Thanyada Rungrotmongkol 2006: Molecular Dynamics and Combined QM/MM Modelling of HIV-1 RT Active Site: Discriminating between Alternative Mechanisms in DNA Polymerization. Doctor of Philosophy (Physical Chemistry), Major Field: Physical Chemistry, Department of Chemistry. Thesis Advisor: Associate Professor Supa Hannongbua, Dr.rer.nat. 139 pages.
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We have investigated the structure and dynamics of the HIV-1 RT active site, by modelling the active conformation of the HIV-1 RT/DNA/dTTP ternary complex. This has included molecular dynamics simulations and combined QM/MM modelling. Three different systems were studied to investigate the effects of different protonation states of dTTP (a deprotonated and two different mono-protonated triphosphate forms), and the effects of different possible protonation state of potentially catalytic aspartate residues (Asp185 and Asp186) were tested. The model of the deprotonated form of dTTP (model A) with the two aspartates in their charged (basic) form seemed to be the most stable and its orientation was in good agreement with crystal structure. The main aim is to study and investigate the details of reaction mechanism in DNA polymerization. We have proposed three reaction steps; Step 1 is the deprotonation of the terminal 3'-OH group of the primer stand; Step2 is the nucleophilic attack of the negatively charged terminal 3'-OH group on Pα atom of dTTP; and Step 3 is the Pα-O3α breaking down to gain the final product complex. Three different base mechanisms (Asp185, Asp186, and dTTP) for H-transfer reaction following by nucleotide addition were estimated with two different semiempirical (AM1 and PM3) QM/MM methods. The most feasible H-transfer reaction was found to proceed in a corresponding reaction path via Asp185 which plays an important role as the catalytic base. Consequently, the nucleophilic attacking on Pα of dTTP (Step 2) leading to the formation of the pentacovalent intermediate and the subsequent Pα-O3α breaking bond of this structure. Step 3 generates the creation of the 3'-5' newly formed phosphodiester and pyrophosphate resulting in the elongation of the primer stand by one new nucleotide and the leaving group, respectively. The activation barrier for overall reaction is energetically closed to 18.4 kcal/mol in which the rate-limiting step is the H-transfer reaction to Asp185 (model A). The critical structures along the reaction pathway were stabilized by the H-bond interactions with Lys65, Arg72 and Asp113 and some tightly bound water molecules. These studies highlight the utility of QM/MM molecular dynamics simulations for calculation of free energy profiles for enzyme reactions. The results provide insight into the structure and interactions of the active site of this important enzyme, with implications for its mechanism, which may be useful in inhibitor design.

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