Mercaptobenzamide inhibitors effects on HIV NCp7 protein: a DFT based structural study

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The action of the Mercaptobenzamide (MB) class of molecules on the HIV Nucleocapsid protein (HIV NCp7), a zinc finger protein, is an issue of relatively recent research interest, relevant to develop a new class of effective and well tolerated HIV antivirals, able to overcome virus escape strategies.
MB molecules are easily and cheaply synthesized and show the ability to unfold the HIV Zinc-finger region, thus avoiding effective viral replication. This effect is not still fully understood, and is highly influenced in addition by the precise composition of MB aromatic ring and chain. Our approach to this biological problem is to adopt a quantum parameter-free scheme based on density functional theory (DFT) to study with atomistic resolution the action mechanism of MB molecules on NCp7 with respect to the role played by each MB functional group. We report and discuss the outcomes of the here proposed DFT simulations with respect to the different final configurational structures obtained.

Introduction

HIV is still a major public health issue with more than 37 million people affected globally.[1,2] The therapeutical schemes being used to attack HIV infection inhibit the essential viral enzymes reverse transcriptase, protease, or integrase.[3]

The principal healing treatment for HIV is the highly active antiretroviral therapy (HAART), in which combinations of drugs simultaneously can target the viral enzymes. Even if infected individuals who adhere to HAART can expect a normal life span, HAART requires regular and lifelong access to costly medication that often impedes many HIV-infected people from receiving the correct treatment. Moreover HAART is affected by viral resistance and may also lead patients to cardiovascular and neurological diseases.

Some resarch groups are interested in the capacity of Mercaptobenzamide(MB)-based molecules to inhibit HIV-1 through the inactivation of HIV Nucleocapsid protein 7, NCp7.[4]

We adopted a quantum ab-initio technique in order to study the action of the Mercaptobenzamide (MB) class of molecules on the HIV NCp7, a zinc finger protein.[5] More specifically, stimulated by some studies on animal models, we were interested in the impact of the binding of these molecules to the structure of the C-terminal HIV NCp7 "zinc-knuckle" motif. This motif binds and stabilizes the viral RNA and is thus essential for viral replication and maturation.[4]

Modeling Protocol

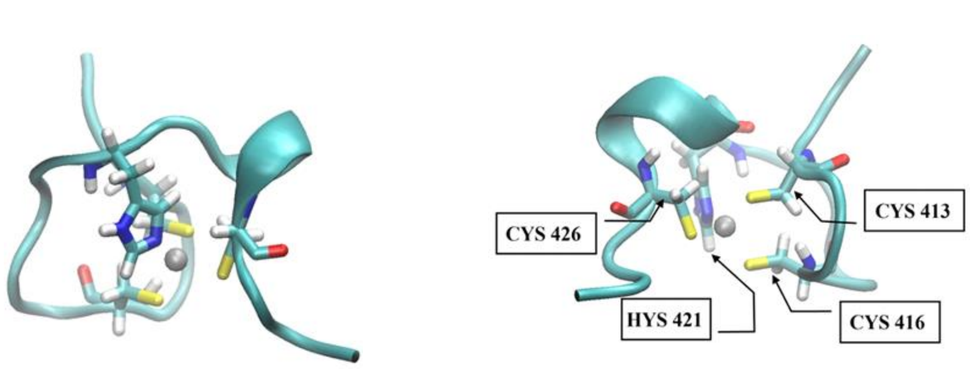
According to our work hypothesis, we developed the following simplified modeling protocol:

•Protein model: we selected model number 1 from PDB NMR structure 2L44 of the C-terminal zinc knuckle of the HIV NCp7. The structure is composed of 18 amino acids representing the zinc knuckle motif Cys3-X2-Cys6-X4-His11-X4-Cys16 corresponding to NCP7 protein amino acids 411-429.

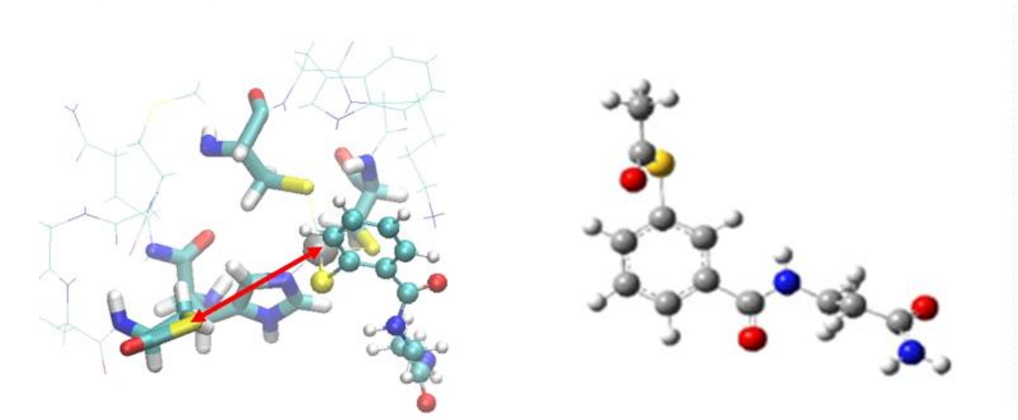
•Ab initio ("parameter free") DFT-based quantum simulation to optimize the MB molecular structure, and its quantum characterization (orbitals, ground state, ...).

