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Interaction between Aedes aegypti CPB1 and viral proteins

Interacción entre Aedes aegypti CPB1 y proteínas virales

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Abstract

The viruses chikingunya (CHIKV), zika (ZIKV) and mayaro (MAYV) are etiological agents of tropical diseases that represent a public health problem. The mechanisms of infection are produced through the Aedes aegypti vector, where a series of viral proteins interact, such as the protein of dengue virus E pro-tein (DENV). Therefore, the identification of surface proteins similar to this allows a better understanding of the internalization mechanisms in their respective vector; that in the future it will facilitate the generation of new therapeutic agents.

The aim of the present work is to analyze, by means of silica methods, proteins similar to the DENV E protein, where the organisms of interest are CHIKV (protein E1), ZIKV (protein E) and MAYV (protein P130) also to propose sites of Interaction between the proteins of the capsid and the intestinal protein CPB1 of the vector. Clustal Omega, I-TASSER, ClusPro online servers and the Protein Data Bank (PDB) database were used for this purpose. In addition to the bioinformatics tools TMPRED and BLAST.

The binding sites, the possible interacting attributes, the types of existing links and their type of environment were elucidated. This study received a history of molecular interaction in silica, with which it can be approached in a guided way, with in vitro or in vivo studies, the problem of diseases such as dengue and zika.

Molecular Coupling (Docking), Aedes Aegypti, Dengue, Zika, Chikungunya, Mayaro, CPB1

Resumen

Los virus chikingunya (CHIKV), zika (ZIKV) y mayaro (MAYV) son agentes etiológicos de enfermedades tropicales emergentes que representan un problema de salud pública. Los mecanismos de infección se producen a través del vector Aedes aegypti, donde interactúan una serie de proteínas virales, como la proteína del virus del dengue E pro-teína (DENV). Por lo tanto, la identificación de proteínas de superficie similares a esta, permite una mejor comprensión de los mecanismos de internalización en su vector respectivo; que en el futuro facilitará la generación de nuevos agentes terapéuticos.

El objetivo del presente trabajo es analizar, mediante métodos in silico, proteínas similares a la proteína E de DENV, donde los organismos de interés son CHIKV (proteína E1), ZIKV (proteína E) y MAYV (proteína P130) también para proponer sitios de interacción entre las proteínas de la capside y la proteína intestinal CPB1 del vector. Clustal Omega, I-TASSER, servidores en línea ClusPro y la base de datos Protein Data Bank (PDB) fueron utilizados para este propósito. Además de las herramientas bioinformáticas TMPRED y BLAST.

Se elucidaron los sitios de unión, los posibles aminoácidos que interactúan, los tipos de enlaces existentes y su tipo de entorno. Este estudio permitió determinar un antecedente de la interaccción molecular in silico, con el que se puede abordar de forma guiada, con estudios in vitro o in vivo, el problema de las enfermedades como el dengue y el zika.

Acoplamiento Molecular (Atraque), Aedes Aegypti, Dengue, Zika, Chikungunya, Mayaro, CPB1

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Introduction

Diseases that are transmitted by vectors, especially mosquitoes, are among the main causes of morbidity and mortality in humans. Aedes aegypti and Aedes albopictus are vectors that can infect human viruses, generating (to name a few diseases) dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), emerging diseases worldwide transmitted by insects that threaten a third of the human population.

During the bite of the female mosquito to a person infected by the DENV, CHIKV, MAYV or ZIKV, it usually contracts, becoming a carrier. Once the virus enters the mosquito system in the blood, the virus spreads from the intestine to the salivary glands of the mosquito and can then transmit the virus to another person while feeding. (Kabra SK, 1999)

That said, it was thought that the internalization method should be shared among different viruses, so the following research question was posed: is there structural convergence between viral proteins whose vector is Aedes aegypti?

Over the years, bioinformatics has generated great theoretical contributions that have subsequently been tested both in vitro and in vivo. That is why it is currently considered an important antecedent for any biological research.

Our work reflects the possibility of interaction of the selected proteins with the protein carboxypeptidase 1 (CPB1) in Aedes aegypti. CPB1 is located in the intestinal cells of the dipterid, which is experimentally defined as participating in the internalization of DENV, where the E protein of this pathogen is anchored to domain II of it. (Hong-Wai Tham 1, 2014).

This work postulates molecular interactions between proteins that are structurally similar to the E protein of DENV, so, we believe that its mechanism of internalization can be carried out by the same receptor protein in Aedes, we speak of CPB1.

Proteins of pathogens such as DENV and ZIKV (Flavovirus), CHIKV and MAYV (Togavirus) were evaluated. Where the inclusion criteria were: to be superficial, structurally conserved domains and sequential homology with the E protein of DENV.

Methodology

Homology of sequences by the algorithm of close neighbors

Initially, the possibilities of analyzing and structurally comparing proteins from different viral organisms were considered, with the purpose of identifying if in addition to "homology" there was functional similarity. However, the point of convergence selected for the analysis of the mentioned viral proteins was the fact of owning the same vector (Aedes aegypty). It is known that these viruses use the aforementioned dipterous as a transport, for which reason they considered the possibility that they use similar internalization mechanisms.

