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An ab initio study of the guanidinium groups in saxitoxin

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Abstract Quantum chemical (Hartree-Fock) calculations were performed on neutral and protonated saxitoxin in order to obtain optimum geometries, rotational energy barriers for the guanidinium ions and proton affinities. For comparison purposes, as model compounds, guanidinium systems in five and six membered rings were also investigated. In addition, DFT (B3LYP) calculations with the 6-31G** basis set were performed and the sodium affinities of the guanidinium groups in saxitoxin were obtained. It was concluded that the inhibition of the sodium channels by the saxitoxin is due to the interaction of the guanidinium group with carboxylate groups from the wall of the channel and not to the binding of the sodium ions.

Keywords Guanidinium group · Rotational barriers · Proton affinities · Sodium affinities

Introduction

Saxitoxin, also known as paralytic shellfish poison, is a small water-soluble molecule naturally synthesized by the marine algal dinoflagellates *Gonyaulax cateralla* [1] and *G. excavate* [2]. In humans, death due to respiratory paralysis occurs within 12 h after ingestion of the poison [1]. The lethal activity of saxitoxin is due to its ability to prevent sodium ions from passing through the membrane of the nerve cells, thus interfering with the transmission of signals along the nerves [3, 4].

The first synthesis of saxitoxin was completed by Kishi et al. at Harvard [5] and later by Jacobi et al. [6]. Figure 1 shows the structure of saxitoxin in its neutral state. One notices the presence of two guanidine groups, one attached to a five-membered ring and the other attached to a six-membered ring. The six-membered ring guanidine is almost entirely protonated, whereas the five-membered ring guanidine can be either protonated or neutral, depending on the pH. Studies involving the pH-dependent activity of saxitoxin indicate that the protonated form of the five-membered guanidine group is directly involved in the blocking of the sodium channels [3]. It is probable that the blocking occurs by the interaction of the guanidinium group with carboxylate groups present in the ion-channel walls. Indeed, the guanidinium–ion interaction with the carboxylate ion shows a strong energy for the charged species [7]. Indeed, it has been reported that the region suitable as a candidate for the selectivity filter of the channel contains two carboxyls: Asp 384 and Glu 942 [8]. Another possibility is the interaction between sodium ions and the lone pair positioned on the exocyclic nitrogen of the guanidine group. Consequently, this work investigates the sodium affinity of the guanidinium groups. However, since the sodium ions are hydrated, this possibility would be less likely to occur.

Several computational studies of saxitoxin have been reported previously, often together with the marine toxin, tetrodotoxin and analogs. In 1989, using DPCILO, CNDO, OPEC and MMP-2 methods, Chen et al. [9] discussed structure–activity relationships (SAR) for toxin binding to sodium channels. Two papers in 1994 included quantum chemical calculations (INDO) for ten saxitoxin derivatives in studies of electronic structures and SAR as well as studies for both saxitoxin and tetrodotoxin (and derivatives) for sodium-channel binding based on electrostatic-potential contour maps and molecular graphics [10, 11]. Semiempirical SCF MO (INDO) calculations were employed in a 1998 study [12] on electronic structure and SAR for saxitoxin and analogs, in comparison with tetrodotoxin. These papers used low calculation levels. Velmer et al. [13] used mass-spectroscopy methods to examine gas-phase dissociation reactions of protonated saxitoxin and neosaxitoxin. They also performed Density Functional Theory calculations at the B3LYP level, for the study of the proton affinities

