JOURNAL OF VIROLOGY, Feb. 1995, p. 695–700 Vol. 69, No. 2
0022-538X/95/$04.00+0

Oligomeric Rearrangement of Tick-Borne Encephalitis Virus Envelope Proteins Induced by an Acidic pH

STEVEN L. ALLISON,* JULIANE SCHALICH, KARIN STIASNY, CHRISTIAN W. MANDL, CHRISTIAN KUNZ, AND FRANZ X. HEINZ

Institute of Virology, University of Vienna, Vienna, Austria

Received 11 August 1994/Accepted 19 October 1994

The flavivirus envelope protein E undergoes irreversible conformational changes at a mildly acidic pH which are believed to be necessary for membrane fusion in endosomes. In this study we used a combination of chemical cross-linking and sedimentation analysis to show that the envelope proteins of the flavivirus tick-borne encephalitis virus also change their oligomeric structure when exposed to a mildly acidic environment. Under neutral or slightly alkaline conditions, protein E on the surface of native virions exists as a homodimer which can be isolated by solubilization with the nonionic detergent Triton X-100. Solubilization with the same detergent after pretreatment at an acidic pH, however, yielded homotrimers rather than homodimers, suggesting that exposure to an acidic pH had induced a simultaneous weakening of dimeric contacts and a strengthening of trimeric ones. The pH threshold for the dimer-to-trimer transition was found to be 6.5. Because the pH dependence of this transition parallels that of previously observed changes in the conformation and hydrophobicity of protein E and that of virus-induced membrane fusion, it appears likely that the mechanism of fusion with endosomal membranes involves a specific rearrangement of the proteins in the viral envelope. Immature virions in which protein E is associated with the uncleaved precursor (prM) of the membrane protein M did not undergo a low-pH-induced rearrangement. This is consistent with a protective role of protein prM for protein E during intracellular transport of immature virions through acidic compartments of the trans-Golgi network.

A common mode of entry of enveloped viruses into host cells involves uptake by endocytosis followed by fusion of viral membranes with endosomal membranes (reviewed in references 21 and 26). The fusion event in these viruses is triggered by the mildly acidic environment in the endosome, which induces a conformational change in the viral fusion protein, converting it to a fusogenic state (36). This conformational change typically results in the exposure of hydrophobic elements that interact with membranes. The exact mechanism by which these structural changes further lead to membrane fusion, however, still remains unclear.

A number of studies provide evidence that flaviviruses enter cells by endocytosis and low-pH-induced fusion (1, 6, 8, 12, 18, 27, 28). These data together suggest that the mechanism of flavivirus entry is analogous to that of orthomyxo- and alphaviruses and requires the acidic environment of the endosome to induce a structural change in the viral envelope leading to fusion with endosomal membranes.

The envelope protein E (50 to 60 kDa) is believed to play a central role in flavivirus entry and fusion. It is the principal target of neutralizing antibodies (17), and some neutralizing monoclonal antibodies (MAbs) directed against this protein are capable of blocking virus-mediated cell-cell fusion (11, 28, 31). Earlier studies have demonstrated that protein E, analogously to other viral pH-dependent fusion proteins (36), undergoes a major irreversible conformational change at a mildly acidic pH, with a pH threshold corresponding to that of fusion (9, 10, 18, 22, 30). This structural transition apparently causes hydrophobic elements on the virion surface to become exposed, as has been demonstrated with a virus aggregation assay (18).

In addition to the envelope protein E, mature flaviviruses contain a capsid protein, C (15 kDa), which is associated with the positive-stranded RNA genome in an isometric nucleocapsid structure, and a second membrane protein, M (7 to 8 kDa). Protein M is initially synthesized as a larger glycosylated precursor, prM, which is associated with protein E in immature intracellular virions and is cleaved by a cellular protease shortly before virus release (3). Cleavage of prM is accompanied by a structural reorganization of the envelope proteins and release of fully active mature virions (34). Recently, we and others presented evidence that protein prM might also play an important indirect role in the regulation of low-pH-induced membrane fusion. Cleavage of prM can be blocked by treatment of infected cells with ammonium chloride or bafilomycin A1, resulting in the release of virus particles containing the uncleaved prM precursor protein. These immature virions are less infectious than mature virions (9, 18, 29, 34) and do not induce cell-cell fusion at an acidic pH (9, 11). The association of prM with protein E in immature virions appears to make the latter protein resistant to the low-pH-induced conformational change required for fusion. This association has recently been shown to stabilize a pH-sensitive epitope on protein E and to prevent viral aggregation at an acidic pH (18). It is therefore currently believed that the function of prM is to protect against premature fusion and virus inactivation by preventing protein E from undergoing structural changes during transport through acidic intracellular compartments.

