydrophobically to target membranes.[6–10] In all three cases, hydrophobic binding to target membranes has been shown to occur through the fusion peptide. Low pH-induced hydrophobic binding of the glycoproteins to target bilayers is an obligate prerequisite for membrane fusion. Hence, the key common step among viral fusion glycoproteins is conversion of their ectodomains from hydrophilic to hydrophobic entities by virtue of an exposed fusion peptide[11] (Fig. 1, step 1).

Although many enveloped viruses, including serious pathogens such as influenza virus, Ebola virus, and West Nile virus, are activated for fusion by exposure to low pH, many others, including serious pathogens such as the human immunodeficiency virus (HIV), herpesviruses, and respiratory syncytial virus (RSV), fuse with cells at neutral pH.[12,13] The current concept is that instead of being activated by low pH, these viruses are activated by specific interaction(s) between the viral glycoprotein(s) and their cognate host cell receptor(s) (Table I). This concept first emerged from studies on

[3] J. Lescar, A. Roussel, M. W. Wien, J. Navaza, S. D. Fuller, G. Wengler, and F. A. Rey, *Cell* **105,** 137 (2001).
[4] F. X. Heinz and S. L. Allison, *Curr. Opin. Microbiol.* **4,** 450 (2001).
[5] C. M. Carr and P. S. Kim, *Cell* **73,** 823 (1993).
[6] C. Harter, P. James, T. Bachi, G. Semenza, and J. Brunner, *J Biol. Chem.* **264,** 6459 (1989).
[7] M. R. Klimjack, S. Jeffrey, and M. Kielian, *J. Virol.* **68,** 6940 (1994).
[8] J. J. Skehel, P. M. Bayley, E. B. Brown, S. R. Martin, M. D. Waterfield, J. M. White, I. A. Wilson, and D. C. Wiley, *Proc. Natl. Acad. Sci. USA* **79,** 968 (1982).
[9] R. W. Doms, A. Helenius, and J. White, *J. Biol. Chem.* **260,** 2973 (1985).
[10] K. Stiasny, S. L. Allison, J. Schalich, and F. X. Heinz, *J. Virol.* **76,** 3784 (2002).
[11] J. M. White and I. A. Wilson, *J. Cell Biol.* **105,** 2887 (1987).
[12] E. Hunter, *in* "Retroviruses" (J. M. Coffin, ed.), p. 71. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1997.
[13] L. D. Hernandez, L. R. Hoffman, T. G. Wolfsberg, and J. M. White, *Annu. Rev. Cell Dev. Biol.* **12,** 627 (1996).

TABLE I
PROPERTIES OF ENVELOPED VIRUS FUSION PROTEINS

Enveloped virus family	Fusion pH	No. of viral proteins needed for fusion	Coiled-coil?/ Type I or type II	No. of receptors	Fusion protein binds liposomes (+/–R)	Virus binds liposomes (+/–R)
Orthomyxovirus	Low	1 (HA)	Yes/I	1	Yes (–R)[a]	Yes (–R)[b]
Togavirus	Low	2 (SFV; E1/E2)	No/II	ND	Yes (–R)[c]	Yes (–R)[d]
Flavivirus	Low	1 (E)	No/II	ND	ND	Yes (–R)[e]
Rhabdovirus	Low	1 (VSV; G)	No/?	ND	ND	Yes (–R)[f]
Bunyavirus	Low	2 (G1/G2)[g]	ND	ND	ND	ND
Arenavirus	Low	ND[h]	ND	1	ND	ND
Filovirus	Low	1 (Ebola; GP)	Yes/I	ND	ND	ND
Retrovirus	Neutral?[i]	1 (Env)	Yes/I	1 (ASLV); 2 (HIV; most strains)	Yes (+R)[j]	Yes (+R)[j]
Paramyxovirus	Neutral	1 or 2 (F and in some cases HN)	Yes/I	1	ND	Yes (+R)[k]
Herpesvirus	Neutral	4 (gB, gD, gH, gL)	ND	1 or 2	ND	ND
Coronavirus	Neutral	2 (S1/S2)	ND	1	ND	ND

Poxvirus	Neutral	ND	ND	ND	ND	ND
Hepadnavirus	Neutral	ND (S)[l]	ND	ND	ND	Yes[m]
Iridovirus	ND	ND	ND	ND	ND	ND

Abbreviations: ND, not determined; R, receptor.

