IL NUOVO CIMENTO **45 C** (2022) 192
DOI 10.1393/ncc/i2022-22192-6

COMMUNICATIONS: SIF Congress 2021

DFT investigation of mercaptobenzamide inhibitors effects on the HIV NCp7 protein: A structural study

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received 30 January 2022

Summary. — The mercaptobenzamide (MB) molecular mechanism of action on the HIV Zn finger Nucleocapsid protein (NCp7), has recently attracted research interest, in order to develop a new class of effective and well tolerated HIV antiviral drugs, able to overcome virus escape strategies. The exact mechanism of MB on NCp7 is still not fully understood, and moreover it is heavy influenced by the specific functional groups in the MB aromatic ring and chain. Our approach to this biological problem is to adopt a computational framework based on the density functional theory (DFT) for the structural study to investigate with atomistic resolution the action mechanism of MB molecules on NCp7.

1. – Introduction

Nucleocapsid protein 7 (NCp7) is a 55-residue protein containing two highly conserved zinc-knuckle motifs [1-3], and recently has attracted attention as a promising target for new antiviral drugs due to its essential role in viral replication and maturation [4, 5]. Indeed, NCp7 plays a key role in some essential replication steps, more specifically in the genome packaging during virion assembly and in the strand transfers during reverse transcription of viral RNA [6]. In this work we analyze the evolution of the tertiary NCp7 structure, performing a DFT structural relaxation, in order to evaluate the effect of the mercaptobenzamide (MB) molecules on the HIV Nucleocapsid protein NCp7 [7]. More specifically, we investigate the effects of the binding of MB on the C-terminal HIV NCp7 Zn-knuckle (see fig. 1) [1-3]. This motif binds and stabilizes the viral RNA and is thus essential for viral replication [2,3,7] and, due to its relevance for virus reproduction, is little or not at all muted across HIV strands and undergoes relatively little mutation upon host infection. For that reasons, the inhibition of NCp7 is a very attractive antiviral mechanism, able to escape the HIV mutation strategies [7-10]. Moreover, recent studies found that MB molecules could be effective inhibitors of the zinc-finger region with IC50 values ranging from 1 to 100 μM [7]. The exact mechanism of action of MB molecules is not fully understood, and the very high sensitivity by little modifications of the molecular

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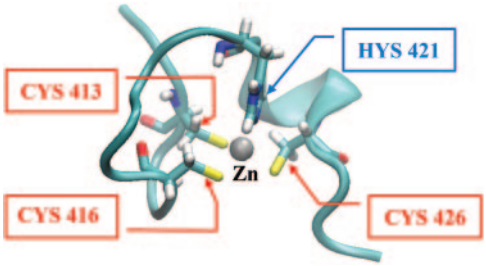

Fig. 1. – Secondary and tertiary structure of the C-terminal Zn-knuckle. Highlighted residues are those coordinated with the Zn atom forming the knuckle. Data taken from PDB file (see ref. [11]).

structure remains unclear. Moreover, some MB forms are active *in vitro* while they lose their effectiveness *in vivo* and even this behaviour is yet poorly understood [2].

Fortunately, a 3D crystal structure of the two coordinated zinc-knuckles in the C-terminal region exists [11] and can be used to build the zinc-finger motif model. From here to the remaining part of the paper, we refer to the specific mercaptobenzamide molecule under investigation using the MB2 identifier acronym reported in ref. [7].

2. – Computational details

We performed DFT [12] structural relaxations on a model of the Zn-knuckle motif of the HIV NCp7 protein, bonded to the MB2 molecule. The coordinates of the protein fragment (18 amino acids) were taken from PDB NMR structure 2L44 [11](1). The starting position of MB2 to bonding NCp7 is chosen in order to guarantee the correct Zn-S bond distance (2.07 Å) and to minimize the steric hindrance of the main chain with respect to the protein residues(2).

Structural relaxations were performed using the Berny algorithm [13] within the Gaussian16 package [14], with the B3LYP exchange–correlation (XC) functional [15, 16] and the 6-31G* basis-set(3) and using the SMD implicit solvent model [17](4). This approach has previously been successfully applied to several organic and inorganic systems of different sizes and complexity [18-24].

3. – Results and discussion

In fig. 1 is shown the structure of the C-terminal part of Nucleocapsid protein 7 (NCp7) which is the target of the present study, highlighting the main residues involved in the Zn-knuckle: CYS426, HIS421, CYS416 and CYS413.

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(1) It is important to note that the simulations involved just the 18 C-terminal residues [11] and the MB2 molecule, because the complete 55 residues NCp7 structure is not yet available.
(2) It is known that the choice of the correct starting position of MB2 is an important problem. For this preliminary study, we make an educated guess about the MB2 position considering the well known affinity of S for transition metals (Zn), the Zn-S bond distance and the steric hindrance of lateral chain. In further research, MD simulations will be performed to estimate the most probable starting configuration of MB2 + NCp7.
(3) Pople basis set with valence double-ζ set augmented and d polarization functions.
(4) To take into account at least the main effects of the typical water environment in living cells.

