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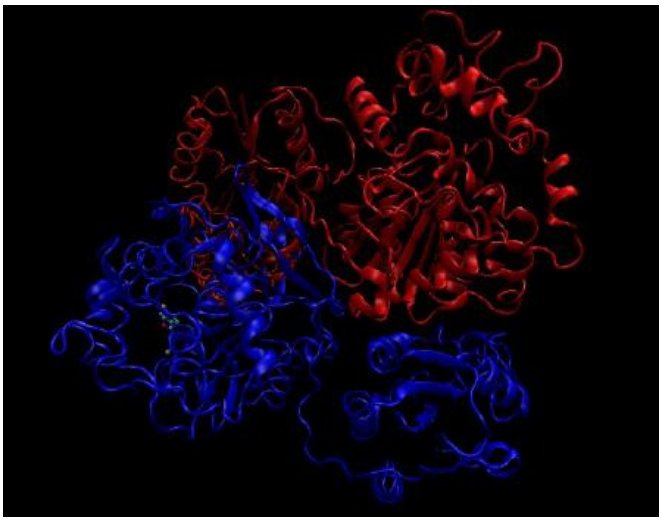

Molecular mechanism and selectivity of epoxide ring opening using a biological catalyst

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A) Introduction.

1. Biological concepts.

The biochemistry is the study of chemical processes within and relating to living organisms. By controlling information flow through biochemical signaling and the flow of chemical energy through metabolism, biochemical processes give rise to the complexity of life. Over the last 40 years, biochemistry has become so successful at explaining living processes that now almost all areas of the life sciences from botany to medicine are engaged in biochemical research. Today, the main focus of pure biochemistry is in understanding how biological molecules give rise to the processes that occur within living cells, which in turn relates greatly to the study and understanding of whole organisms.

Biochemistry is closely related to molecular biology, the study of the molecular mechanisms by which genetic information encoded inDNA is able to result in the processes of life. Depending on the exact definition of the terms used, molecular biology can be thought of as a branch of biochemistry, or biochemistry as a tool with which to investigate and study molecular biology.

Much of biochemistry deals with the structures, functions and interactions of biological macromolecules, such as proteins, nucleic acids, carbohydrates and lipids, which provide the structure of cells and perform many of the functions associated with life. The chemistry of the cell also depends on the reactions of smaller molecules and ions. These can be inorganic, for example water and metal ions, or organic, for example the amino acids which are used to synthesize proteins. The

mechanisms by which cells harness energy from their environment via chemical reactions are known as metabolism. The findings of biochemistry are applied primarily in medicine, nutrition, and agriculture. In medicine, biochemists investigate the causes and cures of disease. In nutrition, they study how to maintain health and study the effects of nutritional deficiencies. In agriculture, biochemists investigate soil and fertilizers, and try to discover ways to improve crop cultivation, crop storage and pest control.

1.1. Biomolecules.

In this moment we know that exist 4 types of biomolecules: Carbohydrates, lipids, proteins and nucleic acids.

The carbohydrates are polyhydroxialdehids or polyhidroxyketones. Their function is energetic or structural. We can divide it according the number of carbonated chains in monosaccharide, disaccharides and polysaccharides.

The lipids are insoluble organics substances in water but soluble in organic solvents. Their function is structural, thermal isolation and energy reserve.

The nucleic acids are macromolecules which have the genetic information and we can localize them in the nucleus cells.

Finally the proteins are macromolecules formed by amino acids or residues.

Amino acid is an organic molecule formed by an amino group, a carboxylic group and a carbonated chain.

Depending on the carbonated chain we have a residue or another one.

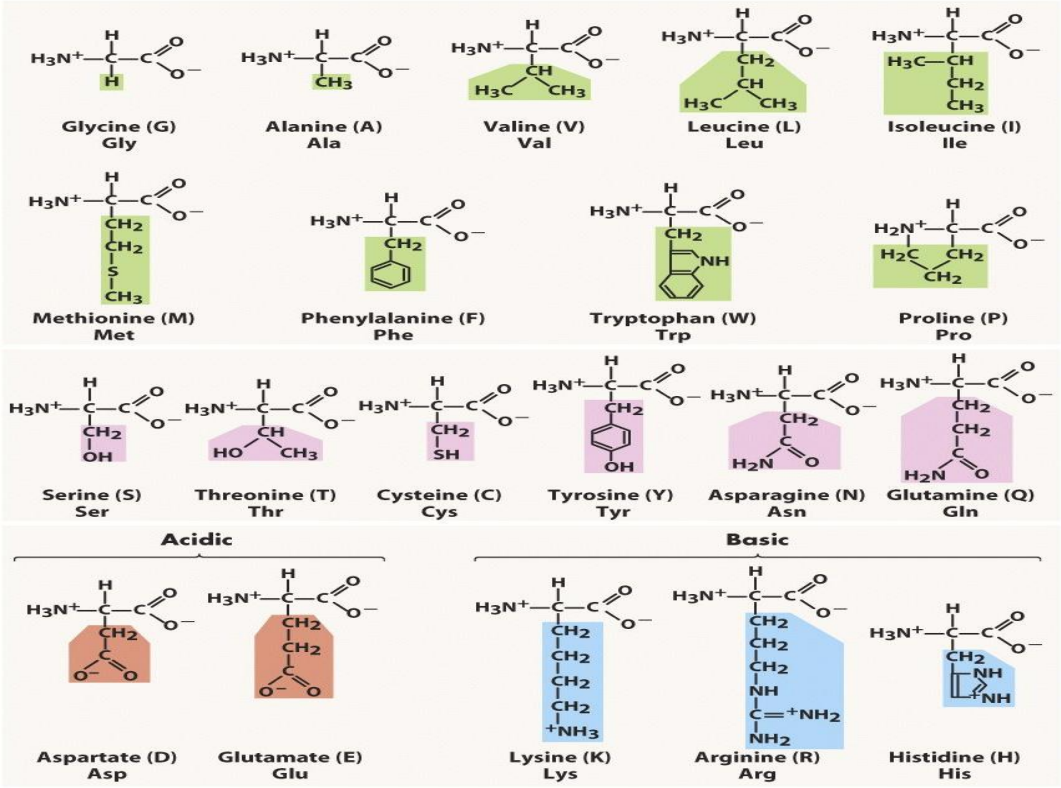


Figure 1: Residues that are present in the nature

The group amino of one residue can react with the group carboxylic from other amino acid forming a peptide bond and finally the joined of a big number of residues form the protein.

Each protein has a different function: Regulation, transport, structural …

Depending on the order of amino acids it will form a protein with a determinate function [(1)].

All the proteins are very important in the nature, but there is a group of protein, the enzymes, which are subject of study because of their catalytic effects that has an important impact in all the organisms because without them, any metabolic rote can't be possible.

1.2. Enzymes.

An enzyme is a protein which catalyzes a metabolic reaction of an organism. If we had not enzymes in our organism, probably we will die because all the metabolic reaction will be very slow.

The enzymes have four characteristics:

- Increase the reaction speed 10^{6}-10^{12} times.
- Their reactions occur in mild conditions.
- They have a big specificity.
- They have a regulation capacity.

How we saw in the figure 1, in the nature exist only 20 amino acids' types and their combinations form the protein. If you have a combination of residues you will have a protein but if you have a different combination you will have another protein.

So the enzyme's structure depends of the amino acids' combination.

In an enzyme there is a part where occurs the catalytic reaction with the substrate (molecule which is going to react), the active site.

There are some enzymes that need a cofactor to carry out the reaction. The cofactor can be an inorganic ion (Fe^{2+}, Mg^{2+}, Mn^{2+} or Zn^{2+}) or an organic complex (coenzyme).

A coenzyme or inorganic ion joined at the enzyme is called prostatic group.

In the figure 2 its well explain the cofactor action in the enzyme

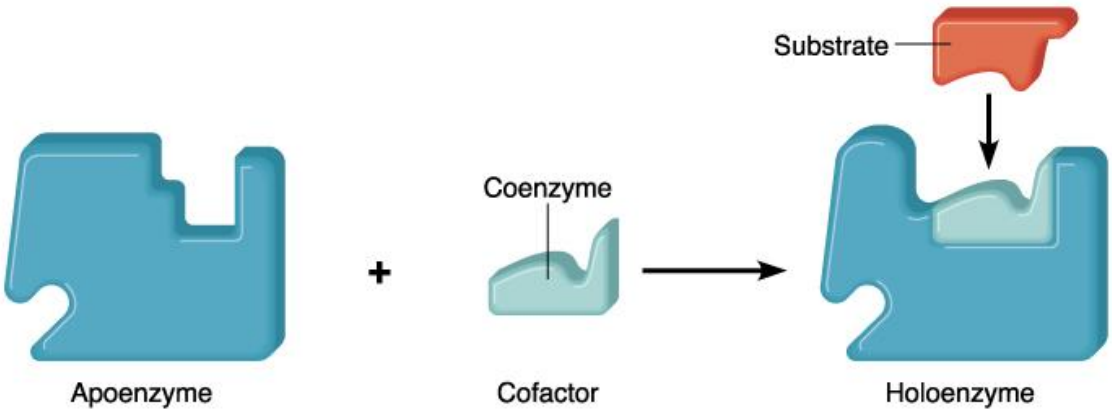


Figure 2: Cofactor action in the enzyme.

