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Toxins by first-principles: Electronic structure mapping structural changes

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Abstract

In this work, we apply first-principles calculations combined with model potential dynamics simulations to investigate structural and electronic aspects of toxins. The dynamics were carried out for ω-conotoxins MVIIA and MVIIC as well as for mutated structures in which tyrosine at position 13, a key residue for the toxin function, was replaced by alanine. An analysis of the electronic structure of specific snapshots shows that structural changes may be mapped in the local electronic behavior of carbonyl groups.

Keywords: Toxin; Molecular dynamics; DFT; Electronic structure; Protein conformation

1. Introduction

An intriguing aspect in biochemistry concerns the relationship between structure and function of proteins, as well as the interplay between these aspects and the electronic structure. Indeed, theoretical works on toxins [1] have shown that the analysis of quantities related to the local reactivity, such as the chemical softness, makes it feasible to find structure–function relationships. Also important is the application of quantum mechanical tools to track the electronic structure features as the system evolves in time, as it has been recently done in the case of the ubiquitin protein [2]. Toxins are particularly interesting because they form very strong and specific complexes with ion channels, and may be used as a probe to investigate their structure [3]. Besides, experiments on mutants are able to pinpoint the most important residues to the toxin function.

An example is given by the ω-conotoxin MVIIA, a N-type calcium channel blocker from the marine snail *Conus magus*. It belongs to a class of toxins whose structures are characterized by the existence of four loops, which are kept rigid by three disulphide bridges, as shown in the ball-and-stick model of Fig. 1a. Its primary sequence is given by:

CKGKGAK-CSRLMYDC-CPGS-CRSGKCG.

Several experimental works have highlighted the importance of residue 13, a tyrosine, for interaction of MVIIA with the channel [4,5]. Indeed, Tyr-Ala mutations in position 13 are responsible for a significant reduction in the toxin activity. Tyr13 is also a key residue in MVIIC [6], a similar ω-conotoxin which is 80% homologue to MVIIA and acts in P/Q-type calcium channels.

The above results suggest that these toxins may undergo important structural changes upon Tyr-Ala replacement, which would be responsible for their loss of functionality. The conformation changes must involve new arrangements of hydrogen bonds within the toxin. How does this process affect (if it does at all) the local electronic structure of the protein? The answer to this question may be helpful in providing a link between electronic structure, conformation and function.

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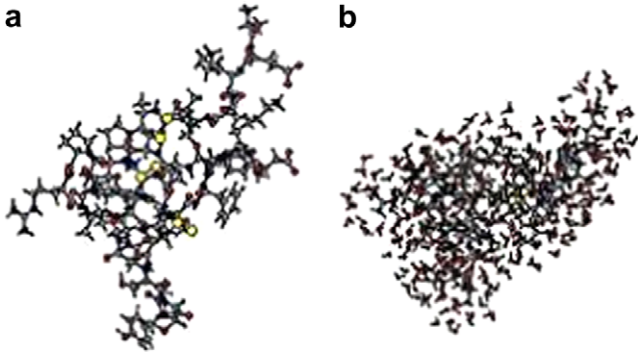

Fig. 1. (a) Ball-and-stick model of the MVIIA toxin. (b) Example of a structure employed in the *ab initio* calculation (a shell with 4 Å of water molecules surrounds the MVIIA toxin). Oxygen, carbon, nitrogen and sulphur are indicated by dark gray (red), light gray, black (blue) and yellow circles, respectively. Hydrogen atoms are the small white circles. The three disulphide bridges are clearly seen in (a). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In this work we address these points by combining model potential molecular dynamics simulations with first-principles calculations. In order to do that we perform a mutation on position 13, replacing a Tyr by an Ala, in both MVIIA and MVIIC, and we compare their structures before and after the molecular dynamics. Next, first-principles calculations are used for geometry optimizations and the determination of electronic properties. We shall show that important structural changes are related to the tyrosine replacement, with significant consequences in the local electronic structure of the toxin.