Once the organisms of interest were defined, they were analyzed using the technique of "homology of sequences by algorithms of close neighbors". The product obtained was a phylogenetic tree. Which is composed of nodes and branches; These nodes can represent either an individual, a species, or a higher grouping and are therefore widely termed taxonomical units. In this case, the terminal nodes represent the species of analysis and are the Operational Taxonomic Units (OTUS). The ordering of the nodes determines the topology of the tree and describes how the lineages have diverged in the course of evolution. The branches of the tree represent the amount of evolutionary divergence between two nodes in the tree and can be based on different measurements.

A tree is completely specified by its topology and the set of all the lengths of the edges. (Woese C., 2000)

Sequential analysis of the proteins of interest with Clustal Omega

Once the proteins of interest for each of the organisms were identified, the similarity of their sequences was analyzed. This was done through the "Clustal Omega" server (http://www.ebi.ac.uk/Tools/msa/clustalo/).

When the homologies were obtained respectively for each protein, the next step was to individually analyze different characteristics such as the existence of transmembrane domains, using the TMPRED tool (http://embnet.vitalit.ch/software/TMPRED_form.html)

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A BLAST of the E1 protein of DENV was made in the PDB and NCBI database obtaining no crystallizable version. Therefore, we proceeded to use bioinformatics tools such as the I-TASSER server (http://zhanglab. ccmb.med.umich. edu/I-TASSER/) which allowed us to make a structural prediction of the mentioned protein.

Molecular docking and search for interacting sites

Once the generated model was obtained and selected, we performed a molecular Docking using the Clus-Pro server (https://cluspro. bu.edu/home.php). At the end of this process we observe and analyze the molecular couplings, selecting the models generated with structurally more stable scores (Balanced, Electrostatic, Hydrophobic and VdW + Elec).

Following the process, the software Swiss-PDBViewer v. 4.1.0 in each one of the proteins of interest were introduced, in order to observe their supposed interacting sites.

Organism	Protein	Localization
DENV	E	None / I-TASSER
MAYV	P130	Q8QZ72 – UniProt
CHIKV	E1	3N42 - PDB
ZIKV	E	None / I-TASSER
Aedes a.	CPB1	None / I-TASSER

Table 1 Location of viral proteins. It shows the IDs in different databases respectively for each protein of interest. "None" refers that there is no crystallographic structure of the protein, for which models were generated by homology.

Results

Homology of sequences by the algorithm of close neighbors

The diagram shows the relationship between the capsid protein sequences of some viruses, where the grouping of the proteins coming from different serotypes of DENV is clearly observed, immediately to this it bifurcates having on the one hand ZIKV with a value of 0.429 of its most immediate node and the CHIKV and MAYV proteins that are part of another branch with a distance of 0.064 and 0.495, respectively.

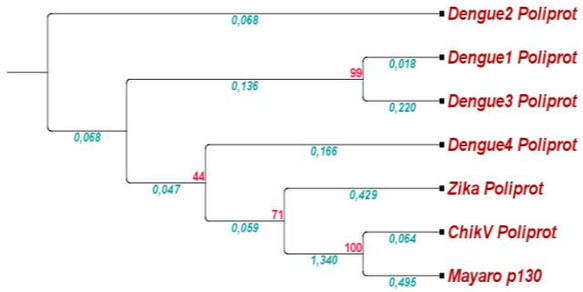

Figure 1 Phylogenetic tree of viral structural proteins. In blue refers phylogenetic distance between the sequences of the proteins to be studied and in red the percentage of times that the calculation generated this same results.

Sequential analysis of the proteins of interest with Clustal Omega

The server was used introducing the sequence of each of the proteins of interest, being the E1 proteins of CHIKV, p130 of MAYV (Togaviridae family), E of DENV and ZIKV (Family Flavoviridae). With what was obtained the following table shows the percentage of homology among all these.

	1	2	3	4	5	6	7
1.DENV1	100						
2.DENV2	72.04	100					
3.DENV3	79.17	66.67	100				
4.DENV4	69.06	69.33	65.48	100			
5.MAYV	17.15	17.52	17.95	18.61	100		
6.ZIKV	57.75	54.50	-nan	56.25	17.65	100	
7.CHIV	16.58	17.60	-nan	18.93	60.32	-nan	100

Figure 2 Homologous-sequential analysis of the proteins of interest. Alignments of biologically significant multiple sequences of amino acid sequence were produced. Evolutionary relationships are seen in percentage.

Molecular docking and search for interacting sites

Complementing the results, we used the TMPRED server (http://www.ch.embnet .org/software/TMPRED_form.html), with the idea of splicing the possible transmembrane sites with the interacting assumptions obtained from the previous study. Figure 3.

Obtaining significantly positive sites in the first 25 aa (being able to be a signal peptide), as well as approximate amino acids at 150, 225, 320 and 400. And sites statistically less significant at 150-200 and 350.