[a] C. Harter, P. James, T. Bachi, G. Semenza, and J. Brunner, *J. Biol. Chem*. **264,** 6459 (1989).

[b] T. Stegmann, F. P. Booy, and J. Wilschut, *J. Biol. Chem*. **262,** 17744 (1987).

[c] M. R. Klimjack, S. Jeffrey, and M. Kielian, *J. Virol*. **68,** 6940 (1994).

[d] R. Bron, J. M. Wahlberg, H. Garoff, and J. Wilschut, *EMBO J*. **12,** 693 (1993).

[e] S. L. Allison, J. Schalich, K. Stiasny, C. W. Mandl, and F. X. Heinz, *J. Virol*. **75,** 4268 (2001).

[f] S. Yamada and S. Ohnishi, *Biochemistry* **25**, 3708 (1986).

[g] The exact role of G2 in fusion is still in debate.

[h] Arenaviruses have two surface glycoproteins with two candidate fusion peptides in GP2 [S. E. Glushakova, I. S. Lukashevich, and L. A. Baratova, *FEBS Lett*. **269**, 145 (1990)]; neither has been characterized.

[i] Previous evidence supports ASLV entry at neutral pH; however, one study (Mothes *et al*.[17]) proposes a two-step entry mechanism requiring receptor binding at neutral pH, followed by exposure to low pH. Most retroviruses (except MMTV) have been shown to fuse at neutral pH.

[j] L. J. Earp, S. E. Delos, R. C. Netter, P. Bates, and J. M. White, *J. Virol.* **77,** 3058 (2003).

[k] Y. S. Tsao and L. Huang, *Biochemistry* **25**, 3971 (1986).

[l] The S protein contains a stretch of amino acids predicted to be a fusion peptide, but has not been further characterized.

[m] E. V. Grgacic and H. Schaller, *J. Virol*. **74**, 5116 (2000).

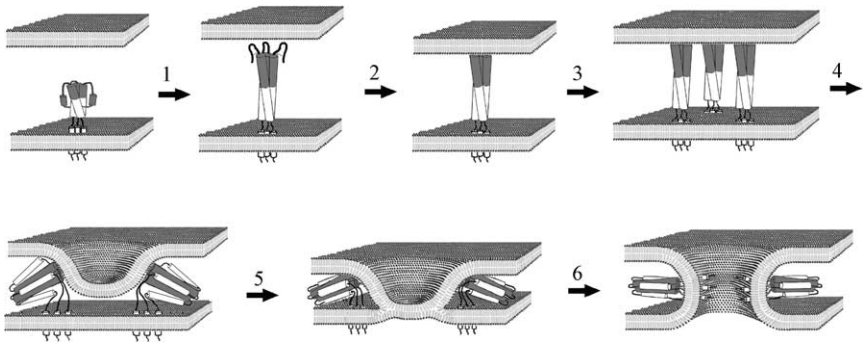

FIG. 1. Proposed model for ASLV-A fusion with target membranes. In the native membrane, EnvA exists in a trimeric, metastable form in which the fusion peptides are buried. The SU domains (not shown for clarity) are thought to act as a clamp, which maintains the TM domains in the metastable state. After engaging its receptor, Tva, at $T \geq 22^{\circ}$ (step 1), EnvA undergoes a conformational change in which the fusion peptides are exposed and can penetrate the target membrane. After penetration of the target membrane by the fusion peptides (step 2), trimers may cluster (step 3). Further conformational changes occur in which EnvA begins (step 4) to form, and then forms (step 5) a six-helix bundle, which mediates lipid mixing of the outer leaflets of the target and viral membranes. Action of the fusion peptides and transmembrane domains on the hemifusion diaphragm would then open the fusion pore (step 6). This model is based on similar models for the influenza HA. In the case of HA, the trigger for step 1 is exposure to low pH.