How we can see in the figure 2, the cofactor change the active site of the enzyme allowing at the substrate react with the enzyme following the model key-lock.

In 1894 Emil Fischer suggested the model key-lock, which said that the substrate and the enzyme fit perfectly like a key and a lock, where the substrate will be the key and the enzyme the lock.

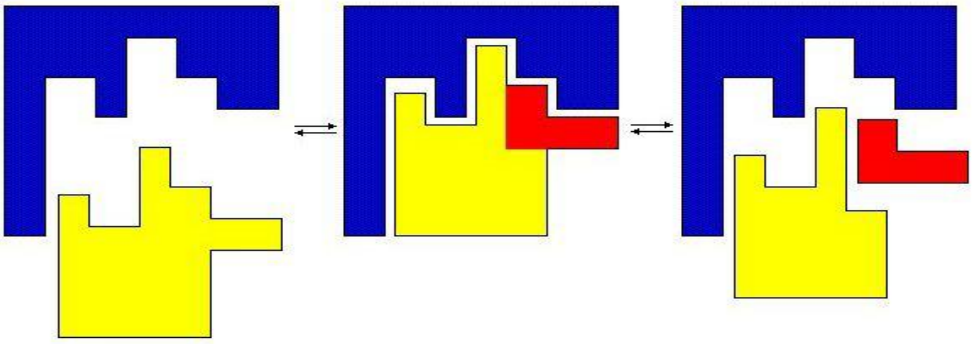


Figure 3: model key-lock of an enzyme suggested by Emil Fischer.

In 1958, Daniel Koshland suggested a modification to the lock and key model, the induced fit model.

How enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with

the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic functions.

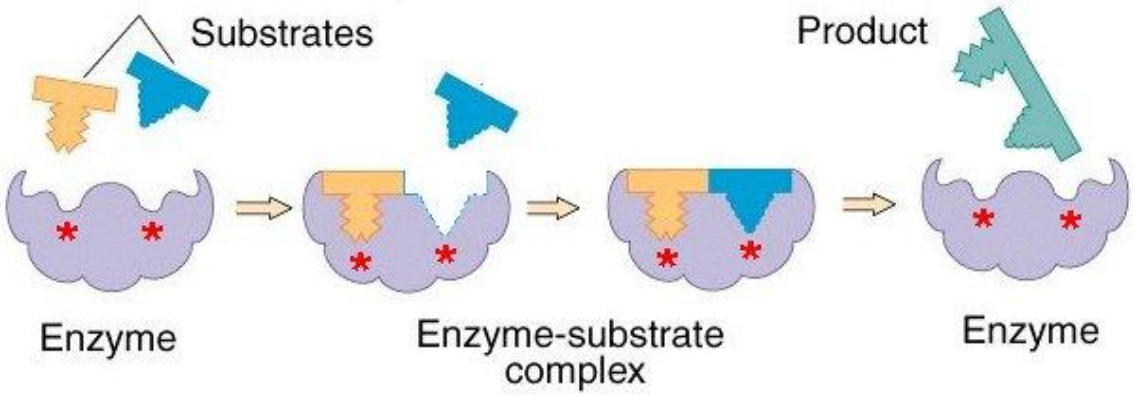


Figure 4: Induced fit model of an enzyme.

1.2.1. Thermodynamics and kinetics aspects in an enzyme.

Enzymes are catalysts that increase the rate of the reaction without altering the balance. They act with low concentrations and don't change the thermodynamics properties, only changes the speed which reach the equilibrium.

The first step in enzymatic catalysis is the union of the enzyme and the substrate to form the complex Enzyme-Substrate (ES).

The substrate binds to the active site using non covalent interactions as:

- Electrostatics links.
- Hydrogen bonds.
- Van der Waals forces.
- Hydrophobic interactions.

The tridimensional form between the enzyme and the substrate can decrease the activation energy of the reaction with factors as:

- The substrate's orientation in the active site.
- The participation of the side chains of amino acids in the complex ES can stabilize the transition states.
- Conformational changes of the enzyme caused by the binding of substrate to the active site induce tension in the molecule and facilitate bond breaking.

Generally an enzymatic reaction starts with the reaction between the enzyme and the substrate and when the substrate is joined with the enzyme it reacts to form the products and finally the enzyme and the products are separated.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

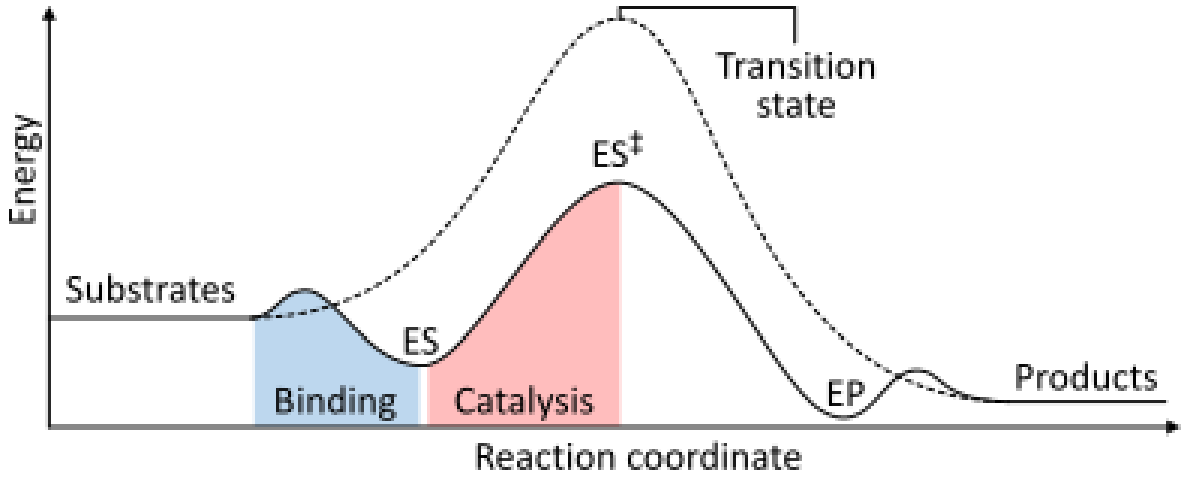


Figure 5: General potential energy profile in an enzyme. The continue line is the energy profile of the enzymatic reaction, and the discontinue line is the energy profile of the reaction without catalyst.

We can observe that have appeared two intermediates: the enzyme with the substrate (ES) and the enzyme with the product (EP).

Moreover we obtain the transition state of the enzyme with the substrate ($ES^{\ddagger}$), which we give us the activation energy of the reaction (Ea). That parameter is essential to calculate the kinetic constant. We can observe also that when the enzyme is not participating in the reaction, the energy of the transition state is higher and that explain why is slower.

We obtain the kinetic constant using the Arrhenius equation:

$$k = Ae^{\frac{-Ea}{RT}}$$

k is the speed constant, A is a constant, Ea is the activation energy, R is the ideal gas constant and T is the temperature.

We can change the form of that equation in the next one:

$$\ln k = \ln A - \frac{Ea}{RT}$$

If we observe this equation we can see that when the activation energy is increasing the constant speed is decreasing.

So we can conclude that when a reaction has a less activation energy it will be faster.

The enzymatic kinetics studies the mechanism, speed and the factors that modified the enzymatic reactions.

In 1913, Leonor Michaelis and Maude L. Menten supposed that the complex formation ES was fast and reversible, while that the dissociation of the product and de enzyme was slow.

With that hypothesis they formulated one of the most important equations in biochemistry, the Michaelis-Menten equation.

$$v = \frac{V_{MAX}[S]}{K_M + [S]}$$

Where:

v= Reaction speed.

[S]= substrate concentration.

V_{MAX}= Maxima enzymatic activity.

K_M= Michaelis constant.