We have used the flavivirus tick-borne encephalitis (TBE) virus as a model system for characterizing structural changes occurring in flavivirus envelope proteins at a low pH. It has been shown previously that the protein E molecules on the TBE virion can be isolated as homodimers either by solubilization of the virus using nonionic detergents (13, 14) or by

* Corresponding author. Mailing address: Institute of Virology, University of Vienna, Kinderspitalgasse 15, A-1095 Vienna, Austria. Phone: 43-1-404-90, ext. 605. Fax: 43-1-43-21-61.

limited proteolysis (16). In each of these previous studies, the dimers were isolated from purified virions at a neutral to slightly alkaline pH and the oligomeric organization of the envelope proteins in the low-pH conformation was not investigated. The purpose of the present work was to investigate changes occurring in the TBE virus envelope quaternary structure in response to the mildly acidic conditions that would be encountered in endosomes. We report here that a mildly acidic pH (6.5 and below) can induce a major change in the interactions of E protein monomers in the viral envelope, leading irreversibly to a quantitative conversion of E homodimers to homotrimers as the fundamental structural unit. We further show that this change, consistent with the proposed protective role of prM during exocytosis, does not occur in immature virions.

MATERIALS AND METHODS

Virus production and purification. The TBE virus prototype strain Neudoerfl (25) was used in all experiments. Virus was grown in primary chicken embryo cells and, unless otherwise indicated, purified from supernatants 48 h postinfection by pelleting and two cycles of sucrose gradient centrifugation as described previously (15). Immature virions were prepared by replacing the culture medium 24 h postinfection with fresh medium containing 20 mM NH_4Cl, replacing this medium 1 h later with fresh NH_4Cl-containing medium, and harvesting 48 h postinfection (18).

Low-pH incubation. Purified virus at a concentration of 150 to 500 μg/ml in TAN buffer (0.05 M triethanolamine [pH 8.0] plus 0.1 M NaCl) or culture supernatant from virus-infected cells was acidified by using a buffer consisting of 0.05 M MES (morpholineethanesulfonic acid), 0.1 M NaCl, and 0.1% bovine serum albumin (BSA) which had been pretitrated to yield the desired final pH when the sample was added. For the pH 8.0 controls, samples were treated with a buffer containing 0.05 M triethanolamine, 0.1 M NaCl, and 0.1% BSA. The virus was incubated for 10 min at 37°C at the low pH and then adjusted back to pH 8.0 by adding a buffer containing 0.1 M triethanolamine, 0.1 M NaCl, and 0.1% BSA. BSA was omitted in the experiments involving cross-linking and gel electrophoresis.

Cross-linking analysis. Purified virus after low-pH treatment was incubated for 1 h at room temperature in the presence or absence of 0.5% Triton X-100 and cross-linked by the addition of dimethylsuberimidate (DMS; Pierce Chemical Co.) to a final concentration of 10 mM. After incubation for 30 min at room temperature the reaction was stopped by adding ethanolamine to a final concentration of 10 mM and incubating the mixture for an additional 15 min at room temperature. Proteins were precipitated with trichloroacetic acid and analyzed by electrophoresis on a sodium dodecyl sulfate (SDS)–5% polyacrylamide gel using a continuous phosphate-buffered system (24).

Sedimentation analysis. Low-pH-treated or untreated virus was solubilized with 0.5% Triton X-100 as described above and analyzed by sedimentation on a continuous gradient of 7 to 20% sucrose in TAN buffer plus 0.1% Triton X-100 with a 1-ml 40% sucrose cushion. Samples were centrifuged for 20 h at 38,000 rpm at 15°C in a Beckman SW40 rotor and fractionated by upward displacement using an ISCO model 640 fraction collector. Samples from the gradient fractions were then either precipitated with trichloroacetic acid and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (23) or denatured by heating at 65°C in 1% SDS for 15 min and tested in a four-layer enzyme-linked immunosorbent assay (ELISA) for quantitation of protein E (19) using SDS-treated purified virus as a standard.

