

Molecular Modelling Approach to Explain the Susceptibility of PMPA for Drug-Resistant HIV-1 Reverse Transcriptase: K65R, Q151M and M184V

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BACKGROUND: 9-*R*-2-Phosphonomethoxypropyl adenine (PMPA) is an acyclic nucleotide phosphonate analogue that has demonstrated efficiency against immunodeficiency virus (HIV). Tenofovir disoproxil fumarate, an oral prodrug form of PMPA, has recently been approved for the treatment of HIV-1 infection. The mutation K65R reduced sensitively the susceptibility with a 3-4 fold resistance. The major advantages of Tenofovir, are no cross-resistance with the multi-drug resistance (MDR) Q151M selected with dideoxynucleotide analogs, or M184 selected with 3TC and FTC. From this observations, we explain by molecular modelling the susceptibility of Tenofovir for this reverse transcriptase (RT) strains in an energetic and structural point of view.

METHODS: The molecular modelling was performed from the crystal structure of the wild-type RT·DNA·2'-deoxynucleoside 5'-triphosphate (dNTP) ternary complex. The construction of the wild-type enzyme was performed from this complex. We have extracted the amino-acids surrounding the catalytic domain within a radius of 25Å. The active conformation of Tenofovir was built from dTTP included in the crystal structure ternary complex. The adenine at the 5 position of template was mutated to thymine and the mutated enzymes structures were obtained by mutation of the selected amino-acids (K65R, Q151M and M184V). The thymidine and the amino-acids side-chains were positioned with GenMol™ (www.3dgenoscience.com) software by local energy minimisation. Then, the Tenofovir was merged to the active site, and the resulting ternary complexes were optimised.

RESULTS: The reduced susceptibility of K65R strain are explained by the strongest electrostatic interactions of the guanidine moiety of Arg65 compared to that of Lys65. This modifications of electrostatic interactions break the Mg²⁺ complexation with the phosphates β and γ . Furthermore, Tenofovir undergoes a slight repositionning in the catalytic site. The Q151M mutation of RT induced no resistance since the 2',3' carbon atoms of dNTP were absent in the structure of Tenofovir, thus avoiding van der Waals interactions with the Met151 side chain. The M184V shows a good correlation in terms of structure and energy. M184V does not induce steric conflict. In fact, the methyl constituent of Tenofovir allows favourable lipophylic interactions with the mutated Val184 side chain. This structural observations are confirmed by the energies interaction between the WT and M184V RT, of -122 and -121 kcal.mol⁻¹ respectively.

CONCLUSIONS: A novel Tridimensional molecular modelling method was developed to explain and partially predict HIV-1 RT drug resistance. This way constitutes an interesting alternative and complementary to the genotypic and phenotypic assays. Further works are in process to extend these first data to other RT mutations like TAMs and others INRTIs.