Theoretical Study and the Mechanism for the Berenil Reduction Reaction

Ferreira JV[1]*, Abreu FC[2], Pavão AC[3] and Nascimento VB[4]

[1]Instituto Federal de Alagoas, Maceió, Brazil

[2]Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Maceió, Brazil

[3]Departamento de Química Fundamental, Universidade Federal de Pernambuco, Recife, Brazil

[4]Departamento de Química, Universidade Federal Rural de Pernambuco, Recife, Brazil

Research Article

*For Correspondence

Joacy Vicente Ferreira, Coordenação de Química - Instituto Federal de Alagoas - IFAL. Rua Mizael Domingues, 75-Centro, Maceió - AL, 57020-600, Brazil, Tel: (082)21267000

E-mail: joacyferreira@hotmail.com

ABSTRACT

In this study, calculations of the berenil reduction reaction have been studied theoretically by using density functional theory. Results of lowest unoccupied molecular orbital and atomic partial charges it was possible to identify the atoms N10 and N11 as is the most likely region for reduction and a mechanism has been proposed.

INTRODUCTION

The Berenil [4,4'-(1-triazene-1,3-diyl)bis(benzamidine) aromatic symmetric diamidine] whose molecular structure is shown in **Figure 1**, is a compound that have significant anti-microbial activity and is used in veterinary medicine as an agent against Trypanosoma and Leishmania [1,2]. It is also applied as ligand for the purification of pDNA from clarified and non-clarified *Escherichia coli* process streams by pseudo-affinity chromatography [3].

With the use of voltammetric techniques it is possible to obtain parameters important in the study of reaction of oxidation and reduction of a molecules, such as the degree of reversibility and the number of protons and electrons involved in the reaction. However, a tool that has been widely used to help in the elucidation of the likely reaction mechanism this is Quantum chemical calculations, through the electrostatic potential derived charges and frontier molecular orbitals: HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) [4], giving an estimate of likely atoms involved process of oxidation or reduction.
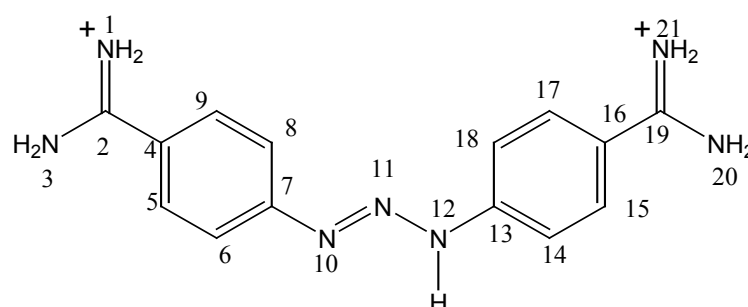

Figure 1. Molecular structure of berenil.

The reduction of berenil on glassy carbon and mercury electrodes examined by cyclic voltammetry showed a unique irreversible reduction with the entry 2e-/2H+ [5,6]. In these two presenting works the authors suggest that the reduction occurs in the triazene function (-N=N-N-) of the berenil molecule. However, they do not indicate exactly which atoms involved and the mechanism of the reduction reaction. For a better understanding of the electrochemical process we conducted density functional theory (DFT) calculations of determining exactly the atoms where occurs the reduction the likely mechanism. A theoretical study similar with semi-empirical quantum chemistry method was used successfully on the proposition of the mechanism of oxidation of the tricyclic antidepressant amitriptyline [7].

COMPUTATIONAL METHOD

The density functional theory (DFT/B3LYP) method [8-10] and 6-31+G(d,p) basis set was used in the optimization of the molecular geometry of berenil, as well as in the calculation of the atomic charges derived from the electrostatic potential and the graphical representation of LUMO (lowest unoccupied molecular orbital) was obtained using the Gaussian 09 program [11].

RESULTS AND DISCUSSION

This study began with the geometric optimization of the berenil in the ground state (+2 charge). The geometric optimization is an important step for obtaining correct of the partial atomic charges and frontier molecular orbitals. The **Table 1** shows a comparison between the calculated inter-atomic distances in the ground state and the experimental values [12-15]. According to these results a planar molecule is expected in agreement to the X-ray spectroscopy [12]. In addition, it is found a good agreement for the calculated and experimental bond distances between the calculated and experimental values.

Table 1. Interatomic Bond Lengths (in Å) for berenil.

