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Understanding of High Pathogenicity of the Avian Influenza Virus H5N1: Why H5 is Better Cleaved by Furin

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The origin of high pathogenicity of the emerging avian influenza H5N1 due to the -RRRKK- insertion at the cleavage loop of the hemagglutinin H5 was studied using molecular dynamics technique in comparison with the non-inserted H5 and H3 bound with furin active site. The cleavage loop of the highly pathogenic H5 was found to bind strongly to the furin cavity, serving as a conformation suitable for the proteolytic reaction. Experimentally, the -RRRKK- insertion was also found to increase the cleavage of hemagglutinin by furin. The simulated data provide a clear answer to the question why inserted H5 is better cleaved by furin than the other subtypes, explaining the high pathogenicity of avian influenza H5N1.

1 Introduction

Proteolytic activation of the hemagglutinin (HA) is essential for viral infectivity and for spread of the avian influenza virus. This process is determined by a cleavage reaction at HA's cleavage site, a conserved arginine, by host proteases. Insertion of the -RRRKK- residues into the low pathogenic avian influenza (LPAI) cleavage site is known to potentially activate infectivity of viruses, i.e., the LPAI viruses, which then become high pathogenic avian influenza (HPAI) viruses, allowing highly virulent strains to be cleaved by furin, an ubiquitously expressed protease.The proposed cleavage mechanism is shown in Fig. 1. Understanding of this fact, why furin cleaves the inserted hemagglutinin strains better than non-inserted strains, is the goal of this study. Therefore, molecular dynamics simulations were carried out for the three complexes, HPH5-FR, LPH5-FR and LPH3-FR. The investigation was focused to intra- and intermolecular interactions and geometries of the substrate-furin complex, potentially involved in the cleavage mechanism.

2 Methodology

The initial model for the HPH5-FR loop (RERRRKKRGL) was built up using the sequence alignments and the atomic coordinates of the X-ray structure (residues 322-331: NVPEKQTQGL) of the HA0 of H3 and dec-RVKR-cmk inhibitor of furin[1] as a template, performed by using the homology module of the Insight II program. For LPAI subtype H5, the initial structure of the cleavage loop (NVPQRETRGL) was constructed using the backbone atoms of the HPH5 loop built previously. The HA$'$s cleavage loop complexed

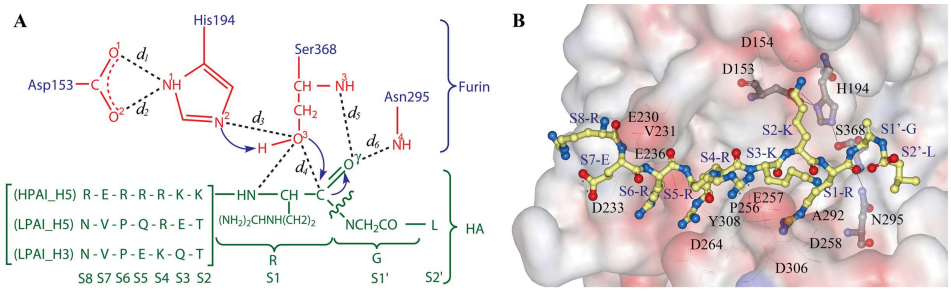

Figure 1. **(A)** Proposed cleavage mechanism of HA by furin and definitions of d_1-d_6. **(B)** Loop of HPAI H5 (ball and stick model) in the electrostatic surface of furin. Blue and red represent positively and negatively charged amino acid residues, respectively.

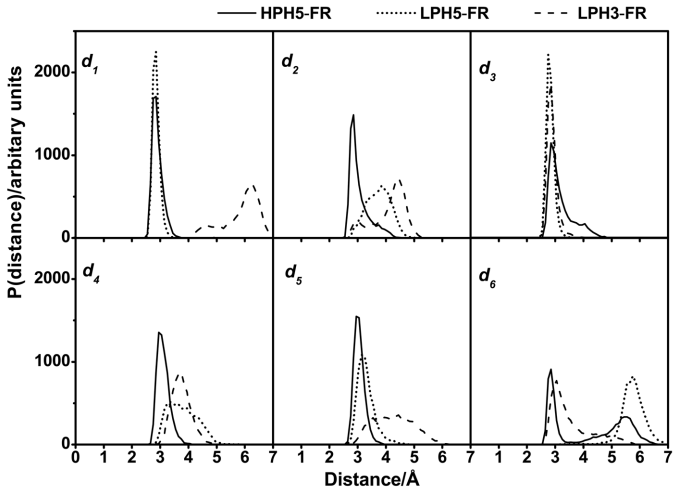

Figure 2. Distributions of the d_1-d_6 distances defined in Fig. 1 for the three simulated systems, sampling from 0.75 to 2.0 ns in MD simulations.