a simple model alpharetrovirus, the avian sarcoma/leukosis virus (ASLV, formerly known as Rous sarcoma virus). The receptor for this virus is a small soluble type I integral membrane glycoprotein called Tva, an acronym denoting that it is the receptor for the avian tumor virus, subtype A.[14] In 1997, it was shown that after binding to the glycoprotein at 4°, a small (83-amino acid) soluble form of the Tva ectodomain, sTva, could induce a soluble trimeric form of the ASLV subtype A (ASLV-A) envelope glycoprotein ectodomain (EnvA-PI) to bind hydrophobically to a target membrane at neutral pH. Binding to target membranes occurred at $T > 22^{\circ}$ and was dependent on the wild-type fusion peptide sequence.[15] Similar results were obtained subsequently by another laboratory.[16] As yet, and most likely because of the relative simplicity of the avian retrovirus system (one viral glycoprotein and one simple host cell receptor; Table I), ASLV EnvA is the only viral glycoprotein that has been proven

[14] P. Bates, L. Rong, H. E. Varmus, J. A. Young, and L. B. Crittenden, *J. Virol.* **72,** 2505 (1998).
[15] L. D. Hernandez, R. J. Peters, S. E. Delos, J. A. Young, D. A. Agard, and J. M. White, *J. Cell Biol.* **139,** 1455 (1997).
[16] R. L. Damico, J. Crane, and P. Bates, *Proc. Natl. Acad. Sci. USA* **95,** 2580 (1998).

to bind to target membranes after a temperature-dependent interaction with its host cell receptor.[15–16a]

Work suggested that ASLV Env is not fully activated for fusion by receptor binding at neutral pH, but rather requires receptor binding at neutral pH (and elevated temperature) followed by exposure to low pH.[17] We have found, however, that neither hydrophobic binding of the subtype A envelope glycoprotein to target membranes, binding of ASLV-A virus particles to target membranes, nor lipid mixing of the viral (ASLV-A) and cellular membranes requires exposure to low pH.[15–16a] Therefore, the critical step of activation of the ASLV-A viral fusion protein occurs at neutral pH. In the remaining sections of this chapter, we describe three assays that we have developed to monitor receptor-induced association of ASLV EnvA with target bilayers: the original assay for monitoring binding of the EnvA ectodomain (EnvA-PI) to target membranes (liposomes), a modified and miniaturized EnvA-PI-liposome binding assay, and an assay to measure binding of intact ASLV-A virus particles to target membranes.

Methods

Preparation of Reagents

EnvA-PI. EnvA-PI is prepared as described previously.[15] Briefly, a stable NIH 3T3 cell line expressing glycosylphosphatidylinositol (GPI)-anchored EnvA [which contains the GPI addition signal from decay-accelerating factor (DAF) in place of the transmembrane domain (TM) and cytoplasmic tail; Gilbert *et al.*[18]] is maintained in Dulbecco's modified Eagle's medium (DMEM)–10% bovine calf serum (supplemental) (HyClone, Logan, UT), 1× each of glutamine, penicillin–streptomycin, and sodium pyruvate, and G418 [0.33 g/500 ml (0.25 g active units); GIBCO, Grand Island, NY]. Sixteen to 18 h before harvest, GPI-anchored EnvA cells are treated with 5 m*M* sodium butyrate to induce EnvA expression. For EnvA-PI-liposome binding procedure I, cells are labeled with NHS-LC biotin. Unlabeled cells are used for EnvA-PI-liposome binding procedure II. GPI-linked proteins are released with phosphatidylinositol-specific phospholipase C (PI-PLC) as described previously.[18] Two cocktails of protease inhibitors are also included in the reaction: (1) phenylmethylsulfonyl fluoride (PMSF, 8.7 mg/ml) and pepstatin A

