Effects of fusion activation on pleomorphicity

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Abstract: *This research investigates Effects of fusion activation on pleomorphicity. The results Statistical analysis were done to further clarify the effects of fusion activation on virus shape. It was concluded that the shape of LCMV native and PICV native up to 900 remain the same even after fusion activation. The shape remained changed and PICV fusion activation remained the same shape as the vesicles. In the case of LCMV fusion activation the shape remains the same whether it was treated with lithium chloride or by lowering the pH. It was further revealed Statistical analysis were done to further clarify the effects of fusion activation on virus shape. It was concluded that the shape of LCMV native and PICV native up to 900 remain the same even after fusion activation. The shape remained changed and PICV fusion activation remained the same shape as the vesicles. In the case of LCMV fusion activation the shape remains the same whether it was treated with lithium chloride or by lowering the pH.*

Key Words: *Effects, fusion, activation*

Introduction.

In this chapter particles were treated with conditions that simulate fusion activation in the glycoprotein. These "fusion activated" particles were then subjected to tests to determine the consequences of fusion activation on the virion core. Virus membrane fusion can take place either at the plasma membrane or at an intracellular location following virus uptake by endocytosis [173] [174]. The fusion reactions of viruses that fuse directly at the plasma membrane are triggered by virus–receptor interactions at neutral pH, as discussed below. By contrast, the fusion of many other viruses is dependent on their internalization by receptor-mediated endocytic pathways such as clathrin-dependent, caveolae-dependent uptake or nonclathrin- dependent, non-caveolae-dependent uptake [174] [175]. Viruses that use such routes frequently have fusion reactions that require exposure to mildly acidic pH within organelles of the endocytic pathway [176]. Although it is unclear why viruses use such a variety of entry pathways, it is possible that those viruses that have evolved to fuse intracellularly gain a selective advantage from releasing their genomes at specific intracellular sites [177].

In order to understand the functional implications of changes in virus protein architecture viral proteins role fusion activation was done. It is not yet known which of arenavirus proteins play a pivotal role in making the particles shape round and which of proteins play a role in making the particles elliptical. In chapter 4 it was concluded that GP and NP play a small but important role in regulating virion shape and size.

In order to understand effects of fusion activation on pleomorphicity and shape and size of the viruses, two populations of arenavirus were treated, one population with lithium chloride and the other population by lowering pH 5.5. As a result GP1 dissociates and GP2 elongated and Z and NP became disorganized. The effects of fusion activation can be seen in figure 5.1.

