Computational Biology and Chemistry 71 (2017) 82–88

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/compbiolchem

Research Article

Evaluation of degradation mechanism of chlorhexidine by means of Density Functional Theory calculations

Michele Aparecida Salvador[a,*], Camila Pinheiro Sousa[b], Simone Morais[c], Pedro de Lima-Neto[b], Adriana Nunes Correia[b], Paula Homem-de-Mello[a,*]

[a] Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Av. dos Estados, 5001, Bloco B, sala 1017, 09210-580, Santo André - SP, Brazil
[b] Departamento de Química Analítica e Físico-Química, Centro de Ciências, Universidade Federal do Ceará, Bloco 940, Campus do Pici, 60440-900, Fortaleza - CE, Brazil
[c] REQUIMTE–LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, R. Dr. António Bernardino de Almeida 431, 4200-072, Porto, Portugal

ARTICLE INFO

Article history:
Received 25 July 2017
Received in revised form 26 September 2017
Accepted 1 October 2017
Available online 3 October 2017

Keywords:
Density Functional Theory
Chlorhexidine
Oxidation mechanism

ABSTRACT

Chlorhexidine (CHD), a germicidal drug, has degradation products that can be hemotoxic and carcinogenic. However, there is no consensus in literature about the degradation pathway. In order to shed light on that mechanism, we have employed Density Functional Theory to study reactants, in different protonation states, products and intermediates involved in the different pathways. Based on free energy values comparison and frontier molecular orbital analysis, we have obtained the most stable structures in each protonation state. CHD in saturated form has HOMO localized in one *p*-chloroaniline, and, due to molecule's symmetry, HOMO-1 has contributions from the other side of the molecule, but mainly from the biguanide portion of the molecule, instead of from the *p*-chloroaniline. For the saturated form, we have studied two possible degradation pathways, starting from the monoprotonated structure, and three pathways starting from the neutral structure. We found out that the mechanisms proposed in literature, whose pathways lead to *p*-chloroaniline (PCA) formation in a smaller number of steps, are more likely than the mechanisms with more intermediate steps or pathways that do not predict PCA formation. Also, based on free energy results, we have found that the formation of another sub-product (PBG-AU) is favorable as well.

1. Introduction

Computational chemistry has been reported as an efficient tool to clarify kinetic and thermodynamic processes involved in synthesis, properties characterization (Matencio et al., 2017; Berzins et al., 2016) and degradation pathways of molecules and structures, especially pharmaceutical drugs (Silva et al., 2017; Rapolu et al., 2015). Its combination with experimental techniques appears as an interesting study field, since degradation products could become their use unfeasible. Indeed, electrochemical behavior and oxidation reactions could be better understood in association with theoretical evaluations (Silva et al., 2017). However, we have not found any computational study about the oxidation mechanism of chlorhexidine (CHD), a widely used drug.

Chlorhexidine (CHD) is a pharmaceutical drug composed by two (p-chlorophenyl) biguanide (PBG) portions bonded by a hexane chain. It is positively charged and reacts with the negatively charged microbial cell surface, destroying the integrity of the cell membrane (NCBI, 2015), therefore being widely used because of its germicidal activity against bacteria, yeasts, and molds (Wang and Tsai, 2001; Jones et al., 1998; Leitune et al., 2010; Fong et al., 2010; Pusateri et al., 2009; Edmiston et al., 2007; Lindblad et al., 2010; Loguercio et al., 2009; Ji et al., 2010). Although the use in mouthwash formulations, toothpaste and disinfectant solutions have been effective, CHD's stability is limited by the appearance of *p*-chloroaniline (PCA), which is its primary degradation product, known as being hemotoxic and quickly absorbed and metabolized, as well as being carcinogenic (Wang and Tsai, 2001; Zong and Kirsch, 2012).

* Corresponding authors.
E-mail addresses: michelesalvador@gmail.com (M.A. Salvador), paula.mello@ufabc.edu.br (P. Homem-de-Mello).

https://doi.org/10.1016/j.compbiolchem.2017.10.001
1476-9271/

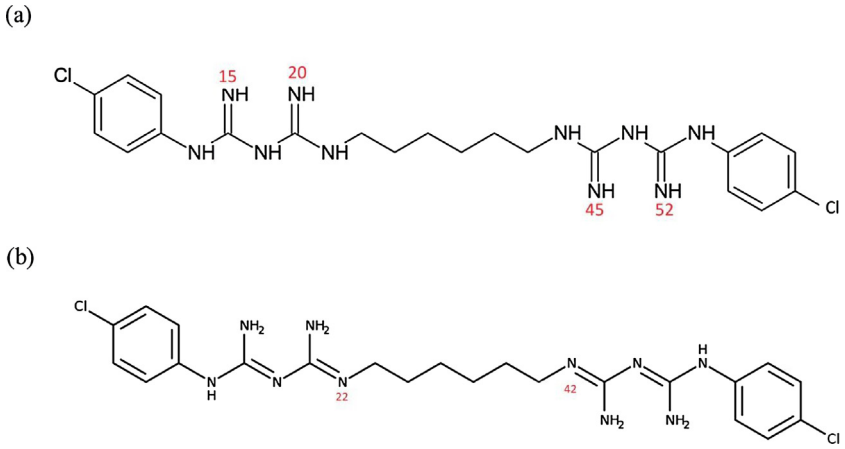

Fig. 1. Two possible neutral structures for CHD: in (a) CHD's saturated chain and in (b) CHD's unsaturated chain. Numbers indicate protonable N atoms.