Epitope analysis. Purified virus preparations were pretreated at various pH values, back neutralized as described above, and diluted in TAN buffer plus 0.1% BSA to a final E-protein concentration of 0.25 μg/ml. These samples were then tested in a four-layer ELISA for reactivity with MAb i2, which is specific for a pH-dependent conformational epitope on protein E (18).

RESULTS

Detection of protein E trimers after acidic pH treatment. To investigate whether the irreversible changes that occur within the TBE virus envelope protein E upon exposure to an acidic pH are associated not only with tertiary structure changes but also with changes in the oligomeric organization of protein E monomers, subunit interactions between envelope proteins on the virion surface were examined by cross-linking with the homobifunctional cross-linker DMS and SDS-PAGE analysis. Since covalent cross-linking of oligomeric glycoprotein complexes with DMS is typically incomplete, as has been observed previously, e.g., with the alphavirus spike heterodimer (5) and the influenza virus hemagglutinin trimer (37), the highest-molecular-weight species in the SDS gel is indicative of the oligomeric state of the complex.

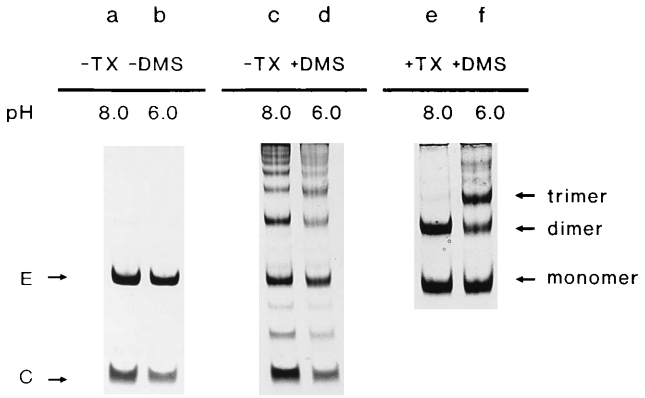

FIG. 1. Cross-linking analysis of native and low-pH-treated TBE virus. After cross-linking with DMS, proteins were precipitated with trichloroacetic acid, separated by SDS-PAGE, and stained with Coomassie blue R-250. Lanes: a and b, no cross-linking; c and d, cross-linking with DMS; e and f, solubilization with 0.5% Triton X-100 (TX) followed by cross-linking with DMS. Positions of the capsid protein (C) and the monomeric, dimeric, and trimeric forms of E are indicated. Protein M is not visible because it runs off the bottom of the gel in this system.

Whole purified virus was preincubated at either pH 8.0 or pH 6.0, back neutralized as described in Materials and Methods, and treated with the cross-linker. Samples were then analyzed by SDS-PAGE as shown in Fig. 1. Cross-linking of whole virus at pH 8.0, consistent with earlier observations (13, 18), gave rise to a continuous ladder of protein E oligomers (Fig. 1, lane c) ranging from monomers to at least hexamers without preferential formation of a particular oligomeric species. After preincubation at pH 6.0, in contrast, a significant relative enrichment in a band corresponding in molecular weight to an E homotrimer was observed, and bands corresponding to the dimeric and tetrameric forms became somewhat diminished in intensity (Fig. 1, lane d). This suggested that a change in the spatial distribution of protein E had occurred on the virion surface.

When virus was pretreated at pH 8.0 or 6.0 and solubilized with the nonionic detergent Triton X-100 before cross-linking, a strong band corresponding in molecular weight to an E dimer was observed with the pH 8.0-treated virus, but the higher oligomeric forms were almost completely absent (Fig. 1, lane e), indicating that protein E in the membrane had been dispersed into detergent-stable homodimers. After pH 6.0 treatment, however, cross-linking of Triton X-100-solubilized E protein gave rise to a band corresponding to a homotrimer (Fig. 1, lane f), indicating that the low-pH treatment had induced an irreversible rearrangement of protein E homodimers to homotrimers and that the newly formed trimer was stable in 0.5% Triton X-100.

