Theoretical Studies on Water–Tetracaine Interaction

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ABSTRACT: The action of local anesthetics (LA) is controversial. There is experimental evidence that the unprotonated form of LA penetrates the axon, while the charged form acts in the intracellular phase. To obtain some insight on the structure of the local anesthetics tetracaine and its pharmacological action, we made calculations using the density functional theory (DFT) method. After those calculations, we performed molecular dynamics (MD) simulations in a p, N, T ensemble, in an aqueous environment, on both unprotonated and protonated forms of the molecule. The radial distribution function was used to study water solvent effects, through the characterization of the affinity of tetracaine to water. The results indicate that the molecule has regions with different degree of hydrophobicity, and the N-terminal of the anesthetic was primarily affected by changes in the protonation state of the anesthetic. The pH-dependent activity of TTC should then be analyzed in view of local changes in different regions of the molecule, rather than in terms of general effects on the hydrophobicity of the molecule as a whole. Int J Quantum Chem 106: 1277–1282, 2006

Key words: density functional theory; molecular dynamics; radial distribution function; local anesthetics; hydrophobicity

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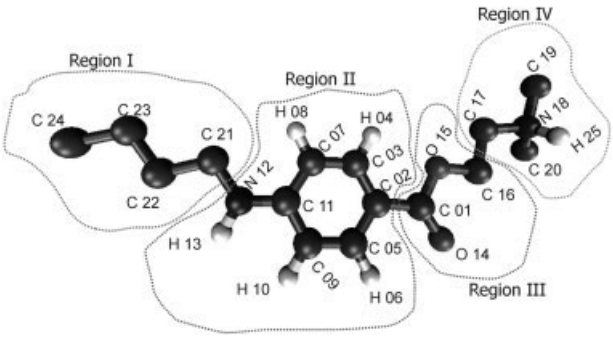

FIGURE 1. Schematic representation of tetracaine in the protonated form, corresponding to the minimal energy structure obtained from DFT calculations. In the unprotonated form, the atom 25 is absent. Region I of TTC is the aliphatic group, containing atoms C24–C21. Region II is the aromatic ring plus the secondary amine, corresponding to atoms C02–H13. Region III is the ester group, from atoms C01–C16. Region IV is the terminal tertiary amine, ranging from atoms C17–H25.

Introduction

The action of anesthetics of local use (LA) has been the object of several experimental studies. The dominant hypothesis is that in the unprotonated form LA crosses the interface of membranes, while the protonated form anchors in the membranes surface. The hypothesis came from the study of physicochemical and pharmacological properties of the molecules, such as their electronegativity, pK_a value, and data from electron paramagnetic resonance (EPR) experiments [1–4]. Detailed structural information about LA is not available from crystallography, mainly because of the difficulty in obtaining crystals of the material using conventional techniques.

Among several LA, tetracaine (TTC) (Fig. 1) is the subject of our attention. TTC is an amino ester unstable in water solution; thus, it is difficult for its structure to be well understood. The molecule has an ester group linking an amino group to an aromatic lipophilic ring. Variation on the amino or aromatic groups changes the chemical activity of the drug. The aromatic ring is soluble in lipids, and this should be important for the penetration of the anesthetic molecule through the lipid bilayer of the nerve cell membrane. In contrast, the amino group is water soluble, making possible to dissolve the molecule in the cellular aqueous environment, allowing TTC to remain in solution on either side of the nerve membrane. The role played by the anesthetic molecule is related to its distribution on both sides of the cell membrane, and this should be dependent on its protonation state. The pK_a of TTC is $>$8.5 [5], indicating that in the physiological environment the concentration of the protonated form is higher than that of the unprotonated form. When the pH decreases the anesthetic action diminish, indicating that the unprotonated form should be preferential to its physiological activity.