[16a] L. J. Earp, S. E. Delos, R. C. Netter, P. Bates, and J. M. White, *J. Virol.* **77,** 3058 (2003).
[17] W. Mothes, A. L. Boerger, S. Narayan, J. M. Cunningham, and J. A. Young, *Cell* **103,** 679 (2000).
[18] J. M. Gilbert, L. D. Hernandez, T. Chernov-Rogan, and J. M. White, *J. Virol.* **67,** 6889 (1993).

(0.5 mg/ml) and (2) leupeptin (1 mg/ml), aprotinin (2 mg/ml), antipain (5 mg/ml), benzamidine (25 mg/ml), soybean trypsin inhibitor (STI, 5 mg/ml), and iodoactamide (50 mg/ml).

Virus. ASLV-A is produced from DF-1 cells that are chronically infected with RCASBP(A)GFP.[19] RCASBP(A)GFP/DF-1 cells are cultured in DMEM–10% FBS and 1× antibiotic–antimycotic. Before harvesting virus, the medium is replaced. After 18 h, the medium is collected, clarified at 1250*g* for 15 min, and concentrated by centrifugation (2.5 h, 4°, at 82,700 *g*) through 5mL 15% sucrose in an SW 28 rotor. Viral pellets are resuspended in 200 μl of 20 m*M* morpholineethanesulforic acid (MES), 20 m*M* HEPES, pH 7.4 (MES–HEPES) overnight at 4°. A fresh preparation of virus is used for each experiment.

Liposomes. L-α-Phosphatidylcholine (egg) (PC; Avanti Polar Lipids, Alabaster, AL) and cholesterol (chol; Sigma, St. Louis, MO) are stored as described previously.[15] For EnvA-PI-liposome binding procedure I,[15] liposomes are prepared as described.[20] They are composed of phosphatidylcholine and cholesterol at a 2:1 molar ratio. More recently, lipids (PC:chol molar ratio, 2:1) are mixed and dried under a stream of nitrogen in a glass test tube. After lyophilization overnight, liposomes are created by the addition of 1 ml of MES–HEPES with vortexing and sonication in a sonicator bath. Liposomes are extruded subsequently through a 0.1-μm pore size filter in an Avanti Mini-Extruder, stored at 4°, and used within 1 week of preparation.

EnvA-PI-Liposome Binding Procedure I

Biotinylated EnvA-PI and soluble receptor, either sTva[21] or sTva47,[22] are incubated on ice for 15 min in a final volume of 60 μl. After addition of 40 μl of liposomes, samples are maintained at 4° or incubated at 37° for 10–30 min. In a clear polycarbonate ultracentrifuge tube, samples are brought to a final concentration of 50% sucrose by the addition of 300 μl of 67% sucrose. The sample is overlaid by a sucrose step gradient composed of 300 μl of 25% sucrose and 200 μl of 10% sucrose. Samples are centrifuged at 200,000 *g* for 3 h at 4° (Fig. 2). Fractions (9 × 100 μl) are collected from the top of the gradient, and samples are immunoprecipitated with anti-DAF, which recognizes the GPI anchor addition signal from decay-accelerating factor (DAF) that is present at the C-terminal end of

[19] M. J. Federspiel and S. H. Hughes, *Methods Cell Biol.* **52,** 179 (1997).
[20] J. White and A. Helenius, *Proc. Natl. Acad. Sci. USA* **77,** 3273 (1980).
[21] J. W. Balliet, J. Berson, C. M. D'Cruz, J. Huang, J. Crane, J. M. Gilbert, and P. Bates, *J. Virol.* **73,** 3054 (1999).
[22] M. Tonelli, R. J. Peters, T. L. James, and D. A. Agard, *FEBS Lett.* **509,** 161 (2001).