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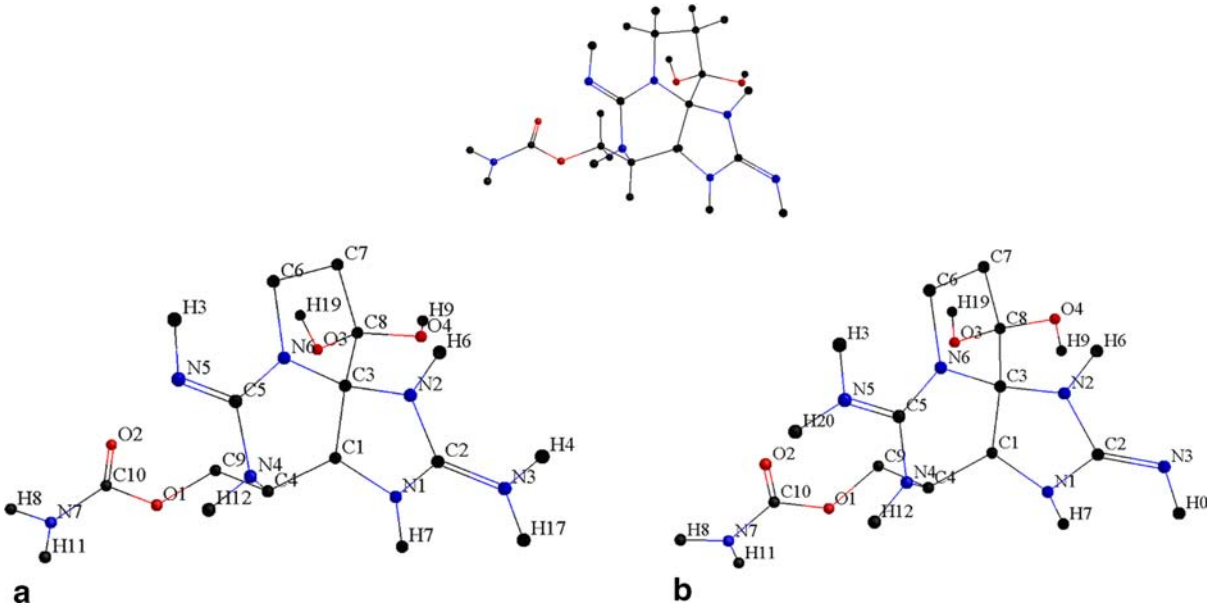

Fig. 1 Calculated structure of compound 1, neutral saxitoxin. **a** Calculated structure of compound 1a, saxitoxin protonated on the guanidine of the five-membered ring. **b** Calculated structure of compound 1b, saxitoxin protonated on the guanidine of the six-membered ring

of saxitoxin and suggested that the protonation takes place at the guanidinium group of the pyrimidine ring.

The present work applies ab initio quantum chemical calculations to obtain, besides the proton affinity of neutral saxitoxin, as mentioned before, the sodium ion affinity for the guanidine groups. The rotational energies of the guanidinium groups are also computed. In addition, guanidinium groups attached to a five-membered ring (shown in Fig. 2) and to a six-membered ring (shown in Fig. 3) are also investigated from the point of view of proton affinities and rotational barriers in order to compare the results with those of saxitoxin.

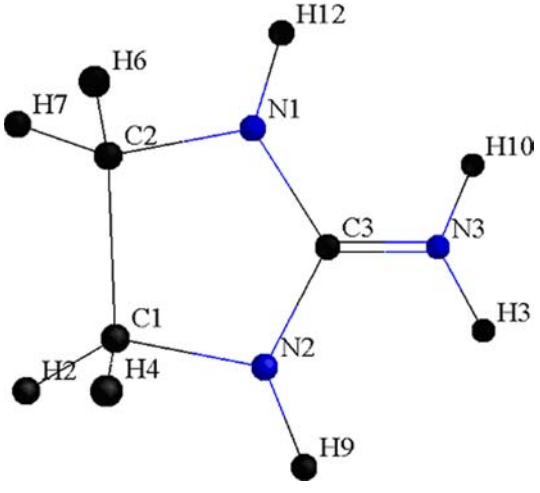

Fig. 2 Calculated structure of compound 2, with the protonated guanidine in a five-membered ring