Bonds	Calculated	Experimental
C-C		
C2-C4	1.47	1.48
C16-C19	1.47	1.49
C4-C5	1.41	1.39
C5-C6	1.39	1.37
C6-C7	1.4	1.38
C7-C8	1.41	1.4
C8-C9	1.38	1.37
C4-C9	1.41	1.4
C15-C16	1.41	1.39
C14-C15	1.38	1.36
C13-C14	1.41	1.39
C13-C18	1.41	1.4
C17-C18	1.38	1.37
C16-C17	1.41	1.39
C-N		
C7-N10	1.41	1.41
C13-N12	1.38	1.39
N-N		
N10-N11	1.26	1.27
N11-N12	1.34	1.33

In order to identify the most probable sites for the reduction reaction of the molecule, a LUMO contour map **(Figure 2)** analysis are obtained for berenil in the ground state. The orbital LUMO provides evidence on the sites of reduction of a molecule as this orbital is responsible for entry of electrons in the molecule.

As can be seen in the **Figure 2**, the orbitals located in the atoms C4, C5, C6, C7, N10, N11, N12, C13, C14, C15 and C16 offer contributions to form the LUMO. Hence, those regions must be directly involved in the reduction process.
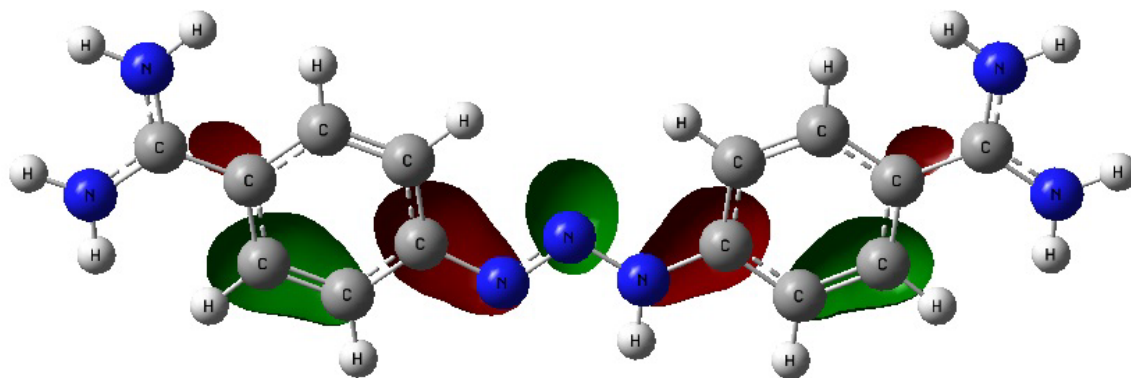
Figure 2. LUMO contour map of the berenil in the ground state.

Table 2. Calculated atomic charges for the berenil molecule in the ground state, reduced form and protonated reduced form.

Atoms	Ground state	Reduced form	Reduced protonated form
N1	-0.83	-0.87	-0.78
C2	0.70	0.53	0.64
N3	-0.84	-0.89	-0.79
C4	-0.11	-0.19	-0.12
C5	-0.06	-0.06	-0.03
C6	-0.21	-0.36	-0.28
C7	0.45	0.49	0.38
C8	-0.19	-0.26	-0.21
C9	-0.10	-0.12	-0.09
N10	-0.40	-0.54	-0.40
N11	0.03	-0.27	-0.23
N12	-0.12	-0.10	-0.43
C13	0.20	0.18	0.40
C14	-0.21	-0.27	-0.32
C15	-0.05	-0.09	-0.01
C16	-0.11	-0.14	-0.14
C17	-0.09	-0.12	-0.11
C18	-0.10	-0.16	-0.13
C19	0.69	0.52	0.65
N20	-0.84	-0.88	-0.79
N21	-0.84	-0.88	-0.78

To realm the evidences observed from the LUMO analysis, electrostatic atomic charges were calculated for three states: ground state (charge +2), reduced form (charge 0) and protonated reduced form (charge +2). This calculation allowed evaluating the change in electron density before and after the reduction of the molecule. The values of the atomic charge calculated are indicated in **Table 2**.

The **Table 2** shows that the inclusion of two electrons in the berenil molecule, leads to an increase in the electronic density of the atoms N1, C2, N3, C4, C6, C8, C9, N10, N11, C13, C14, C15, C16, C17, C18, C19, N20 and N21. As C4, C6, C8, C9, C13, C14, C15, C16, C17 and C18 atoms, are located in aromatic rings, the delocalization of charges in these regions explains the variations found out. Yet, in C2 and C19 atoms, although there is a considered increase in the electronic density after addition of electrons, they are not expected to represent sites of reduction, since, in accordance to the **Figure 2** they do not contribute to the formation of the LUMO. It is observed in **Table 2**, the largest variations in charge density occurred in the N11 (0.03 in the ground state and -0.27 after reduction) and the N10 (-0.40 in the ground state and -0.54 after reduction). So, the first step in the mechanism is the entrance of an electron in the atom N11 and the other in the N10. After the entry of electrons, the protonation occurs with the entry of H^+ ions in atoms N10 and N11 **(Figure 3)**. We see in **Table 2** that the protonation caused a decrease occurred in the electron density of the atoms N11 (-0.27 in the reduced molecule and -0.23 in the protonated molecule) and N10 (-0.54 in the