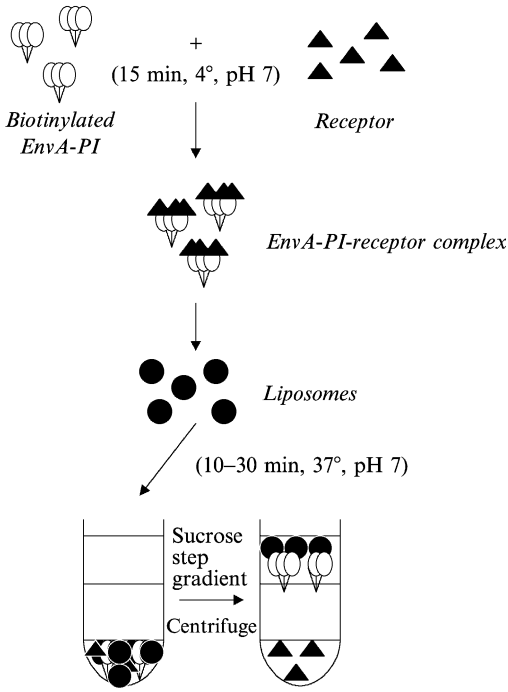

FIG. 2. EnvA-PI-liposome binding procedure I. Biotinylated EnvA-PI is incubated with soluble receptor (sTva47) on ice for 15 min. Liposomes are added and samples are shifted to 37° for 10–30 min. Samples are overlaid with a sucrose step gradient, centrifuged, and fractionated as described in Hernandez *et al.*[15]

EnvA-PI. This is done in a final volume of 400 μl (after the addition of lysis buffer). Samples are subjected to sodium dodecyl sulfate (SDS)–12.5% polyacrylamide gel electrophoresis (PAGE), transferred to nitrocellulose, and probed with streptavidin–horseradish peroxidase (HRP). Blots are developed by enhanced chemiluminescence (ECL).[15]

Results. When incubated with soluble receptor at 37°, EnvA-PI colocalizes with liposomes in the top three fractions of the gradient. EnvA-PI that has not been exposed to soluble receptor remains at the bottom of the gradient. See, for example, Fig. 1 in Hernandez *et al.*[15]

EnvA-PI-Liposome Binding Procedure II

Twelve microliters of EnvA-PI and 0.29 μg of sTva47 are incubated in the bottom of a TLA100 centrifuge tube (7 × 20 mm) (343775; Beckman Coulter, Fullerton, CA) on ice for 15 min. Eight microliters of liposomes

are added, and samples are either kept at 4° or incubated at 37° for 10 min. Tubes are then placed on ice, and the sample is brought to a final concentration of 50% sucrose by adding 60 μl of 67% sucrose. A step gradient is then layered on top of the sample with the addition of 60 μl of 25% sucrose and 60 μl of 10% sucrose. All sucrose solutions are made weight per weight in PBS (without Ca^{2+} or Mg^{2+}). Samples are centrifuged at 197,000 *g* for 1 h at 4° in a TLA100 rotor (Fig. 3A). Six 30-μl fractions are collected from the top of the gradient, boiled in 30 μl of sample buffer containing dithiothreitol (DTT), and subjected to SDS–12% PAGE. After transferring to nitrocellulose, fractions are probed for the location of EnvA-PI, using an antibody to the N terminus of the gp37 subunit (anti-Ngp37). Blots are developed by ECL.

