Research Article

Study of Interaction between Dipeptidyl Peptidase-4 and Products Extracted From the Stevia Plant by Molecular Modeling

Hicham Ayachi, Meriem Merad, Said Ghalem*
Laboratory of Natural and Bioactive Substances (LASNABIO), Department of Chemistry, Faculty of Science, University of Tlemcen 13000, Algeria.
***Corresponding author's E-mail:** s_ghalem2002@yahoo.fr

ABSTRACT

Knowledge of enzymes is crucial because they are the ones that catalyze most of the chemical reactions in living organisms. Among these enzymes we chose the dipeptidyl peptidase-4 Our work is a study of the molecular interaction between the enzyme dipeptidyl peptidase-4 (DPP-4) and substrates (Stevioside and Rebaudioside A extract of the plant Stivia). Different molecular modeling tools are used to perform this work (molecular mechanics, molecular dynamics and molecular docking) or home predicts how a small molecule (drug) can bind to a receptor (protein) whose 3D structure is known. Our goal of research is to study the inhibition of dipeptidyl peptidase-4 (DPP-4) by molecular modeling methods. The introduction of bulky groups causes a conformational rearrangement within the active site pocket which probably will increase complementarity and therefore activity. Other hand, the total interaction energy of the inhibitor is 1491.86 Kcal/mol. The results obtained from this work, into which the inhibition of dipeptidyl peptidase-4 (DPP-4) by molecular modeling methods was elucidated, allow us to conclude that Stevioside presents a more optimised inhibition of dipeptidyl peptidase-4 (DPP-4).

Keywords: Dipeptidyl peptidase-4 (DPP-4), Enzyme, ES interaction, Molecular modeling MM, DM, and Docking.

INTRODUCTION

Diabetes is a complex disease, its pathophysiological mechanisms of genetic and geneses have complications determinism. This is a heterogeneous group of metabolic diseases whose main feature is a chronic hyperglycemia resulting from a lack of secretion, insulin action, or both abnormalities.[1]

Hyperglycemia is associated most often with external symptoms more or less suggestive of disease severity. In addition to acute complications (hyperglycemia, ketoacidosis, hyperosmolar syndrome), chronic hyperglycemia degenerates more or less serious complications.[2]

For years, diabetes has been defined in different ways. Even today, it is impossible to give a single etiologic or pathogenic or clinical definition. Based on current knowledge, it is a chronic disease caused by inherited or acquired by insufficient production of insulin in the pancreas, which is a gland whose secretion is discharged into the duodenum or jejunum.[3]

The type 2 diabetes is a disease characterized by chronic hyperglycemia, that is to say, by too much glucose (sugar) in the blood. This disease usually occurs in adults advancing age and affects more obese or overweight people.[4]

This is a polyuria-polydipsia syndrome by hyperglycemia. Three kinds of interrelated processes involved in hyperglycemia: insufficient insulin secretion, Resistance of muscle and adipose tissues to the action of insulin.[5]

MATERIALS AND METHODS

Dipeptidyl peptidase-4 (DPP-4)

DPP 4 is the short name for an enzyme known as dipeptidyl peptidase 4. Dipeptidyl peptidase 4 breaks down two gut hormones known as incretins. Incretins are produced in the intestine when food is eaten, and they stimulate insulin secretion, which lowers blood glucose levels. In the disease known as type 2 diabetes, there is not enough insulin, or it is less effective, and blood glucose levels rise. Drugs to treat type 2 diabetes have been developed which inhibit DPP 4, preventing incretin breakdown and prolonging insulin secretion, increasing its effect.[6]

The enzyme known as DPP 4 is found in a number of different sites in the human body, including the cells that line small arteries in the gut. It is sometimes referred to as dipeptidyl peptidase IV (DPP IV). Part of the structure of a protein known as CD26 is identical to dipeptidyl peptidase 4.[7] CD26 is found in the membranes of many different cells, including T cells in the immune system, which it may help activate. The DPP 4 section of the molecule protrudes on the outside of the cell.

The inhibitors of DPP-4

We have chosen as inhibitors for inhibition of DPP-4: Rebaudioside A and Stevioside (these products extracted from the Stevia plant).

