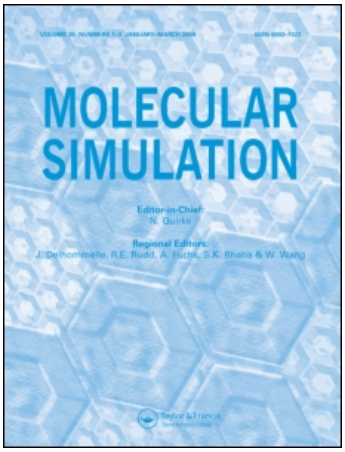

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Density functional and molecular dynamics simulations of local anesthetics in 0.9% NaCl solution

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We have made density functional calculations and molecular dynamics (MD) simulations to investigate the structure and pharmacological action of local anesthetics: tetracaine, procaine and lidocaine. The MD simulations were made in a NPT ensemble, in a 0.9% NaCl solution, on both unprotonated and protonated forms of the molecules. The radial distribution function was used to study solvent effects in different regions of the molecules. Although all three anesthetics have different degrees of hydrophobicity, the amino-terminals were the mostly affected by the protonation yielding hydrophilic regions. The charged amino-esters present hydrophilicity on the ester as well as amine terminals. Cl^- from the solvent solution forms hydrogen bonds via protonated hydrogen attached to nitrogen, yielding neutral molecules, which could, in principle, penetrate the membranes and loose Cl^- to act in the protonated form. Density functional theory calculations indicated a change in the electrostatic potential and showed that Cl^- weakly binds to the amine hydrogen, what suggests it is a favorable interaction and supports the existence of the hydrochloric forms of these local anesthetics.

Keywords: Molecular dynamics simulations; Local anesthetics; Tetracaine; Lidocaine; Procaine

1. Introduction

The physico–chemical properties of local anesthetics (LA) such as tetracaine (TTC), procaine (PRC) and lidocaine (LDC) (figure 1) have been of considerable theoretical and experimental interest. TTC and PRC are amino-esters composed of an ionizable amine, a polar group and an aromatic lipophilic ring. The amino groups should be able to dissolve in the cellular environment and remain on either side of the nerve membrane. The aromatic ring is soluble in lipids which is important for penetration through the lipid bilayer of the nerve cell membrane. For amino-amide LDC, an amide connects the lipophilic aromatic ring to an amine with an extra nitrogen in the carboxyl group (C═O).

In particular, it is believed that the charge state, hydrophobicity, hydrophilicity as well as the effects of solvents play important roles in pharmaceutical properties which determine whether the anesthetic is capable of going through the cellular membrane, shutting down the ions pathway, or remain attached to the surface of the cellular membrane determining the potency and extension of the anesthetic [1–5].

Apparently, the uncharged form is more capable of going inside membrane, meanwhile, the charged molecule remains attached on the interface. The anesthetic effect could come from both forms, but in different ways. It is possible that the organization of the charged form would help its docking on the ion channel, shutting it down. Meanwhile, the uncharged LA could disarrange the lipids from the membrane, thus changing the channel conformation and leading to an anesthetic effect. Therefore, there are two hypothesis: the first one refers to the binding between transmembrane protein (ion channel) and drug; the second one refers to the interaction between LA and membrane lipids, changing their structure and closing the ion channel [4–8].

In this work we have used density functional theory (DFT) and molecular dynamics (MD) simulations to investigate TTC, PRC and LDC with different protonation