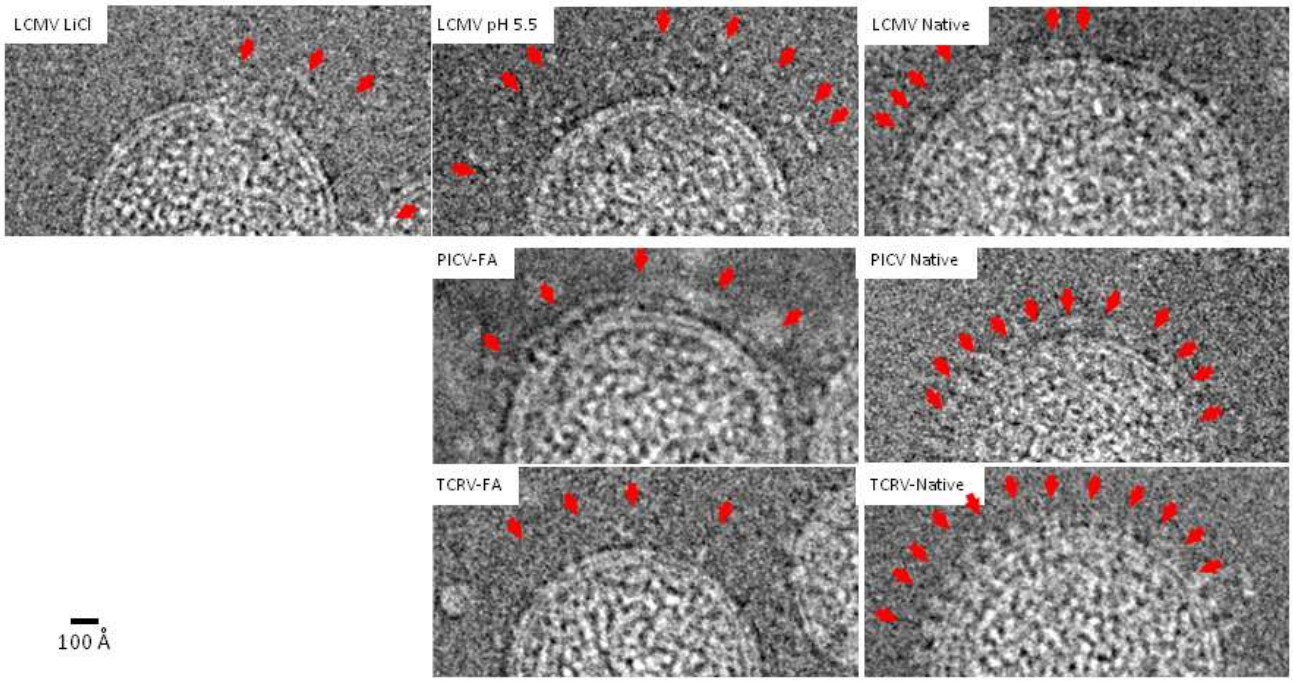

Figure.5.1 Initiated by acidity or strong salts. GP-1 dissociates from the virion and NP remains inside the virion GP-2 elongates Z and NP become disorganized but internal density does not change. Arrow shows GP on the virion of native virus and arrow in the fusion activated viruses shows GPs displaced but just far from the virion.

5.2 Fusion Activation Affects Pleomorphicity:

In order to understand the proteins' role in virus shape and size, the longest and shortest diameter of Fusion-activated LCMV were measured. As shown in figure 5.1 fusion activation shifted the shape curve rightward and had a flattening effect, corresponding to the presence of fewer round virions and more elliptical virions. As shown in figure 5.2 the size distribution of virions remained essentially unchanged after fusion activation suggesting that the observed change in shape was not the result of virion-virion fusion. Figure 5.1 and 5.2 also demonstrate that both low pH and high salt conditions produce the same effect on virion shape. Vesicle shape on the other hand was not strongly effected between method of fusion activation although this observation was based on a small sample size (date not shown).

This might be due to changes upon fusion activation by acidic pH or Lithium chloride as GP-1 dissociates from the virion causing particles to appear "spikeless",GP-2 changes shape and appears longer is no longer visible near the viral membrane and NP remains inside the virion (see figure 5.2). As it was hypothesized, GP has an important role in forming the shape of virus particle they are.

An interesting and unexpected effect of fusion activation was the disappearance of the strong correlation between size and shape that was observed in native LCMV.

As shown in figure 5.3, the correlation between size and shape is virtually abolished after fusion activation.