A special feature of the enzymatic reactions is the behavior of the rate against the concentration of substrate. Thus, according to the Michaeliz-Menten equation:

When [S] << K_M, the rate depends almost linearly on [S], and [S] is rate limiting.

When [S] >> K_M, the enzyme reaches a saturation limit by the substrate.

These two cases can be plotted as shown in figure 6.

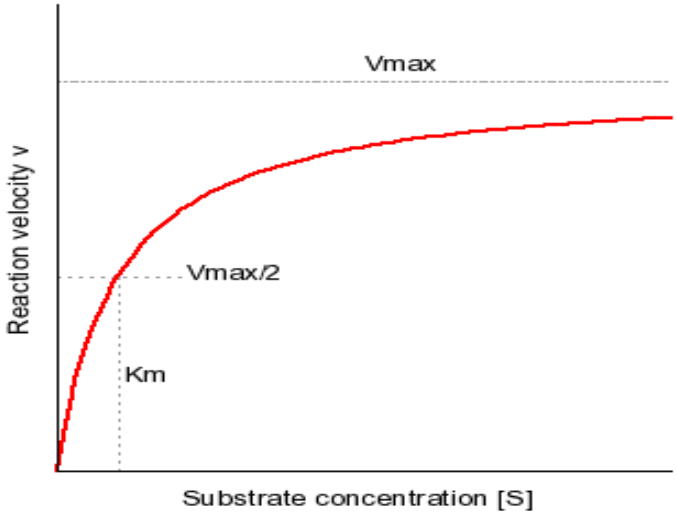


Figure 6: Michaelis-Menten equation representation.

2. Computational concepts.

Computational chemistry is a branch of chemistry that uses computer simulation to assist in solving chemical problems.

Questions commonly investigated computationally are:

-Molecular geometry: The shapes of molecules (bond lengths, angles and dihedrals.)

-Energies of molecules and transition states: What isomer is favored at equilibrium and how fast is a reaction.

- The interaction of a substrate with an enzyme.

-The physical properties of substances: the strength and melting point of a polymer depend on how well the molecules fit together and how strong the forces between them are [(2)].

2.1. Potential energy surface (PES).

A **potential energy surface** (PES) is a function of the electronic energy of a molecular system that depends on the set of possible relative position of its nuclei. The surface might define the energy as a function of one or more coordinates; if there is only one coordinate, the surface is called a *potential energy curve.* The calculus is based on the Born-Oppenheimer approximation.

There are different methods to calculate a PES:

- **Molecular mechanics (MM)**: This model represents a molecule as a group of balls (atoms) and springs (bonds). If we know the normal spring lengths and the angles between them, and how much energy

it takes to stretches and end the springs, we can calculate the energy of a given collection of balls and springs. Changing the geometry until the lowest energy is found enables us to do a geometry optimization.

This method can be applied to any system depending on the selected mathematical function and the parameters, but it is not possible to treat processes where electronic reorganization or electronic transfer takes place.

- **Ab initio calculations**: This method solves the Schrödinger equation for a molecule and gives us the molecule's energy and wavefunction. They can be applied to any structure of the PES as well as excited states. Their only limitation is the computational cost, as a consequence of previous limitations, it is difficult to treat the environment with high accuracy
- **Semiempirical methods (SE):** This method is also based on the Schrödinger equation. The program draws on a kind of library of integrals that was compiled by finding the best fit of some calculated entity like geometry or energy to the experimental values. They are much cheaper than the ab initio methods, being able to treat up to 1000 atoms.

Potential energy surface is very useful because it is a good tool to see the relation between the potential energy and the molecular structure.

The potential energy is a function V(r) of 3N-6 or 3N-5 (if the molecule is lineal) variables.

All the structures can be defined by the vector X.

$$X\equiv (x_1, y_1, z_1, x_2, y_2, z_2,\ldots,x_N, y_N, z_N)$$

x_I, y_I, z_I are the Cartesians coordinates of the atom I in our system [3].

If we have a diatomic molecule as the oxygen (O_2), we can compare macroscopically with two balls held together by a spring.

The molecule in equilibrium has energy, but when the spring stretches or shrinks, its energy is also changing, following the next diagram.

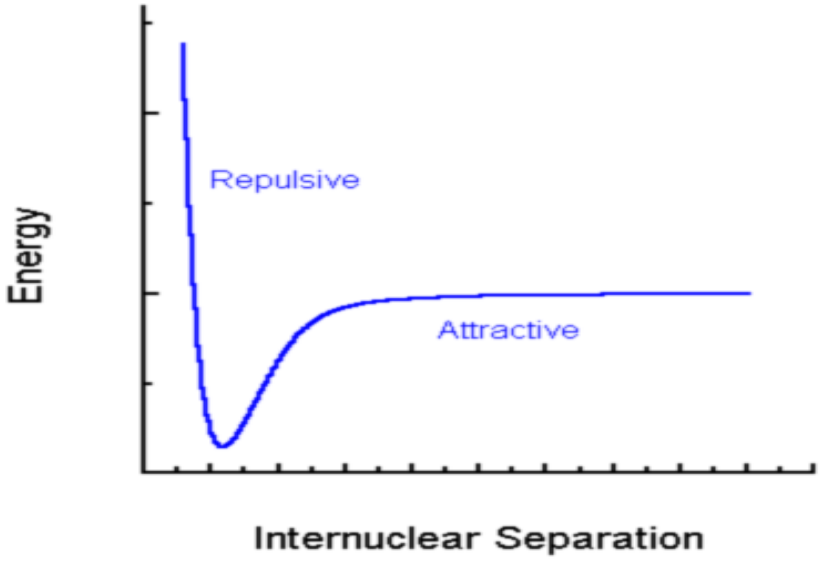


Figure 7: Potential energy surface of an oxygen molecule in front distance r

Since the model is motionless while we hold it at the new geometry, this energy is not kinetic and so is by default potential.

When we have more than one coordinate, we can control more parameters.

For example, if we have the next reaction:

$$H_a + H_bH_c \rightarrow H_aH_b + H_c$$

To control this reaction using computational methods we need the parameters of the R_{ab} and the R_{ac} to obtain the next PES.

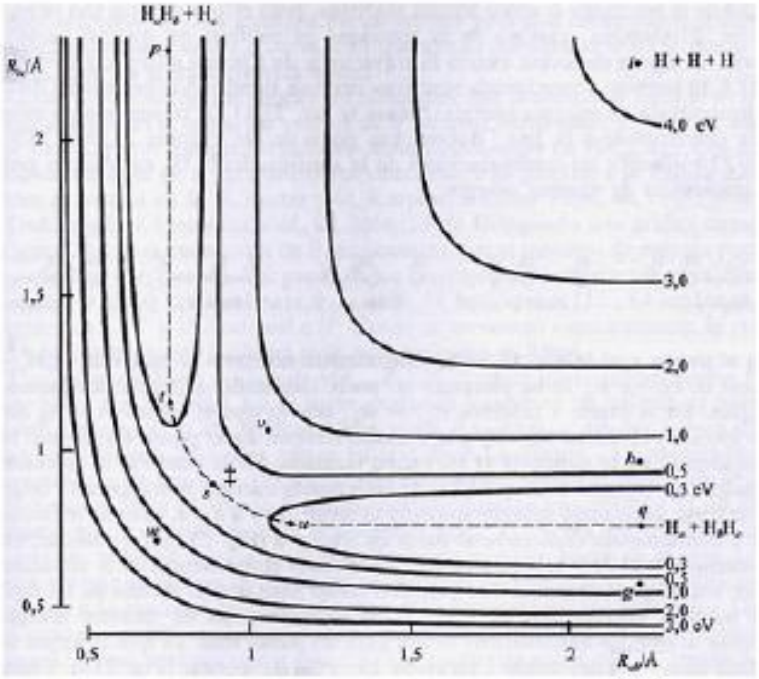


Figure 8: Potential energy surface of a reaction with three atoms.

There are two main concepts that we need to describe prior the study of the PES: the gradient and the Hessian.

The gradient is a vector composed of the first derivatives of the potential energy with respect to the positions of the atoms.

$$\overrightarrow{g_i} = \frac{\partial U}{\partial \overrightarrow{r_i}} \qquad\qquad i = 1, \dots, N$$

Being N the number of atoms.

The Hessian is a symmetric square matrix which has as elements the second derivatives of the energy with respect to the displacement of two coordinates for all the atoms.

$$H_{ij} = \frac{\partial^2 U}{\partial \vec{r_i} \partial \vec{r_j}}$$

When the Hessian is diagonalized, the iegenvalues equation can be solved:

$$F\vec{u_i} = \lambda \vec{u}$$

Where F is the diagonalized Hessian matrix, $\vec{u_i}$ is the vector that represents the curvature principal axes and represents the normal modes and λ are the eigenvalues.

