Hydrostatic Pressure Induces the Fusion-active State of Enveloped Viruses*

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Enveloped viruses must undergo membrane fusion to deliver their genome into the host cell. We demonstrate that high pressure inactivates two membrane-enveloped viruses, influenza and Sindbis, by trapping the particles in a fusion-intermediate state. The pressure-induced conformational changes in Sindbis and influenza viruses were followed using intrinsic and extrinsic fluorescence spectroscopy, circular dichroism, and fusion, plaque, and hemagglutination assays. Influenza virus subjected to pressure exposes hydrophobic domains as determined by tryptophan fluorescence and by the binding of bis-8-anilino-1-naphthalenesulfonate, a well established marker of the fusogenic state in influenza virus. Pressure also produced an increase in the fusion activity at neutral pH as monitored by fluorescence resonance energy transfer using lipid vesicles labeled with fluorescence probes. Sindbis virus also underwent conformational changes induced by pressure similar to those in influenza virus, and the increase in fusion activity was followed by pyrene excimer fluorescence of the metabolically labeled virus particles. Overall we show that pressure elicits subtle changes in the whole structure of the enveloped viruses triggering a conformational change that is similar to the change triggered by low pH. Our data strengthen the hypothesis that the native conformation of fusion proteins is metastable, and a cycle of pressure leads to a final state, the fusion-active state, of smaller volume.

Enveloped viruses utilize regulated membrane fusion to introduce their genomes in the cytoplasm of the host cell. The fusion is mediated by surface envelope proteins of the virus in response to a trigger (1, 2). Once triggered, the fusion process leads to a conformational change that promotes the interaction of a specific sequence (fusion peptide) with the target membrane and initiates membrane fusion. Membrane fusion is crucial in other biological functions such as myotube formation, fertilization, and trafficking of endocytic and exocytic vesicles within eukaryotic cells (1, 3). Many enveloped animal viruses have been studied as models for understanding the mechanism of membrane fusion. While the fusion proteins of many viruses reveal significant similarity in their putative fusogenic conformation, such as those of influenza virus (hemagglutinin HA2), human immunodeficiency virus (gp120 protein), Moloney murine leukemia virus (TM protein), and Ebola virus (GP2 protein) (4), the events of membrane fusion for other virus families (e.g. Flaviviridae and Togaviridae) are beginning to be understood. Alphavirus and the flavivirus fusion proteins appear to have evolved from a common ancestor (5) and possess a similar new class of membrane fusion proteins that do not form coiled-coils (6–8).

Sindbis and influenza are enveloped viruses that first enter a cell by endocytosis and then fuse with the cellular membrane in response to acidic conditions. Sindbis virus is the prototype of the Alphavirus genus, Togaviridae family. The Alphavirus spike is composed of E1 and E2 transmembrane subunits arranged as 80 trimers of E1/E2 heterodimers. Upon exposure to mildly acidic pH, the E1/E2 dimer dissociates, and E1 undergoes conformational changes that result in exposure of a previously hidden fusion peptide with the formation of a highly stable, trypsin-resistant E1 homotrimer (9–11). E1 then associates with the target membrane by insertion of the fusion peptide, and membrane fusion is triggered. Structural predictions suggest that, unlike spike proteins of the influenza virus class, alphavirus E1 does not refold into an extended α-helical coiled-coil during fusion (4).

One of the best known representatives of the fusion process is the influenza virus. The HA of influenza virus is a homotrimeric glycoprotein located on the outer surface of the virus particle. HA is first synthesized inside the cells as a fusion-incompetent precursor (HA0) that is proteolytically cleaved to the mature, two-chain HA1/HA2 native state. HA binds to a cell-surface receptor, sialic acid, which results in the adsorption of the virus onto the cell surface. After binding to its receptor, the virus is endocytosed by the cell. As the pH within the endosome decreases, the HA undergoes an irreversible conformational change that exposes the hydrophobic N terminus of HA2, the fusion peptide. The crystal structures of HA in the native and fusogenic structures have been determined (12–14). It has been suggested that the native, nonfusogenic state of HA

The abbreviations used are: HA, hemagglutinin; bis-ANS, bis-8-anilino-1-naphthalenesulfonate; N, native; FG, fusogenic.

