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Folded Conformations of Maitotoxin

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Abstract We used classical molecular mechanics and dynamics simulations to sample the conformational space of maitotoxin, the largest and most lethal natural product. Among the set of minima obtained, the five conformers with the lowest energies show folded structures with an intramolecular hole. We found the folding mainly due to hydrogen bonds that fasten the mobile zones of the molecule. The hole on each MTX conformer is big enough to interact with ions, such as Ca^{2+} or Na^{+}, which could be involved in its toxic effects observed on all cell types.
Keywords: Maitotoxin, conformational analysis, hydrogen bonds.

Resumen Se estudia el espacio conformacional de la maitotoxina por medio de mecánica y dinámica molecular clásicas. La maitotoxina es hasta ahora el producto natural con mayor número de átomos y con mayor toxicidad que se conoce. De todas las estructuras encontradas como mínimos, las cinco con menor energía muestran una estructura plegada que incluye una cavidad intramolecular. El plegamiento se debe, en buena medida, a la formación de puentes de hidrógeno que anclan las zonas móviles de la molécula. En el contexto de su actividad biológica, se discute la hipótesis de que la molécula pueda interactuar con Ca^{2+} y Na^{+}.
Palabras clave: Maitotoxina, análisis conformacional, puentes de hidrógeno.

Introduction

Marine toxins are natural products of dimensions only surpassed by DNA and polypeptides. Most of these toxins are polycyclic ethers synthesized by unicellular microalgae known as dynoflagellates. These microorganisms are present in red tides in massive quantities; they are eaten by fish and shellfish, leading to seafood intoxication in humans through the food chain. A variety of symptoms can be developed as a consequence of seafood intoxication, such as diarrhea, as well as neurotoxic, amnesic, or paralytic poisoning. If all of the previous symptoms are present, then the poisoning is called ciguatera [1]. Two marine toxins are known to produce ciguatera: the ciguatoxin (CTX) and the maitotoxin (MTX).

MTX is the largest marine toxin (3422 Da) and the most poisonous, 50 ng/kg [2], known so far. It was extracted in 1976 by Yasumoto *et al.*, from the tahitian surgeonfish, *maito* [3] and it is produced by the dynoflagellate, *Gambierdiscus toxicus*. The overall structure of MTX contains 32 rings and 99 elements of stereochemistry (98 stereogenic centers and one trisubstituted double bond). Its stereochemistry (see Fig. 1) was discovered in 1996 by Zheng *et al.* [4] and confirmed by Kishi [5]. Structurally speaking, MTX comprises four extended fused-ring systems termed as polyether ladders [6] and its spatial conformation is unknown. The elucidation of the ensemble of possible spatial conformations of MTX is a big challenge due to its large size and large number of internal rotors (red boxes in Fig. 1).

Previous findings [7, 8] show a MTX conformation with the hydrophobic section rigid and folded while the rest of the structure is almost fully extended. Recently, two new structures with some stereochemistry changes have been proposed for the GHIJK rings (see Figure 1) domain [6,9]. In spite of the recent efforts to establish the MTX conformation, the first structure proposed [5] is still the most useful.

It has been found that MTX shows a wide variety of biological effects, such as hemolytic, and cytotoxic [10]. However, the main action of MTX is the activation of Ca^{2+} entry to the cell [11, 12, 13, 14, 15, 16, 17]. This effect is not related to an ionophoretic activity [18, 19], but it appears to involve the activation of Ca^{2+}-permeable, nonselective cation channels. Toxins with structural similarities to MTX, like brevetoxins (BTX) and palytoxin (PTX) have specific targets on the plasma membrane: the sodium voltage gated channels and the Na/K-ATPase, respectively [20, 21, 22, 23]. However, in spite of the efforts, the MTX receptor is still unknown.

The aim of this work is to apply standard force fields and molecular mechanics techniques to give insights on the three dimensional structure of MTX and possible implications on its biological activity.