There are some zones in a PES where we can localize a local minimum. Those minimums correspond to saddle points.

One task very important in computational chemistry is to find the structure and energy of certain molecules and find the transitions state of a chemical reaction because you need that information to confirm since a reaction is possible or not.

2.2. Transition state theory.

In the figure 5 we saw that the transition state is the system which is formed by the enzyme and the substrate and we could also observe that is the structure that has as energy the activation energy. So it's very important to find the transition states in computational chemistry because you need to find its energy to know if the reaction will be fast or slow.

So the question is how do we find the transition states?

A transition state is a first-order saddle point on the potential energy surface. In addition to this mathematical condition, in order to be a true transition state a first-order saddle point must fulfill come chemical conditions. Essentially, a transition state is the highest energy point along

a continuous line connecting reactants and products. If there is more than one point fulfilling that condition, the true transition state should be that of lowest energy. Therefore, the complete determination of a transition state requires some kind of chemical reasoning, which implies an interpretation of the different stationary points found on a potential surface.

From the mathematical point of view locating transition states is a much more complicated task than finding minima. Newton type methods work rather well for minimization, in the sense that usually they are able to go to the minimum even from rather far starting points. This is not true for transition states, since the radius of convergence is much smaller. In order to reach the saddle point one should start from a sufficiently close geometry. Optimizations to transition states are also more sensitive to the choice of coordinates than locations of minima.

In the case of transition states it is usually helpful to associate the reaction path to a coordinate, and this is generally achieved operating with internal coordinates. Internal coordinates incorporate bond distances, bond angles and torsion angles. The use of internal coordinates has also the advantage of automatically removing the translational and rotational degrees of freedom.

The most commonly employed method for locating transition states is a Newton-Raphson approach in which the energy is maximized in one direction and minimized along all other directions. In order to be successful the starting point must have the correct curvature. In any case it is crucial to have a good starting point, to compute the initial Hessian, and to have a good updating of the Hessian. In difficult cases it might be

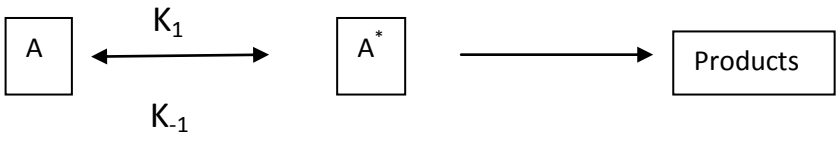
even necessary to compute the Hessian at each point during the optimization. In some cases, when employing augmented Hessian methods, it is enough that the lowest eigenvalue (even positive) point to the correct direction.[8]

When there is a minima and the Hessian matrix at this point is diagonalized, all the eigenvalues obtained are positive. This means that an infinitesimal displacement of the geometry of the system along the direction defined by any of its eigenvectors, will lead to an increase in energy. The minima are the reactants, products and intermediates

Whereas, when there is a first-order saddle point and the Hessian matrix is diagonalized, all the eigenvectors obtained are positive except one. In this case, if we perform an infinitesimal displacement along the eigenvector corresponding to the negative eigenvalue, the energy will decrease. However, if the displacement is along the eigenvector associated to a positive eigenvalue, the energy will increase.

The eigenvector associated to the negative eigenvalue is known as the transition vector and indicates the direction from the transition structure to reacts and products.

In the chemical reaction:



The reaction rate is:

$$v_{reaction} = v[A^*] = \frac{K_B T}{h}[A^*]$$

Taking into account the definition of the equilibrium constant, K_1, and its relationship with the Gibbs' free energy:

$$K^* = \frac{[A^*]}{[A]}$$

$$\Delta G^* = -RTlnK^*$$

We can obtain the concentration of the species in the transition state:

$$[A^*] = [A]e^{\frac{-\Delta G^*}{RT}}$$

Comparing our equation we obtain the rate constant for our reaction:

$$k = \frac{k_B T}{h} e^{\frac{-\Delta G^*}{RT}}$$

We can estimate the reaction rate because we obtain the free energy of all the structures.

3. The soluble epoxide hydrolase (sEH).

3.1. The substrate.

An epoxide is an eter cyclic that have a specific reactivity because of its high polarizability in the bond carbon-oxygen and the high tensions that present the ring [4].

Those compounds are present in our organism or they can be originated of drugs metabolic routes. Some of them are responsible of damage in the cells because they can react with the DNA and the proteins [4-6].

3.2. An hypothesis of the reaction mechanism of sEH.

Epoxide Hydrolase are enzymes that transform catalytically the epoxide compounds forming 1,2-diols or glycols and they are present in all our organism.

There are five known mammalian epoxide hydrolases: microsomal and soluble epoxide hydrolases, leukotriene A4 hydrolase, cholesterol epoxide hydrolase and hepoxilin epoxide hydrolase [(6)].

In this work we will focus in the soluble epoxide hydrolase (sEH).

The sEH is highly concentrated in the kidneys and its inhibition is a potential treatment for many diseases how, kidney failure, high blood pressure and atherosclerosis. We can use also this enzyme in the synthesis of enantiopurs diols using a water molecule, and epoxides, which are important synthons in organic synthesis [(4-6)].

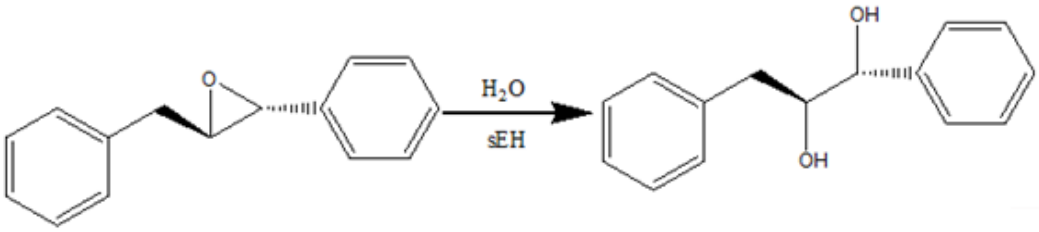


Figure 9: Global Reaction of the epoxide in the enzyme soluble epoxide hydrolase

How I said before, the epoxide hydrolase form part of the epoxide hydrolases family. The enzymes that are part of this family have a common triad whose will be very important to make the catalytic reaction. Generally this triad is formed by a nucleophyl, a histidine and an

acid [6]. This triad has a lot of importance because the nucleophyl act with the histidine, making it more o less acidic.

Moreover the sEH has two tyrosines which have been proved that have an important effect in the catalytic reaction because an investigator group from Amsterdam mutated the enzyme changing the tyrosines by phenylalanine and they saw that the enzyme lost the 90% of reactivity [6].

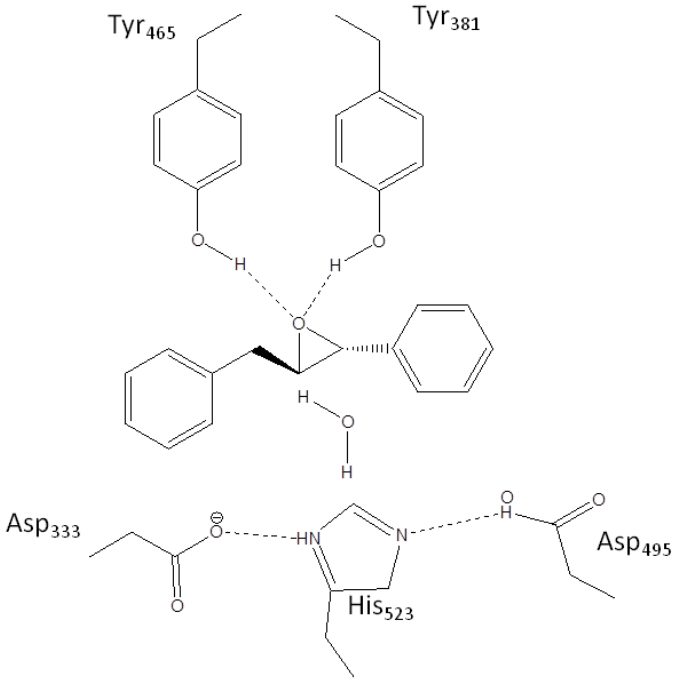


Figure 10: Active site of the enzyme

The substrate epoxide is polarized by two tyrosine residues, which form a hydrogen bond with the oxygen's epoxide.

The selectivity of the ring-opened reaction is influenced be the next factors:

- The proximity of the nucleophile.

- Electronic stabilization of the transition state.
- Hydrogen bonding to two active site tyrosine residues.

