ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 01, pp.20339-20342, January, 2022

DOI: https://doi.org/10.24941/ijcr.42016.01.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IN SILICO ANALYSIS OF THE CONFORMATIONAL CHANGES OF CEFAMANDOLE INSIDE AND OUTSIDE THE ACTIVE SITE OF THE B-LACTAMASE K73A FROM *MYCOBACTERIUM TUBERCULOSIS*

Tejeda Rosales María Elena

Computational Chemistry Laboratory, Pharamceutical and Biological Chemistry Department, Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México

ARTICLE INFO

Article History:
Received 07th October, 2021
Received in revised form
16th November, 2021
Accepted 14th December, 2021
Published online 28th January, 2022

Key Words:
In silico analysis
Computational Chemistry
Computational Biochemistry.

**Corresponding author:*
Nema Ram

ABSTRACT

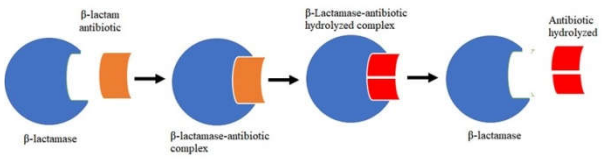
Experimentally it is not possible to calculate the energy nor the structure of the substrate during an enzymatic reaction. With the aid of *in silico* analysis, it is possible to follow the energetic and structural change of the conformers (products and substrates) that are involved in an enzymatic reaction. In the present work, an *in silico* analysis of the Cefamandole's structural and energetic change was conducted; both, inside and outside the active site of the β-lactamase K73A from*Mycobacterium tuberculosis*. The energy for each conformer was determined by molcular mechanics. Cefamandole's non-hydrolyzed energy, before entering the active site of β-lactamase K73A, was of 333.16 kJ/mol; inside the active site the value changed to 342.06 kJ/mol. The energy of the hydrolyzed Cefamandole inside the active site was of 323.86 kJ/mol; while outside of the active site the energy was 321.76 kJ/mol. The energy surge of the non-hydrolyzed Cefamandole inside the active site is due to a conformational change required to occupy the active site. The decrease in energy of the hydrolyzation makes the reaction irreversible. Hydrolyzed Cefamandole outside of the active site has the lowest energy, which explains the escape from the active site.

Citation: ***Tejeda Rosales María Elena.*** **2021.** "In silico analysis of the conformational changes of Cefamandole inside and outside the active site of the β-Lactamase K73A from Mycobacterium tuberculosis.", *International Journal of Current Research*, 14, (01), 20339-20342.

INTRODUCTION

β-lactam antibiotics are one of the most important classes of antibacterial agents in clinical use in present.These compounds disrupt bacterial cell wall biosynthesis by irreversibly inhibiting D, D-transpeptidases, which are the enzymes responsible for the last step in thebiosynthesis of cell wall peptidoglycan (1). The β-lactamase are enzymes which can hydrolyzeβ-lactam antibiotics (penicillin, cephalosporin, monobactam and carbapenem related antibiotics) and represent one of the principal mechanisms of antimicrobial resistance. The first descriptions of these enzymes were made shortly after the use of penicillin began. With the rise and repetitive use of new β-lactam antibiotics, new variants of β-lactamases arose, which have been classified under diverse criteria. (2). For any β-lactamase, regardless of their classification, the result of their reaction is the irreversible hydrolysis of the β-lactam ring, inhibiting the antibiotic activity of these molecules. The global reaction that the β-lactamase catalyzes is portrayed in Figure 1.

Figure 1. Reaction catalyzed by a β-lactamase enzyme.



It has been experimentally determined that β-lactamases have a multistage reaction kinetics (3) but empirically, it is not possible to know or isolate the chemical structure that the substrate acquires within the active site during an enzymatic reaction. It is also not possible to calculate experimentally the conformer energy of a substrate in the active site (4). With *in silico* methods, it is feasible to extract the substrate from the crystallographic structures of the active site, and to follow the geometric and energetic change of each of the conformers (products and substrates) involved in the enzymatic reaction.