Isolation of trimeric E proteins by sucrose gradient centrifugation. To study the sedimentation behavior of the low-pH-treated and detergent-solubilized protein E complexes, whole virus that was preincubated at either pH 8.0 or pH 6.0 as in the previous experiment was solubilized with 0.5% Triton X-100 and analyzed by rate zonal centrifugation in a sucrose gradient containing 0.1% Triton X-100. It has been shown previously that under these conditions the neutral form of protein E sediments as a dimer (14). Whole virus that had been boiled for 1 min in 1% SDS to completely dissociate the protein E

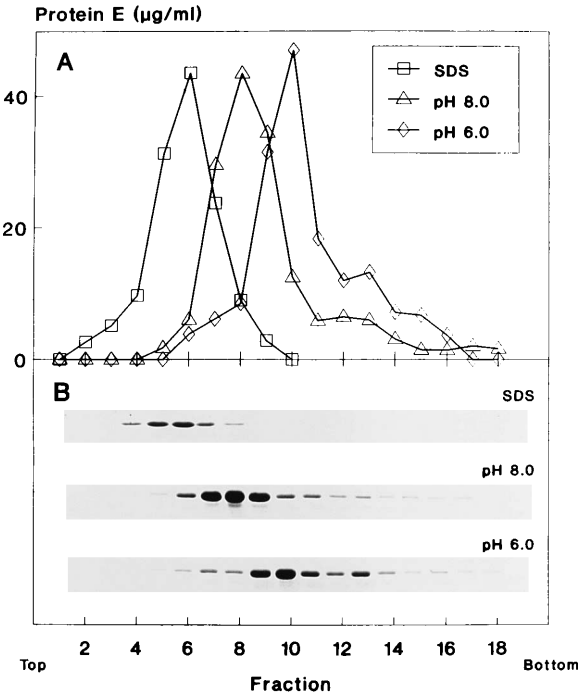

FIG. 2. Sedimentation analysis of detergent-solubilized protein E complexes. Purified TBE virus pretreated at pH 8.0 or 6.0 was solubilized with 0.5% Triton X-100, and detergent-stable protein E complexes were analyzed by sedimentation in 7 to 20% sucrose gradients containing 0.1% Triton X-100. Protein E monomers produced by boiling in 1% SDS were run in a parallel gradient as a sedimentation standard. (A) Quantitation of protein E in gradient fractions using four-layer ELISA. (B) Protein E from the same fractions stained with Coomassie blue after trichloroacetic acid precipitation and SDS-PAGE. Top row, SDS treatment; middle row, pH 8.0; bottom row, pH 6.0.

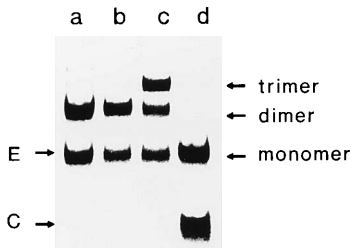

FIG. 3. Cross-linking analysis of protein E complexes isolated by sucrose gradient centrifugation. Protein E complexes produced by Triton X-100 solubilization of purified TBE virus pretreated at pH 8.0, 7.4, or 6.0 were isolated by sucrose gradient centrifugation as for Fig. 2. Peak fractions were pooled together and analyzed by cross-linking as for Fig. 1. Lanes: a, pH 8.0; b, pH 7.4; c, pH 6.0; d, whole TBE virus without cross-linking. The positions of the capsid protein (C) and the protein E monomer, dimer, and trimer are shown.

subunits (13) was also used as a sedimentation standard for the E monomer. Quantitation of protein E in the gradient fractions (Fig. 2A) revealed that each of the pH 6.0-, pH 8.0-, and SDS-treated viruses produced a single homogeneous peak containing the majority of the total protein E, but the three forms of E differed in their sedimentation velocities. The monomeric form of E, as expected, sedimented most slowly, with the pH 8.0 dimer sedimenting somewhat faster (Fig. 2A). The material from the pH 6.0-treated sample clearly sedimented even faster than the pH 8.0 dimer, suggesting that low-pH treatment had caused an increase in the molecular weight of the protein E complex. Small amounts of still-larger aggregates containing protein E as well as unconverted dimers could also be observed for the pH 6.0 fractions (Fig. 2). Protein M did not cosediment with the E dimer or trimer at either pH under these conditions (data not shown), and it remains to be determined what involvement, if any, protein M might have in this process.