Rebaudioside A (Inhibitor 1)

Rebaudioside A is an intense sweetener of natural origin. It is obtained by extracting leaves Stevia rebaudiana Bertoni. It is used to impart a sweet taste to foods or used in tabletop sweeteners.[8]
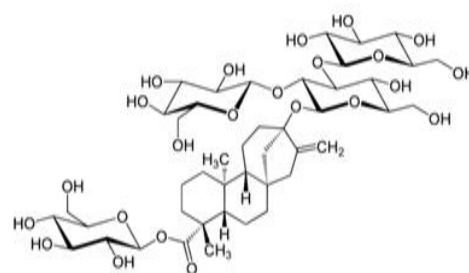
Stevia

Products extracted from the Stevia plant


Rebaudioside A - Reb A

Physico-chemical properties

- Nomenclature rebaudioside A
- Gross Chemical formula $C_{44}H_{70}O_{23}$
- Molecular weight 967.03
- Sweetness on 350-450 times that of sucrose
- Solubility Soluble in water and ethanol
- Melting point 242-244°C.[9]

Stevioside (Inhibitor 2)

Stevioside is a natural sweetener extracted from leaves of Stevia rebaudiana (Bertoni). It is a glycoside known for its intense sweetness, and for this reason, its use as a sweetener.

Stevioside is a major product of the extraction of Stevia.[10]
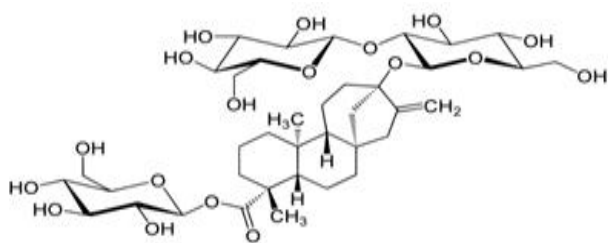
Stevioside

Physico-chemical properties

- Nomenclature Stevioside

- Gross Chemical formula $C_{38}H_{60}O_{18}$

- Molecular weight 804.872

- Sweetness on 300 times that of sucrose

- Solubility Soluble in water and ethanol

- Melting point 196-198 °C.[10]

Preparation of the enzyme

Downloading of the Dipeptidyl peptidase-4 (DDP-4) was made from the database **Bookhaven Protein Data Bank (access code 3F8S).**

The calculations for this study are:

1- A molecular mechanics calculation (optimization of molecular geometry).

2- A molecular dynamics calculation performed for all the starting structures (DPP-4 alone inhibitors).

a- The positioning of the inhibitors was in the active site of DPP-4 using molecular docking.

b- Once the complex (DPP-4-inhibitor) is formed, it will adopt the most stable conformation corresponding to the lowest energy level, and it is this conformation that will be sought during the molecular dynamics simulations performed for all the complexes formed.

RESULTS AND DISCUSSION

The discussion of results of calculation was based on the results of the interaction energy between DPP-4 and the inhibitors. The three dimensional structure of (DPP-4) was obtained by X-ray diffraction with a resolution (2.43 Å).[10] Note that the Dipeptidyl peptidase-4 crystallizes as a monomer (Figure 1), with 1456 residues and 11926 atoms. With the reducing effect of molecular modeling, we simplified the model of the enzyme by removing water molecules; this allowed us to obtain the following model (Figure 2). This model includes amino acids forming the active site (Figure 3).
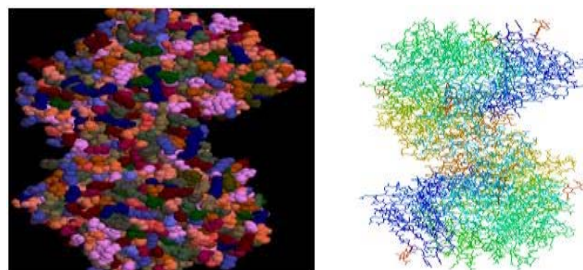