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is in a metastable conformation, separated from the lower free energy state ( fusogenic conformation ) by a kinetic barrier ( 13 – 15 ). The barrier is surpassed when the fusion events are triggered by virus–cell interaction.

In our studies we tested how the exposure of hydrophobic domains induced by high pressure can convert the envelope proteins from the metastable state to the fusion-active state. Because pressure is a mild perturbation when compared with denaturants and high temperature ( 16 – 19 ), it elicits no dramatic changes in structure of the whole particle. However, in both cases, the viruses are inactivated when their spike proteins are displaced from the metastable state by high pressure. Recent efforts to inhibit the virus fusion-active state have been demonstrated ( 20 , 21 ). The use of pressure to trigger the fusion-active state may be useful to the design of new antivirus drugs and vaccines.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Bis–8-anilino–1-naphthalenesulfonate ( bis–ANS ) and 12–1- pyrene–dodecanedicarboxylic acid ( PyC12 ) were purchased from Molecular Probes ( Eugene, OR ). The following lipids used to make synthetic vesicles were ordered from Avanti Polar Lipids: dioleoylphosphatidylcholine, N–( 7–nitrobenz–2–oxa–1–3–diazol–4–yldiacetyl) phosphatidylethanolamine, and lissamine–rhodamine–phosphatidylethanolamine ( rhodamine ). All other reagents were of analytical grade.

The Sindbis virus spectroscopic experiments were performed at 25 °C in buffer containing 50 mM Tris, 150 mM NaCl, 0.1 mM EDTA ( pH 7.5 ). For influenza virus the experiments were performed at 37 °C in buffer containing 50 mM Hepes, 150 mM sodium chloride, 0.1 mM EDTA ( pH 7.4 ).

**Sindbis Virus Purification and Infectivity**—Sindbis virus ( strain AR339 ) was grown in BHK–21 cells ( baby hamster kidney ). The cells were maintained and cultured as monolayers in Dulbecco’s modified Eagle’s medium ( Sigma Chemical Co. ) supplemented with 10% fetal bovine serum and 3% tryptose phosphate and grown at 37 °C. The supernatant was collected by centrifugation at 190,000 × g for 1.5 h at 37 °C with a multiplicity of infection of 1. The supernatant was collected and cleared of cellular debris, and 40% polyethylene glycol 8000 in 2 M NaCl was added and rocked overnight at 4 °C. Virus was collected by centrifugation at 18,000 × g for 1 h and resuspended in a small volume of 50 mM Tris, 150 mM NaCl, 0.1 mM EDTA buffer ( pH 7.5 ).

Virus was applied to the top of a linear 15–40% ( w/v ) sucrose density gradient and centrifuged for 1.5 h at 190,000 × g. Banded virus was harvested and dialyzed overnight at 4 °C against 50 mM Tris, 150 mM NaCl, 0.1 mM EDTA buffer ( pH 7.5 ). Sindbis infectivity was evaluated by plaque assay as described previously ( 22 ). Sindbis virus concentrations for the infectivity assays varied from 0.05 to 2.0 mg/ml.

**Mayaro Virus**—Mayaro virus was propagated and purified as described previously ( 23 ). The concentration of Mayaro virus was between 0.05 and 0.5 mg/ml.

**Sindbis Virus**—Sindbis virus ( strain AR339 ) was grown in BHK–21 cells ( baby hamster kidney ). The cells were maintained and cultured as monolayers in Dulbecco’s modified Eagle’s medium ( Sigma Chemical Co. ) supplemented with 10% fetal bovine serum and 3% tryptose phosphate and grown at 37 °C. The supernatant was collected by centrifugation at 190,000 × g for 1.5 h at 37 °C. Banded virus was harvested and dialyzed overnight at 4 °C. Virus was collected by centrifugation at 190,000 × g for 4 h at 4 °C, purified on a continuous sucrose gradient ( 5–40%), and stored at 0 °C.

**Influenza Virus Preparation and Hemagglutination Assays**—Influenza virus ( strain A/ Udorn 307/72, H3N2 ) was grown for 48 h at 35–36 °C in the allantoic cavity of 10–day-old embryonated chicken eggs. The allantoic fluid was collected, and cell debris was removed by a low speed spin ( 4,000 × g for 3 min at 4 °C ). The virus was pelleted by spinning the allantoic fluid at 100,000 × g for 45 min, then resuspended in TE buffer ( 20 mM Tris, 2 mM EDTA, pH 8.4 ), and banded on a continuous 20–60% sucrose density gradient in TE buffer, pH 8.4. Banded virus was dialyzed overnight at 4 °C against TE buffer, pH 8.4.