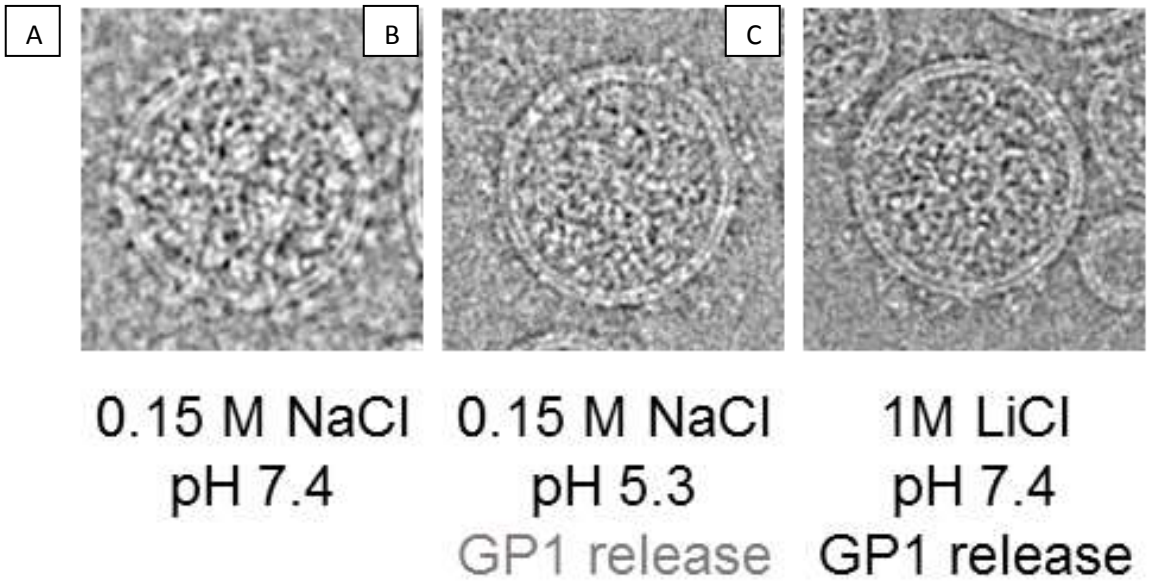

Figure 5.2.LCMV particles from right to left. (A) Normal LCMV particle (B) GP 1 releases from fusion affection by lowering pH (C) and this particle is treated with lithium chloride [178].

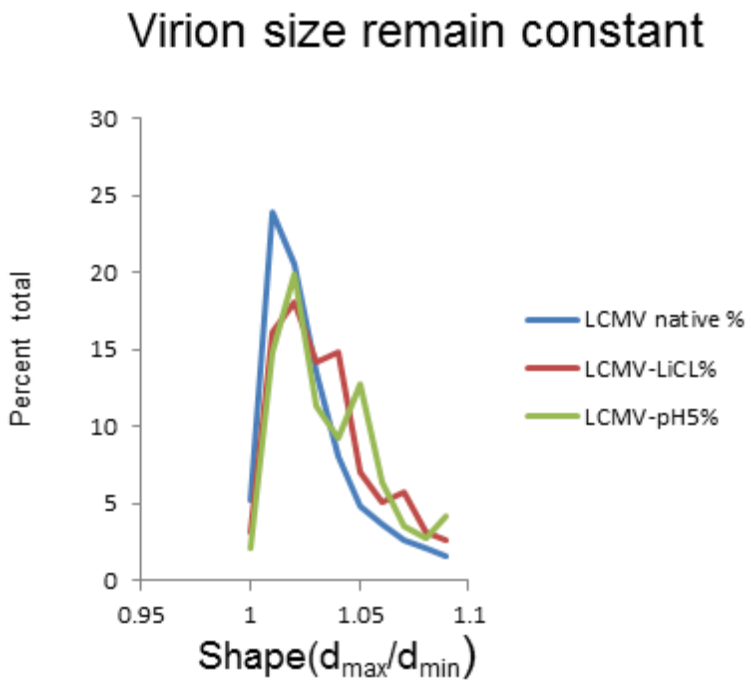

Figure 5.3 Virion size remain constant.

