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Structural basis for the role of LYS220 as proton donor for nucleotidyl transfer in HIV-1 reverse transcriptase

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ABSTRACT

Biochemical studies by Castro et al. have recently revealed a crucial role for a general acid in the catalysis of nucleic acid transfer in distinct classes of polymerases. For HIV-RT LYS220 was identified as proton donor. This was unanticipated from a structural point of view, since in all ternary crystal structures of HIV-RT LYS220 are too distant from the active site to fulfill this role. In this work molecular dynamics simulations were used to reveal the dynamics of HIV-RT and to provide structural evidence for the role of LYS220. During a 1 μs molecular dynamics simulation LYS220 migrates toward the active site and occupies several positions enabling direct and water mediated proton transfer towards pyrophosphate. A combination of quantum mechanical and molecular mechanics methods was used to validate the different modes of interaction.

1. Introduction

HIV reverse transcriptase is a critical enzyme in the HIV life cycle, catalyzing the transcription of the viral RNA to DNA. It is also a target for numerous drugs. Detailed knowledge of the catalytic mechanism is vital to rationally develop nucleoside like drugs that cause chain termination [1]. The active site and reaction are schematically represented in Fig. 1. Nucleophilic attack on the phosphorus atom of the ribonucleoside triphosphate by the primer 3′-hydroxyl leads to the formation of a phosphodiester bond and release of pyrophosphate. Two Mg^{2+} ions are present in the active site. Mg^{2+} A lowers the pKa of the primer's 3′-hydroxyl to facilitate the initial proton transfer [2,3]. Mg^{2+} B has a structural role, it stabilizes the negative charge in the transition state and might help the release of pyrophosphate [4,5]. A second proton transfer involves the protonation of triphosphate [6].

The acceptor for the first proton transfer is still unknown. Potential acceptors are ASP186 or water. The donor of the second proton transfer was recently identified by Castro et al. by sequence alignment and confirmed by biochemical studies [7]. It was found that LYS220 fulfills this role in HIV-RT. Moreover, lysine was revealed to function in several classes of polymerases as general acid. However, structural evidence for the role of LYS220 is missing since it is not situated near the active site in any of the ternary crystal structures of HIV-RT[8–10]. In Fig. 2 the position of LYS220 in the crystal structure is shown (the position of LYS220 in the crystal structure is indicated by the blue color, the code of the crystal structure used is 1RTD). The side chain nitrogen of LYS220 is located more than 1.5 nm from the active site. This crystal structure cannot give a structural justification for its role in the catalysis. Molecular dynamics simulations in this work reveal the dynamics of this residue and provide the necessary structural evidence.

2. Methods

2.1. Molecular dynamics

2.1.1. Model building

The simulation was started from the 1RTD crystal structure [10]. The p66 unit, including DNA was used for the simulations. The base of the entering tymidine triphosphate was modified to an adenine and the complementary adenine base (E5) was changed into a thymine by an inverse fitting procedure using Quatfit (Quatfit program in CCL software archives).

The AMBER 99SB force field was used for the DNA and the protein [11]. It was verified that the DNA backbone parameters α and γ stayed close to their canonical value, since transition to unrealistic values is a known problem for free DNA simulations in the AMBER force field [12]. The triphosphate parameters were taken from Meagher et al. [13]. Protonation states were calculated using the H++−server [14] and confirmed by the PROPKA server [15]. The 3′-hydroxyl group of the primer strand is missing in the crystal structure and was added

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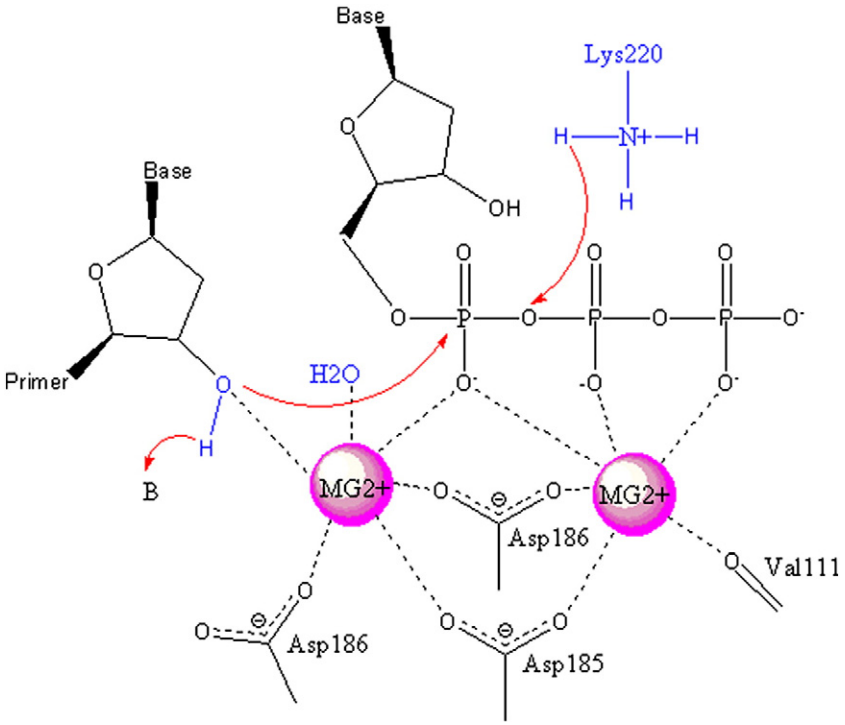