In this work, we suggested that the opening of the ring can be originated by the attack of a water molecule, which can enter there because the active site of the enzyme is hydrophylic.

The reaction has two steps, the first step consist in the water attack direct.

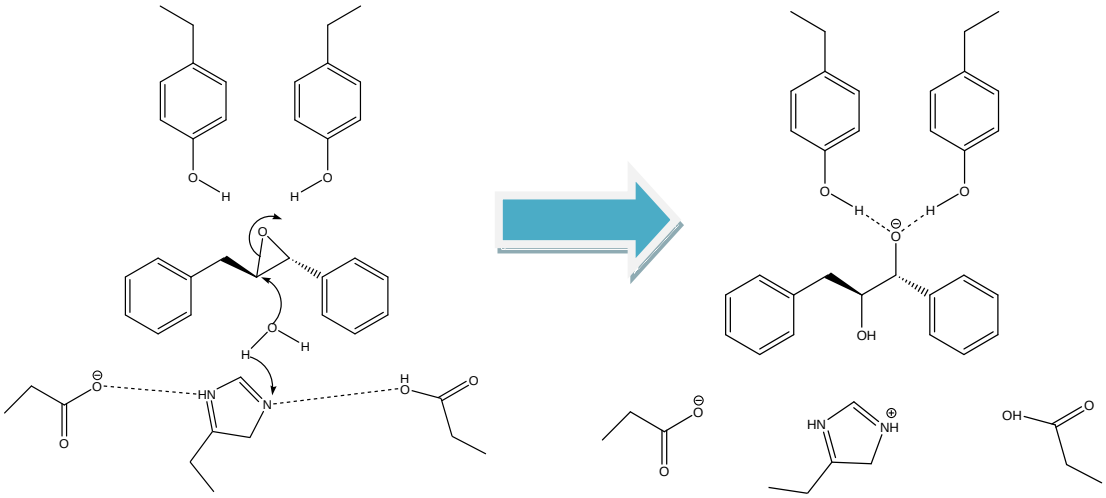


Figure 11: direct attack of the water at the substrate in the enzyme sEH.

The water molecule attack at the epoxide carbon and after that form a hydroxile group of the diol because of the second proton of the water goes to the histidine.

In the second step the proton of the histidine that proceeds of the water goes to the substrate.

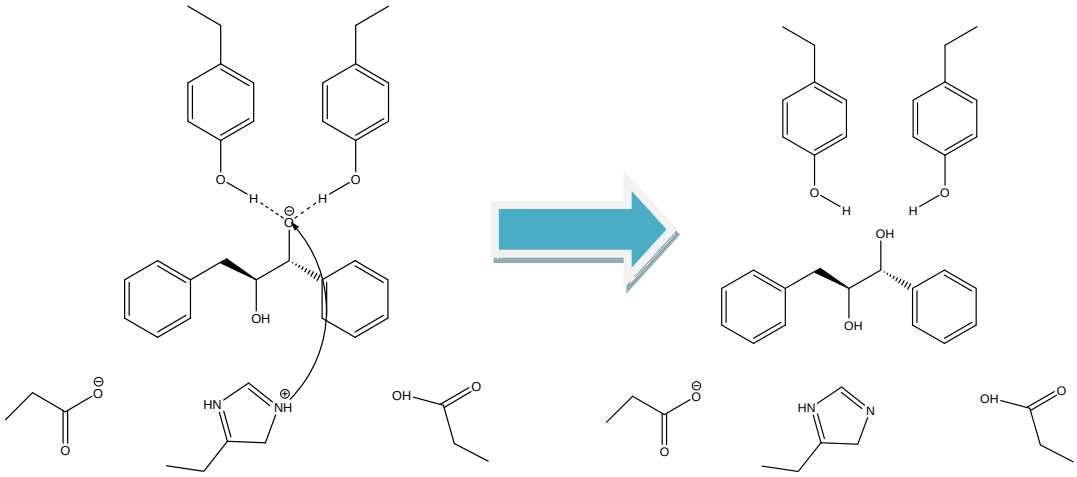


Figure 12: Transfer of the histidine proton at the oxygen anion.

Those reactions correspond at the water attack to the carbon 2, but with the objective to study the selectivity, we have to study also the reaction when the water attacks to the carbon 1.

B. Objective.

The objective of this work is to study the molecular mechanism of the hydrolysis of an epoxide catalyzed by sEH by means of quantum methods with a reduced model of the enzyme and to explore the selectivity of the enzyme. The epoxide to be hydrolyzed is the trans-2-Benzyl-3-phenyloxirane (t-DPPO) as depicted in Figure 13.

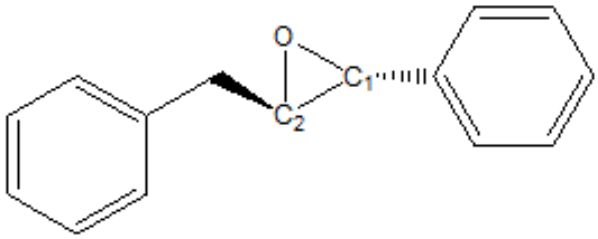


Figure 13: Trans-2-benzyl-3-phenyloxirane (t-DPPO)

C. Experimental part.

1. Set up molecular model.

The first thing that we must to do when we are going to study a reaction with a computational method is make the model that is going to be study.

The system in our case is the active site of the soluble epoxide hydrolase (sEH). For obtain that system, we appeal at the Protein Data Bank (PDB). This website is a place where there are a lot of enzymes and you can get them for do any study of them.

In figure 14 it is the reduce model that simulate the active site of the enzyme with an inhibitor.

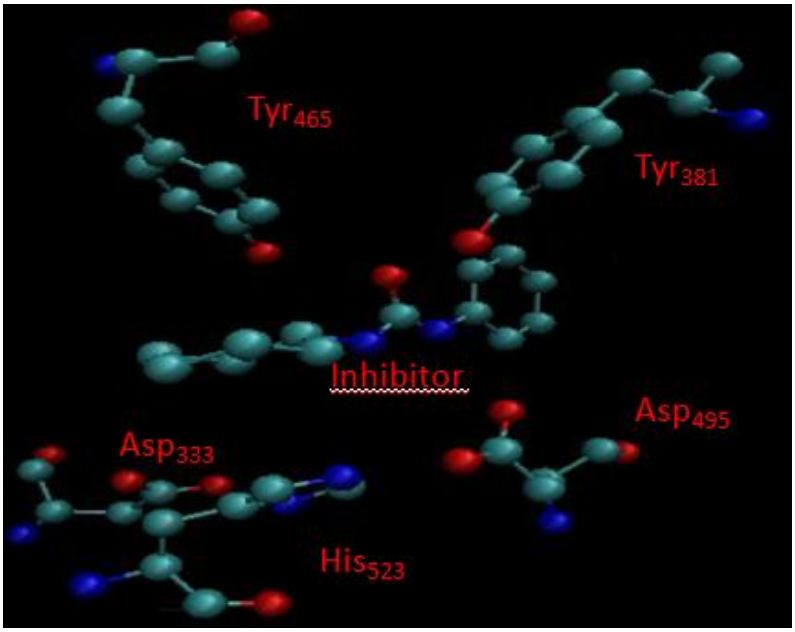


Figure 14: Active site of the soluble epoxide hydrolase downloaded from PDB (Code: 1EK1)

In figure 14 we can see perfectly the tyrosines, the histidine and the aspartates, but the substrate is not the molecule that we proposed to study, that molecule corresponds at an inhibitor.

We can also observe that there are not protons and water molecule, but that is not a problem because you can modify the system until you get it the target system.

In that case, we made the next changes:

- We added all the protons' system and the water molecule that will react with our substrate
- We eliminated of the residues the groups amine and carboxylic because they don't participate in the reaction and you can reduce the calculus eliminating those functional groups.
- We modified the inhibitor because we are going to study the reaction of the enzyme sEH with an epoxide.

In addition to that, we have to know if our residues are or not protonated. To make that, we use the program propka[7].

In that website you can see what are the standard pKa in solution of the residues and the pKa that the residues have in the enzyme. In the next table you can observe the pKa of the different residues that participate in the reaction:

Residue	Standard pKa in solution	pKa of the residue in the enzyme
Histidine 523	6.5	1.23
Aspartate 495	3.8	9.1
Aspartate 333	3.8	10.31

table 1: Standard pKa of the residues in solution and the pKa of the residues in the enzyme as computed with PROPKA3.0[(8)]

If the pKa of the residue in the enzyme is smaller than the pH, your residue will be dedeprotonated and if your pKa of the residue in the enzyme is bigger than the pH, your residue will be protonated.