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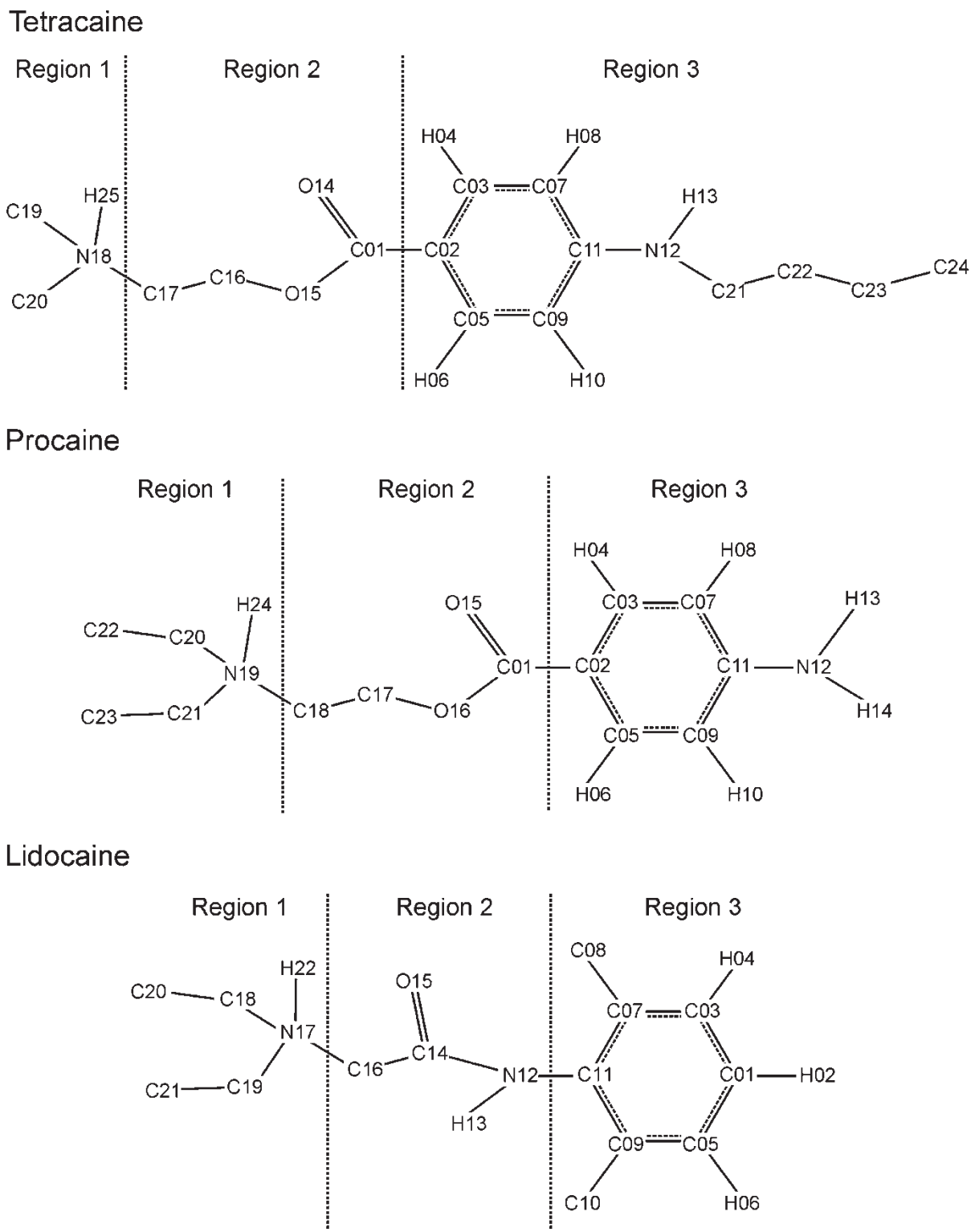

Figure 1. Schematic representation of the LA in the protonated form and with numberated atoms. 814 × 1027 mm (72 × 72 DPI).

states and concentrations of NaCl solutions. We determined radial distribution functions $g(r)$ for groups of atoms in order to examine hydrophobicity, hydrophilicity as well as the interaction of the LA with the NaCl solvents. The MD simulations also indicate a stable interaction between Cl^- of the solution with hydrogen of protonated form of the local anesthetics.

2. Methodology

The computer models of LDC, PRC and TTC were constructed based on molecular structures deposited on CAS (Chemical Abstracts Services—http://www.cas.org) from American Chemical Society. For each LA, two forms were built: protonated and a deprotonated. The optimized

molecular geometries were obtained by quantum mechanical (QM) calculation with the DFT/B3LYP method and the 6-31G** basis set using Gaussian03 package [9–10]. The atomic charges were fitted to reproduce the molecular electrostatic potential through the ChelpG scheme [11].

The polarization effects of solvent on LA [11] were evaluated on TTC using the Onsager continuum solvation model [12]. The obtained charges for each atom were very close to the charges calculated with the simpler methods (DFT/B3LYP without Onsager model) which neglect polarization effects. As the later calculation is time consuming, it was not repeated for the other LA.

The MD studies were made using the GROMACS package [13]. For LA and ions the GROMOS 43a1 [14–15] parameter set was used with modifications to LA partial atomic charges, bond angles and distances, according to the *ab-initio* results. The LA were placed in the center of a cubic periodic box, 4.5 nm length, and hydrated with two distinct solutions, one with pure SPC water model [16], and other with 0.9% NaCl (27 NaCl molecules in ~3000 water molecules varying little with LA) in a total of 12 simulation systems. This salt concentration falls into the range of concentration that have been applied in pharmacological experiments [17].