This in order to analyze, from a structural and energetic point of view, the overall reaction catalyzed by a β-lactamase.

MATERIALS AND METHODS

The K73A β-lactamase form *Mycobacterium tuberculosis* was obtained and crystalographically resolve by Tremblay, LW, Xu, H y Blanchard, JS with a resolution of 1.22Å (5). In the reported crystallographic structure, there is a non-hydrolyzed Cefamandole molecule in the enzyme's active site. In another crystallographic structure, a Cefamandole moleculewas hydrolyzed in the β-Lactam ring inside the active site of β-Lactamase K73A (5). The *Mycobacterium* tuberculosisβ-Lactamase K73A described in the Protein Data Bank with the numbers PDB3N8S y PDB3NY4 were used in this work to analyze the energetic and structural change of Cefamandole in the active site; before and after being enzymatically hydrolyzed in the -Lactam ring. The geometric visualization and the conformers extraction of the K73Aβ-Lactamase from *Mycobacterium tuberculosis,* were realized with the open access software ArgusLab 4.0.1 from Planaria Software LLC (6).

The molecular mechanics calculations to obtain the energy and minimum energy structures of the various Cefamandole conformers were performed on a 64-bit PC with Windows 10 operating system, Intel Core i7-5500 processor at 2.40 GHz with 4 cores and with 12 Gb of RAM memory; using the Yasara Bioinformatics software 30.2081-2982 version 20.7.4. IMBM University of Graz Austria (7). Cefamandole has a condensed formula $C_{18}H_{18}N_6O_5S_2$ and a molecular mass of 462.5 g / mol (8). The structure of non-hydrolyzed Cefamandole outside the active site was obtained from Pub Chem (open chemistry database at the National Institutes of Health), under CID: 456255(8), the minimum energy structure and its energy were determinedusing Yasara Bioinformatics software. Non-hydrolyzed Cefamandole within the active site, was extracted from theK73Aβ-lactamase in the Protein Data Bank with the number PDB3NY4(9)and the conformer hydrolyzed in its β-Lactam ring within the active site, was extracted from the structure deposited in the Protein Data Bank with the number PDB3N8S(10). A copy of the hydrolyzed conformer of the active site was made; the minimum energy structure and its energy were determined for this conformer, which was considered as the structure of Cefamandole hydrolyzed outside the active site of β-lactamase K73A.

RESULTS

In order to carry out a structural analysis of the conformers, Figure 2 shows the method in which the atoms of Cefamandole will be numbered.

Figure 2. Cefamandole molecule numeration

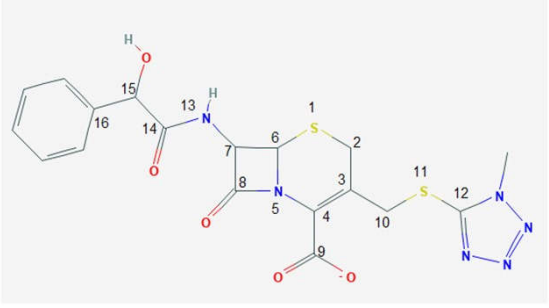


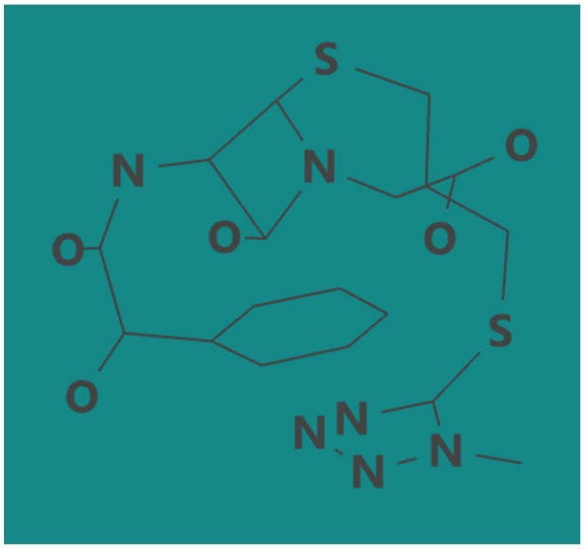
Table 1 shows the *in-silico* energies for the different Cefamandole conformers, obtained by molecular mechanics.