If we observe the table 1 we appreciate that the histidine will be protonated and the aspartates no, but we protonated the aspartate 333 because it acts as nucleophile.

When we have done all the modifications, we have to optimize the system i.e. we have to calculate the most stable conformation, the lowest energy conformation.

When you are optimizing in gas phase probably you will obtain a wrong structure because how you are working in gas phase the atoms move freely and you obtain unwanted molecules.

Moreover I said before that the tyrosines of the system are very important in the reaction because they form hydrogen bonds with the epoxide, so the orientation of their hydrogen and the oxygen don't have to change when you optimized.

To solve those problems we have to put constraints i.e. we have to fix the coordinates of some atoms. If you do that, the atoms that you have fixed will not move in the optimization.

When you optimized using constraints you have to return to optimize the structure without constraints.

When we make all this we obtain our system optimized, which we'll use to study all the reactions.

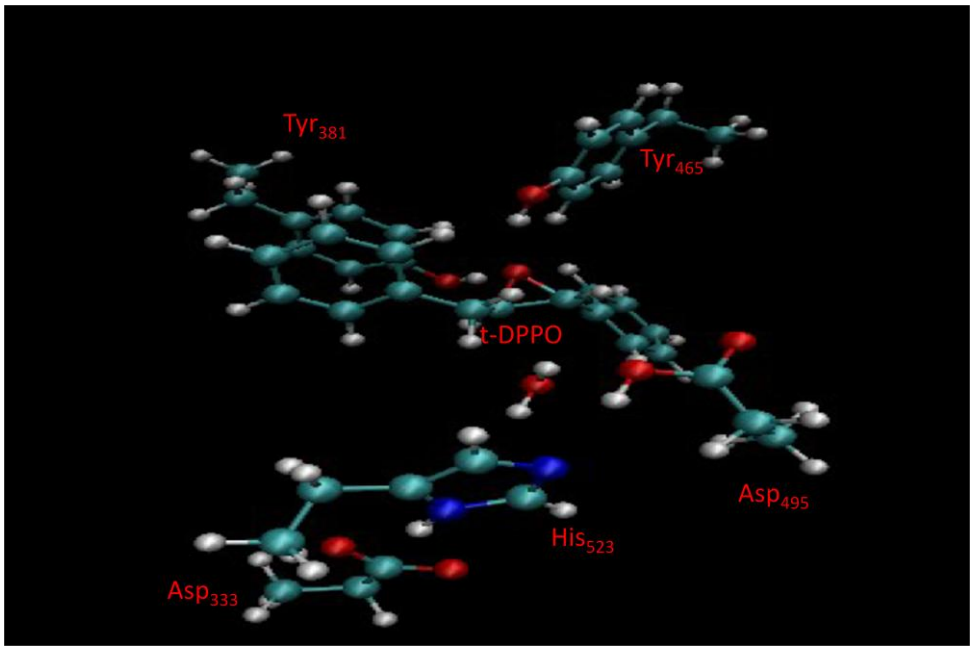


Figure 15: optimized active site

2. Direct water attack.

In this case the water attack directly at the epoxide carbon, but how the epoxide is not symmetric we have to study two different attacks:

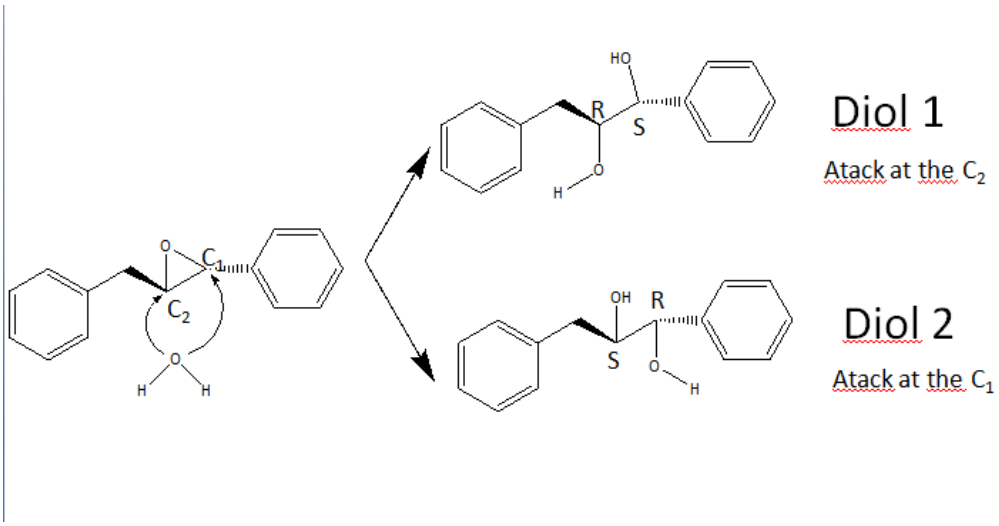


Figure 96: Different products that we can obtain when the water attack directly at the epoxide.

To see this reaction, we will make a PES which we control two antisimetric distances, the distance from the O^{epox} to the C^{epox} minus the distances

from the C^{epox} and the O^{water} and the distances from the N^{his} to the H^{water} minus the distances from the O^{water} to the H^{water}.

3. Proton transfer from the histidine.

The proton transfer from the histidine to the oxygen is the second step of the reaction.

In this case we make the same that the first step to calculate the distances, we apply two antisimmetric distances because the distances of the water's proton with the histidine's nitrogen and the proton with the epoxide oxygen are changing. So we calculate the $d(H^{water}\text{-}N^{his})\text{-}d(H^{water}\text{-}O^{epox})$.

D. <u>Results and discusion.</u>

1. Attack at the carbon 2

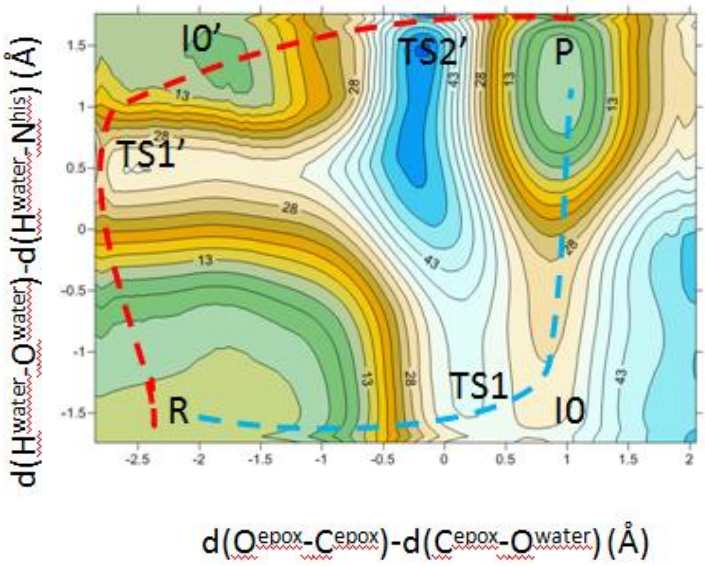


Figure 17: Potential energy surface of the direct attack of the water at the carbon 2

In that PES we can observer that the reaction has two pathways. The first one (color blue) the Reactive (R) goes to an intermediate (I0) in where the water has joined to the carbon. How in the PES there is not a saddle point

between the I0 and the I1 we made another PES from the structure I0 controlling only the antisimmetric distance d(H^{water}-O^{water})-d(H^{water}-N^{his}).