In order to determine the oligomeric states of the detergent-stable complexes observed in the sedimentation analysis, Triton X-100-solubilized protein E oligomers were isolated by sucrose gradient centrifugation as in the previous experiment from virus pretreated at pH 8.0, 7.4, or 6.0, and peak fractions containing protein E were analyzed by DMS cross-linking and SDS-PAGE. The protein E oligomers formed at both pH 7.4 and pH 8.0 yielded sedimentation profiles identical to that shown for pH 8.0-treated virus in Fig. 2A, and the pH 6.0-treated virus peak again sedimented faster, appearing in the same fractions as in Fig. 2A. Cross-linking analysis of the peak fractions from the pH 8.0- and pH 7.4-treated virus yielded only dimers and monomers, whereas the pH 6.0-treated virus also yielded a single additional strong band corresponding in molecular weight to a protein E trimer (Fig. 3). Since this material sedimented as a single peak that was clearly distinguishable from the dimer and monomer peaks, it was concluded that protein E at pH 6.0 was probably almost exclusively trimeric. Because the material from the pH 8.0 and 7.4 gradients appeared to be identical, consisting only of E homodimers, it appears that the dimeric form of E represents the native state at a physiological pH and the trimeric state is attained only after exposure to a low pH.

To rule out the possibility that the formation of dimers and trimers of E was in some way due to the manner in which the virus was handled during the purification procedure, the acidification, back neutralization, Triton X-100 treatment, and sedimentation analysis were also carried out with a fresh supernatant harvested at an early stage from an infected cell culture that was not subjected to any further purification steps. In this experiment BSA (1 mg/ml) was included in the medium as a stabilizer. The unpurified virus in these supernatants behaved identically to the purified virions, yielding the expected dimer and trimer peaks at pH 8.0 and 6.0, respectively (data not shown).

The dimer-to-trimer transition and loss of a pH-sensitive conformational epitope occur at the same pH threshold. It has been shown previously that incubation of TBE virus at a mildly acidic pH results in an irreversible conformational change which can be monitored by the loss of reactivity with MAb i2 in a four-layer ELISA (18). MAb i2 recognizes an epitope present on the neutral, but not the acidic form of protein E. In order to compare the pH threshold of this change in antigenic conformation with that of the protein E dimer-to-trimer transition, samples of purified TBE virus were pretreated at pH 8.0, 6.6, 6.5, 6.2, and 6.0 as described in Materials and Methods, the pH was adjusted back to 8.0, and the same samples were analyzed both by sedimentation analysis after solubilization with 0.5% Triton X-100 and by four-layer ELISA using MAb i2. As shown in Fig. 4, most of the protein E sedimented as a dimer at pH 8.0 and 6.6 and as a trimer at pH 6.2 and 6.0. Trimerization was essentially complete at 6.0. At pH 6.5 a mixed population of dimers and trimers was observed, suggesting that the threshold for the dimer-to-trimer transition is at or near pH 6.5. Analysis of the same samples in the four-layer ELISA with MAb i2 (Fig. 5) showed that the epitope for MAb i2 was still fully intact at pH 6.6 but that some loss of reactivity was already observable at pH 6.5, after which the binding activity then continued to decrease sharply until at least pH 6.0. These results show that both transitions occur at the same

