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Research article

The viral transmembrane superfamily: possible divergence of Arenavirus and Filovirus glycoproteins from a common RNA virus ancestor

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Abstract

Background: Recent studies of viral entry proteins from influenza, measles, human immunodeficiency virus, type I (HIV-1), and Ebola virus have shown, first with molecular modeling, and then X-ray crystallographic or other biophysical studies, that these disparate viruses share a coiled-coil type of entry protein.

Results: Structural models of the transmembrane glycoproteins (GP-2) of the Arenaviruses, lymphochoriomeningitis virus (LCMV) and Lassa fever virus, are presented, based on consistent structural propensities despite variation in the amino acid sequence. The principal features of the model, a hydrophobic amino terminus, and two antiparallel helices separated by a glycosylated, antigenic apex, are common to a number of otherwise disparate families of enveloped RNA viruses. Within the first amphipathic helix, demonstrable by circular dichroism of a peptide fragment, there is a highly conserved heptad repeat pattern proposed to mediate multimerization by coiled-coil interactions. The amino terminal 18 amino acids are 28% identical and 50% highly similar to the corresponding region of Ebola, a member of the Filovirus family. Within the second, charged helix just prior to membrane insertion there is also high similarity over the central 18 amino acids in corresponding regions of Lassa and Ebola, which may be further related to the similar region of HIV-1 defining a potent antiviral peptide analogue.

Conclusions: These findings indicate a common pattern of structure and function among viral transmembrane fusion proteins from a number of virus families. Such a pattern may define a viral transmembrane superfamily that evolved from a common precursor eons ago.

Background

Findings in a number of laboratories have indicated that the transmembrane (TM) proteins of a number of RNA viruses have common structural and functional elements critical for virus entry. These include a hydrophobic re-

gion designated a "fusion peptide", usually at or near the amino-terminus generated by cleavage of a precursor protein, together with fibrous structure defined by two antiparallel alpha helices. These general principles appear to apply to the Orthomyxoviruses, Paramyxovirus-