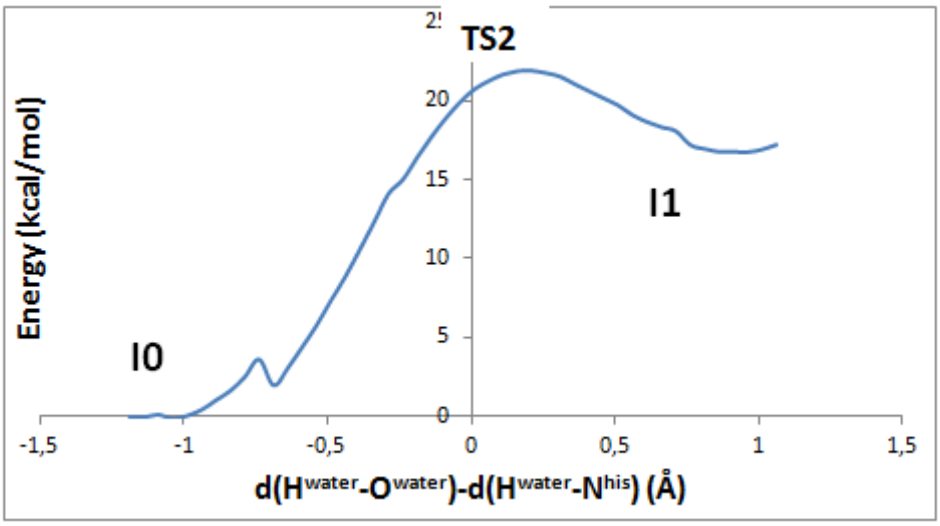
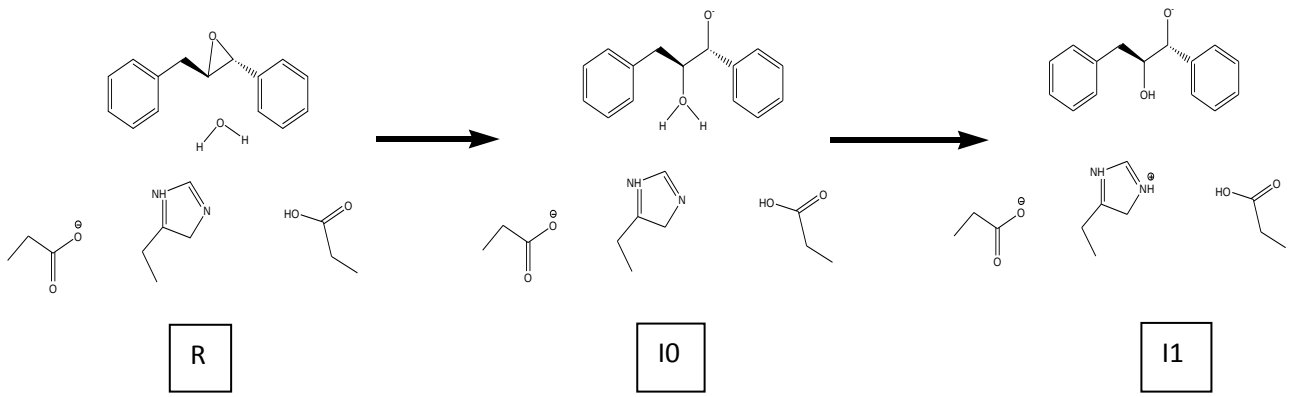


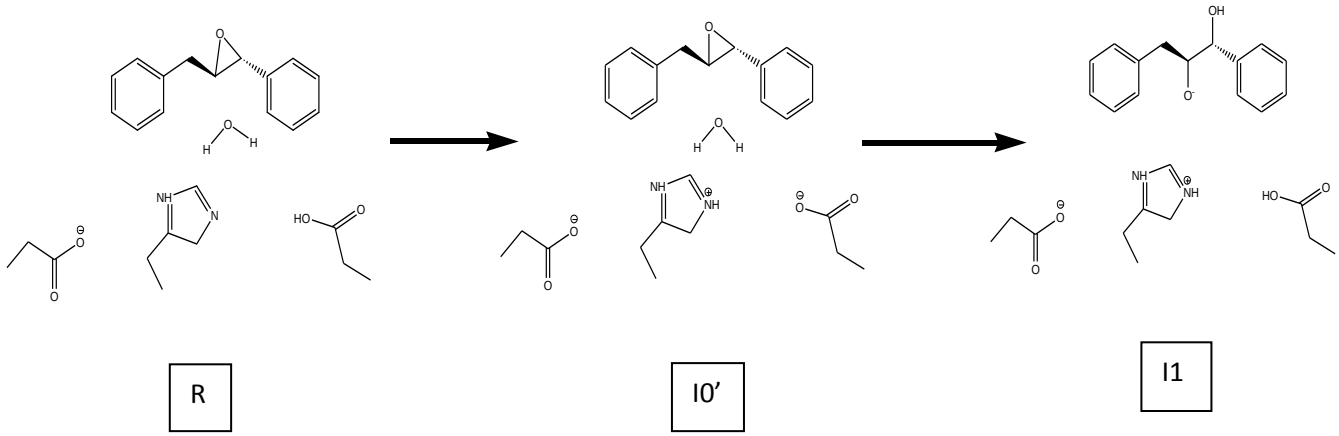
Figure18: Potential energy surface of the water proton transfer to the histidine.

In the second one (color red) the Reactive (R) goes to the intermediate (I0') in where the water proton has been transferred but the results show us that the proton from the aspartate is transferred also to the water. After that the water attack at the carbon and the aspartate proton return at its original position forming the intermediate (I1)

<u>Pathway 1</u>



Pathway 2



If we see the PES we can conclude that the pathway with minimum energy is the pathway 1 because the TS2' is more energetic that the TS2.

When we optimizated the intermediate I1 we make another PES controlling the antisimetric distances d(H^{water}-N^{his})-(H^{water}-O^{water}).

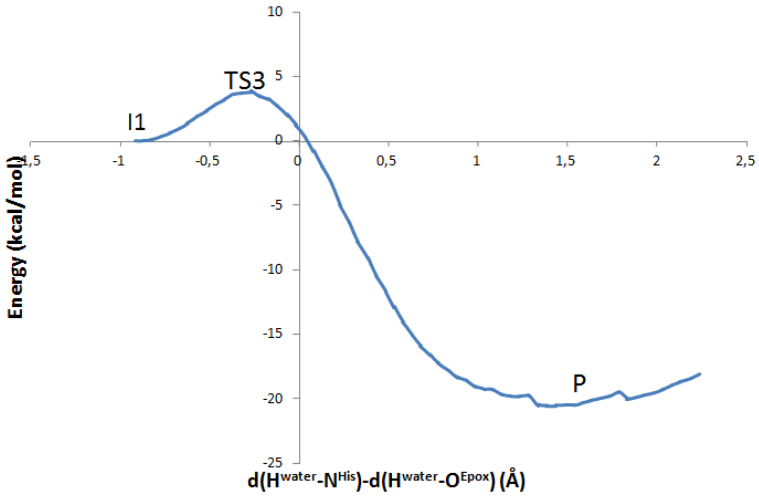


Figure19: Potential energy surface of the histidine proton transfer to the oxygen ion.

In the figure 19 we are calculating the PES of proton histidine transfer to the oxygen ion.

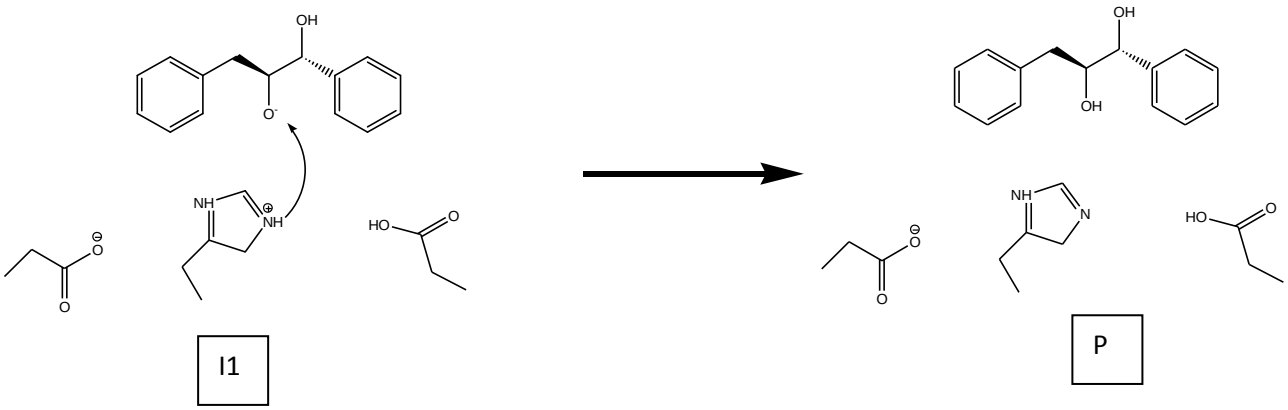


Figure20: Histidine proton transfer at the oxygen.

When we have obtained all the PES and we optimized all the intermediates and the products we are ready to build the energy profile

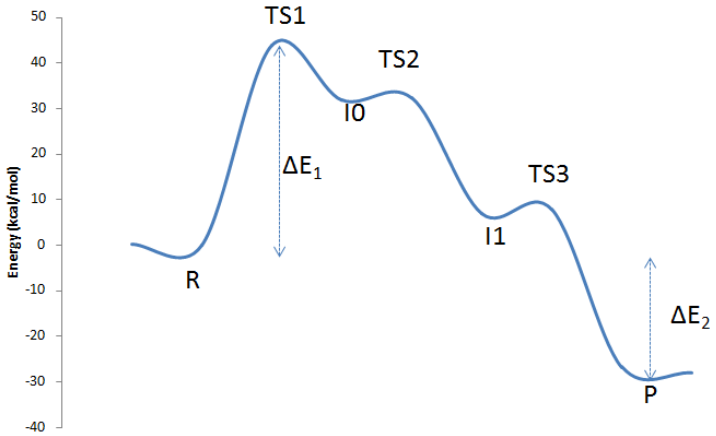


Figure 21: Energy profile attacking at the carbon 2.