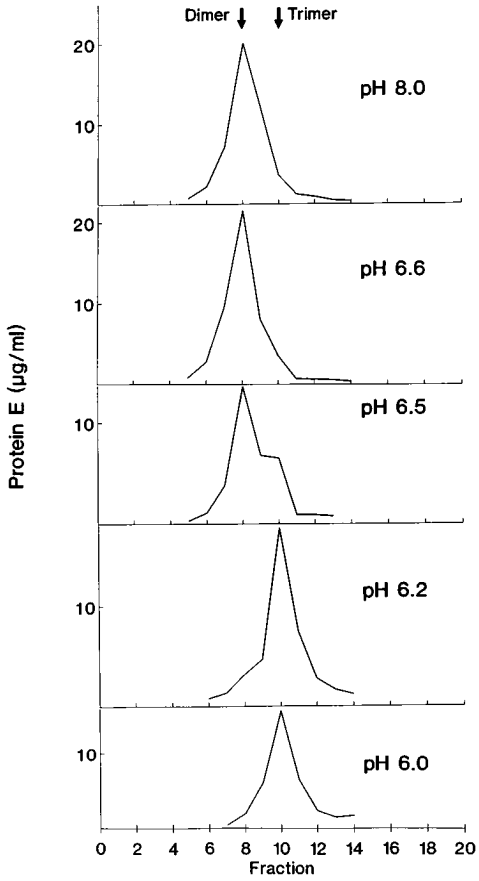

FIG. 4. pH dependence of trimer formation. Samples of TBE virus pretreated at various pH values were solubilized in 0.5% Triton X-100 and subjected to sucrose gradient centrifugation as for Fig. 2. Sedimentation profiles of Triton X-100-stable protein E complexes were determined by four-layer ELISA as for Fig. 2A. The positions of the protein E dimer and trimer peaks are indicated.

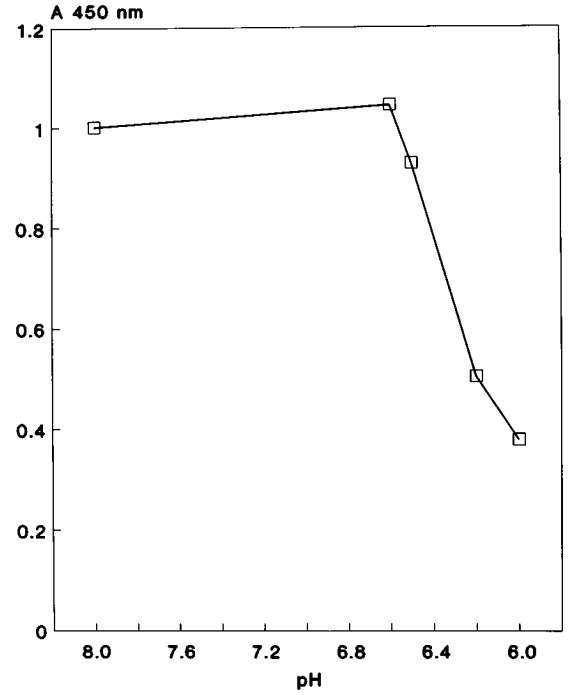

FIG. 5. pH dependence of the conformational change in protein E. The pH-treated virus samples in Fig. 4 were tested by four-layer ELISA for reactivity with MAb i2, which preferentially recognizes the native, rather than the low-pH, form of protein E.

pH threshold and suggest that the same structural alteration occurring at pH 6.5 and below might be responsible for both the loss of conformational epitopes and the rearrangement of the oligomeric structure.

In a time course experiment, purified virus was treated at pH 6.0 for 30, 60, 120, 180, 300, or 600 s and aliquots were either back neutralized or immediately solubilized in 0.5% Triton X-100 at pH 8.0. The back neutralized samples were then analyzed by four-layer ELISA with MAb i2, and the oligomeric structure of the solubilized samples was determined by sedimentation analysis. Both the conformational change and the oligomeric rearrangement were complete within the first 30 s, and no further changes were observed at the later time points (data not shown).

Protein E in immature virions does not undergo structural rearrangement at a low pH. Treatment of TBE virus-infected cells with ammonium chloride during the late phase of infection leads to the release of virions in which the prM protein, the precursor of protein M, is incorporated but not cleaved to the mature form (11, 18). Solubilization of these virions with Triton X-100 has been shown to give rise to a population of heterooligomeric forms containing E and prM (18). In addition to being less infectious than mature virus, these immature virions have been shown to be resistant to low-pH-induced conformational changes and induction of fusion activity (11, 18). We therefore predicted that the presence of uncleaved prM might also prevent the formation of E trimers at a low pH. To address this question, purified immature virions were preincubated at pH 6.0 or 8.0 and back neutralized in the same way as described above for mature virions. This material was then solubilized with Triton X-100 and analyzed by sucrose gradient sedimentation (Fig. 6). As expected, immature virus at pH 8.0 yielded a relatively broad peak of protein E-containing material, presumably due to multiple detergent-stable interactions between E and prM monomers. The pH 6.0-treated immature virus gave a nearly identical sedimentation profile (Fig. 6), with no appearance of an E homotrimer peak at