For hemagglutination, chicken red blood cells were washed three times in phosphate-buffered saline. The red blood cell final concentration was 1% ( v/v ). A 96–well plate was used to incubate the purified influenza virus with the red blood cells as described previously ( 24 ). Influenza virus concentrations used in the hemagglutination assays were between 0.05 and 0.5 mg/ml.

**Circular Dichroism Spectroscopy**—CD spectra were obtained with a Jasco–710 spectropolarimeter using a quartz cuvette of 0.1–cm path length. Spectra were the average of four scans, and the buffer base lines were subtracted. The concentration of Sindbis virus was 100 μg/ml.

**RESULTS**

**Pressure-induced Exposure of Hydrophobic Domains in Enveloped Viruses**—The fluorescence yield of the probe bis–ANS increases by up to 100–fold when it is transferred from water to a nonpolar environment as may occur when it binds to hydrophobic domains in proteins ( 29 ). When a protein is partially disorganized, hydrophobic segments may be exposed, leading to bis–ANS binding with an increase in fluorescence. More recently, bis–ANS has been used to follow changes in the conformation of an enveloped virus as it assumes the fusion–active state ( 30, 31 ). Bis–ANS has been used to follow changes in the capsid protein in the disassembly pathway ( 23, 32, 33 ). Fig. 1, A and B, show the changes in bis–ANS fluorescence of influenza virus as the pressure is increased. The increase in bis–ANS fluorescence with pressure ( Fig. 1A ) indicates the appearance of sites of hydrophobic character at which bis–ANS is binding. Fig. 1B shows a blue shift ( increase in the energy of emission ) in the spectral center of mass of bis–ANS attached to the pressurized influenza virus, consistent with binding of the probe to a nonpolar domain. Fig. 1C shows that a blue shift also occurs when Sindbis virus is pressurized in the presence of bis–ANS. These results indicate an increase in exposure of hydrophobic sites on the surface of influenza and Sindbis virus envelope proteins. Previous studies correlated the binding of bis–ANS to the formation of the fusion–active state of influenza virus ( 30, 31 ). The increase in bis–ANS binding to influenza virus with pressure clearly indicates population of the fusogenic state. After return to atmospheric pressure, the intensity of bis–ANS remained high for 3 h. There was a small decrease in the fluorescence intensity over the period of 24 h, but the spectrum remained blue-shifted, indicating binding to nonpolar domains.

**Virus Inactivation by High Hydrostatic Pressure and Conversion into the Fusion–Active State**—Sindbis virus suspensions were pressurized at 2.5 kbar for different time periods, and its infectivity was measured in terms of plaque–forming units ( Fig. 2A ). Incubation for 8 h at 2.5 kbar reduced the infectivity by 0.6:0.6:4.5 ( 25 ). For the preparation of liposomes the mixture was dried under a stream of nitrogen. Dried lipid films were hydrated in 2 ml of HNE buffer ( 5 mM HEPES, 150 mM NaCl, and 0.1 mM EDTA ( pH 7.4 )). Extrusion was done 10 times through stacked polycarbonate filters with a pore size of 0.2 μm ( Nucleopore, Inc., Pleasanton, CA ). Lipid concentrations of liposomes suspension were determined on the basis of phospholipid analysis ( 26 ).

For the fusion assay, a previously described protocol was used ( 25 ). The fluorescence was monitored by exciting the sample at 465 nm, and emission was measured at 535 nm in a ISSPC fluorometer ( ISS Inc., Champaign, IL ). The lipid–mixing end point was defined as the fluorescence emission at 535 nm on addition of Triton X–100 to 1%.
Pressure inactivation of influenza virus was also observed as measured by hemagglutination assays (Fig. 2). The effects of high pressure on Sindbis and influenza viruses were also characterized by following changes in tryptophan fluorescence (spectral center of mass and light scattering). Fig. 3A shows the change in the spectral center of mass as a function of pressure (up to 3.0 kbar) for Sindbis virus. Sindbis undergoes a relatively small change in tryptophan emission under pressure (~200 cm⁻¹), and it is almost completely reversible upon return to atmospheric pressure (open circle). The changes in light scattering are also small (Fig. 3B), indicating that the particles do not suffer extensive denaturation under pressure. After decompression, the light scattering is reversible (open square), also suggesting recovery of a structure of the same size as the native structure. In contrast, high concentration of urea produced much greater changes (Fig. 3). Although intrinsic fluorescence and light scattering changes suggest that the particles remain intact after pressurization, it was observed that pressure induces virus inactivation (Fig. 2, A and B).