The figure 21 show that the formation of the diol from the epoxide t-DPPO carry out in three steps.

2. Attack at the carbon 1.

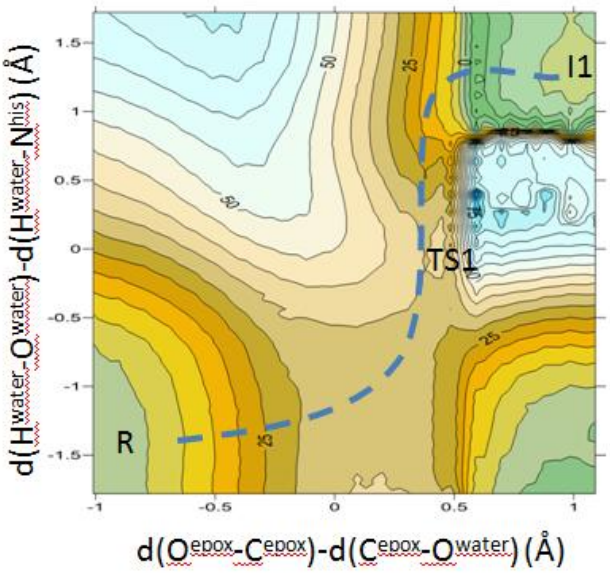


Figure 22: Potential energy surface of the direct attack of the water at the carbon 2.

In this case we made the same calculus that the attack at the carbon 2, but the aspartate 495 transferred its proton at the histidine, so we eliminated the residue aspartate 495 because it doesn't participate in the reaction, obtaining finally the PES from the figure 18.

In the figure 22 we can observe that there is only one transition state, so there is a pathway.

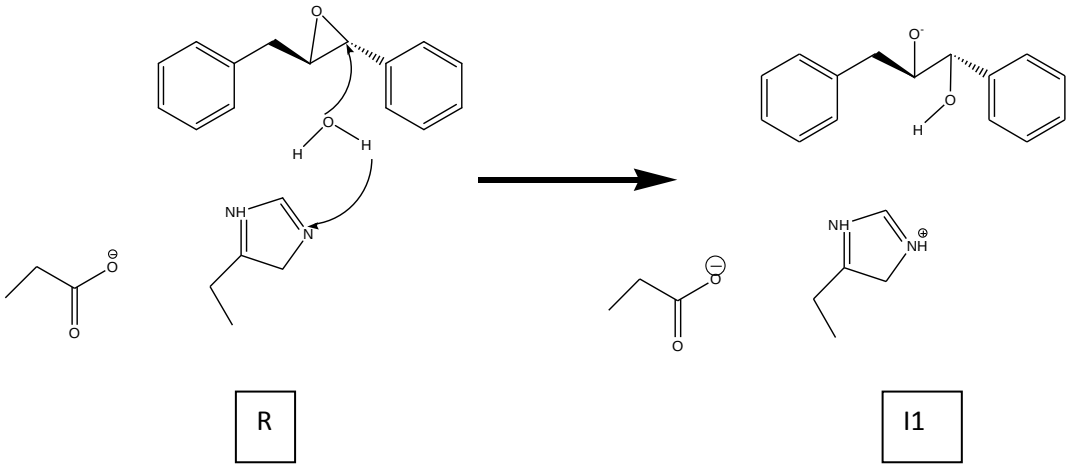


Figure 23: Pathway when the water attacks at the carbon 1.

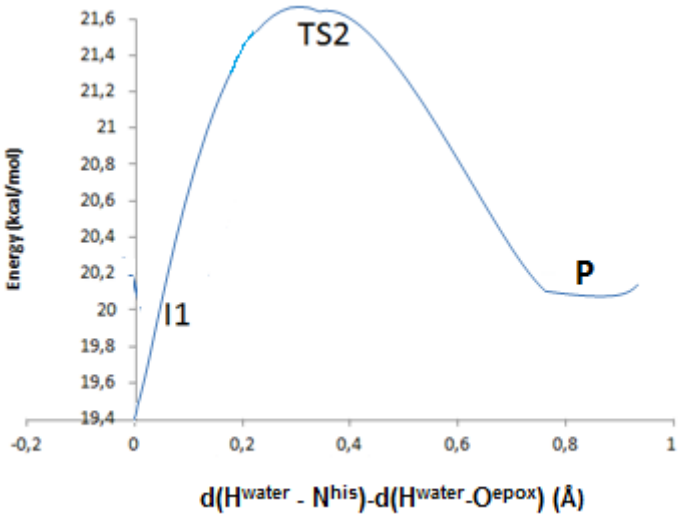


Figure 24: Potential energy surface of the histidine proton transfer to the oxygen charged negatively in the substrate.

In the PES of the figure 24 we are studying the next reaction.

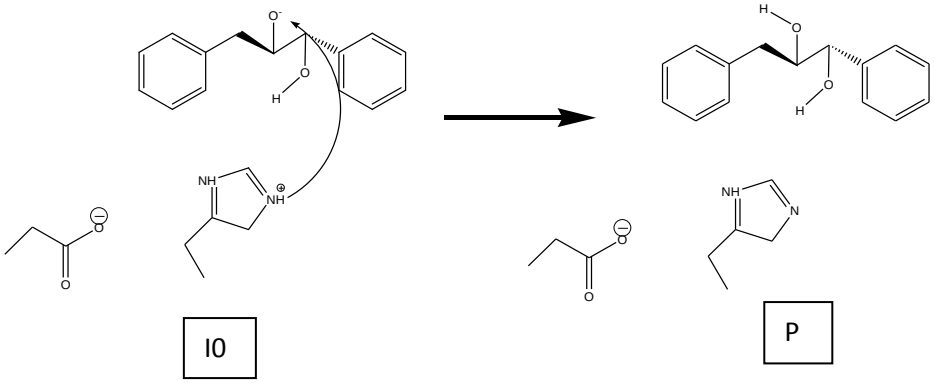


Figure 25: Histidine proton transfer to the oxygen anion of the substrate.

Finally we can build the energy profile of the global reaction.

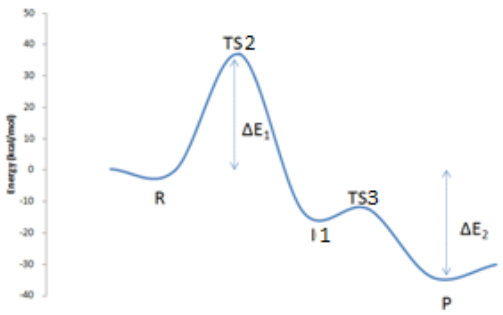


Figure 26: Energy profile attacking at the carbon 2.

E. Conclusion.

In conclusion, we have study the molecular mechanism of the hydrolysis of the epoxide trans-2-benzyl-3-phenyloxirane in a reduced model of the enzyme soluble epoxide hydrolase

When the water is attacking at the Carbon 1 the reaction has two steps, the first one is the bond formation between the Carbon and the oxygen of the water, producing at the same time de water proton transfer at the histidine. The second step is the transfer of the histidine proton at the oxygen anion of the substrate. While when the water attacks at the Carbon 2 the pathway of the reaction has three steps. The first step is the join of the epoxide carbon with the oxygen's water, the second step is the transfer of a water proton to the histidine and finally the last step is the proton histidine transfer to the oxygen anion of the substrate.

With the objective to see the different selectivity of both reaction (attack at the carbon 1 and the carbon 2) we have to study the figures 21 and 26.

The next table summarizes the relative energies of the different stationary point structures to reactants:

Energy (kcal/mol)	**Attack at the Carbon 1**	**Attack at the Carbon 2**
Reactive (R)	0	0
TS1		48.87
I0		33.91
TS2	42.97	37,36
I1	-16.15	6.36
TS3	-12.14	9.87
Product (P)	-34.03	-28.63

table 2: Relative energies of each structure that appears in both reactions.

We can conclude that the attack at the carbon 1 is more selectivity than the attack at the carbon 2 because its first transition state has 6 kcal/mol less than the TS1 of the attack at the Carbon 2.

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