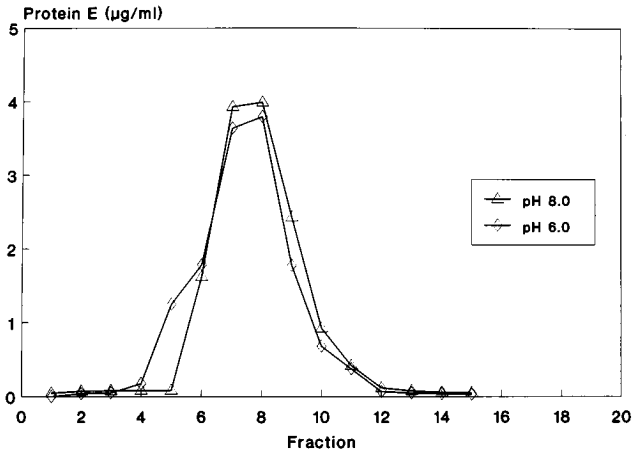

FIG. 6. Sedimentation analysis of Triton X-100-solubilized low-pH-treated immature virions. Immature virions containing uncleaved prM protein were pretreated at pH 8.0 or 6.0, solubilized in 0.5% Triton X-100, and subjected to sedimentation analysis as for Fig. 2A.

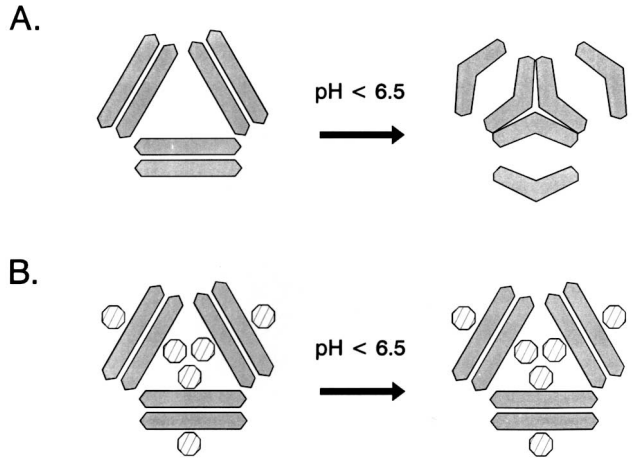

FIG. 7. Schematic representation of the proposed structural rearrangement of the TBE virus envelope at a low pH. Shaded bars, protein E; hatched octagons, protein prM. (A) The native envelope structure is organized as a network of closely packed homodimers which, when exposed to the mildly acidic environment of the endosome, adopt a new conformation and a structural configuration in which trimeric contacts are strengthened at the expense of dimeric ones. In this state, structural elements required for membrane fusion would become exposed or activated. (B) Protein prM prevents low-pH-induced structural changes in protein E and oligomeric rearrangements, thereby protecting the virus from premature fusion and inactivation during intracellular transport through acidic compartments.

around fraction 10. These data show that immature virions are more acid resistant than mature virions and suggest that the interactions between proteins E and prM in immature virions prevent the structural rearrangement leading to the formation of E trimers.

DISCUSSION

The results presented in this article together with those of earlier studies show that the envelope proteins of TBE virus undergo a significant structural rearrangement upon exposure to a mildly acidic environment. Although the causal relationships have not yet been rigorously established, it appears likely that a single low-pH-induced alteration in protein E gives rise to changes in a number of measurable structural and functional properties of the virus. Preincubation at a low pH results in the loss of certain conformational epitopes, a reorganization of protein E homodimers into homotrimers, and exposure of hydrophobic elements on the E-protein surface. These structural changes are irreversible and appear to occur at or below a pH threshold of 6.5. This pH threshold also corresponds well to the observed pH thresholds of TBE virus-induced cell-cell fusion (11), virus fusion with artificial membranes (32), loss of infectivity and hemagglutination activity (18), and virus aggregation due to increased hydrophobicity (18).

A model correlating these phenomena is depicted schematically in Fig. 7. In this model the surface of the native TBE virion at a neutral pH is proposed to consist of a lattice of relatively closely packed protein E dimers which can be joined together to form higher oligomeric structures by chemical cross-linking. A conformational change and structural reorganization at a low pH would cause some intersubunit interactions, particularly those stabilizing the E homodimer, to be disrupted while simultaneously creating new contacts to stabilize the trimer. The new conformation of the trimeric form would then allow important functional domains necessary for fusion to be presented at the protein surface, where they could presumably interact with target membranes. This process is apparently blocked by the presence of uncleaved prM in immature virions, which prevents both trimerization and the transition to a fusion-active state.