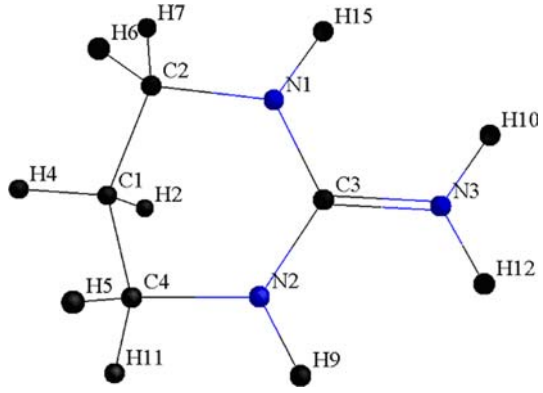

Fig. 3 Calculated structure of compound 3, with the protonated guanidine in a six-membered ring

Methods and results

The Spartan ES 04 computer program [14] was used to perform ab initio (Hartree-Fock) calculations, with the 6-31G* split-valence set with polarization functions. The calculations were performed on saxitoxin, saxitoxin protonated at the guanidine attached to the five-membered ring and on saxitoxin protonated at the guanidine attached to the six-membered ring. These species are shown in Fig. 1a and b. The molecules were geometry-optimized and thus their lowest energies were obtained. In order to determine the energy of rotation of the guanidinium groups (the exocyclic NH_2^+ moiety), a dihedral angle comprising one of the hydrogens of the guanidinium group, the nitrogen to which it is attached, the carbon and a second (endocyclic) nitrogen to which the carbon is attached, was frozen at different values, as shown in Table 1. This angle, H4N3C2N1, is called angle α. The other parameters of the molecule, including the position of the other

Table 1 Energy (au) as dependent on the dihedral angle α for saxitoxin figuring the guanidinium ion attached to the five-membered ring (structure 1a) and solvation energy (kcal mol^{-1})

α	Energy	Solvation energy
0.69 (optimum structure)	−1069.61121	69.59
20.57	−1069.61053	69.08
40.57	−1069.60801	68.21
60.57	−1069.60345	67.38
90.38	−1069.59537	68.80

guanidinium hydrogen, were allowed to optimize. Since a previous group of calculations [15] that the structures of saxitoxin featuring different orientations of the guanidine exocyclic NH exhibit different energies, the lower-energy orientation was chosen, as shown in Fig. 1, for all the calculations. The energies thus obtained are shown in Table 1. Table 2 shows similar results for the species with the guanidinium ion attached to the six-membered ring, with the dihedral angle H3N5C5N6 called angle β. Tables 3 and 4 show the results of applying this procedure to the rings shown in Figs. 2 and 3. These tables also display the solvation energies of the different structures, obtained by a mixed molecular mechanics/quantum mechanics molecular dynamics method, as implemented in the Spartan ES 04 program [14].

Tables 5 and 6 show the optimized geometries of some relevant parameters in the two protonated saxitoxins, structures **1a** and **b**. Tables 7 and 8 show the relevant optimized parameters for structures **2** and **3**.

The proton affinities of different entities are obtained as the difference between the energy of the protonated species and the energy of the neutral species. These results are shown in Table 9. Table 10 shows the barrier to rotation of the guanidinium groups in the compounds investigated.

Table 2 Dihedral angle β-dependent energies (au) and solvation energies (kcal mol^{-1}) of structure 1b

β	Energy	Solvation energy
−6.15 (optimized structure)	−1069.62155	63.62
19.85	−1069.61928	64.04
39.85	−1069.61514	65.02
59.85	−1069.61026	63.96
89.85	−1069.60414	61.73

Table 3 Energies (au) and solvation energies (kcal mol^{-1}) of structure 2

H10N3C3N1	Energy	Solvation energy
0.00	−281.42671	60.53
20.59	−281.42605	60.46
40.41	−281.42338	60.78
60.22	−281.41862	59.60
90.00	−281.40979	60.98