In any given solution of anesthetic, the molecular structure shifts between two forms that exists in an equilibrium dependent on the exact pH of the solution. In the present work, we examine the structural characteristics of TTC in different protonation states, obtained from calculations using the density functional theory (DFT) method. The results from quantum mechanical calculations were used to perform molecular dynamics (MD) simulations of TTC in the presence of water solvent molecules. To study the interaction of the molecule with the aqueous solvent, we examined the radial distribution function (rdf), referred to as $g(r)$. Despite the fact that $g(r)$ was calculated for all the atoms analyzed in MD simulations, we made the option to plot the $g(r)$ function for groups of atoms representative of different regions of TTC. We could then identify possible regions of high or low affinity for water molecules. The results allow us to foresee the behavior of the two forms of the TTC in the physiological environment nearby the cell membrane.

Materials and Methods

Initial geometry for TTC was established starting from its structural formula, imposing the planarity of the benzene ring for the two possible protonated and unprotonated forms. For quantum mechanics calculations we chose the DFT B3LYP at level 6-31G** [6–9] in the Gaussian package [10] and the calculations, made after choosing the chelp G method, provided the charges [11] corresponding to the ground lowest-energy conformations structures for both protonated and unprotonated forms of TTC and the geometry used as the starting configuration for MD simulations. The dynamical studies were carried out using the Gromacs computational package [12] in the NPT ensemble in a water environment (water SPC216), fixing the number of molecules N, the isotropic pressure at 1 bar, and the temperature T of the system at 300 K[13,14]. In the Gromacs package, the groups CH_3 and CH_2 were

treated as united atoms and the other atoms were explicitly represented.

The simulations were run for a total of 500 ps dynamics, and the Gromacs parameters for charge, interatomic distances, and bond angles were modified so that the initial structure for MD simulations corresponded to the results of quantum mechanics calculations. The parameters for the united atoms CH_3 and CH_2 were obtained adding the charges of the corresponding carbon and hydrogen atoms, and the positions, bond angles, and adjacent atoms are the same as those of the carbon.

Results

The results for atomic electronic charge obtained from quantum mechanical calculations (Table I) indicate that most atoms of TTC have only small modifications due to the protonation of the N-terminal. It is noticeable that the proton charge added to TTC in the protonated state is distributed throughout the molecule, so that all atoms negatively charged in the unprotonated molecule had the addition of some positive charge, decreasing its absolute value. The modification near the N-terminal is more dramatic, so that the Nitrogen changes its charge from negative to positive. In minor extent, the ester and the aliphatic chain are also affected (Table I).

TABLE I

Atomic charges for tetracaine obtained from DFT calculations using the chelpg option in Gaussian 03 package.*

Atom		Charges	
No.	Type[a]	Protonated	Unprotonated
1	C	0.65843	0.70798
2	C	−0.20553	−0.20597
3	C	−0.02105	0.01118
4	HC	0.08243	0.07277
5	C	0.01830	0.02076
6	HC	0.09935	0.08706
7	C	−0.25057	−0.27783
8	HC	0.11994	0.11608
9	C	−0.31150	−0.32759
10	HC	0.14134	0.13001
11	C	0.45804	0.46540
12	N	−0.62812	−0.68337
13	H	0.32542	0.32500
14	O	−0.50671	−0.53851
15	OA	−0.42920	−0.47951
16	CH_2	0.31663	0.30522
17	CH_2	0.11781	0.20775
18	N0	0.03401	−0.46019
19	CH_3	0.19885	0.12843
20	CH_3	0.20681	0.12843
21	CH_2	0.25163	0.25615
22	CH_2	0.25163	0.25615
23	CH_2	0.05802	0.06705
24	CH_3	−0.01955	−0.03748
25	H	0.28275	

* Numbers of atoms refer to convention adopted in Figure 1.
[a] Atom type convection: C: bare Carbon; HC: Hydrogen bound to Carbon; N: peptide Nitrogen; H: Hidrogen not bound to Carbon; O: carbonyl Oxigen; OA: hydroxyl Oxigen; CH_3, CH_2 aliphatic CH_3 or CH_2 group.