Methodology

We used molecular mechanics (MM) and molecular dynamics (MD) [24,25] procedures with the extensible systematic forcefield (ESFF) developed by Shi *et al.*[26], as implemented in the InsightII program [27]. The ESFF is based on simple rules that define the values of the parameters of the force field for different atomic interactions in the molecule (bonded and nonbonded interactions). These rules depend on the characteristics of the involved atoms, such as electronegativity, ionization potential, etc., and it was designed to include atoms from H to Rn. The energy expression for this force field is:

$$E_{pot} = \sum_b D_b \left[1 - e^{-\alpha\left(r_b - r_b^0\right)}\right]^2 \tag{1a}$$

$$\left.\begin{aligned} &+ \sum_a \frac{K_a}{\text{sen}^2\theta_a^0}\left(\cos\theta_a - \cos\theta_a^0\right)^2 \quad \text{(normal)} \\ &+ \sum_a 2K_a\left(\cos\theta_a + 1\right) \quad \text{(linear)} \\ &+ \sum_a K_a^{\theta_a}\cos^2\theta_a \quad \text{(perpendicular)} \\ &+ \sum_a \frac{2K_a}{n^2}\left(1 - \cos\left(n\theta_a\right)\right) + 2K_a^{-\beta\left(r_{13} - \rho_a\right)} \quad \text{(equatorial)} \end{aligned}\right\} \tag{1b}$$

$$+ \sum_\tau D_\tau\left(\frac{\text{sen}^2\theta_1\text{sen}^2\theta_2}{\text{sen}^2\theta_1^0\text{sen}^2\theta_2^0} + \text{sgn}\frac{\text{sen}^n\theta_1\text{sen}^n\theta_2}{\text{sen}^n\theta_1^0\text{sen}^n\theta_2^0}\cos\left[n\tau\right]\right) \tag{1c}$$

$$+ \sum_o D_o\phi^2 \tag{1d}$$

$$+ \sum_{nb}\left(\frac{A_iB_j + A_jB_i}{r_{nb}^9} + 3\frac{B_iB_j}{r_{nb}^6}\right) \tag{1e}$$

$$+ \sum_{nb}\frac{q_iq_j}{r_{nb}} \tag{1f}$$

where (1a), (1b), and (1c) depict the bond distance energies, the bond angle energies, and the energy of the torsion angles, respectively, (1d) represents the out-of plane interactions, and (1e) and (1f), describe the non bonded interactions (van der Waals and coulombic, respectively). This force field does not include crossed terms, which reduces the computational cost of the calculation.

Taking into account that MTX is water soluble, the two Na^+ ions from the molecule are dissociated. Therefore, our calculations do not include these atoms and the total charge of the system is –2.

To determine the $[MTX]^{2-}$ conformation, an initial MM calculation was performed, and then we used the simulated annealing procedure [28] with 200 ps MD calculations at 1 fs time step. The first MD calculation was carried out at 700 K, from where three minima were selected. Each minimum was used in MD calculations at 500 K, giving another three minima per job. These nine structures were then used as input for a simulation at 300 K, from where four minima per job were selected. These minima yield a total of 36 structures, which were optimized by MM. A second simulated annealing cycle was applied to the lowest energy structure obtained from the first cycle. The initial temperature for the new cycle was 1000 K, and the minima at 1000, 700, and 500 K were selected for MD calculations at 700, 500, and 300 K, respectively. Overall, we performed five simulated annealing cycles (starting at 1000 K), with approximately 35 MD calculations each.

In order to obtain a representative sample of the minima, we selected first the structures with the lowest energies, and then, we eliminated those with similar conformations and slightly higher energies. The resulting samples were used in the series of simulations described above.

A total of 490 atoms (164 C, 256 H, 68 O, and 2 S) were involved in every MD calculation, and the real time spent by each one of them was about 8 hrs. in a SGI Octane. No solvent effect was used.

Results and Discussion

The lowest energy structures are shown in Figure 2. These and subsequent conformations of higher energy were found to be folded structures. We search for a non-folded structure, but even when the initial structure is fully stretched out, the MD calculation only yielded folded ones. This characteristic is due to the intramolecular hydrogen bonds formed during the simulation. Indeed, the folding process is governed by the appearance of hydrogen bonds in the marked red regions of Fig. 1 that fasten the mobile joints between the rigid ring moieties. It is important to emphasize that the small energy differences between the most stable structures (less than 3 kcal/mol) do not allow identify the global minimum among them. This is so, because a methodol-