Table 4 Energies (au) and solvation energies (kcal mol^{-1}) of structure 3

H10N3C3N1	Energy	Solvation energy
0.00	−320.47199	57.40
20.75	−320.47154	56.86
40.38	−320.46957	56.27
59.78	−320.46601	55.82
90.38	320.45872	55.05

Table 5 Some optimized parameters of structure 1a (length in Angstroms and angles in degrees)

α	Parameter	
0.69	H17N3C2N2	1.64
	H4N3C2	121.34
	H17N3C2	121.34
	H4N3H17	117.32
	H4N3	0.996
	H17N3	0.996
	N3C2	1.320
	C2N1	1.316
	C2N2	1.317
20.57	H17N3C2N2	−4.31
	H4N3C2	120.12
	H17N3C2	120.15
	H4N3H17	116.18
	H4N3	0.997
	H17N3	0.997
	N3C2	1.325
40.54	H17N3C2N2	−2.91
	H4N3C2	118.01
	H17N3C2	117.57
	H4N3H17	113.52
	H4N3	0.999
	H17N3	0.998
	N3C2	1.336
	C2N1	1.315
	C2N2	1.312
60.57	H17N3C2N2	5.29
	H4N3C2	116.03
	H17N3C2	114.93
	H4N3H17	110.96
	H4N3	1.001
	H17N3	1.000
	N3C2	1.351
	C2N1	1.310
	C2N2	1.310
90.38	H17N3C2N2	150.11
	H17N3C2	112.80
	H4N3C2	112.60
	H17N3H4	108.87
	H4N3	1.004
	H17N3	1.002
	N3C2	1.374
	C2N2	1.308
	C2N1	1.301

Additional calculations were performed with the Density Functional Theory (DFT) method, at the B3LYP level, with the 6-31G** basis set, which adds *p*-orbitals to the hydrogen atoms. These calculations were done using the Titan program [16] and were used to determine the structure of neutral saxitoxin, saxitoxin

Table 6 Some optimized parameters of structure 1b (length in Angstroms, angles in degrees)

β	Parameter	
−6.15	H20N5C5N4	−32.19
	H3N5C5	120.61
	H20N5C5	117.75
	H3N5H20	116.54
	H3N5	0.995
	H20N5	0.999
	N5C5	1.326
	C5N6	1.317
	C5N4	1.329
19.85	H20N5C5N4	−29.34
	H3N5C5	118.01
	H20N5C5	115.23
	H3N5H20	111.51
	H3N5	0.997
	H20N5	0.999
	N5C5	1.335
	C5N6	1.315
	C5N4	1.329
39.85	H20N5C5N4	−17.54
	H3N5C5	116.02
	H20N5C5	114.50
	H3N5H29	109.21
	H3N5	0.999
	H20N5	0.999
	N5C5	1.344
	C5N6	1.314
	C5N4	1.326
59.85	H20N5C5N4	−2.93
	H20N5C5	113.5
	H3N5C5	113.5
	H3N5H20	107.71
	H3N5	1.000
	H20N5	1.003
	N5C5	1.358
	C5N6	1.310
	C5N4	1.325
89.85	H20N5C5N4	27.05
	H3N5C5	109.4
	H20N5C5	111.55
	H3N5H20	107.82
	H3N5	1.003
	H20N5	1.008
	N5C5	1.383
	C5N6	1.300
	C5N4	1.324

Table 7 Some optimized parameters of structure 2 (length in Angstroms and angles in degrees)

