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Human Rhinovirus VPG Peptide: Insights about HRV Replication

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Abstract

Human rhinovirus (HRV) is the major etiologic agent of the common cold. Currently three species of HRV are described: A, B and C, and apparently there are differences between the clinical presentation in disease dependent of the species. Viral replication is an important aspect of the viral respiratory disease pathogenesis, and in this context VPg peptide plays a key role in replication. The aim of the present study is to provide preliminary information about possible differences in structure and function of VPg peptide that can affect viral replication. Different in silico analysis were used to evaluate the peptide behavior of the HRV species. Amino acid alignment, radius of gyration and root mean square deviation/fluctuation were carried out. An interesting aspect observed during the analysis is that apparently the species C peptide has more positively charged residues (four residues) compared to the species A and B (3 residues). This fact could enhance the binding of VPg C specie with the HRV RNA cis-replication element (cre). The analyzes of the radius of gyration and RMSD/RMSF apparently indicate that the VPg peptide of C species has a higher tensegrity. Based on the presented results, we can conclude that the structural behavior of the VPg peptide species C is different from the species A and B. This difference may reflect a different replicative behavior, which can ultimately affect the clinical presentation of the patient infected with HRV C.

Introduction

Human rhinovirus (HRV) is the major etiologic agent of the common cold [1,2]. Currently three species of HRV are described: A, B and C, and apparently there are differences between the clinical presentation in disease dependent of the species [3]. HRV C has been associated with more severe infection, especially among children under five years [4,5]. The disease caused by HRV different species of is not well understood and consequently the understanding of pathogenesis associated with different clinical phenotypes is important for the future treatments development and patient clinical management.

Viral replication is an important aspect of the viral respiratory disease pathogenesis, and in this context VPg peptide plays a key role in replication [6]. Two important studies demonstrate the role of VPg on HRV replication describe that inserted mutation in the VPg polypeptide precursor (3AB gene) results in defective RNA synthesis [6].

Objective

The aim of the present study is to provide preliminary information about possible differences in structure and function of VPg peptide that can affect viral replication.

Material and Methods

Selection and analysis of the sequences obtained from genetic database

Twenty eight sequences of VPg peptide were selected from international database Gen Bank [7]: 10 sequences of a species; 10 of the B; 8 of the C species. The Bio Edit program v7.2.5. Was used to perform the multiple alignment using CLUSTAL-W [8] in order to verify possible changes between the sequences. Predictions of secondary structure were made using PSIPred servers [9] and Predict Protein [10]. The Predic Protein server [10] was also used to determine possible post-translational modifications and possible interaction sites in proteins.

VPg in silico peptide modeling

There is little experimental structural information of the VPg peptide, since these proteins have a highly hydrophobic region and consequently the production in the laboratory is difficult. The prediction of the tertiary structure of peptides VPg species A, B and C were made by molecular modeling technique using I-TASSER server [11].

Simulations of VPg peptide in solution

In order to evaluate the structural characteristics of VPg

peptides, simulations of VPg structures in 150 mM NaCl solution were carried out. All simulations were performed in solution with GROMACS package version 4.5.5 [12] using the field strength GROMOS53a6 [13]. For the simulation of peptides systems in water three cubic boxes of 6 nm sides were built, one for each model of the VPg peptide structure containing ~ 7000 water molecules and ionic concentration of 150 mM NaCl. The water Model used was SPC "Simple Charge Point" [14]. The optimizations of the systems were performed with change of 5000 steps of Steepest Descent and Conjugate Gradient.

The N-terminus of each protein in model of the study was treated as a positively charged group (NH3 +) and negatively charged C-terminus (COO-), since these regions are present at pH 7.0. It was first performed for each template a 20 ps (picoseconds) simulation position restriction of the peptide structure of atoms to allow relaxation of the ions and the solvent, then 300 ns (nanoseconds) simulation was performed for data acquisition. The integration step for the motion equations was 2 fs (femtoseconds). Two different temperatures were chosen for the simulation: 306 K (33°C) to mimic an upper respiratory tract environment and 310 K (37°C) to mimic a lower respiratory tract environment. The pressure was kept constant at 1 bar. For the pressure and temperature parameters the algorithm of Berendsen was used [15] with time constants of 1.0 ps and 0.1 ps. Periodic outline conditions were imposed on the simulations with the cutting radius equal to 1.4 nm and a neighbors list was updated every 5 integration steps. The LINCS algorithm [16] was used to restrict the protein covalent bonds. The long-range electrostatic interactions were treated using the method SME (particle mesh Ewald) [17] at 1 nm cutting radius. To calculate the electrostatic contributions of short-range and Van der Waals interactions we used cutting radius of 1.4 nm.