reduced molecule and -0.40 in the molecule after the protonation), simultaneously the protonation, occurs a rearrangement of the electron stabilizing the N10-N11 bond.
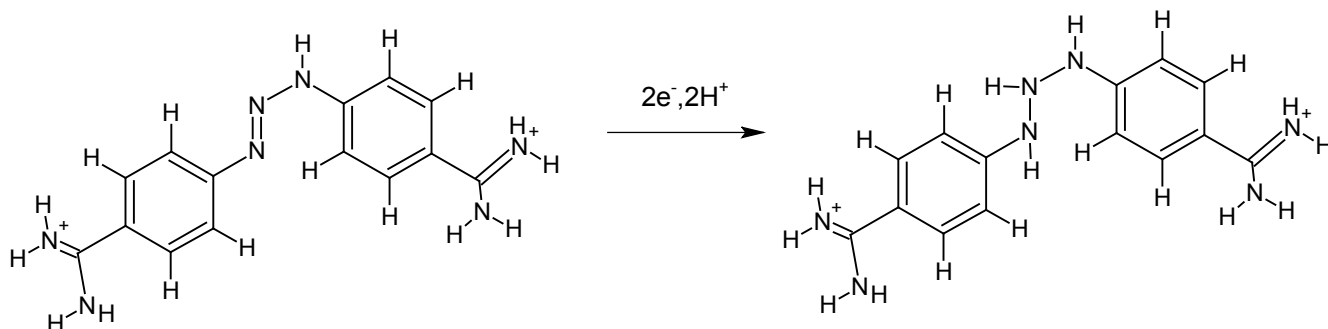

Figure 3. Berenil reduction mechanism.

CONCLUSION

The LUMO contour map of the berenil molecule shows a large contribution of atoms C4, C6, C7, C8, N10, N11, N12, C13, C14 and C16 to the formation of this orbital indicating that these atoms would be the possible sites for reducing the molecule. The electrostatic atomic charges of the berenil molecule in the ground state and after the reduction, reveals large variations in charge densities for the N10 and N11 atoms, indicating these atoms as the site of reduction in the molecule. A possible mechanism for the reaction of reduction of berenil was suggested.

REFERENCES

1. Burri C, et al. Topoisomerases in kinetoplastids. Parasitol Today. 1996;12:226-231.

2. Clausen PH, et al. Polymerase chain reaction and DNA probe hybridization to assess the efficacy of diminazene treatment in Trypanosoma brucei-infected cattle. Parasitol Res. 1999;85:206.

3. Nunes CC, et al. Purification of plasmid DNA from clarified and non-clarified Escherichia coli lysates by berenil pseudo-affinity chromatography. J Chromatogr B 2012;904:81.

4. Clare BW. Frontier orbital energies in quantitative structure-activity relationships: A comparison of quantum chemical methods. Theor Chim Acta 1994;87:415-430.

5. Abreu FC, et al. The Application of DNA-Biosensors and Differential Scanning Calorimetry to the Study of the DNA-Binding Agent Berenil. Sensors 2008;8:1519-1538.

6. Roig A, et al. Voltammetric behavior of berenil. J Pharmaceut Sci 1993;82:251-253.

7. Toledo, RA, et al. Estudo eletroquímico e químico-quântico da oxidação do antidepressivo tricíclico amitriptilina. Quím Nova. 2005;28:456-461.

8. Kohn W, Sham L. Self-Consistent Equations Including Exchange and Correlation Effects. J Phys Rev. 1965;140:1133.

9. Becke AD. Density-functional thermochemistry. III. The role of exact exchange. J Chem Phys. 1993;98:5648-4652.

10. Lee C, et al. Development of the Colle-Salvetti conelation energy formula into a functional of the electron density. Phys Rev B. 1988;37:785-789.

11. Gaussian 09, Revision A1. 2009.

12. Walton AR, et al. Studies on 1,3-Diaryltriazene Analogues of Berenil: Molecules with Potential GC Base-Pair Selectivity. Acta Crystallo Sec B: Structural Science 1991;47:771-775.

13. Gabelica V, et al. Interaction between antitumor drugs and a double-stranded oligonucleotide studied by electrospray ionization mass spectrometry. J Mass Spectrometry 1999;34:1328-1333.

14. Reinert KE. DNA multimode interaction with berenil and pentamidine; double helix stiffening, unbending and bending. J Biomol Struc Dynam. 1999;17:311-331.

15. Conte MR, et al. Interaction of minor-groove-binding diamidine ligands with an asymmetric DNA duplex. NMR and Mol Model Stud. 1995.