The effects in the molecule solvation properties due to the modifications in the electronic structure upon protonation/deprotonation were examined through the $g(r)$ function, which describes the average solvent density $\rho(r)$ at a distance r from a given solute particle. The presence of an atom at the origin of the reference system excludes other particles at distances smaller than the radius of the first coordination shell corresponding to the first maximum in $g(r)$. The presence of the first coordination shell tends to exclude particles at distances closer than the radius of the second coordination shell, where $g(r)$ has another maximum. The oscillatory form for $g(r)$ persists until r is larger than the range of correlation between the particles. At distances larger than the correlation length, for uncorrelated particles, $g(r)$ is equal to one. Considering the solvent as water molecules, values of $g(r)$ of >1.0 indicate that the atom or group of atoms in the region under consideration is hydrophilic, and values off <1.0 indicate that the analyzed region is hydrophobic.

To make analysis of $g(r)$ more comprehensible, we divided the molecule in four groups of atoms (Fig. 1). The region I is the aliphatic chain corresponding to atoms 21, 22, 23, and 24 in Figure 1; the region II contains the secondary amine (atoms 12 and 13) and the benzene ring (atoms 02–11); the ester group (atoms 01, 14, 15, 16) forms the region III; and the region IV consists of the tertiary amine, made by atoms 17, 18, 19, and 20, plus the hydrogen atom (atom 25), in the protonated molecule. This last region of TTC molecule has peculiar properties and we used independent plots of $g(r)$ functions for CH_3 atoms and the hydrogen atom added to the molecule.

The plots of $g(r)$ show that solvation properties of regions I, II and III of TTC are not significantly modified with protonation/deprotonation (Figs. 2–4). Figure 2 shows that the plots for the region I of unpro-

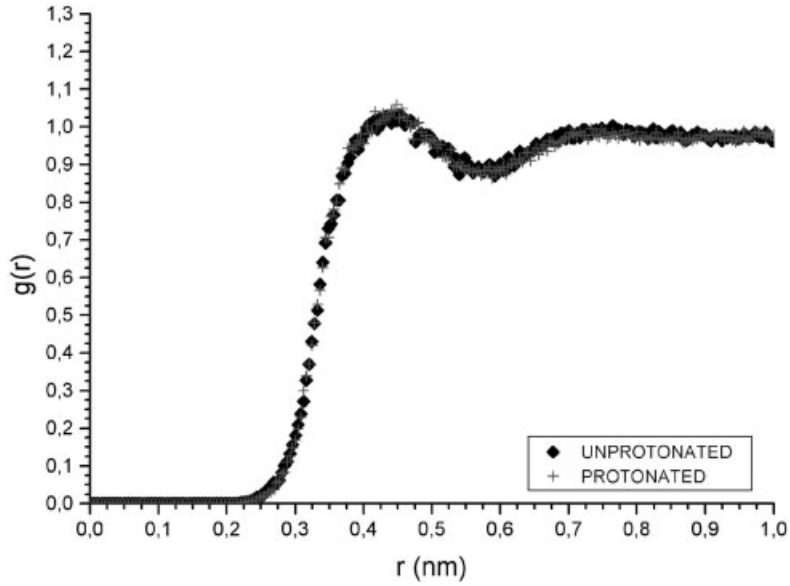

FIGURE 2. Plot of radial distribution function for the aliphatic region (Region I) of TTC. +, protonated TTC; ◆, unprotonated TTC.

tonated TTC (curve ◆) and protonated TTC (curve +) are similar so that the traces are indistinguishable. The $g(r)$ function has a peak with intensity ~1.1, at a distance of 0.4 nm (Fig. 2). These values indicate a weak hydrophilic character in that region of TTC.