The simulations were carried out in the NPT ensemble and the systems were thermodynamically coupled in every step to a 300 K bath and pressure coupling was at 1 bar, with Berendsen models [18]. Non-bonded interactions were considered to a "cut-off" of 1 nm, and the simulation time-step was set to 2 fs. An initial energy minimization was carried out followed by a 2 ns simulation to equilibrate all systems before a production run of 5 ns.

The radial distribution function (RDF), know as $g(r)$ function, is defined as the average radial density of a certain observable to a distance r from an origin that provide an insight regarding the local structure of the surrounding media such as hydration shells for a solvated molecule [19]. The $g(r)$ function can be used to identify hydrophilic and hydrophobic groups and regions of affinity to the solvent as well.

The structure for LA–Cl^- complex for QM calculations was produced by placing the Cl^- atom in the same bond plane and 0.3 nm away from amine hydrogen, distance derived from MD, and optimized by B3LYP/6-31G**. Constraints were applied to maintain the protonation state of the amine. The MD simulation of this complex was carried out after a 1 ns run with position restraints (force constant = 1000 kJ/(mol nm^2)) to TTC–Cl^- complex followed by a production run of 1 ns in the same conditions described above.

3. Results and discussion

The charge distribution for the investigated LA indicates a larger variation in amine nitrogen for the charged molecules which is a result of the extra hydrogen that attaches to nitrogen (N18, N19, and N17 in figure 1 to TTC, PRC and LDC, respectively). The negative charge of N for the LA in the neutral form assumes a positive charge when the local anesthetics are charged, as showed in our previous work [8].

The relation between solvent and LA was analyzed using the calculation from the RDF of the dynamics. LA can be divided into some regions (figure 1). The region 1 is a tertiary amine (dimethylamine on TTC and diethylamine on PRC and LDC), the second region is ester for some anesthetics, like TTC and PRC, and an amide for LDC. The region 3 is singular for each anesthetic but an aromatic ring is a common feature in all LA. The first region is of particular interest as the protonation site of molecules and has important features such as coordination of water shells and affinity to different portions of biological membranes, and will be more investigated for all three LA.

3.1 Region 1

The $g(r)$ of water for the amine hydrogen shows a reasonable difference in the intensity of the first peak (figure 2). This intensity is lower in NaCl simulations, suggesting its presence around this region or even a disturbance on the water coordination shell around this amine hydrogen. To investigate a possible interaction between the proton and Cl^- we monitored their minimal distance through simulation (figure 3). The distance distribution shows that the Cl^- ion may occasionally interact with the amine hydrogen, for all LA. The close distances occur within short lifetimes, probably due to weak binding between Cl^- ion and the LA [20–22] and a limitation of classical mechanics to reproduce electronic effects that would stabilize this interaction. To override this limitation and check the possibility of a stronger interaction we performed QM calculations using DFT B3LYP/6-31G** for more precise data.

The geometry optimization indicated for TTC, a H25-Cl^- distance of 0.168 nm, the ChelpG charge for Cl^- was $-0.63e$ and the H25 changes from $0.28e$ to $0.12e$. For PRC the H24–Cl^- distance was 0.180 nm, the charge for Cl was $-0.64e$ and for H24 changes from $0.30e$ to $0.12e$. For LDC the H22–Cl^- distance was 0.185 nm whereas the H13–Cl distance was 0.223 nm, the charge for Cl^- was $-0.61e$ and the H22 changes from $0.12e$ to $0.065e$. The charge distribution and the smaller distance among these atoms suggest the existence of an interaction for these groups.

In the QM calculation with Cl^-, the three LA folded in different ways to weakly bind to the hydrogen that protonates the LA (figure 4). For TTC, only the amine terminal is slightly rotated to permit the H25–Cl^- bond. In PRC the amine rotated around 180° to accommodate the H24–Cl^- bond. In LDC this same region also rotated around 180° for the H22–Cl^- bond and formed an additional H13–Cl^- bond (2.23 Å). The conformational changes lowered the internal energy of the LA compared