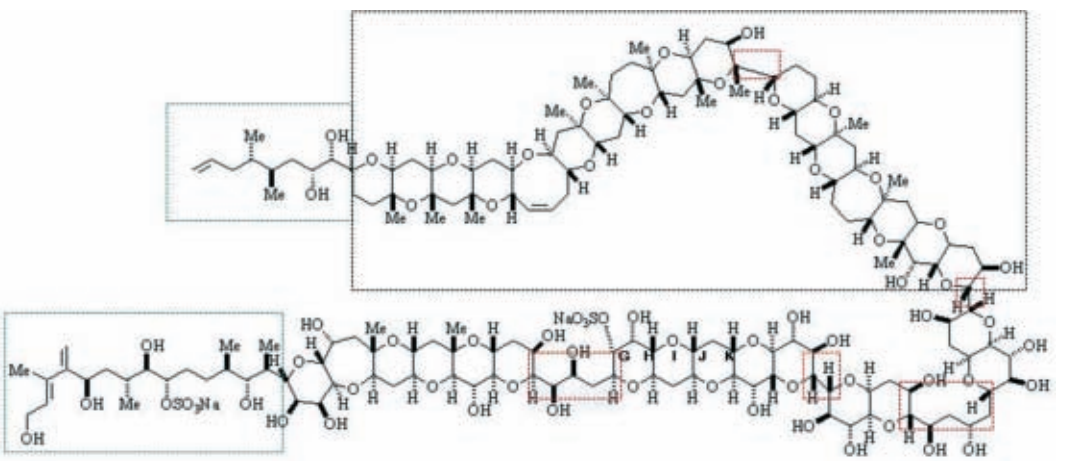

Fig. 1. Maitotoxin ($C_{164}H_{256}O_{68}S_2Na_2$). Internal rotors are shown in red boxes; external rotors are shown in green boxes. The black box shows the hydrophobic zone of the molecule, whereas the rest of the molecule is hydrophilic.

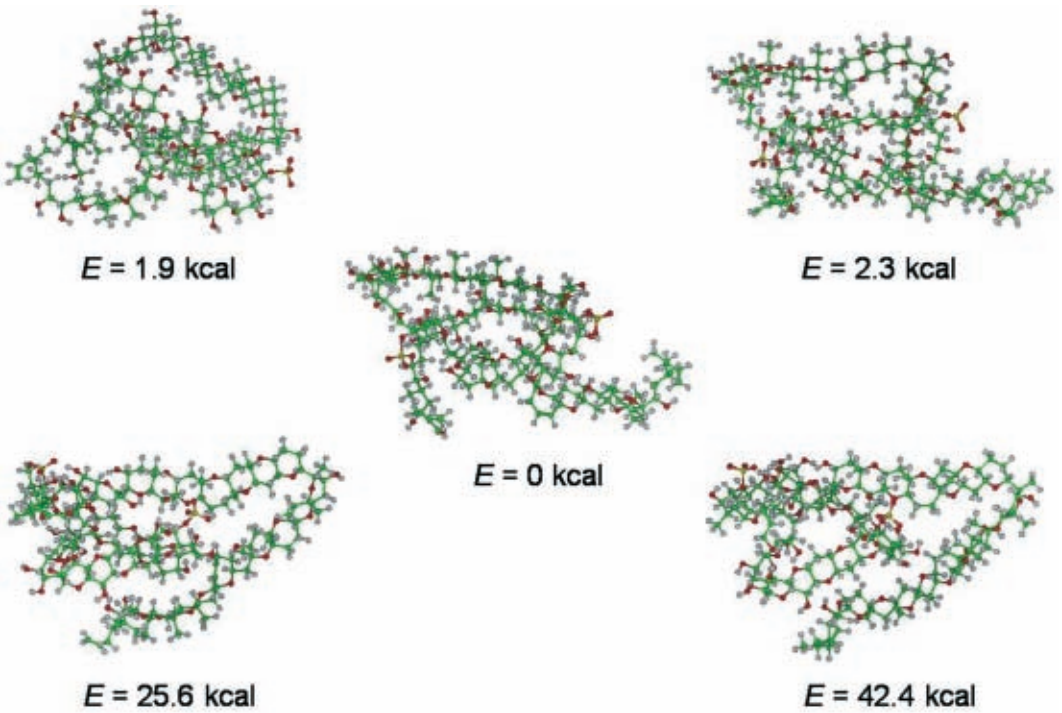

Fig. 2. Low energy structures for $[MTX]^{2-}$, the relative energy with respect to the lowest is shown for each conformer.

ogy based on a force field avoids such degree of precision; in particular, hydrogen bonds are detected and its geometrical structure qualitatively described but the energy of such interaction is not treated with the appropriate accuracy to favor such structures. Besides this limitation, an important issue is the fact that the low energy structures are folded.

Another conformational characteristic shows both sulphonate groups pointing at the outside of the structure. These groups are localized in the hydrophilic section of the structure (see Fig. 1), which is also the most folded segment. In contrast, the most stretched segment is the hydrophobic section (black box in Fig. 1).