The region II is clearly hydrophobic: the $g(r)$ function of the unprotonated molecule (curve ◆ in Fig. 3) does not present any peak, and its intensity is <1.0 even at distances to water molecules as large as 1.0 nm. Since $g(r)$ determines the average density of water molecules at a coordinate r relative to this region, we could say that region II is hydrophobic. The same hydrophobic character is present in the protonated molecule (curve + in Fig. 3), where the

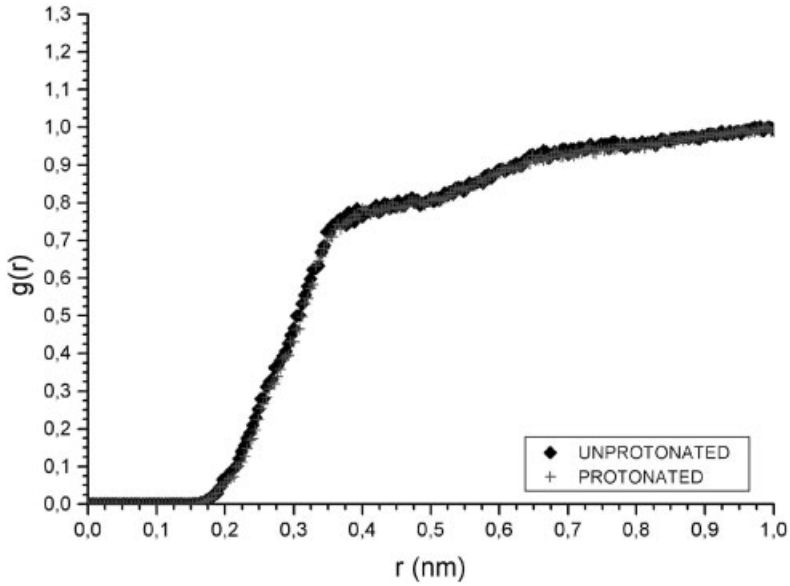

FIGURE 3. Plot of radial distribution function for the secondary amine and the benzene ring (Region II) of TTC molecule. +, protonated TTC; ◆, unprotonated TTC.

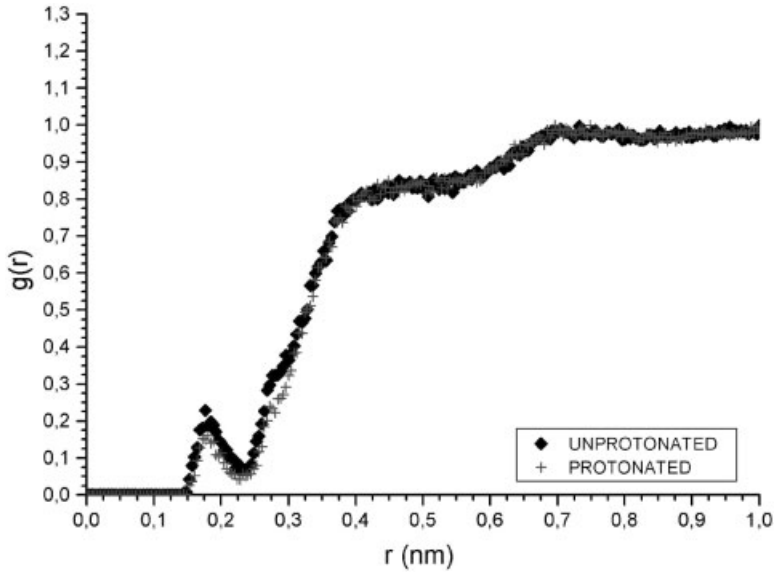

FIGURE 4. Plot of radial distribution function for the ester group (Region III) of TTC molecule. +, protonated TTC; ◆, unprotonated TTC.

intensity of $g(r)$ function takes values near to 1.0 only at distances near 1.0 nm from water molecules. The plots for the region III shows that the ester group of TTC has the same hydrophobic character both in protonated as unprotonated forms (Fig. 4), the differences being irrelevant to the behavior of the molecule in the aqueous environment. The small "peaks," with intensities of <0.20 in unprotonated and protonated TTC, at ~ 0.18 nm from water solvent, are due to carboxyl oxygen, and have minor effects in the hydrophobic/hydrophilic behavior of the molecule.