Table 1. Energies of the different conformers of Cefamandole.

Conformer	Energy (kJ/mol)
Non-Hydrolyzed Cefamandole outside the active site	333.16
Non-Hydrolyzed Cefamandole within the active site	342.06
Cefamandole hydrolyzed within the active site	323.86
Cefamandole hydrolyzed outside the active site	321.76

The structure of the non-hydrolyzed Cefamandole before entering the active site of the β-lactamase K73A,Figure 3, has an energy of 333.16 kJ / mol.

Figure 3. Structure of non-hydrolyzed Cefamandole before entering the active site of K73A β-lactamase.



According to the energy results, the conformational arrangementfor the Cefamandole molecule to occupy the active site requires an increase in energy of 8.9 kJ / mol. The changes occur mainly in the dihedral angles C_3-C_{10}-S_{11}-C_{12}, C_6-C_7-N_{13}-C_{14} and C_6-C_7-N_{13}-C_{14}, see Table 2.

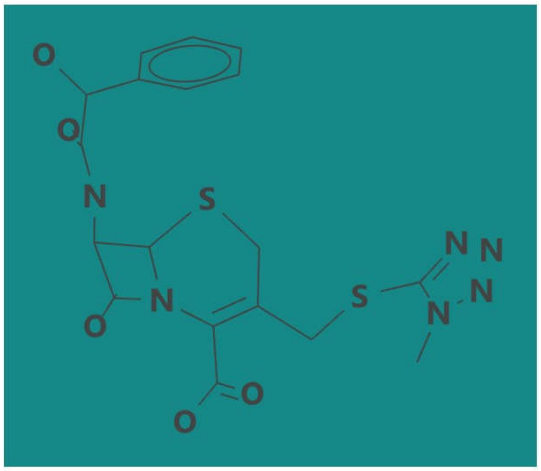
Table 2. Change in dihedral angles of Cefamandole outside and inside the active site.

Dihedral angles	Outside active site	Inside active site
C_3-C_{10}-S_{11}-C_{12}	321.76.98	-122.68 °
C_6-C_7-N_{13}-C_{14}	103.94	86.51 °
N_{13}-C_{14}-C_{15}-C_{16}	93.53	55.72 °

Once carried outthe hydrolysis of the β-lactam ring (breaking of the C_8-N_5 bond), regardless of the mechanism by which it occurs, the energy of the hydrolyzed Cefamandole within the active site of the β-lactamase K73A decreases to 323.86 kJ / mol. The energy of the hydrolyzed Cefamandole inside the active site is lower than that of the non-hydrolyzed conformer inside and outside the active site, see Table 1. The hydrolysis of the β-lactam ring, due to the breaking of the C_8-N_5 bond, which is essentially the enzymatic reaction that catalyzes β-

lactamase K73A,releases the tension of this functional group and generates a decrease of 18.2 kJ /mol, see Figure 4.

Figure 4. Non-hydrolyzed Cefamandole within the active site.