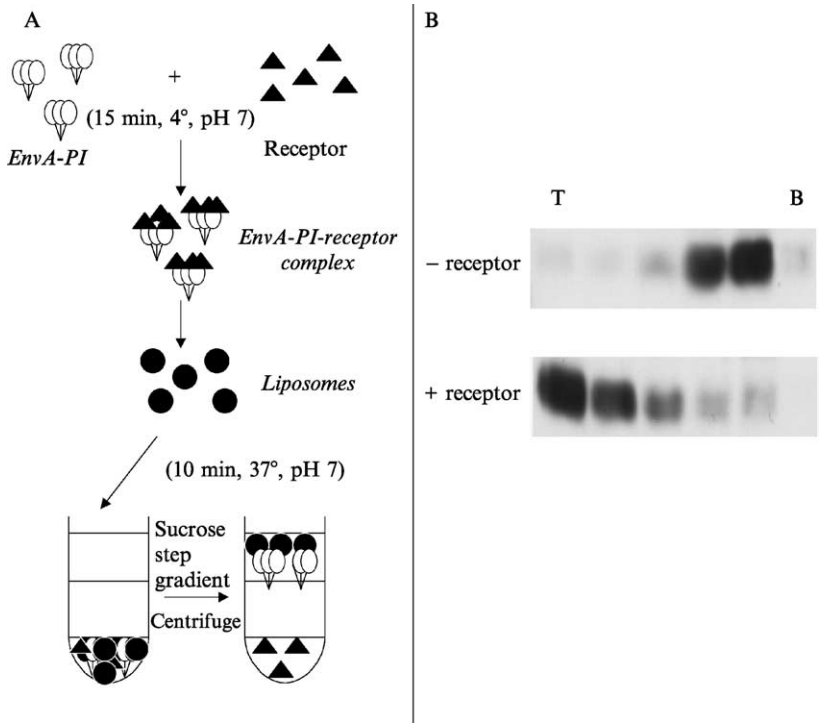

FIG. 3. EnvA-PI-liposome binding procedure II. (A) EnvA-PI is incubated with sTva47 on ice for 15 min. Liposomes are added, and samples are incubated at 37° for 10 min. Samples are overlaid with sucrose step gradients and centrifuged, fractionated, and subjected to SDS–PAGE, as described in Methods. (B) In the absence of receptor at pH 7 and 37°, EnvA-PI stays in the bottom of the sucrose gradient *(top panel)*. When incubated with receptor at pH 7 and 37°, EnvA-PI floats to the top fractions of the gradient with liposomes *(bottom panel)*.

Results. When exposed to sTva47 at 37°, EnvA-PI floats to the top of the gradient with liposomes, similar to the results seen with procedure I.[15] In the absence of sTva47, EnvA-PI remains in the bottom of the gradient (Fig. 3B), while the liposomes float to the top fraction (data not shown).

Virus–Liposome Binding Assay

In an Eppendorf tube, 25 μl of RCASBP(A)GFP and 0.5 μg of sTva47 are mixed on ice and incubated for 15 min. Liposomes (25 μl) are added, and samples are kept at 4° or incubated at 37° for 30 min and then placed on ice. Sucrose step gradients are created in Beckman Coulter polycarbonate centrifuge tubes (7 × 20 mm): 25 μl of 50% sucrose is overlaid with 75 μl of 25% sucrose and 50 μl of 20% sucrose. A 50 μl sample is layered on top of the gradient, and samples are centrifuged at 197,000 *g* for 1 h at 4° in a TLA100 rotor. Three fractions are collected from the top of each gradient such that each fraction contains an interface between sucrose concentrations (fraction 1, 75 μl; fraction 2, 50 μl; fraction 3, 65 μl) (Fig. 4A). Samples are boiled in 3× sample buffer containing DTT (fraction 1, 37.5 μl; fraction 2, 25 μl; fraction 3, 32.5 μl). Of each fraction, 25% is loaded onto an SDS–12% polyacrylamide gel and subjected to electrophoresis. The gel is then transferred to nitrocellulose, and probed with a polyclonal antibody to the matrix (MA) protein (obtained from V. Vogt, Cornell University, Ithaca, NY). Blots are developed by ECL.