The folding in the $[MTX]^{2-}$ minima produces an intramolecular hole. Figure 3 shows this pattern in the lowest energy conformation (the view in this picture was changed with respect to that in Fig. 1). This hole has an irregular shape but it is wide enough (around 5 Å) to contain small molecules or metallic ions; for instance, the radii for Ca^{2+} and Na^{+} are 0.99 and 0.97 Å, respectively.

$[MTX]^{2-}$ induces an elevation of Ca^{2+} and Na^{+} influx in cells, and as a result a strong depolarization of their membranes; such effect is related to non selective channels and is altered by the presence of some ions in the extracellular environment. The increase of inwardly Na^{+} currents produced by $[MTX]^{2-}$ takes place only when extracellular Ca^{2+} is present [16]. An additional effect is related to Na^{+}: reducing the extracellular concentration of this ion, dramatically increases the influx of Ca^{2+}; and the complete removal of extracellular Na^{+} results in faster and stronger Ca^{2+} influx after $[MTX]^{2-}$ application [16]. The existence of cavities in the folded structure of $[MTX]^{2-}$ could be important for the interaction of the toxin with cations, since such holes could serve as a trap for them. Therefore, changes induced by extracellular Ca^{2+} and Na^{+} in the activity of $[MTX]^{2-}$ suggest the interaction of these ions with a $[MTX]^{2-}$ binding site. If there were a competition among these ions for the $[MTX]^{2-}$ binding site such that,

$$[MTX]^{2-} + Ca^{2+} \rightarrow [MTXCa]$$

and

$$[MTX]^{2-} + Na^{+} \rightarrow [MTXNa]^{-};$$

and the $[MTXCa]$ specie were responsible for the activity of MTX, thus, when Na^{+} ions were present, the competition could diminish its concentration. In addition, Ca^{2+} would be required to "activate" MTX. This hypothesis opens the possibility to further theoretical and experimental analysis. Along this line, it would be also important to include solvent effects to observe the impact of them in the folded pattern. As the hydrophilic sites in the folded molecule in vacuum are in outer region of the molecule, one may expect small changes in the folded pattern when the solvent is present. However this has to be verified with the inclusion of a solvent.

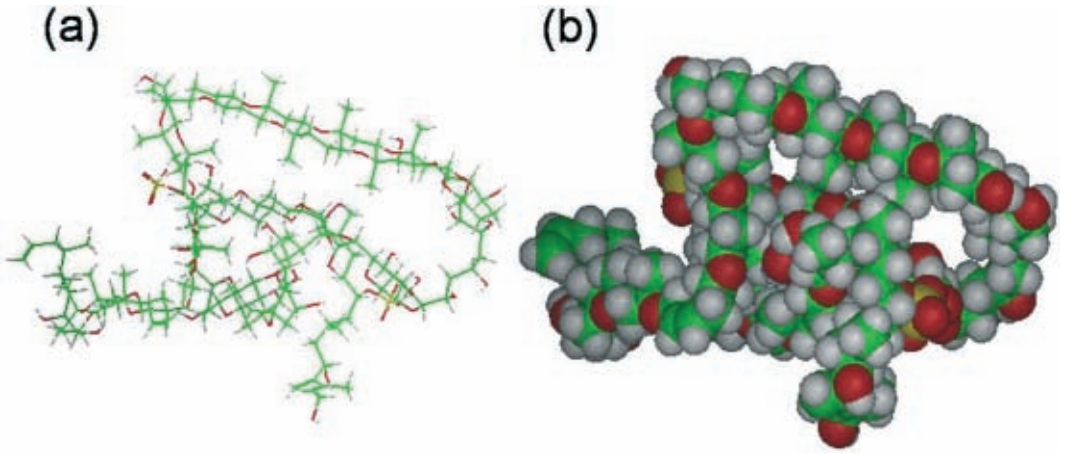

Fig. 3. (a) Intramolecular hole shown in the lowest energy conformation. **(b)** van der Waals surface.

Conclusions

We found a folded most stable conformation for the $[MTX]^{2-}$ specie. The folding process involves the formation of hydrogen bonds in the flexible moieties of the structure. In all the minima of the potential energy surface obtained in this study, the sulphonate groups are localized on external sites, and contain an intramolecular cavity with a size wide enough to interact with calcium or sodium ions. This MTX folding is shown for the first time in the literature and is a good starting point for studying the detailed interaction of the toxin with possible plasma membrane receptors. We propose the hypothesis that Ca^{2+} and Na^{+} could compete to bind $[MTX]^{2-}$ to form the active MTX.

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