H8N3C2N1	Parameter	
0.00	H10N3C2N2	0.0
	H8N3C2	121.4
	H10N3C2	122.5
	H8N3H10	117.2
	H8N3	0.996
	H10N3	0.996
	N3C2	1.319
	C2N1	1.319
	C2N2	1.319
20.59	H10N3C2N2	−5.77
	H8N3C2	120.15
	H10N3C2	120.10
	H8N3H10	115.86
	H8N3	0.997
	H10N3	0.997
	N3C2	1.323
	C2N1	1.321
	C2N2	1.320
40.41	H10N3C3N2	−2.51
	H8N3C2	118.51
	H10N3C2	117.75
	H8N3H10	113.37
	H8N3	0.999
	H10N3	0.998
	N3C2	1.333
	C2N1	1.317
	C2N2	1.318
60.22	H10N3C2N2	−5.49
	H8N3C2	116.57
	H10N3C2	115.25
	H8N3H10	110.89
	H8N3	1.001
	H10N3	1.000
	N3C2	1.348
	C2N1	1.314
	C2N2	1.313
90.00	H10N3C2N2	27.73
	H8N3C2	113.88
	H10N3C2	112.37
	H8N3	1.004
	H10N3	1.002
	N3C2	1.373
	C2N1	1.305
	C2N2	1.311

protonated at the five-membered ring, and at the six-membered ring. In addition, saxitoxin featuring a sodium ion attached to the exocyclic nitrogen of the five-membered ring (structure **4**) and with a sodium ion attached to the exocyclic nitrogen of the six-membered ring (structure **5**) are also investigated with the B3LYP method. The energies of the protonated saxitoxins featuring angles α and β of 90° were calculated. These results are shown in Tables 11 (energies), 12 (proton affinities) and 13 (rotational barriers).

Discussion

As seen from Tables 1, 2, 3 and 4, the lowest energies of all the species investigated correspond to the guanidinium ion positioned so that the NH_2 group is coplanar with the central carbon and one of the other nitrogens attached to it. Indeed, when total optimization of the molecule is performed, these are the configurations that are obtained. This is due to the delocalization of the π-electrons on the CN bond, leading to *Y*-aromaticity, as described by Gund [17]. As the NH bond of the guanidinium is rotated to afford dihedral angles of 20, 40, 60 and 90°, the energy rises, as shown in Tables 1, 2, 3 and 4, followed by changes in the parameters of the molecule, as shown in Tables 5, 6, 7 and 8. Such changes include especially the fact that the rotated amine group ceases to be planar, indicating that the nitrogen atom changes hybridization from sp^2 to sp^3 . Other changes concern the lengthening of the exocyclic NC bond, indicating that there is no more π-electron delocalization

Table 8 Some optimized parameters of structure 3 (length in Angstroms, angles in degrees)

H10N3C3N1	Parameters	
0.00	H12N3C3N2	0.0
	H10N3C3	121.57
	H12N3C3	121.57
	H10N3H12	116.84
	H10N3	0.995
	H12N3	0.995
	N3C3	1.330
	C3N1	1.321
	C3N2	1.321
20.75	H12N3C3N2	4.34
	H10N3C3	120.18
	H12N3C3	120.23
	H10N3H12	115.52
	H10N3	0.997
	H12N3	0.997
	N3C3	1.335
	C3N1	1.319
	C3N2	1.320
40.25	H12N3C3N2	−0.30
	H10N3C3	118.22
	H12N3C3	118.25
	H10N3H12	113.57
	H10N3	0.998
	H12N3	0.997
	N3C3	1.345
	C3N1	1.317
	C3N2	1.317
59.78	H12N3C3N2	8.00
	H10N3C3	116.15
	H12N3C3	115.83
	H10N3H12	111.28
	H10N3	1.001
	H12N3	0.999
	N3C3	1.360
	C3N1	1.313
	C3N2	1.314
90.38	H12N3C3N2	−34.30
	H10N3C3	112.47
	H12N3C3	113.24
	H10N3H12	109.11
	H10N3	1.004
	H12N3	1.002
	N3C3	1.385
	C3N1	1.305
	C3N2	1.311

Table 9 Proton affinity of the guanidine groups of different structures (kcal mol^{-1}), at HF/6-31G* level

Structure	Proton affinity
1a	257.23
1b	263.72
2	256.87
3	262.90

on it. These aspects of the guanidinium ion rotation have been discussed previously [18, 19]. However, one of the additional points of this work is to determine the differences between the saxitoxin guanidinium entities and the simple five-membered and six-membered rings with guanidinium groups attached.