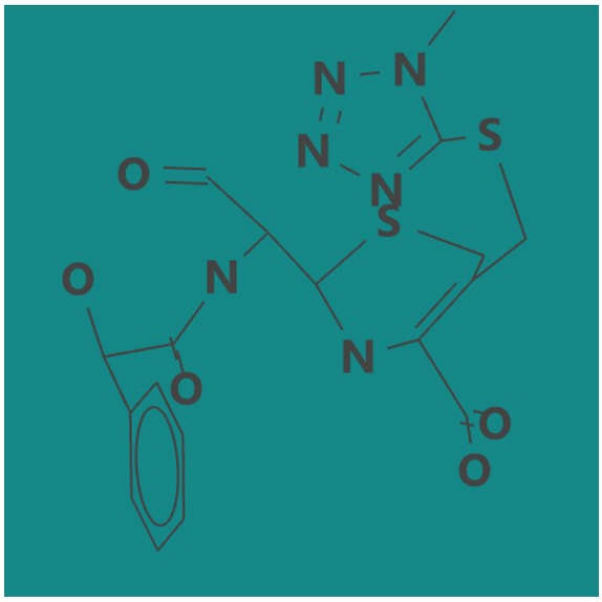


In Table 3, the angles of the β-lactam ring are presented, before and after hydrolysis. The hydrolysis of the cyclic amide of Cefamandole, eliminates the tension and decreases the energy of the conformer. The breaking of the C_8-N_5 bond, generates an important conformational change, that completely modifies dihedral angles C_3-C_{10}-S_{11}-C_{12}, C_6-C_7-N_{13}-C_{14} and C_6-C_7-N_{13}-C_{14}, and changes the position of the tetrazole and phenylrings, see Figure 5. The hydrolyzed Cefamandole outside the active site has an energy of 321.76 kJ/mol.

Table 3. Angles of the β-lactam ring

Angle	Non-hydrolyzed β-lactam ring	Hidrolyzed β-lactam ring
N_5-C_6-C_7	88.73 °	106.99 °
C_6-C_7-C_8	83.01 °	108.19 °
N_5-C_7-C_8	95.59 °	

Figure 5. Hydrolyzed Cefamandole within the active site.



Graphic 1 is a graphical representation of the energy of the different Cefamandole conformers during the general reaction points that β-lactamase K73A catalyzes.

The decrease in the energy of Cefamandole when the C_8-N_5 bond of the β-lactam ring is hydrolyzed, explains why the reaction is irreversible. Hydrolyzed Cefamandole outside the active site has the lowest energy of all the conformers, which would explain the escape from the active site of K73A β-lactamase.

Graphic *1*. Cefamandole conformer energy

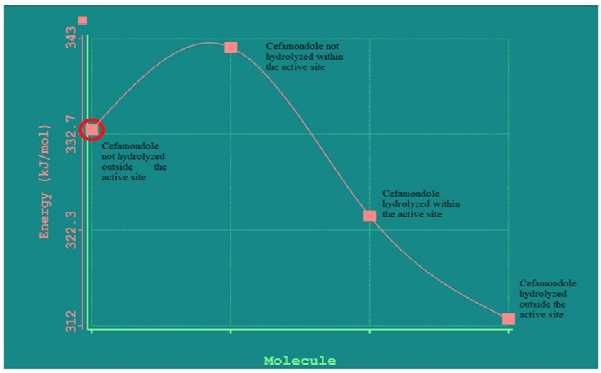
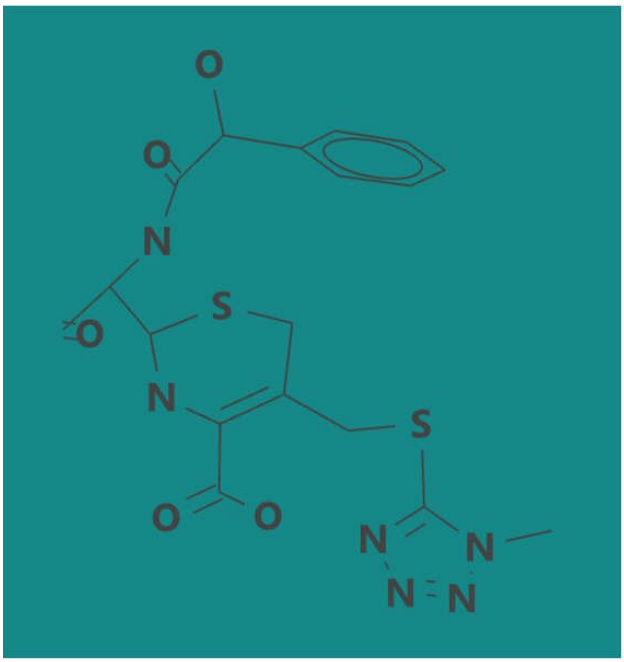


Figure 6. Hydrolyzed Cefamandole outside the active site.