The changes in the spectral center of mass and in the light scattering induced by high pressure on influenza virus are also small and seem to be reversible, leading the particles to a final configuration that is similar to the structure of native virus (Fig. 3, C and D). However, pressure-treated influenza virus lost hemagglutination activity (Fig. 2B) and could not infect cells in culture and chicken embryos. Pressure inactivation has been described for several animal enveloped viruses, such as vesicular stomatitis virus (34), simian immunodeficiency virus (35), and human immunodeficiency virus type 1 (36).

Concentration Dependence and Changes in Secondary Structure—Table I shows that the $p_{50}$ (pressure that promotes half of the changes in tryptophan fluorescence) did not undergo any change that is significantly above the error of the measurement when the concentration was varied for Sindbis virus, Mayaro virus, an Alphavirus, and influenza virus. We also tested the concentration dependence for the reduction in infectivity (Sindbis and Mayaro viruses) and hemagglutination (Table I). For

![Figure 1](http://www.jbc.org/)

**Fig. 1.** Pressure-induced binding of bis-ANS to enveloped viruses and viral inactivation. Sindbis and influenza viruses were incubated with bis-ANS probe and subjected to increasing pressure. A, effect of pressure on bis-ANS fluorescence emission of influenza virus (50 μg/ml) (●). The open circle represents the spectral area after return to atmospheric pressure. B, the spectral center of mass of bis-ANS as it binds to influenza virus (50 μg/ml). The open circle represents the spectral center of mass of bis-ANS after return to atmospheric pressure. Influenza virus final concentration was 50 μg/ml. C, the spectral center of mass of bis-ANS as it binds to Sindbis virus (60 μg/ml) (▲). The open triangle represents the spectral center of mass of bis-ANS after return to atmospheric pressure. The bis-ANS was excited at 360 nm, and the emission was measured from 400 to 600 nm. Bis-ANS final concentration was 2 μM for Sindbis virus and 4 μM for influenza virus. The lines in A, B, and C are nonlinear fittings. D, fraction of change in bis-ANS center of mass for influenza virus and for Mayaro virus (▲) at the virus concentrations of 50 μg/ml (open circles) and 500 μg/ml (open squares). The values after return to atmospheric pressure are also shown for 50 μg/ml (●) and 500 μg/ml (■). A.U., arbitrary units.
subjected to pressure at 2.5 kbar for up to 8 h, incubation time under pressure (2.5 kbar). The virus suspensions were plaque-forming unit (PFU), hemagglutination unit (HU).

The secondary structure was not reduced by high-pressure treatment (Fig. 3). The CD spectrum is complex because of the presence of several proteins. However, it can be seen that the helix component. The pressure effects on enveloped viruses can be complex since the presence of several proteins. However, it can be seen that the secondary structure was not reduced by high-pressure treatment (Fig. 3E). In fact, it appears that there is a slight increase in the α-helix component. The pressure effects on Sindbis were compared with treatment with 8.0 M urea and with low pH (Fig. 3E). In contrast to pressurization, exposure to low pH substantially reduced the content of secondary structure, consistent with a difference in virus conformation (Fig. 3E). As recently shown by Carr et al. (25), exposure to low pH tends to denature the virus, first abolishing the infectivity and subsequently the fusogenic activity. For the same virus concentration, 8 M urea reduced the ellipticity value at 222 nm by more than 50%, indicating substantial denaturation of most of the proteins in the virus particle. Heat denaturation of the native and pressurized viruses were followed by changes in circular dichroism and revealed that the two particles underwent a similar cooperative transition (not shown).