There are different proposed structural forms of neutral CHD, as shown in Fig. 1, being (a) the neutral form with a saturated chain and two pairs of nitrogen atoms bonded to the chain by unsaturated bonds (NCBI, 2015; Anon.,2017) as presented by Wang and Tsai (2001), and (b) the neutral form with an unsaturated chain, as proposed byVan Oosten et al. (2014), and Blackburn et al. (2007). Bharatam et al. (2005) represented CHD with unsaturated bonds as its neutral structure (Fig1(b)), and attributed its chemical, biochemical, and therapeutic activities to the electron distribution in the system.

The commercial form (Fig. 1(a)) of the drug is doubly positively charged and available in acetate, gluconate and hydrochloride forms (Ha and Cheung, 1996). As can be seen for both neutral proposed structures of CHD, this is a symmetric molecule, and different forms of protonation have been reported in acidic conditions, with two (Anon., 2017) or four (NCBI, 2015) protonated nitrogen atoms. Wang and Tsai (2001) suggested that each PBG portion has two nitrogen atoms with unsaturated bonds, leading to two protonable sites.

Different pathways to CHD's degradation in acidic medium have been proposed (Edmiston et al., 2007; Anon., 2017; Van Oosten et al.,2014); even though Ha and Cheung (1996) reported degradation pathways under alkaline conditions, we focused on acidic media since most of the experimental work was done under this condition. In acidic conditions, CHD's degradation lead to PCA formation (Edmiston et al., 2007; Anon., 2017; Van Oosten et al., 2014), but *p*-chlorophenyl-biguanidine (PCPG) and phenylbiguanidine have been reported as degradation products as well, being the latter a photolysis product (Zong and Kirsch, 2012). Revelle et al. (1993) reported the identification of 11 degradation products by High-Performance Liquid Chromatography and Mass Spectrometry (HPLC–MS), six of them representing new compounds, and various degradation mechanisms were proposed for different stress conditions. They suggested that the main decomposition product under thermal stress is the so-called impurity G (referred as PBG-G by Wang et al.).

Taking these apparently conflicting experimental results into account, in this work we aim to shed light on the oxidation process of chlorhexidine, starting from different neutral forms, and by considering various protonation sites. In a previous work (Sousa et al., 2017) we performed quantum mechanical calculations simulations and free energy studies to support electrochemical studies on CHD oxidation, focusing on the adsorption process. Here, we extend that study in order to give a molecular insight about the effects of the protonation states on degradation pathway of CHD. The evaluation of the stability of intermediates and products of this degradation process can aid for example, in developing new formulations and drug delivery systems (Fernandes et al., 2014).

2. Methodology

We have studied different states of protonation for CHD and also possible intermediates and products of degradation proposed in literature, mainly in references (Zong and Kirsch, 2012; Ha and Cheung, 1996), namely:(*p*-chlorophenyl)urea (PCPU), (*p*- chlorophenyl)guanidine (PCPG), [(*p*-chlorophenyl)amidino]urea (PBG-AU), and N-amidino-N'-(*p*-chlorophenyl)urea (PBG-APU). We have employed Density Functional Theory (DFT), because it is the methodology that best predicts electronic and structural properties of molecular systems, allowing even the study of reaction mechanisms at a feasible computational cost (Gino DiLabio and Otero-de-la-Roza, 2014; Fernandes and Joa, 2007; Capelle, 2006; Geerlings, 2003).

In this way, we have optimized the geometries of proposed structures by means of B3LYP hybrid functional and 6-311G(d) basis set. Frequency calculations were carried out to ensure structures obtained are minima in the potential energy surface. From these calculations, we have obtained also free energies of formation of each compound (reactant in different protonation states, as well as possible intermediates and products) to evaluate the oxidation mechanisms. Time-Dependent Density Functional Theory (TD-DFT) calculations with B3LYP/6-311G(d) were carried out to obtain the frontier molecular orbital energies. All calculations were performed with Gaussian 09 package (Frisch et al., 2010). The values of Root Mean Square Deviation (RMSD), that quantifies the differences among geometries, was calculated using VMD software (Humphrey et al., 1996). MarvinSketch tool (ChemAxon, 2015), was used to estimate the pKa of the different

Table 1
Molecular orbitals energies (obtained via TD-DFT Calculations) and free energy of formation (ΔG) (a.u.) for CHD neutral and charged species.

	saturated CHD	unsaturated CHD
LUMO	−0.04288	−0.03583
HOMO	−0.20759	−0.21442
HOMO-1	−0.21709	−0.21778
HOMO-2	−0.23463	−0.21942
ΔG (neutral)	−2324.249669	−2324.246721
ΔG (charge +1)	−2324.002500	−2324.007668
ΔG (charge +2)	−2323.675495	−2323.704822