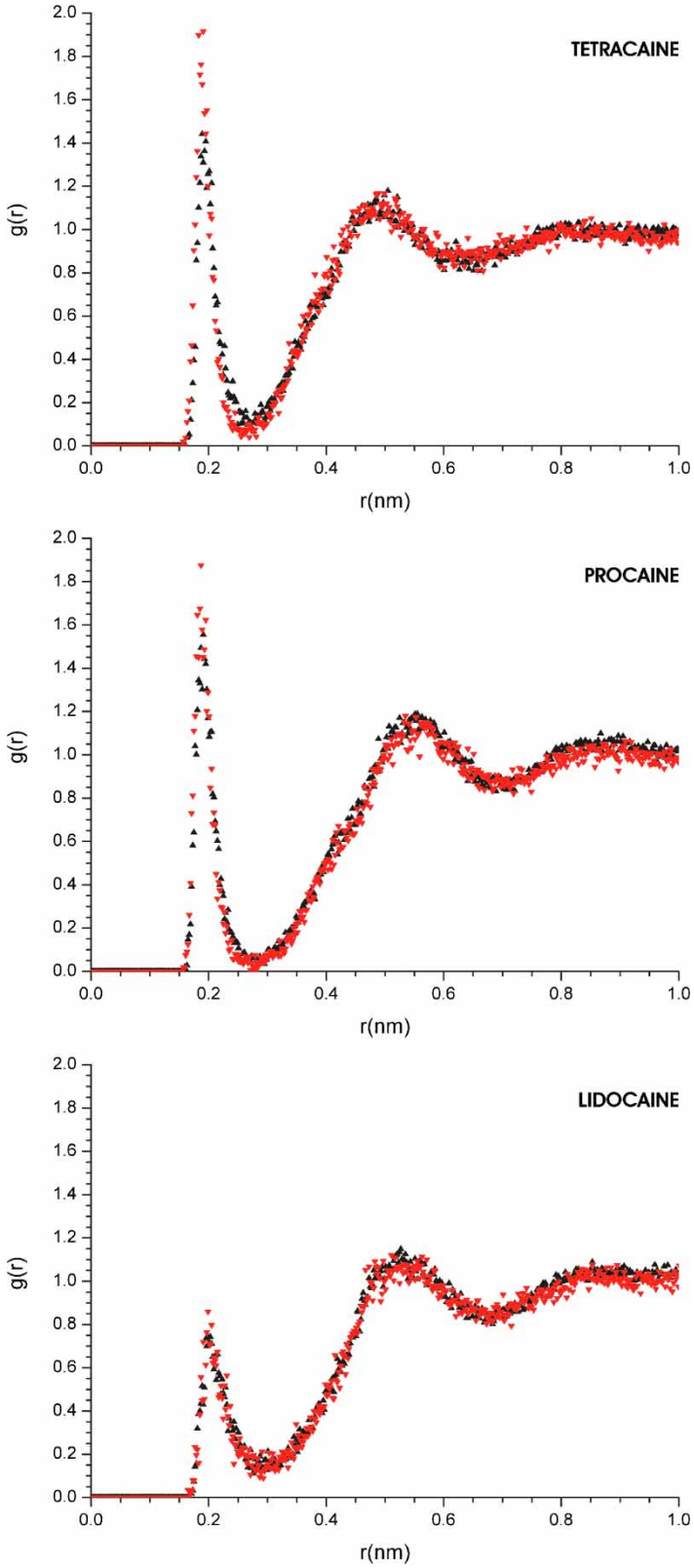

Figure 2. Plots of $g(r)$ for the hydrogen atom involved in the protonation of LA molecules without NaCl (▼), with 0.9% NaCl/water solution (▲). 650 × 1208 mm (72 × 72 DPI).