RMSD and RMSD

The value of the root mean square deviation (RMSD) is a measure of the average distance between atoms of a molecule structure relative to a reference structure, which may be the initial structure. In protein conformations studies the RMSD is calculated by overlaying a structure with any frame of reference. The overlapping is performed with all atoms or with only the main chain atoms (N, Cα and C). The RMSD may be calculated by the following equation:

$$RMSD(t_1,t_2)=\left[\frac{1}{M}\sum_{i=1}^{N} m_i \parallel r_i(t_1)-r_i(t_2)\parallel^2\right]^{\frac{1}{2}}$$

Where $m=\sum_{i=1}^{N} m_i$ and $r_i(t)$ represents the atom position i in time t.

The root mean square fluctuation (RMSF) calculates the fluctuations of atomic positions during a certain trajectory. From the calculation of RMSF you can also get a .pdb file with RMSF values converted to beta factor values. The factor beta is a color spectrum representation which region is more or less stable during the simulation, the most stable regions depicted in blue and red regions represents lower stability.

Radius of gyration (Rg)

To analyze the compression of a structure, the radius of gyration of a structure along its trajectory is calculated. The can be calculated by the following equation:

$$Rg=\left(\frac{\Sigma_i \parallel r_i \parallel^2 m_i}{\Sigma_i m_i}\right)^{\frac{1}{2}}$$

Where m_i the atom is mass, i and r_i is the position of the atom i in relation to the mass center of the molecule. In the present study the Rg was used to analyze the structural behavior of VPg peptides in solution.

Results

In the sequences alignment of the three species (A, B and C) some mutations were observed, especially the species with C higher number of mutations throughout the sequence [18]. It was also noted that amino acids near the N-terminal region are more conserved among inter and intra species (Figure 1).

Species A presents six mutations positions (positions 10, 11, 12, 13, 18 and 20). Of these, three (positions 11, 12 and 18) occur between amino acids of same characteristic, and three between amino acids of different characteristics (positions 10, 13 and 20). The comparison of the isoelectric point values (pI) of the HRVs A sequences was performed between sequences 66, 89, 16, 55, 07, 89 and 01 versus sequences 28, 80 and 101. The figures indicated a slight change of value pI between these two groups of sequences being 10:27 to HRVs the 66, 89, 16, 55, 07, 89 and 01 and pI 9.99 to HRVs the 28, 80 and 101. The values indicate a small alteration in the pI value between these two groups of sequences with 10.27 to first group and 9.99 to the second.

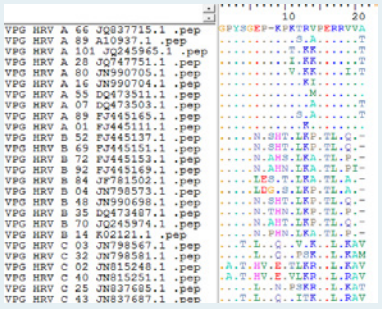

Figure 1: Alignment of the HRV VPg peptide amino acid sequences. The number after each species represents serotypes and after serotype represents the Gen Bank accession number.

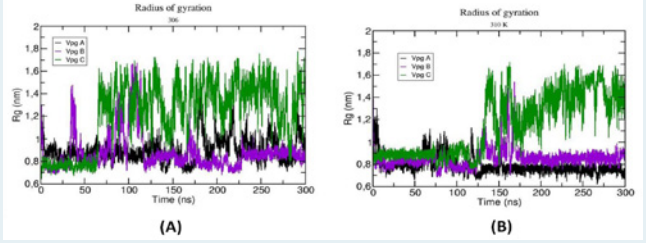

Figure 2: radius of gyration of VPg peptide in simulation time function. Simulations in water with 150 mM NaCl at 33°C/306 K (A) and 37°C/310 K (B). The increase in the value of the radius of gyration indicates a structural model change to a more linear conformation.

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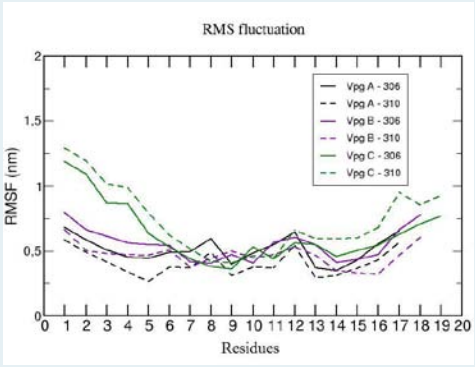

Figure 3: Root mean square fluctuation position of the VPg residue models of serotype A, B and C. Water simulations with 150 mM NaCl at 33°C (306 K) and 37°C (310 K). Higher values of fluctuations indicate regions with the highest degree of freedom.