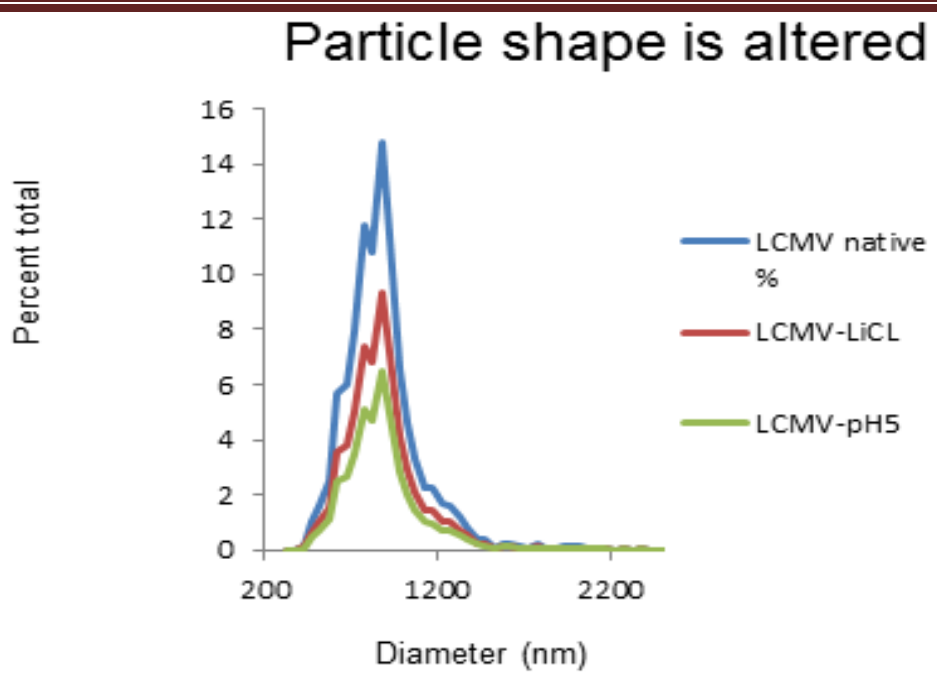

Figure 5.4 Particle shape is altered.

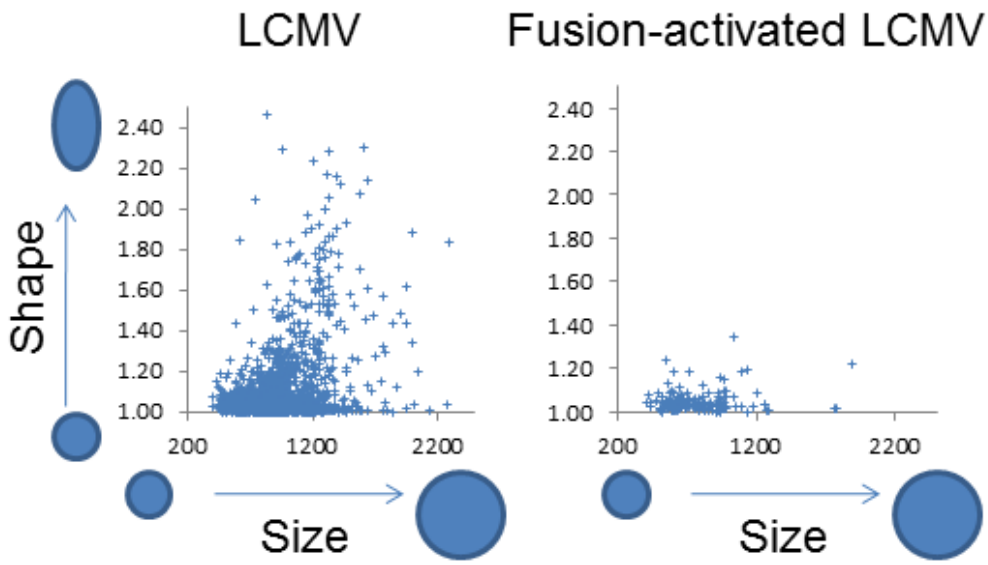

Figure 5.5 Positive +ve number shows size is related and Negative –ve number shows size is not related.

Statistical analysis were done to further clarify the effects of fusion activation on virus shape. It was concluded that the shape of LCMV native and PICV native up to 900 remain the same even after fusion activation. The shape remained changed and PICV fusion activation remained the same shape as the vesicles. In the case of LCMV fusion activation the shape remains the same whether it was treated with lithium chloride or by lowering the pH.

Table 5.1. Showing average shape, standard deviation and P values of statistical comparison of LCMV Native, LCMV LiCL, LCMV-pH5, PICV Native, PICV FA and vesicles all.