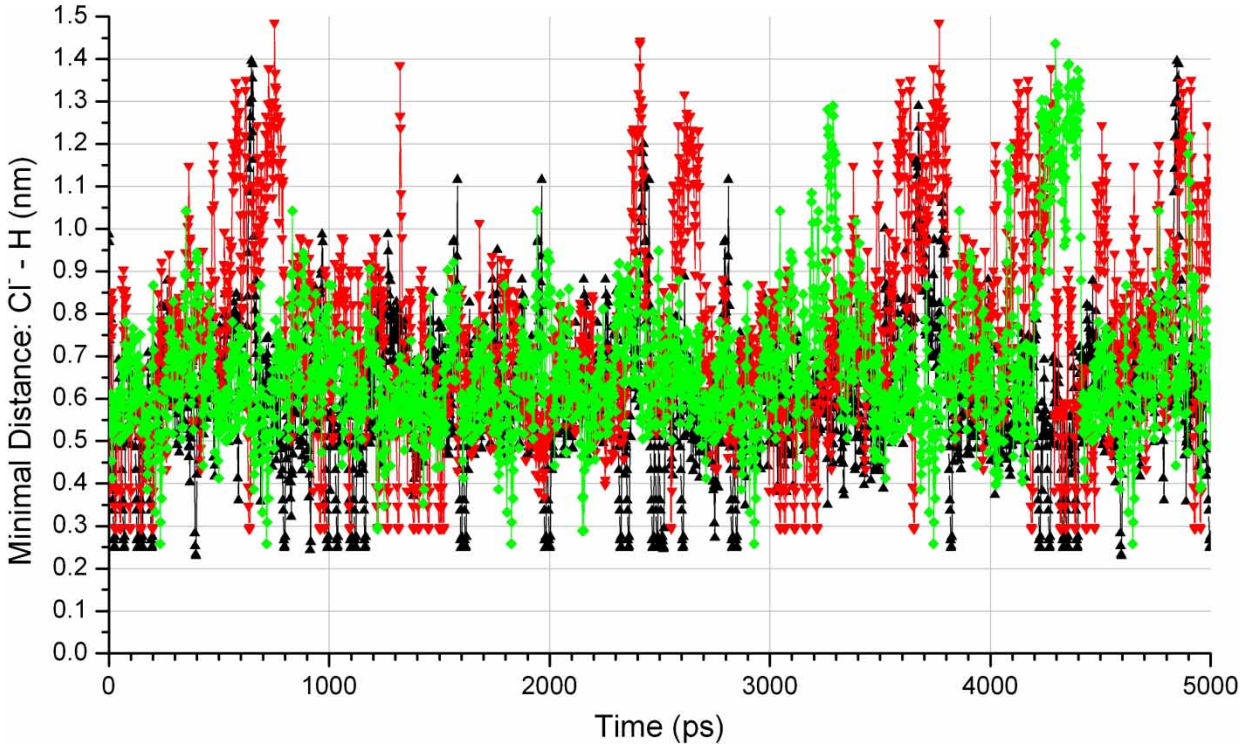

Figure 3. Plots of distance between the closest Cl^- to the hydrogen atom that protonated the LA—tetracaine (▲), procaine (▼) and lidocaine (◆). 1212 × 850 mm (72 × 72 DPI).

to simulations without Cl^-, what suggests it is a favorable interaction and supports the existence of hydrochloric forms of these local anesthetics.

Using the new conformation and charge values we performed a MD only for TTC to test how long will Cl^- be connected to the drug in a purely classical dynamics. For this system a 1ns MD simulation with position restraints for LA–Cl complex was carried out followed by an unrestrained 1ns MD simulation. The distance between Cl^- and H25 does not remain constant with CL^- going away from the LA in the first MD steps. In this simulation we are treating the system classically and not imposing any other constraints to the reproduce LA—Cl bond interaction. As only the non-covalent interactions are considered the charge difference between the two groups is not sufficient to stabilize the system.

3.2 Region 2 and 3

Analogous to our previous work of anesthetics in pure water solution [8], the three LA investigated in this paper are mostly hydrophobic or weakly hydrophilic in regions 2 and 3, the RDF which is indistinct from the water solvent analysis (data not show). In general the protonated forms of LA are slightly more hydrophilic than the deprotonated molecules. Region 3, characterized by the aromatic ring, is clearly hydrophobic for all three LA in both the protonated and deprotonated forms in saline solution as well as in water [3].

4. Conclusions

It is noticeable from this work that the presence of NaCl affects the protonated form of the LA turning then into slightly more hydrophilic molecules. DFT and MD calculations are useful to describe the interactions and predict practicable pharmacological trends for the LA with solvent solutions and assist the development of models to explain whereas the LA penetrates the cell membrane or remain attached to its surface, and determine the potency and length of the anesthesia. The investigated LA showed a different degree of hydrophobicity, hydrophilicity and affinity to the solvent solution depending on the charge of the molecules, but the presence NaCl affects only the water shell around of amine-terminal of protonated molecule. It is possible that, for being a polar region, it is more susceptible to binding to NaCl and the probability of a local distrubance in the water coordination shell is greater than in others portions' of the molecules.