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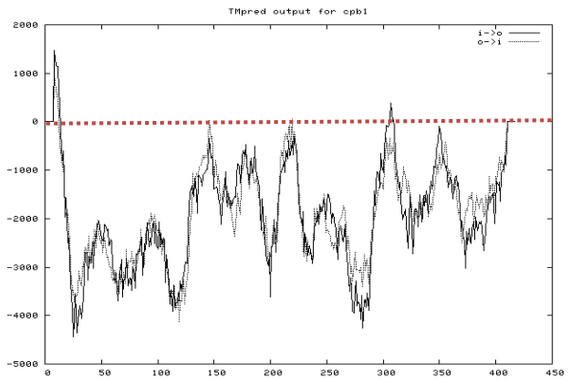

iFigure 3 Possible transmembrane sites of CPB1. The algorithm is based on statistical base analysis, a database of natural transmembrane proteins. Sequences of amino acids close to zero (dotted line) will be the statistically most likely to be transmembrane sites.

We used standard E protein in DENV, which Hong-Wai T. and Vinod R.M.T in 2014 reported their interacting amino acids with CPB1 protein in *Aedes aegypti*.

We managed to reproduce these same results in our work, apart from the fact that in parallel we obtained new ones with the different proteins mentioned.

The joining energies being the following: DENV (-203.3), MAYV (-157.3), ZIKV (-210.4) and CHIKV (-247.0).

The results of homology of sequences by means of the algorithm of close neighbors revealed the similarity between the analyzed proteins, existing between each one of the sequences "possible common ancestors", which explains the possibility of having similar functions, such as the inter-nalization to the Aedes enterocyte in the same way, that is, through CPB1.

We were able to reproduce the previously published results on the interaction between the E protein of DENV and CPB1 in Aedes, which confirms the good manipulation of the bioinformatic data.

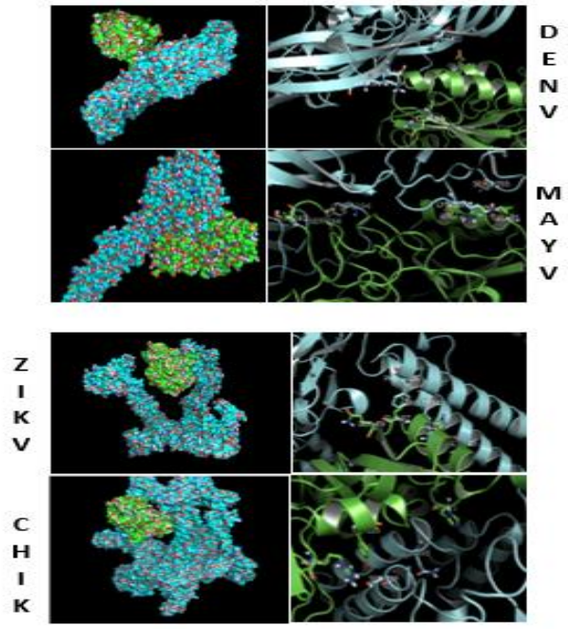

Figure 4 Molecular docking of viral proteins vs CPB1 of Aedes aegypti. In green CPB1 is shown and in blue the respective proteins of the different viruses.

Viral Protein	aa CPB1		aa Viral Protein	
E (DENV)	GLN	89	THR, ASN, THR	66,67,68
	GLU	43	LYS	122
P130 (MAYV)	GLU, ARG	35,42	LYS	1060
	ASN	39	SER	1055
	SER	244	TYR	1194
E1 (CHIKV)	GLN	89	ARG	2162
	GLU	87	PHE	2436
	GLN	85	ARG, VAL	2894, 2890
E (ZIKV)	HIS	21	---	---
	TYR	225	GLN	465

Table 2 Proposal of interacting amino acids. List of interacting aa in CPB1 and in the viral proteins evaluated.

Discussion

We analyzed the interacting sites of CPB1 with the different proteins, obtaining that between the E protein of DENV and the E1 protein of CHIKV there are very similar couplings, as well as the p130 protein of MAYV and the E protein in ZIKV.

Therefore, the importance of the prediction of transmembrane helices of CPB1, which demonstrates the possibility of multiple binding sites is noteworthy. In addition, the existing interactions between the viral proteins and CPB1 conserve in all cases hydrophobic and polar characters, which are indispensable in the stability and viability of the proposed models.

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Conclusions

The E protein in DENV is coupled to CPB1 in a similar way in terms of interaction type compared to the other protein models reported here. However, the binding sites were not homogenous, suggesting multiple binding sites in CPB1. The next step will be to evaluate the dimeric or trimeric conformations (depending on the case) of the different viral proteins, with the idea of simulating in a more realistic way the existing interactions in nature.

The binding sites reported here may be used in the future for the development of CPB1 blockers, thus avoiding the excessive propagation of said viruses, and in turn improving public health strategies.

In silico methods provide several advantages for the resolution of biological problems, because through them it is possible to observe molecurlarly a macrophenomeno, in addition to reducing costs (with the use of free servers and academic sofwares) and time in basic science.

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