CONCLUSION

TheK73A β-lactamase from *Mycobacterium* tuberculosis, described in the Protein Data Bank with the numbers PDB3N8S and PDB3NY4 were used in this work to analyze the energy and structural change of Cefamandole in the active site, before and after itsβ-Lactam ring was enzymatically hydrolyzed. The energy of the non-hydrolyzed Cefamandole outside the active site of the β-lactamase K73A was 333.16 kJ/mol. The conformational arrangement required for the Cefamandole molecule to occupy the active site requires an increase in its energy. The energy of non-hydrolyzed Cefamandole within the active site was 432.06 kJ/mol. Once carried out, the hydrolysis of the β-lactam ring, regardless of the mechanism by which it occurs, the energy of the hydrolyzed Cefamandole within the active site of the K73A β-lactamase decreases to 323.86 kJ/mol.

The energy of the hydrolyzed Cefamandole inside the active site is lower than that of the non-hydrolyzed conformer inside and outside the active site. Hydrolyzed Cefamandole outside the active site has the lowest energy of all the conformers, which would explain the exit from the active site ofK73Aβ-lactamase.

The energy distribution of the structures is: Non-hydrolyzed Cefamandole within the active site>Non-hydrolyzed Cefamandole outside the active site>Cefamandole hydrolyzed within the active site>Cefamandole hydrolyzed outside the active site.

Acknowledgements

We want to acknowledge the contribution and support of Juan Francisco Sánchez Tejeda and Manuel Guillermo Sánchez Tejeda in the creation of this article.

REFERENCES

1.Meliá C, Ferrer S, Moliner V, Bertran J. 2015. Theoretical studies of the hydrolysis of antibiotics catalyzed by a metallo-β-lactamase. Archives of Biochemistry and Biophysics 582:116–126

2.Silveira MC, Azevedo da Silva R, Faria da Mota F, Catanho M, Jardim R, R Guimarães AC, de Miranda AB. 2018. Systematic Identification and Classification of β-Lactamases Based on Sequence Similarity Criteria: β-Lactamase Annotation. Evol Bioinform Online 14:117693431879735

3.Frau J, Donoso J, Muñoz F, Blanco FG. 1992. Theoretical calculations of β-lactam antibiotics. III. AM1, MNDO, and MINDO/3 calculations of hydrolysis of β-lactam compound (azetidin-2-one ring): β-LACTAM ANTIBIOTICS. III. J Comput Chem 13:681–692

4.Kingsley LJ, Lill MA. 2015. Substrate tunnels in enzymes: Structure-function relationships and computational methodology: Protein Tunnel Structure-Function Relationship. Proteins 83:599–611

5.Tremblay LW, Xu H, Blanchard JS. 2010. Structures of the Michaelis Complex (1.2 Å) and the Covalent Acyl Intermediate (2.0 Å) of Cefamandole Bound in the Active Sites of the Mycobacterium tuberculosis β-Lactamase K73A and E166A Mutants. Biochemistry 49:9685–9687

6.ArgusLab. http://www.arguslab.com/ arguslab.com/ Argus Lab.html. Accessed 9 Jul 2021

7.Krieger E, Vriend G 2014. YASARA View—molecular graphics for all devices—from smartphones to workstations. Bioinformatics 30:2981–2982

8.PubChem Cefamandole. https://pubchem.ncbi.nlm.nih.gov/compound/456255. Accessed 9 Jul 2021

9.Bank RPD RCSB PDB - 3NY4: Crystal Structure of BlaC-K73A bound with Cefamandole. https://www.rcsb.org/structure/3NY4. Accessed 9 Jul 2021

10.Bank RPD RCSB PDB - 3N8S: Crystal Structure of BlaC-E166A covalently bound with Cefamandole. https://www.rcsb.org/structure/3N8S. Accessed 9 Jul 2021

****