2. Method

Our calculations are performed as follows. For the molecular dynamics we employ the GROMACS package [7] and the force field gromos96 [8]. The NMR experimental structures are used as the starting point of the dynamics. The coordinates of the MVIIA and MVIIC toxins may be found in the Protein Data Bank (PDB) under the codes 1FEO [9] and 1CNN [10], respectively. Each toxin is placed in a box with periodic boundary conditions whose walls are at least 11 Å distant from the peptide and solvated by Single Point Charge (SPC) water molecules [11]. A first energy minimization is performed to remove close contacts, followed by a position restrained dynamics in which the toxin is kept fixed. Next, the actual dynamics is performed at an average temperature of 300 K by means of a coupling to a external bath [12], a time step of 2 fs and for a total time of 7 ns. For long-range electrostatics, the particle mesh Ewald (PME) summation [13] is applied. A 1.0 nm cutoff is used for van der Waals interactions. After that, the final configuration of the dynamics is relaxed, still employing the gromos96 force field. For the first-principles calculations, we use this resulting geometry, keeping only water molecules which have distances up to 4 Å from the toxin, as we show in Fig. 1b for the MVIIA toxin. The protein structure was fully relaxed using an ab initio formalism based on the pseudopotential [14,15] density functional theory [16] as implemented in the SIESTA code [17,18]. The basis set for the wavefunction expansion is composed of pseudo-atomic orbitals of finite range [17,18] and the force tolerance in the relaxation is set to 0.2 eV/A. The electronic structure is characterized by the determination of the local density of states (LDOS), as explained below.

3. Results and discussion

We begin by describing some of the hydrogen bond rearrangements, which take place as a consequence of the Tyr-Ala mutation. For this purpose, Fig. 2 is handy. It shows the toxin before (left) and after (right) the replacement. The water molecules are omitted for clarity. On the left, it is clearly seen that an important role played by Tyr13 is to establish a link between loops 2 and 4 due to its interaction with loop 4 residues. As a consequence of this interaction, residue Ser19 is able to interact with the C-terminus of the toxin. Upon replacement of Tyr13 by Ala13, the distance between loops 2 and 4 increases, and residue 19 no longer reaches the C-terminus, which now is hydrogen bonded to loop 1 (more specifically, to residue 5, a glycine) as it can be seen in Fig. 2, on the right. In order to show that these changes are not characteristic of a particular snapshot, we depict the time evolution of this behavior in Fig. 3 (upper panel). The solid black line represents the minimum distance between residues 13 and 23, which belongs to loop 4, as a function of time, whereas the dashed red line represents the same quantity for Ala13-MVIIA. Both simulations start in the same configuration. Note that MVIIA rapidly evolves towards a conformation in which residue 13 keeps an average minimum distance from residue 23 of around 4 Å, while for Ala13-MVIIA this average distance is 7 Å. Fig. 3 (lower panel) shows the minimum distances between residues 19 and 26 for MVIIA and Ala13-MVIIA (solid black line and dashed red line, respectively). It is clearly seen that Ser19 is hydrogen bonded to the C-terminus of the toxin in MVIIA, since the average minimum distance is around 2 Å and the deviation from

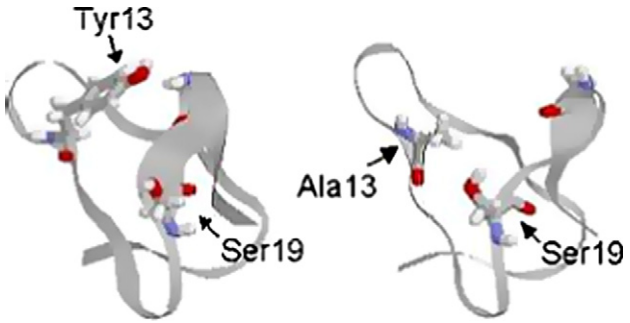

Fig. 2. MVIIA toxin after 7 ns of simulation. On the left, the toxin without mutation, and, on the right, the toxin with alanine replacing a tyrosine at position 13.