Theoretical study of the interaction between Zidovudine (and novel derivatives) and human serum albumin

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Human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS), a disease that affects the immune system and produces its inability to cope with infections and other pathological processes. This disease, discovered in the United States in 1981, nowadays affects men, women and children around the world.

Until now, no cure has been found due to the fact that the standard methodology for developing vaccines cannot be used. Given this situation, AIDS is treated with antiretroviral cocktails, in which Zidovudine (AZT) is included. AZT was the first drug approved for AIDS treatment by the Food and Drug Administration (FDA) in 1986. Despite its effectiveness, AZT has significant adverse effects, many of them associated to low plasma protein binding, including the human serum albumin (HSA).

Hence, obtaining AZT prodrugs, with higher affinity for HSA, is a key strategy to increase the effectiveness of the drug. The main strategy commonly used is the chemical modification by making use of functional groups of diverse nature, at the 5'-OH position of the molecule, thus producing the AZT derivatives.

HSA has different functions in the human body, but this work focuses on the transport function. The importance of HSA, in connection with transport, is the ability to bind, solubilize and transport both endogenous and exogenous substances. In the latter group, there are several drugs, for instance AZT.

HSA is present in the body in its pure form (ASHp) and complexed with fatty acids (ASHAG). Owing to the fact that both species exhibit different biodistribution, the studies aimed at obtaining a detailed description of the topology of the site in HSA (ASHp and ASHAG) where AZT binds. This was done in order to determine the molecular aspects that lead to different affinities of the AZT derivatives, for both species of HSA, establishing a qualitative (structural) and quantitative (energetic) relationship between the chemical structure and the fraction of the bound drug.

In order to design AZT derivatives with increased affinity for HSAp and HSAAG, molecular modeling methodologies, docking and molecular dynamics were applied based on the crystallographic structures of both species of HSA.

In the first instance, the crystallographic structure PDB 3B9L was used, in which HSAAG is complexed with AZT and Miristate (Myr). The crystal structure of AZT molecule was extracted from this complex. The structures of the rest of the ligands: acid AZT (AZT-Ac) and AZT derivatives complexed with the amino acids leucine (AZT-Leu), isoleucine (AZT-iLeu), phenylalanine (AZT-Phe), valine (AZT-Val) and tryptophan (AZT-Trp) were obtained by means of molecular modeling techniques, in order to find their optimized geometries, in their minimum energy conformations.

Firstly, a geometry optimization was applied to all the structures resulting from the binding of AZT with each amino acid, and secondly, a calculation of RESP charges was carried out using the Gaussian 03 software. Then, a simulated annealing method (global optimization) was applied to the ligands so as to obtain the minimum energy conformations, subsequently used for molecular docking. In relation to the receptors, molecular dynamics (1 ns) were applied to refine amino acid side chains positions. The crystallographic structure of HSAp used was PDB 1BM0.
The docking stage was implemented using AutoDock Tools 1.5 and AutoDock 3 software suites. The theoretical studies were performed on the protein primary binding site (Sudlow Site I). An almost superimposed structure on the crystallography was achieved for AZT, thus validating the model.

Two possible orientations, for the ligands of ASHₚ complexes, were identified (supported by energy calculations done later). In the favorable position, the base ring is located within the hydrophobic cavity, and the oxalyl bridge carbons are close to the Lys residues of the hydrophilic cavity. Since the Lys residues are positively charged, this orientation could favor the stabilization of the negative charges of the derivatives that are situated in the carbons mentioned before. In addition, in the unfavorable orientation, the oxalyl bridge is located inside the hydrophilic pocket. This region limits the ligand growth, i.e. the ligands with amino acids larger than Leu cannot adopt that position even if it is favorable, due to the steric hindrance in the cavity.

In relation to ASHₐG complexes, all ligands are positioned differently. This could be caused by the Myr molecule located in the binding site, thus preventing the access to that site. All the AZT-Ac molecules are situated in the primary binding site and one structure is located very close to AZT. Additionally, subsequent docking studies with different parameters converged on the same structure. For this reason, it can be said that the structure reflects the docking interaction with ASHₐG in the body. The docking studies of the rest of the derivatives are currently being analyzed to compare the results with each other and extend the analysis with the molecular dynamic results.

The molecular dynamics of the complexes were performed using implicit solvent, obtaining 2 ns of simulation for each complex. As a result, all the complex simulations show a reasonable behavior, because they tend to stabilize.

The energy calculations derived from the MM_PBSA module of AMBER10 package exhibit a positive electrostatic energy value for the complexes formed by AZT derivatives with HSAₐG, which suggests that the amino acids charges originate an electrostatic repulsion with the fatty acids polar heads. This repulsion is in agreement with the observed decrease in the experimental affinity [1] between the AZT derivatives and HSAₐG, when compared to that of the AZT-ASHₐG complex. Since, as AZT is not ionized, it does not generate any electrostatic repulsion with the fatty acids.

Negative electrostatic energy values were obtained for the majority of the complexes formed with ASHₚ due to the fact that the negatively charged amino acids tend to stabilize with the positively charged residues, located on the Sudlow Site I.

Further studies will be focused on molecular dynamics with explicit solvent (and longer time) in order to obtain results that are closer to the human body physiology. On the other hand, methylated derivatives are expected to be modeled so as to neutralize their charges and compare them with the previous results.

**References**