Table 10 Rotational barriers of the guanidinium groups (kcal mol^{-1}) at HF/6-31G* level

Structure	Barrier
1a	9.94
1b	10.92
2	10.62
3	8.33

Table 11 Energies of the species investigated by the DFT (B3LYP) method with 6-31G** basis set

Structure	Energy (au)
1	1075.49713
1a	1075.92038
1b	1075.92561
4	1237.66862
5	1237.66348

Table 12 Proton and sodium affinities for the structures 1a, 1b, 4 and 5, with the B3LYP (6-31G**) method

Structure	
1a	Proton affinity 257.69 kcal mol^{-1}[a]
1b	Proton affinity 258.31 kcal mol^{-1}[a]
4	Sodium affinity 56.63 kcal mol^{-1}
5	Sodium affinity 53.41 kcal mol^{-1}

[a] The values include zero-point vibrational energy as calculated for the model five and six-membered rings, respectively

Table 13 Rotational barriers of the guanidinium group at DFT (B3LYP) level calculations (kcal mol^{-1})

1a	6.12
1b	8.11

The pyramidalization of the exocyclic NH_2 group when one of the NH bonds is rotated to form dihedral angles of 90° with the NCN plane is very similar for all structures examined, as can be seen from Tables 5, 6, 7 and 8. The exocyclic NC bond length is larger in structure **1a** than in **1b**. The same phenomenon may be seen in structures **2** and **3**, suggesting that the guanidinium ion attached to the six-membered ring features a longer exocyclic NC bond. As the guanidinium group is rotated, this bond length gradually increases with the increase of the dihedral angle, reaching its maximum value at 90°, where its single-bond character is most pronounced. As for the optimized structures, the NC attached to a six-membered ring is longer than the one attached to the five-membered ring.

The guanidine group attached to the six-membered ring features a higher proton affinity than the one attached to the five-membered ring, in agreement with the results of Sleno et al. [13]. There do not appear to be differences in the calculated proton affinities for the

simple five-membered and six-membered rings guanidines chosen as models, compared to the respective five-membered and six-membered rings incorporated within saxitoxin itself.

As seen from Table 10, the highest rotational barrier is featured by structure **1b** but no significant trend is observed.

When DFT (B3LYP) calculations are performed, the proton affinities of structures **1a** and **b** show the same trend. However, the value of the proton affinities are slightly higher than at the HF/6-31G* level. Table 12 shows the proton affinities including the zero-point energies.

As seen also from Table 12, the sodium affinities are very small, as expected. It is thus unlikely that sodium-channel blocking would occur via sodium ions bound by the saxitoxin molecule. It is more probable that the guanidinium group binds to carboxylate groups in the channel, as discussed in the introduction. To achieve the binding, it might be necessary for the group to rotate and one notices that the rotational barrier of the five-membered ring is lower than that of the six-membered ring. This effect might contribute to the fact that the five-membered ring guanidinium saxitoxin [1] is suggested to inhibit the sodium channel more than the six-membered ring structure [2].

It can also be seen from Tables 10 and 13 that the rotational energies predicted at the DFT (B3LYP)/6-31G** level are smaller than those predicted at HF/6-31G*.

Conclusions

The fact that the proton affinity of the guanidinium attached to the six-membered ring is higher than that of the guanidinium group attached to the five-membered ring is in agreement with previous calculations [13]. It can also be seen that the five-membered ring guanidinium is more flexible with respect to rotation.

The sodium affinities are quite low so they preclude significant binding, especially since the sodium ions would presumably be hydrated. Accordingly, it is most likely that the inhibition of the sodium channels is the result of the guanidinium ion binding to the carboxylate groups in the channel wall.

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