With quantum data we propose the following model, Cl^- attaches to hydrogen of the charged species, neutralizing the molecule charge helping it to penetrate the membrane. The LA–Cl^- interaction, known as hydrochloric form, is seen in various experimental procedures [20–22]. It is also known that it is easier for the neutral species to penetrate the membrane [5–6] and this would be facilitated by the bonding of H and Cl^-. After penetrating the membrane, we expected that the anesthetic separates from the Cl^-, regaining its charged form to interact with ion channels inside the cell membrane.

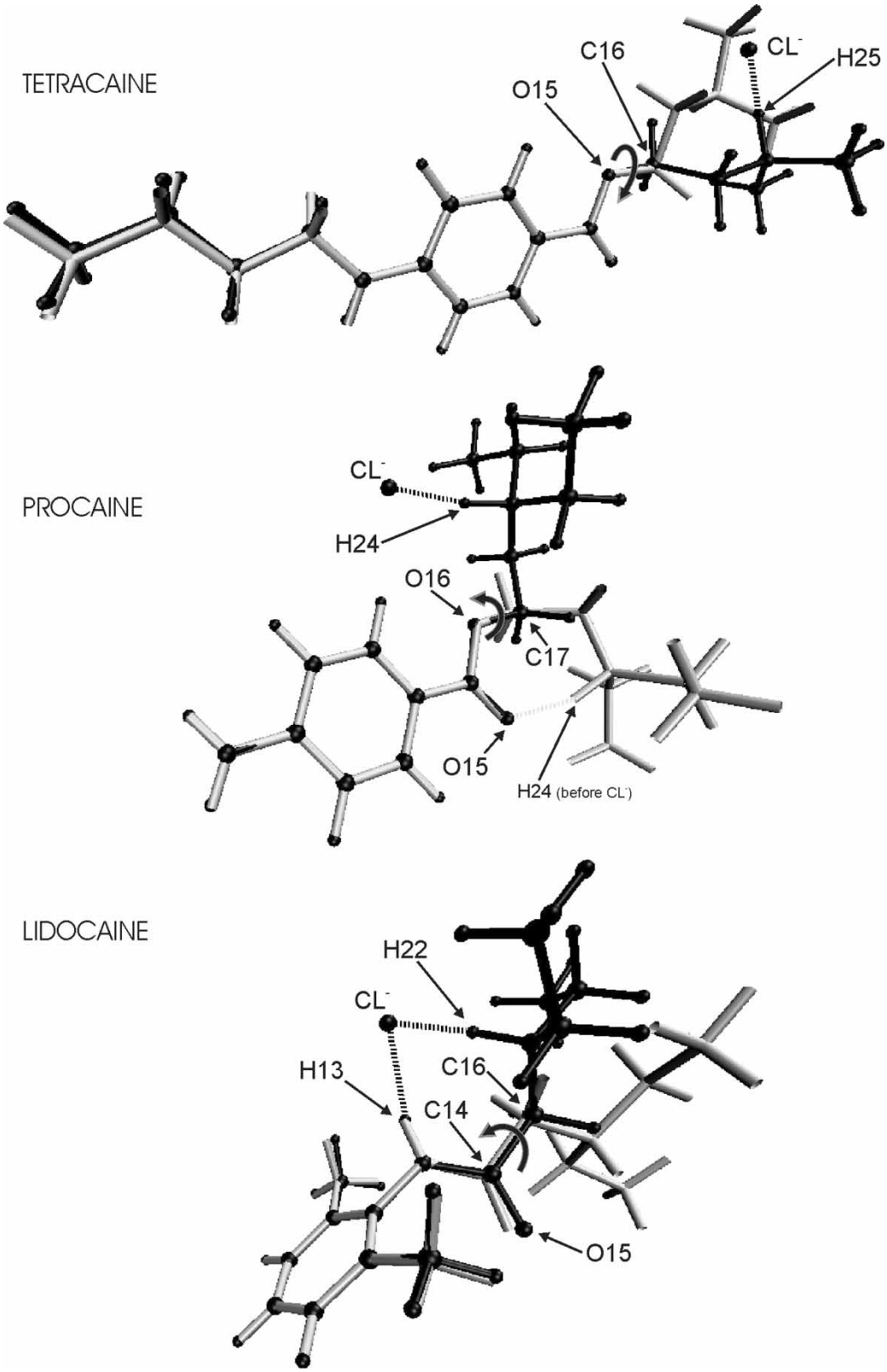

Figure 4. Caption from DFT B3LYP/6-31G** calucation. In gray we have the output file without Cl^- and in black the output with Cl^-. 546 × 782 mm (72 × 72 DPI).

Acknowledgements

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