with furin, generated by all HA heavy atoms of S1-S4, were superimposed with the crystal structure of the dec-RVKR-cmk inhibitor while the HA backbone atoms of S5-S8 and $S1' - S2'$ were superimposed with the crystal structure of the HA0 loop of H3. MD simulations for the HA's cleavage loop complexed with furin, HPH5-FR, LPH5-FR and LPH3-FR, were carried out using the SANDER module of AMBER 7.

3 Results and Discussion

To search for detailed information on molecular level, selected structural parameters (d_1-d_6 defined in Fig. 1) were plotted in Fig. 2. The structures of the complexes are in detail described in three regions, the arrangement of the catalytic triad, the attachment of the catalytic Ser368 to the reactive S1-Arg, and the formation of the oxyanion hole. From

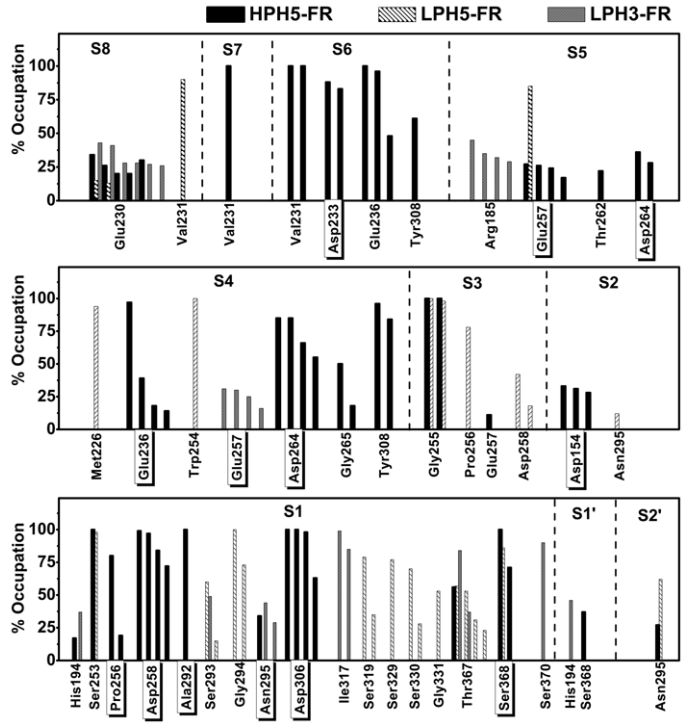

Figure 3. Percent occupations of hydrogen bonds between furin and the ten HA residues where the residues with a box around the label represent experimentally detected bonds for the inhibitor-furin complex.

the result, a sharp peak was found only in the HPH5-FR complex and occurred at suitable distances, signifying the rigidity of the complex which thus serves as a more appropriate configuration for the nucleophilic attack. In contrast, for the other two complexes the peaks show a broad distribution and occure at significantly larger distances.

To assess the stability of the hemagglutinin loop binding into furin protease, percentage and number of hydrogen bonds between each HA residue and the active site residues of the target enzyme, furin, were evaluated and plotted in Fig. 3. Considering the role of the -RRRKK- insertion more hydrogen bonds and a higher percentage occupation between the S2-S6 residues of HA and the surrounding residues of furin were found for HPH5-FR in comparison with the two LPAI systems. This means that the -RRRKK- insertion can directly help to hold the substrate in place.

4 Conclusion

In conclusion, the -RRRKK- insertion in the HPH5-FR, in particular the two arginines at S4 and S6 positions helps directly to hold the HA's cleavage loop in place by forming strong hydrogen bonds between residues of HA and furin. This consequently leads to an active conformation of the HPH5-FR complex suitable for the acylation reaction and is the primary source of high pathogenicity of the avian influenza viruses subtype H5N1.

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