In contrast to the others, region IV, which corresponds to the tertiary N-terminal, changes significantly with protonation/deprotonation. That region is only slightly hydrophilic in the unprotonated molecule and has high hydrophilicity when TTC is protonated. The $g(r)$ function of the amine C-terminal atoms display this change in the affinity to water: in the protonated molecule there are peaks with intensity greater than the normalized value at distances of 0.35 nm (curve + in Fig. 5). In contrast, in the unprotonated molecule the peak of $g(r)$, present at same distance, have intensities near to or less than the normalized values (curve ◆ in Fig. 5).

Conclusions

The methods employed in our calculations proved useful to describe the non-homogeneous character of TTC with respect to its affinity to the water solvent molecules. We could identify different regions of the molecule with varied degree of hydrophobicity and the modifications in the affinity to water caused by changes in its state of protonation. The unprotonated TTC has a predominantly hydrophobic character, clearly present not only in the aromatic ring, but also in the ester chain, while some small affinity to water is present in the region of the aliphatic chain and the tertiary N-terminal. On the other side, the protonated molecule acquires hydrophilic character not only in the aliphatic chain terminal but also in the tertiary N-terminal.

Despite the possible specific interaction of TTC with membrane proteins, the differences in hydrophobicity between protonated and unprotonated forms are relevant to the interaction with the cell membrane. The results show that the TTC molecule in a water/membrane interface should behave differently, depending on its state of protonation: the neutral molecule will insert into the lipid phase of the membrane, keeping the aliphatic terminal pointing to the more polar head groups; the protonated TTC will have the central aromatic ring and ester group penetrating into the nonpolar region of the membrane, and the terminals anchored in the surface. The results are consistent with the proposal of a mechanism of action pH dependent. When both forms are present in equilibrium at the water/membrane interface, the protonated form would penetrate less into the membrane, compared to the

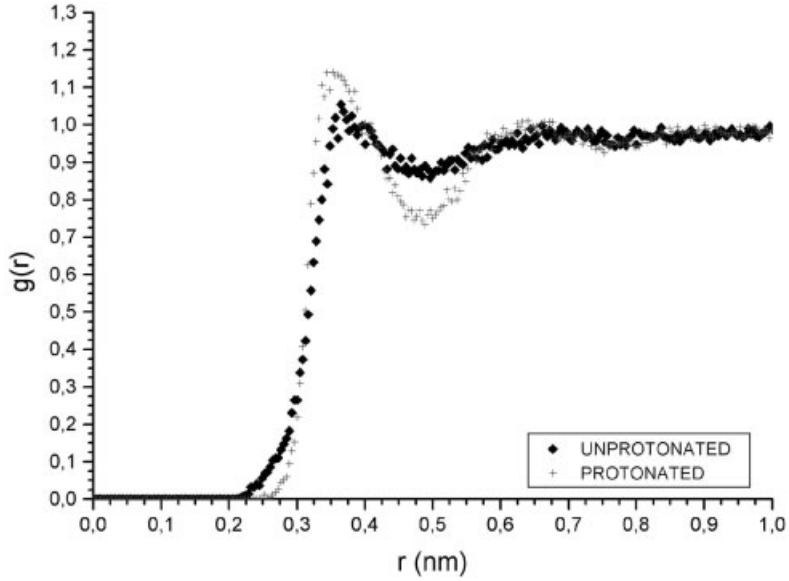

FIGURE 5. Plot of radial distribution function for the distances between CH_3 and water solvent for region IV. +, protonated TTC; ◆, unprotonated TTC.

unprotonated form that can go further inside the bilayer. However, the decrease in the anesthetic action of the molecule lowering the medium pH, more than a result of general changes in the degree of penetration of TTC into the membrane, can be related to the positioning of both aliphatic and N-terminals of the anesthetic in the polar region in the surface of the membrane.

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