Results. When ASLV-A particles are mixed with liposomes and incubated at 37° in the absence of sTva47, most of the ASLV-A sediments to the bottom (third) fraction of the gradient, on top of the 50% sucrose cushion (Fig. 4B, top). In contrast, if ASLV-A is preincubated with sTva47 (at 4°), before the incubation with liposomes at 37°, the virus particles are largely retained in the top two fractions of the gradient (Fig. 4B, bottom). An initial study employing this assay has been conducted.[16a]

Conclusions

The assays described here have proven their utility for studying receptor (Tva)-induced activation of ASLV EnvA.[15,16a] They should also be useful for studying other receptor-activated viral fusion proteins. When one viral glycoprotein and one "simple" host cell receptor are involved, it should be possible to develop assays directly analogous to those described above for studying Tva-induced binding of the EnvA ectodomain (EnvA-PI) to target membranes. A general prerequisite for a fusion protein/target membrane binding assay is a soluble and correctly oligomeric form of the viral fusion protein ectodomain. The simplest host cell

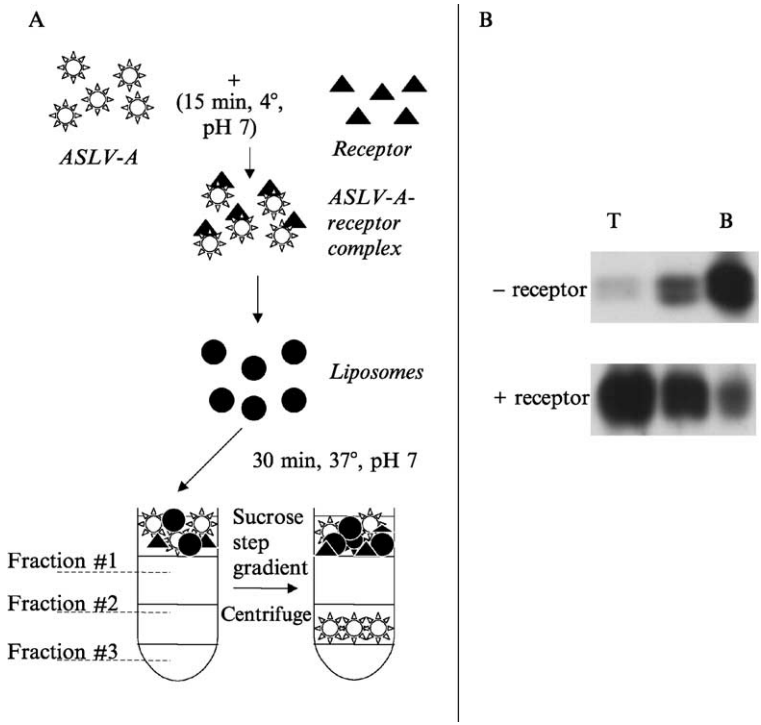

FIG. 4. Virus–liposome binding procedure. (A) ASLV-A is incubated with or without sTva47 on ice for 15 min. Liposomes are added, and samples are incubated at 37° for 30 min. Samples are placed on top of a sucrose step gradient and centrifuged, fractionated, and processed as described in Methods. (B) In the absence of receptor at pH 7 and 37°, ASLV-A pellets to the bottom of the gradient *(top panel)*. When incubated with receptor at pH 7 and 37°, ASLV-A is largely retained in the top fraction of the gradient *(bottom panel)*.

receptors that would be amenable to this type of analysis are type I or type II integral membrane proteins (i.e., proteins whose ectodomains are tethered to the host cell membrane at only one end, either N or C terminal). Soluble versions of the ectodomains of these receptors, produced by genetic engineering or proteolytic release (Fig. 5, image 1), could then be used to trigger the cognate fusion protein. The methodology could, similarly, be applicable to multimembrane-spanning host cell receptors when the functional part of the receptor is tethered at only one end (Fig. 5, image 2) or where an ectodomain loop (Fig. 5, image 3) preserves enough structure to function as a soluble analog, perhaps by generating a cyclic peptide analog of the loop. The same ''receptor reagents'' (Fig. 5) could be employed for intact virus particle/target membrane binding assays (Fig. 4).