**Fusion of Pressure-treated Influenza Virus with Lipid Vesicles**—The influenza virus fusion assay relies on the measurement of lipid mixing between virus and liposomes. Lipid vesicles were prepared with two incorporated fluorescent lipid derivatives, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)diacetyl phosphatidyethanolamine (fluorescence donor) and rhodamine-phosphatidylethanolamine (fluorescence acceptor) as described in earlier studies (25). On excitation of the donor fluorophore there is a resonance energy transfer to the acceptor fluorophore. The efficiency of energy transfer is dependent on the surface densities of fluorophores in membranes. When the fusion event takes place and mixing between labeled and unlabeled lipids happens, the density of fluorophores decreases causing a decrease in transfer efficiency. Thus an increase of the fluorescence intensity of the donor can be used to monitor the fusion process (25). The mechanism of influenza virus membrane fusion induced at low pH has been demonstrated in earlier studies that monitored the fusion activity of viral HA (15). As expected, we observed that the influenza virus membrane fusion activity was present after lowering the pH to 5.8 (Fig. 4). Recently it has been shown that heat and urea induce a conformational change, at neutral pH, that is similar to the fusogenic state induced by low pH (25). We find that high-pressure treatment elicits a dramatic fusion activity of influenza virus particles at neutral pH (Fig. 4). These results agree with the binding of bis-ANS (Fig. 1).

**Fusion of Sindbis Virus with Erythrocyte Ghosts after Virus Pressurization**—To test the ability of Sindbis virus to fuse with membranes after pressure treatment, Sindbis virus was labeled biosynthetically with pyrene-labeled fatty acid as described previously (10). The fusion assay relies on the measurement of lipid mixing between viral and ghost membranes (Fig. 5A). The pyrene fluorescence spectrum exhibits characteristic peaks at 389 and 410 nm (monomer fluorescence) and at 470 nm (excimer fluorescence). Excimers represent complexes between an excited state and a ground state probe molecule. Therefore, the pyrene excimer-to-monomer fluorescence ratio is proportional to the concentration of the pyrene probe. During the fusion between pyrene-labeled Sindbis virus and erythrocyte ghosts, the dilution of the pyrene phospholipids into the ghost target membranes can be monitored as a decrease in the pyrene excimer fluorescence. Both control and pyrene lipid-labeled virions had identical infectivity titers (data not shown).

Fig. 5 (B and C) confirms earlier findings that Sindbis virus associates with membranes in vitro in a pH-dependent manner. The two lower curves in Fig. 5B show that pyrene phospholipid in the Sindbis virus at low pH or after pressurization (at neutral pH) undergoes a decline in excimer/monomer ratio on interaction with ghosts indicating membrane fusion that was complete by 30 min. The low pH curves were also performed at pH 5.8 and 5.5, and the results show lower curves compared with the low pH values used in our studies (data not shown). By comparison, the pyrene excimer/monomer ratio showed very little change in interaction of native Sindbis virus with ghosts at pH 7.5 for a period as long as 60 min. Fig. 5C shows the values after 60 min for three experiments for each condition described in Fig. 5B.

**DISCUSSION**

Pressure effects on enveloped viruses can be complex since they depend on a number of protein-lipid, protein-protein, and protein-nucleic acid interactions (19, 37). Sindbis and influenza viruses do not dissociate under pressure as demonstrated by fluorescence spectroscopy and light scattering (Fig. 3) where high pressure is much milder than high concentrations of urea. Nevertheless, pressurized virus is very different from native virus when infectivity is measured. Our article shows that these two different enveloped viruses are inactivated by pressure with the main change occurring in the envelope protein.
that undergoes a transition to the fusogenic state.

Recent studies have demonstrated intermediate states in the assembly and disassembly pathway of many viruses, and protein-nucleic acid interactions appear to remain intact under pressure (32, 37–39). Our results with Sindbis and influenza viruses show that although pressure can preserve most of the physical properties of the envelope, it causes exposure of hydrophobic domains that populate the fusion-active state that may account for the pronounced inactivation. Although CD spectra were different for the pressure- and acid-induced conformations of Sindbis virus, both states induced fusion.

Recent studies have shown that any destabilizing reagent will cause the same conformational change and subsequently promote membrane fusion in enveloped viruses (25). High pressure was used in this study to characterize pressure-induced conformational changes that lead to the fusogenic state similar to that characterized at acid pH (Figs. 4 and 5).