•Ab initio DFT quantum simulation of MB interacting with the Zn-finger motif in vacuum and with implicit water. The MB sulfur atom have been placed close to the zinc coordination atom.

•Analysis of the distance variations between the cysteine sidechain sulfur atoms, histidine nitrogen atom and the central coordinating zinc atom during the DFT runs is performed. To evidence structural changes, particularly motif unfolding, we also analysed the variations of distances between the NCp7 backbone C-alpha atoms relative to the histidine and cysteines that define the motif.



In the above Figure is reported within two different perspectives the Nucleocapsid protein 7(NCp7) which is the target of the present study. Reported in the right panel are also the main residues forming the protein, namely CYS426, HYA421,CYS416,CYS413.



In the above Figure: On the left the final structure of the unfolded NCp7 + MB inhibitor (implicit water simulation). Highlighted the final distance of Zn-CYS 426 after the structural relaxation. On the right panel the MB inhibitor.

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Quantum MD Simulation

Parameter-free (ab-initio: AI) molecular dynamics (MD) based on density functional theory (DFT) computations were performed using the Gaussian simulation package.[6] Geometry optimizations have been obtained using the Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid electronic exchange–correlation (XC) functional, in combination with the 6–31 G* electronic basis-set, a valence double-ζ set augmented with d polarization functions.

The B3LYP exchange-correlation (XC) functional has stable behavior, with some well-documented limitations. The AIMD structural optimization of the system was performed without imposing symmetry constraints. The procedure here followed has been successfully tested for different systems, ranging from organic and inorganic clusters of different dimension and grade of complexity. [7-11]

Notice that, being mainly interested to the protein structure evolution, we were able to do a simulation with a total number of atoms quite larger than for DFT standard simulations. Consider that we were able to perform either simulations with implicit water and also relaxation with the presence of real water molecules.

Results and Discussion

To have a more deep and quantitative insight in the effects of the MB inhibitor we report in the following Table the distances between the Zn atom and the residues of the NCp7 protein in the different simulation schemes here adopted.

In the first column the experimental data, in the second colum the results of the stability check performed in vacuum. In paretheses the percentage of deviations with respect to the experiment. The distance between the Zn atom and the CYS 426 group shows a consistent enlargement (more than 30%). In the third column data after the simulation with the MB inhibitor. In this particular case the above distance is more than five time larger than the experiment, demonstrating the relevant active action of the inhibitor.

The fourth column reports the effect of the MB in a more realistic scenario, i.e. with the presence of implicit water in the simulation. The distance between Zn atom and the CYS 426 is more than three times larger with respect to the starting one. Interestingly this enlargement is strongly hampered in the case the simulation is started from the use of a MB inactivated inhibitor (5° column).

Table	NCp7 [Å] (Exp.)	NCp7 [Å] (Vacuum)	NCp7 + MB [Å] (Vacuum)	NCp7 + MB [Å] (H_2O impl)	NCp7 + MB [Å] (Inactive form–H_2O impl)
$d_{Zn\ Cys\ 413}$	2.30	2.34 (+ 1.2 %)	2.42 (+ 5.2 %)	2.38 (+ 3.5 %)	2.35 (+ 1.2 %)
$d_{Zn\ Cys\ 416}$	2.27	2.25 (< 1 %)	2.44 (+ 7.5 %)	2.38 (+ 4 %)	2.40 (+ 6 %)
$d_{Zn\ Cys\ 426}$	2.37	3.17 (+ 34 %)	13.03 (~ 5.5)	8.45 (~ 3.6)	3.24 (+ 37 %)
$d_{Zn\ Hys\ 421}$	2.05	1.88 (8.3 %)	2.03 (1 %)	2.03 (1 %)	2.04 (< 1 %)

Conclusions

The effect of MB molecules upon HIV Zinc-finger region NCp7 has been analyzed with the use of a quantum parameter-free scheme.

Our approach to this biological problem is to adopt a method based on density functional theory (DFT) to study with atomistic resolution the action mechanism of MB molecules on NCp7 with respect to the role played by each MB functional group.

We found that the distance between the Zn atom and the CYS 426 group could show a consistent enlargement. In the presence of the MB inhibitor that distance could be further enlarged, demonstrating the active action of that inhibitor. This enlargement is strongly hampered in the case the simulation is started from the use of an inactivated MB inhibitor.

We believe the results presented here and their future development will be useful for rational drug design, including discovering interesting targets and understanding molecular action mechanisms.

Moreover due to recent emerging results we plan to continue and deepen this line of research by explicitly including the acetyl group and by the use of Molecular Dynamics simulation techniques to explicitly solvate the system. In this way we hope to attack distinct protein equilibrium states, with distinct roles on antiviral action mechanism.

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