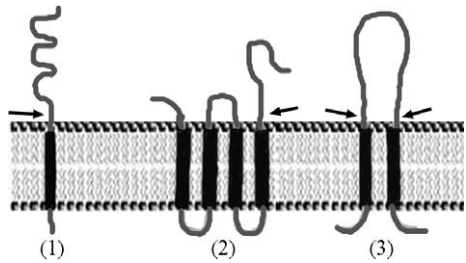

FIG. 5. Receptor species for use in liposome binding assays. (1) A type I or type II integral membrane protein with a functional domain that is tethered to the membrane at only one end; (2) a multimembrane-spanning protein that possesses a functional domain tethered to the membrane at one end; (3) a multimembrane-spanning protein with an extracellular loop that retains or can be engineered so as to retain enough structure to serve as a functional domain when cleaved from its transmembrane domains. Arrows indicate possible sites at which to cleave the functional receptor domain from the parent protein. Alternatively, secreted functional domains could be produced by genetic engineering or, where applicable, could be mimicked by synthetic peptides.

When a single obligate receptor does not meet the above-cited criteria (as is the case for most strains of HIV), it may be necessary to reconstitute one receptor (e.g., the chemokine receptor for HIV) into target liposomes. For HIV, a soluble ectodomain fragment of CD4, as well as the target liposomes containing the chemokine receptor, could then be used to recapitulate the target membrane binding step of the fusion reaction (Fig. 1, step 2). In addition, HIV Env presents another challenge in the development of a membrane binding assay: an envelope glycoprotein possessing two subunits that are not covalently associated. Because the gp120 and gp41 subunits of HIV Env are not associated in such a way, it has been shown that the gp120 subunit is shed after binding to its receptor (CD4).[23] Therefore, it is necessary to take this into consideration when making a soluble form of this glycoprotein.

In the case of most paramyxoviruses, two viral glycoproteins are necessary for optimal fusion, the actual fusion protein (F) and the cognate ''HN'' protein, which contains host cell receptor binding activity. For such systems, it may not be possible to induce binding of an isolated fusion protein ectodomain to target liposomes even in the presence of a correctly oligomeric soluble form of the HN protein; the fusion and HN proteins may need to be in a precise configuration *vis-à-vis* one another in the plane of a membrane for the HN protein to be able to activate the F protein. In

[23] M. Thali, C. Furman, E. Helseth, H. Repke, and J. Sodroski, *J. Virol.* **66,** 5516 (1992).

this case it may be easiest, at least for initial purposes, to measure receptor-induced binding of intact viral (or subviral) particles to target membranes (Fig. 4A). This would, however, require a soluble form of the host cell receptor as described above. We note that, in addition to our studies demonstrating binding of ASLV-A particles to target liposomes, several low pH-triggered viruses have been demonstrated to bind to liposomes, either as whole virus particles or as subviral particles; these cases include Sindbis virus, TBE, and influenza (Table I).

In summary, the key principle that underlies the operation of enveloped virus fusion proteins is conversion of the fusion protein ectodomain from a hydrophilic to a hydrophobic entity by virtue of an exposed fusion peptide (Fig. 1, step 2). In many cases (Table I) this event appears to be induced by binding to the host cell receptor under fusion-permissive conditions (e.g., physiologic temperature). Derivatives of the assays that we have described in this chapter should prove useful in monitoring this critical unifying event of receptor-induced fusion: receptor-induced binding of the fusion protein to target membranes. It is possible to do this with either the free (oligomeric) fusion protein ectodomain or with intact viral particles or subviral particles containing the viral fusion protein. Once a target membrane binding assay is established, it should provide a powerful tool with which to dissect further requirements for a key step in the fusion cascade: hydrophobic binding of the fusion protein to the host cell bilayer (Fig. 1, step 2).