Virus		Avg Shape		SD	T test native and FA vs vesicle	T test Native vs Fusion activated
LCMV Native	0-900	1.04	±	0.06	1.1×10^{-7}	----------
	all	1.07	±	0.17	0.20	
LCMV-LiCL	all	1.05	±	0.05	9.9×10^{-5}	0.01
LCMV-pH5	all	1.05	±	0.05	3.6×10^{-4}	2.3×10^{-5}
PICV Native	0-900	1.03	±	0.05	8.3×10^{-10}	----------
	all	1.03	±	0.05	1.1×10^{-9}	
PICV-FA	all	1.08	±	0.08	0.53	3.6×10^{-12}
Vesicles	all	1.08	±	0.23	----------	----------

The effects of fusion activation on the distribution of virions shapes, as opposed to the effect on the mean shape described above, is presented in figure 5.6. In this figure, data was sorted according to size and the average shape of cohorts of 5% of the dataset each was calculated. This visualization confirms the observation above that virions become uniformly slightly deformed after fusion activation in both the surrounding and elliptical populations. The mechanism for this change is not clear from this data.

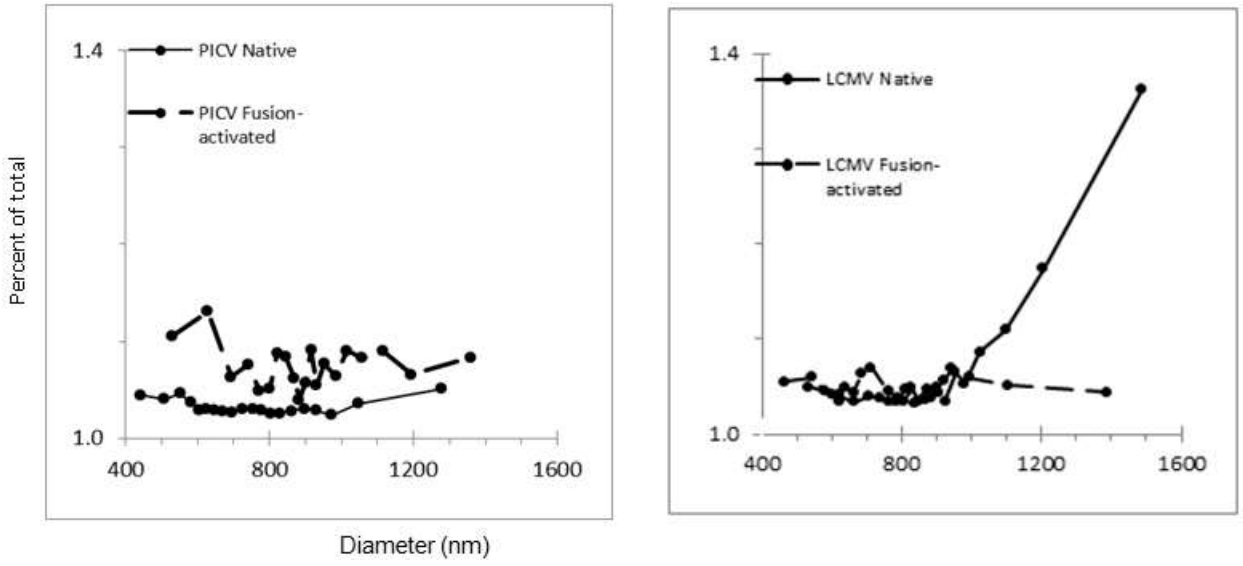

Figure 5.6. Scatter plot showing the variation in size of (right) PICV native and PICV fusion activated and (left) LCMV native and LCMV fusion activated.

Fusion activated virus becomes almost the same shape as the vesicles, and size is no longer related to shape. Correlation coefficient results show that fusion activation does not affect virus shape (See table 5.1).

5.3 Discussion:

According to Barble Kaufmann *et al* [179] study, cryo-electron microscopy (cryo-EM) was used to examine West Nile Virus (WNV) of of the family Flaviviridae complexes with E16 antigen binding fragments (Fab) after exposure to low pH. The virions were trapped irreversibly as a pre-fusion intermediate with the E glycoprotein/Fab layer expanded radially outwards leaving an ~60 Å -wide gap between the lipid bilayer and the outer protein shell. These structural data suggest that the low pH-triggered formation of fusion-active E homotrimers on the viral surface is preceded by the outward extension of the E stem region [179].