Fig. 1. Schematic representation of the reaction and active site of HIV-RT. Nucleophilic attack on the-phosphorus atom of the ribonucleoside triphosphate by the primer 3′-hydroxyl leads to the formation of a phosphodiester bond and release of pyrophosphate. Catalytic Mg^{2+} cations and their coordination shell are represented. Parts not present in the active site of the crystal structure but which occur after modeling (3′-hydroxyl) and MD-simulations (water and lys220) are colored blue.

using tleap [16]. The AMBER topology and structure file were converted to GROMACS using amb2gmx [17,18].

2.1.2. Simulation setup

A dodecahedral simulation box was added. The dimensions were set to the diameter of the system (largest distance between 2 atoms) plus twice 0.8 nm. The system was solvated with 38349 TIP3P water molecules [19]. To neutralize the system 32 sodium ions were added. The total system consists of 125 661 atoms. The GROMACS simulation package [20] was used to simulate the neutralized solvated system. Long-range electrostatics were computed with the particle mesh Ewald method [21]. The non-bonded cutoff was set to 1.1 nm. A Fourier spacing of 0.12 nm was used. All covalent bonds were constrained using P-LINCS [22]. A timestep of 2 fs was used.

The system was first minimized using the steepest descent method, followed by a slow heating of the system from 0 to 300 K during 500 ps. Next the system was subjected to 500 ps of equilibration at 300 K, to equilibrate the energy. The system temperature was controlled by the velocity rescaling thermostat [23] and the pressure was controlled using the Parinello-Rhaman barostat [24]. Finally the system was simulated in a production run of 1 μs.

2.2. QM/MM optimizations

2.2.1. Methods

The QM/MM calculations were done with a two-layer ONIOM [25–27] approach. The selected model system (Fig. 3) was treated with density functional theory (DFT) with the B3LYP [28] functional and DGDZVP basis set [29,30], the rest of the system was treated with the AMBER 99SB force field. The QM model was mechanically embedded [31] in the MM system. The boundary between the 2 regions was treated using link hydrogen atoms. This combination of the AMBER force field and B3LYP has proven its use in many studies [32,33]. For the optimization the macro/microiterative scheme with quadratic coupling was used [34]. The calculations were done using Gaussian 09 [35].

2.2.2. Model system

Three clusters of interactions between the active site were determined by molecular dynamics simulations. Representative snapshots were chosen and optimized. First, the fully solvated structure was optimized using the AMBER99SB force field followed by aQM/MM optimization of the system with a water shell. The water shell consists of all water molecules within 0.5 nm of the system or

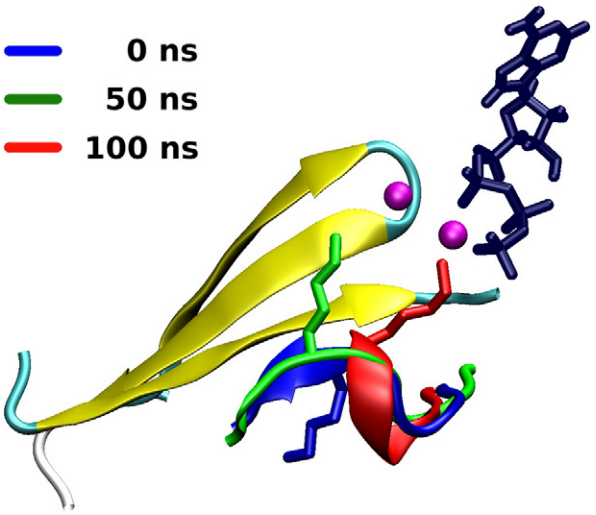

Fig. 2. Position of LYS220 at different times with respect to the active site in HIV-RT. With 0 ns the crystal structure. Adenine triphosphate (dark blue) and Mg^{2+} of the active site are shown together with the structural domain containing LYS220.