The species B presented 8 mutations at positions 6, 7, 8, 9, 10, 14, 19 and 20. Of these mutations, 4 are between amino acids of same characteristics (positions 9, 10, 14 and 20) and 4 between amino acids with different characteristics (positions 6, 7, 8 and 19). The pI of the sequences were calculated by Prot Param resulting in a pI of 10.0 for HRV B 4 and 84, slightly lower when compared to other sequences of the same species that presented pI equal to 10.29.

In the analysis of species C 11 mutations wsere found (positions 2, 4, 6, 7, 8, 10, 11, 12, 13, 18 and 20), 4 between same characteristics amino acids (positions 2, 4, 13 and 18) and 7 between amino acids with different characteristics (positions 6, 7, 8, 10, 11, 12 and 20). The pI values of the sequences HRV C 02 and 40 were compared with the values of the other sequences. The calculated values were 9.7 for the sequence HRV C 02 and 9.98 for HRV C 40 sequence, while for the other sequences values ranged from 10.28 to sequences (HRV C 15, 25, 26 and 43), 10.45 to sequence C HRV 03 and 10.0 to sequence of HRV C 32.

The HRV C sequences presented higher variation of pI values, as well as higher number of mutations. All species showed mutations, especially in the central region. These mutations in all serotypes were between amino acids with different characteristics. However, the species A and B proved to be the most highly conserved in the N-terminal region compared to C specie. Studies have shown that the VPg peptide is connected to the 5' terminal poliovirus RNA by a phosphor diester bond with the tyrosine residue [19]. All serotypes presented the tyrosine residue (Y) at the 3'conserved position. The Predict Protein software also indicated possible interaction sites with other proteins. For all three species, these sites were predicted in the N-terminal regions of VPg peptide, where tyrosine residue is present. The following sequences (accession number JN990704.1, FJ445151.1, JN798581.1) were chosen for structural and functional analyzes. To check this preliminary prediction analyzes the software Predict Protein (https://www.predictprotein.org/)were used. Regarding the secondary structure, the high percentage of coil structures is present in all three species (85% for species A, species B to 100% and 75% for type C). The server also indicated a high value of exposed residues (90%) for the species A and B, however this analysis could not be performed for the species C. The models for the species A and B showed more linear configuration, confirming the exposed amino acids.

Other analyzes conducted through the Predict Protein online software indicated the presence of interaction sites in proteins at positions 1, 12, 13 and 17 to species A, positions 1, 3, 5, 18, 19 and 20 for the species B, and positions 1 and 5 for the species C. The positions 1 was predicted as interaction site with other proteins for all species, but not appear in species A, and the position 5 was also predicted as the site of interaction with proteins in species A and B. These two positions appear at the N-terminal regions that were high conserved among the three species.

Simulations of VPg peptide in solution

The Figures XX and XX respectively indicate the radius of gyration and the average position of the atoms of each residue in model simulation time function. These data indicate that all peptides have identical behavior in both temperatures which mimic the upper respiratory tract (33° C or 306 K) and for temperatures that mimic the lower respiratory tract (37° C or 310 K). The VPg peptides of the species A (black) and B (purple) presented more similar structural features as compared to the species C (green).

The results present in Figure 15 shows the difference between the species A and B when compared to serotype C. Both species A (black lines) and species B (purple line) showed constant fluctuations of amino acids along the whole length of the chain while the C species (green lines) showed the most flexible N-terminal region. This result was observed for both temperatures.

Discussion

An interesting aspect observed during the analysis is that apparently the species C peptide has more positively charged residues (four residues) compared to the species A and B (3 residues). This fact could enhance the binding of VPg C specie with the HRV RNA cis-replication element (cre). The analyzes of the radius of gyration and RMSD/RMSF apparently indicate that the VPg peptide of C species has a higher tensegrity [20,21], therefore this peptide presents better balance when subjected to different tensions and compressions forces. Due to better tensegrity a hypothesis based on the results obtained in the present study is that the VPg peptide of C specie may have an improved ability to adapt to different conditions, particularly with respect to upper and lower respiratory tract temperature variation. The results of the computational analysis indicate that the VPg peptide of species C is structurally different from the other two species, A and B, which may possibly reflect functional differences.

Conclusion

Based on the presented results, we can conclude that the structural behavior of the VPg peptide species C is different from the species A and B. Both species (A and B) have the similar structural behavior. This difference may reflect a different

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replicative behavior, which can ultimately affect the clinical presentation of the patient infected by the HRV C.

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