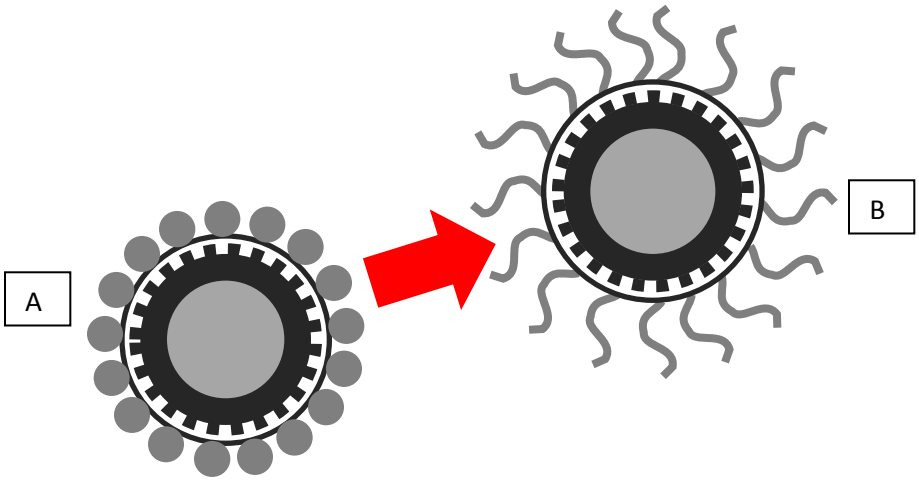

Figure 5.7 Showing the organization of arenavirus GPC in native and fusion activation.(A) Normal particle remains (B) GPC changes shape during fusion activation – this may lead to disorganization of Z and NP.

According to Di Simone *et al* [180] that membrane fusion activity of the glycoprotein (GP) complex of LCMV is activated in a low pH dependent fashion. These experiments revealed that after exposure to acid pH the LCMV glycoprotein spike complex underwent irreversible conformational change in which GP-1 was dissociated from the virion, conformational epitopes on GP-1 were lost, and sequestered epitopes on GP-2 became exposed. Further, LCMV infectivity was irreversibly inactivated by exposure to acidic pH (<6.0), likely due to the loss of GP-1 and conformation changes in GP-2. Here it has been shown for the first time that structural changes in the virion core of arenaviruses can also be detected, and that these changes alter virion shape (see figure 5.7).

According to Kielian *et al* [181] virus membrane fusion protein drive the fusion reaction by undergoing a major conformational change that is triggered by interaction with the target cell. The specific trigger depends on the virus for example influenza viruses, alphaviruses and flaviviruses which are an identical example of viruses that fuse upon exposure to low pH in the endocytic pathway or in the test tube [173, 176] [182, 183]. By contrast fusion of HIV-1 happens at neutral pH and is triggered by the sequential interaction of the virus fusion protein with the receptor CD4 and coreceptor such as CCR5 or CXCR4 members of the 7 TM domain chemokine receptor family [184] [185]. According to Da Poian [186] glycoprotein G of rhabdoviruses fusion mechanism is different from the well-known classes of 1 and 2 fusion protein as per their review [186].

Conclusion

The current study revealed that cryo-electron microscopy (cryo-EM) was used to examine West Nile Virus (WNV) of of the family Flaviviridae complexes with E16 antigen binding fragments (Fab) after exposure to low pH. The virions were trapped irreversibly as a pre-fusion intermediate with the E glycoprotein/Fab layer expanded radially outwards leaving an ~60 Å -wide gap between the lipid bilayer and the outer protein shell. These structural data suggest that the low pH-triggered formation of fusion-active E homotrimers on the viral surface is preceded by the outward extension of the E stem region.

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