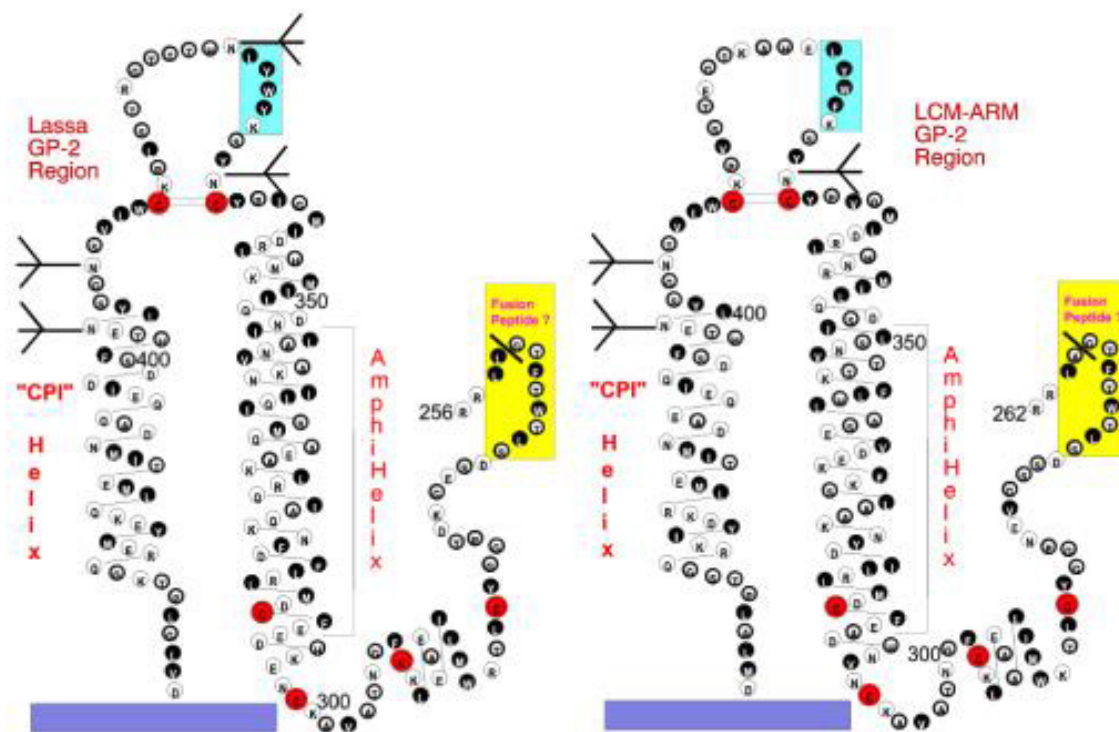


Figure 1
Models of the Transmembrane Proteins GP-2 of Lassa Virus and LCMV. Projections of the model structure for GP-2 of Lassa and LCMV are shown in parallel with one another, based on the consensus structures for algorithms of both viral amino acid sequences. Proposed helices are shown in helical net projection with sequential amino acids connected by solid lines. Proposed disulfide linkages are indicated by double lines. Hydrophobic amino acids are grouped with a solid background; neutral amino acids with a heavily outlined circle; hydrophilic amino acids with a light circle; glycosylation sites indicated by tridents. A heavy solid line indicates the point of proteolytic cleavage of the GP-C precursor protein to yield GP-1 and GP-2. Cysteines are highlighted by larger red circles. The surface membrane of the virus is indicated by a solid purple rectangle, the amino-terminal hydrophobic region by a yellow rectangle, and the conserved B-cell epitope by a blue rectangle. The two proposed antiparallel helices are labeled "AmphiHelix" for the extended heptad repeat, and "CPI Helix" for the charged, pre-insertion helix.

acids substituted is generally conserved. In particular, while none of the central 4 heptad amino acids (underlined and in bold) are identical in each virus, in all cases the hydrophobic character of the heptad repeat is maintained.

The apical domain is the only region to be glycosylated, also in line with a number of TM proteins including that of HIV-1 and other Retroviruses. The apical sequence, particularly the peptide KFWYL in LCMV or KYWYL in Lassa, defines a broadly-cross reactive antibody epitope shared by these viruses [18] that is in precisely the same topographical location as the broadly-reactive apical epitope (positions 598-609, LGIWGCSGKLIC) that has been finely mapped in HIV-1 [19]. Also like that in HIV-1, it is responsive to multimer conformation, and increasingly exposed after receptor binding that results in release of the binding subunit, GP-1 [13].

The second helical region has properties similar to that of the Retroviruses and Filoviruses, in that it is highly charged (30%) and amphipathic, with its helicity possibly stabilized by multiple ion pairings of acidic and basic residues, as first noted for the corresponding region of HIV-1 [3]

Although Lassa fever and Ebola viruses represent different virus families, both helices share an unexpectedly high sequence homology. The first lies in the amino-terminal half of the extended amphipathic helix. As shown in a concentric helical wheel projection in Figure 2A, when the helices are oriented with respect to the exclusion of charge and the heptad repeats for each sequence, 9 identical or highly similar amino acids (50%) may be aligned in each sequence.

The carboxy-terminal helical region also has properties in common with the similarly located helices in both Las-

Conclusions

The most likely explanation for such high levels of similarity among Arenaviruses and Filoviruses would be divergence of both of these agents from a common viral ancestor. Since both virus families exhibit type variation over large areas coupled with stability among isolates within a more limited geographical area over considerable periods of time (the Arenaviruses being the more widespread) such divergence must have occurred eons ago. The potential importance of such apparent conservation in the biology of these agents is underscored by noting that of the corresponding peptide sequences within the TM superfamily of proteins, that for HIV-1 forms the center of a peptide analogue shown to inhibit fusion in the nanomolar range [20].

Modeling studies begun in the late 1980s have thus revealed a number of common and sequence motifs, subsequently shown in several cases to have homologous biological roles in infection, that were not otherwise apparent in studies of sequence homology. These models may lead to a common strategy of antiviral inhibition preventing entry of virus into host cells that is broadly applicable over a broad range of very diverse virus families.

Materials and Methods

Molecular Modeling

Sequences used for this analysis were LCMV - ARM (Genbank P09991) and Lassa, Josiah (Genbank P08669), and are numbered from the initiation methionine. Detailed models of the Arenavirus GP-1 proteins were determined by the methods of Gallaher et al. previously described [3,4,21] A consensus of several independent structural algorithms is used, and compared for different GP-2 sequences to test the consensus. The resulting model is an average consensus of the algorithms for these two sequences. Models are projected in helical net or helical wheel projections also as previously described.

Peptide Synthesis and Circular Dichroism

A peptide corresponding to amino acid positions 326-355 of LCMV-ARM-4 (Genbank VGXPLM) in single letter code, NKAALSFKFEDVESALHLFKTTVNSLISDQ, with an additional histidine at the N terminus was synthesized by standard BOC chemistry using double coupling and HF cleavage. The peptide was purified by reverse phase HPLC on a C-18 column and the peptide's weight confirmed by mass spectroscopy. The peptide was selected as predicted by the Lupas algorithm [22] to have a greater than 90% probability of forming a heptad repeat in the native protein structure. Peptide samples for circular dichroism (CD) were prepared at 0.1 mg/ml concentrations in either 1 mM NaCO₃, pH 7.2 (Neutral) or in

100 mM MES, pH 5.5 (Acid). In spectra recorded with TFE, the TFE was present as 45% of solution volume. CD spectra were recorded from 300-180 nm with 0.5 nm steps with a pathlength of 0.1 cm and at 4°C. Final values were determined using the average of 15 spectra which were correlated with baseline spectra of buffer samples. A characteristic alpha helical spectrum was apparent for the peptide when placed in TFE with a positive peak at 195 nm ($\Theta = +43000$) and a second minimum peak at 210 nm ($\Theta = -25000$).

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