Several lines of evidence indicate that flaviviruses use the pathway of endocytosis and low-pH-induced fusion to enter cells. It has been shown in electron microscopy studies that after binding to cells, the virus is internalized in coated vesicles (6, 27). Infection can be blocked by treatment of cells with acidotropic agents that raise the pH of intracellular compartments (1, 6, 8, 12, 18, 28). Under these conditions virus particles accumulate in endosomes (6) and do not get uncoated (8). Cell-cell fusion of C6/36 mosquito cells (4, 9, 11, 12, 28, 31) or artificial liposomes (7, 32) can be induced by exogenously added flavivirus after a brief treatment at a mildly acidic pH, but fusion is not observed at a neutral pH. In the present study we found that the structural transition to the proposed fusogenic state occurs rapidly and is mostly complete at pH 6.2, suggesting that flavivirus fusion, like that of alphaviruses, might take place in early endosomes (26).

Previous studies with TBE virus (11, 18) and other flaviviruses (9, 29, 34) have shown that the low-pH-induced conformational change in E as well as infectivity, fusion activity, and hemagglutination activity are all inhibited by the presence of uncleaved prM protein in virions. We have shown here that the presence of prM also apparently prevents E homotrimer formation at a low pH. This finding is consistent with the hypothesis that one of the principal functions of prM is to render the envelope protein structure of newly synthesized virus resistant to low-pH-induced changes during transport through acidic compartments of the secretory pathway, thereby preventing inactivation of the virus due to premature activation of functions necessary for entry (9, 18). It cannot be determined from this study, however, whether E trimerization itself is physically blocked by the presence of prM or if prM indirectly interferes with trimerization by inhibiting a necessary conformational change occurring prior to trimer formation. It also remains to be established whether trimerization itself is a prerequisite for fusion or rather a physical consequence of the change to the fusogenic state.

In this study we confirmed earlier observations that protein E from TBE virus is initially dimeric in its neutral form and further showed that incubation at a low pH irreversibly converts it to a trimer. Isolation of trimeric forms of protein E from another flavivirus, West Nile virus, has also been reported (34, 35). In those studies, however, trimers were obtained from the virus at pH 8.0 without preincubation at a low pH. The apparent discrepancy between the TBE virus and West Nile virus studies might merely reflect subtle differences in the way that the envelope proteins of different flaviviruses interact. Recent X-ray crystallographic data obtained with dimeric protein E ectodomain fragments produced by trypsin cleavage of the neutral form of TBE virus suggest that TBE virus protein E, in contrast to influenza virus hemagglutinin and several other viral fusion proteins, does not form a spike structure but might instead form a surface matrix involving multiple subunit interactions (29a). In agreement with this model, we confirmed earlier observations that higher oligomeric forms of protein E can be obtained by cross-linking, suggesting that E-protein dimers are closely packed on the virion surface. If flavivirus envelopes are organized in a lattice structure in which dimeric interactions are particularly favored in TBE virus at a neutral pH (but not necessarily in other flaviviruses), an irreversible conformational change at a low pH might serve to stabilize trimeric interactions at the expense of both dimeric and interdimeric interactions. More detailed analyses of the surface

structure of TBE virus and other flaviviruses will be needed to clarify this point.

The occurrence of a low-pH-induced oligomeric rearrangement at the pH of fusion is not unique to TBE virus. Similar findings have been reported recently for another enveloped virus, the alphavirus Semliki Forest virus (2, 20, 33). Fusion of this virus with target membranes is preceded by a transition from a heterooligomeric form to a homotrimeric form of the fusion-active envelope protein E1. This rearrangement, like that of TBE virus, is irreversible.

More detailed molecular analyses of the protein E and virion surface structures will be needed to elucidate the structural basis of the low-pH-induced rearrangements in the TBE virus envelope and how they contribute to the mechanism of flavivirus entry and fusion.

ACKNOWLEDGMENTS

We gratefully acknowledge Angela Dohnal and Walter Holzer for providing excellent technical assistance.

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