Studies with Semliki Forest virus have demonstrated that the low pH environment of the endosome leads to a dissociation of the E1/E2 heterodimer and a concomitant trimerization of the E1 subunits (6, 7, 10). An important consequence of the conformational change is the exposure and outward movement of fusion peptides. This was first recognized for influenza virus HA: a hydrophobic N-terminal peptide hidden in the trimeric structure is exposed after low pH activation and relocated by 10 nm toward the top of the spike structure where it interacts with the target membrane (13, 14).

Although the structural motifs are quite different in Sindbis and influenza viruses, pressure appears to lead both equally to the fusogenic state. Our results give additional support to the metastability model where pressure first induces exposure of
The two buried surfaces add up to 2177 Å

izable residues implicated in the N

with nonpolar residues (HA2 residues 1–10), and several ion-

Pressure- and acid-induced membrane fusion of influ-

enza viruses to liposomes. Fusion activities of different samples of influenza virus were measured by means of lipid mixing between virus and labeled liposomes. The virus sample was pressurized at 3.0 kbar for 2 h at pH 7.4. After return to atmospheric pressure, the sample was added to a cuvette containing the liposome suspension at pH 7.4 (○). Acid-induced activity was measured by incubating the virus at pH 5.8 and adding the sample to the lipid suspension (△). Influenza virus samples incubated at pH 6.2 did not present fusion activity (△). All experiments were carried out at 37 °C. Virus concentration was 1 mg/ml. N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)diacyl phosphatidylethanolaminate-labeled liposomes were excited at 465 nm, and the emission was measured at 535 nm.

the hydrophobic surfaces that are less energetically costly. If a kinetic barrier separates the metastable, native state from the stable form, pressure will induce the conversion depending on the difference in volume between the native (N) and the fusogenic (FG) states. In Fig. 6, we sketch free energy and volume diagrams for the conversion between the N and FG conformations. Although the transition state (T) for the conversion has higher energy, our data indicate that it has a lower volume. Because pressure induces the conversion from N to FG, the sign of the change is clearly negative. However, the changes in bis-ANS fluorescence were too fast to allow us to determine the value of the activation volume change.

It is not surprising that a volume change would occur due to the major refolding of the secondary and tertiary structure of the HA1/HA2 molecule upon conversion to the fusogenic state (14, 25). The recent elucidation of the structure of the ectodomain of the hemagglutinin precursor HA0 showed that the proteolytic cleavage results in structural rearrangements that can explain why the protein becomes labile and sensitive to pressure (40). A water-exposed cavity in HA0 becomes filled with nonpolar residues (HA2 residues 1–10), and several ionizable residues implicated in the N → FG transition are buried. The two buried surfaces add up to 2177 Å², and the previously wet cavity in HA0 becomes nonpolar. This change makes it sensitive to both pH and pressure. Cavities have been implicated in protein metastability in several systems (32, 41–43). The influenza virus system has the unique property of having a water-penetrated cavity in the precursor protein, which eliminates water on proteolytic cleavage, originating the pH and pressure sensitivity. Recently we found that the maturation/cleavage of flock house virus capsid converts the coat protein into a metastable conformation, whereas a cleavage-defective

mutant particle coat protein reversibly reassembles into particles. The mature capsid is more sensitive to pressure than the cleavage-defective, immature particle (42).

The breakage of E1/E2 contacts in Sindbis followed by trimerization of E1 (10) would also lead to a net decrease in volume. Pressure shifts any association/dissociation (or folding/unfolding) reaction toward the smaller volume whether in monomeric proteins (17, 44, 45), in oligomeric proteins (16, 18, 37), in protein-DNA complexes (46–49), or in the conversion of proteins into the amyloidogenic or aggregated state (50, 51). In all these cases, pressure appears to produce its effects by in-
FIG. 6. Gibbs free energy and volume diagrams for enveloped viruses. A, free energy diagram showing the conversion between the N state and FG state. The transition intermediate state is represented by T, B, the volume change diagram for the native, intermediate, and fusogenic states. 

producing the penetration of molecules of water into the interior of the protein structure (52, 53), which facilitates the population of the protein structure (52, 53), which facilitates the population

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43. Focused on a potential for fusion complexes for human immoductivity virus vaccine development. Since high pressure is able to produce fusion-intermediate states, which are similar to that at low pH in endosomes (alphaviruses and influenza virus) or that produced by binding to receptors (such as in human immunodeficiency virus), there is a great potential to utilize the pressure approach to produce vaccines.

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