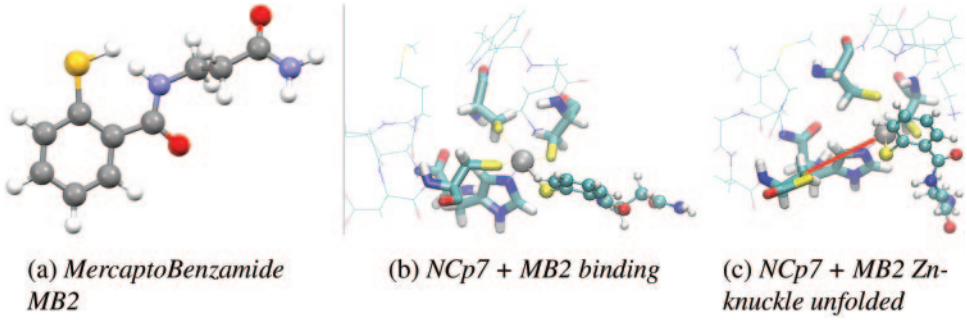

Fig. 2. – (a) Mercaptobenzamide molecule under investigation. (b) The MB molecule (ball stick visualization) bonded with the Zn-knuckle complex. (c) Final structure after simulation of NCp7 bonded with MB2 inhibitor. The new enlarged distance between Zn and Cys 426 that shows the unfolding of the Zn-knuckle is highlighted in red.

Figure 2 shows the mercaptobenzamide inhibitor (a) and the starting structure of NCp7 and MB2 inhibitor bonded to the Zn-knuckle complex (b). We have also performed calculations in case of an active and a non-active MB2 inhibitor form. The non-active form is obtained by replacing two H atoms instead of the first O atom in the main lateral chain. In fig. 2(c) we show the effect of the active form of MB2 inhibitor to the NCP7 Zn-knuckle. To have a quantitative insight in the effects of the MB2 inhibitor we report in table I the distances between the Zn atom and the residues of NCp7 forming the pocket, obtained in different simulation conditions.

From the obtained results we can observe a large increase of the CYS426-Zn distance after the simulation with active MB2 (see table I, Columns 1 and 4). Considering that all the 4 coordinations between Zn and CYS 413, 416, 426 and HIS 421 are needed to maintain the Zn-knuckle folded and active [7], the increase of CYS426-Zn distance can be interpreted as a possible evidence of the unfolding, and the consequential inactivation of the Zn-Knuckle. On the other hand, a simulation performed with NCp7 only (without MB2) shows that the same distances remains almost unchanged in the case of simulation

TABLE I. – *Distances between the Zn atom and the groups of the NCp7 protein in the different conditions. In the first column the experimental data of NCp7 after reference ref.* [11]*, in the second column the results of the optimization of the only NCp7 performed in vacuum. The third column shows the results for the simulation in the case of NCp7+ MB2 inhibitor performed in vacuum. The fourth column reports the data for the simulation of NCP7 + MB2 inhibitor using an implicit water model. The last column shows the data for NCp7 + MB2 inhibitor modified in an inactive form (using an implicit water model).*

	NCp7 (exp.) [Å]	NCp7 (vac.) [Å]	NCp7 + MB2 (vac.) [Å]	NCp7 + MB (H_2O) [Å]	NCp7 + MB2 Inactive MB2 (H_2O) [Å]
$d_{\text{Zn-CYS413}}$	2.30	2.34 (+1.2%)	2.42 (+5.2%)	2.38 (+3.5%)	2.35 (+1.2%)
$d_{\text{Zn-CYS416}}$	2.27	2.25 (<1.0%)	2.44 (+7.5%)	2.38 (+4.0%)	2.40 (+6.0%)
$d_{\text{Zn-CYS426}}$	2.37	2.71 (+13%)	13.03 ($\sim \times 5.5$)	8.45 ($\sim \times 3.6$)	2.80 (+17.0%)
$d_{\text{Zn-HIS421}}$	2.05	1.88 (−8.2%)	2.03 (−1%)	2.03 (−1.0%)	2.04 (<−1.0%)

in water environment and undergoes just a small increase in the simulation in vacuo (probably ascribable to the lack of the stabilizing effect of the water around the protein). If we compare the data in table I in the case of simulation with inactive MB (table I —Col 5) with the experimental distances in NCp7 (table I —Col 1) we found just a small increase of the Zn-CYS 426 and Zn-CYS 416 distances but the structure of the knuckle remains almost unchanged and stable.

4. – Conclusions

We have analyzed the effect of the MB2 molecule upon the Zn-knuckle region of HIV NCp7, within a DFT-based full quantum framework. Our simulations demonstrate that the main effect of the MB2 inhibitor is the unfolding of the Zn-knuckle on the NCp7 protein, even in the presence of water. Other simulations, conducted with small modifications to the MB2 structure, show that this behavior can be hampered by small structural changes in the MB2. The results can be useful for rational drug design, discovering new interesting targets and understanding molecular action mechanisms of a promising class of drugs.

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