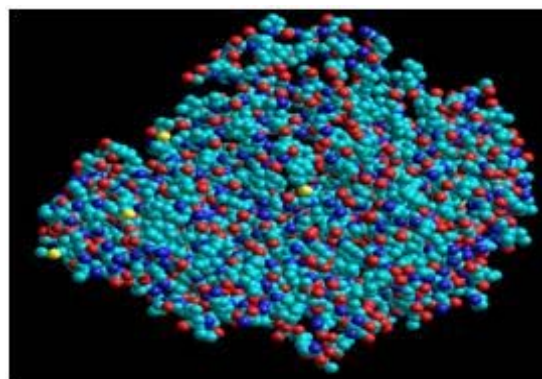**Figure 1:** The monomeric form of Dipeptidyl peptidase-4 (DPP-4)

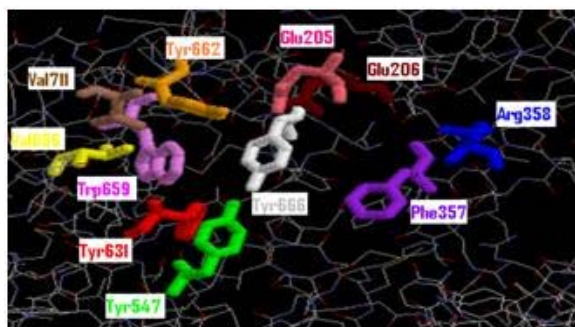**Figure 2:** Simplified model of DPP-4

Figure 3: Amino acids within the active site

Optimization of Dipeptidyl peptidase-4

The optimization of the geometry of Dipeptidyl peptidase-4 was performed using the force field Amber99.[11] implemented in the software Hyperchem7.5 professional version. The main chain was maintained rigid while the side chains are flexible. This approximation enables the side chains of the protein to detect more easily the position in which interactions are most favourable.

The value of the optimization energy is: E_{Opt}= - 4184.0639 Kcal/mol

Optimization of substrates

Construction and optimization of ligand were made by the EMO program (an energy molecule) (Table1).

Molecular Dynamics

Molecular dynamics of Dipeptidyl peptidase-4

We started the dynamics by a system initialization: at t = 0 we have r (t) = 0, that is the initial structure, previously minimized. Then we heated the system up to 300 K for 1000 steps with a step of integration of 1 fs. At 300 K, there is equilibrium: the speeds are adjusted to maintain a constant temperature (there is an exchange between kinetic and potential energy). Then, there is production of conformations. Time simulation of molecular dynamics was 100 ps. Figures 4 represent the results of molecular dynamic calculations of inhibitors and the complex (enzyme-ligand) trained. According to the curves that were plotted by the software Origin 6.0 we note the stability of complexes formed and inhibitors.

Docking of substrates and building complexes

The next step, after the construction of the ligand, is the positioning of this molecule in the active site of Dipeptidyl peptidase-4 (DDP-4). For this, we used the Molecular Docking Module using software Hex4e.[12] Once the ligand-receptor complex is formed, it will adapt the most stable conformation, i.e. the lowest energy level.

Molecular dynamics of the complexes

Once the complexes are formed, we will perform a geometry optimization and molecular dynamics calculation to find the most stable conformation.

Table 1: Results obtained using the program EMO

Steric energy (KJ/mol)	Elongation	Valence	Torsion	VDW.	Electro.	Solvent	Total
Inhibitor1	279.59	789.42	225.35	641.61	-157.3	.00	1778.66
Inhibitor2	39.50	146.04	191.59	308.52	-119.04	.00	566.17

Table 2: Distances between the active site amino acids and groups of inhibitors

Distances(Å)	Tyr 547	Tyr 631	Val 656	Trp 659	Val 711	Tyr 662	Tyr 666	Glu 206	Glu 205	Phe 357	Arg 358
Inhibitor1	10.94	18.35	23.43	37.74	12.78	33.17	32.57	23.71	30.13	23.87	12.38
Inhibitor2	3.04	4.50	7.41	12.45	2.65	11.67	10.52	3.89	4.24	2.71	5.37

Table 3: Summary of energies (kcal / mol)

	$E_{complex\ total}$ E-S	$E_{total\ substrate}$ S	$E_{vdw\ du\ complex}$ E-S	$E_{vdw\ substrate}$ S	E_{intera} VDW	E_{intera} Total
Inhibitor1	8730.77	1778.66	-149.97	641.61	-1281.41	6623.34
Inhibitor2	2386.80	566.17	-574.33	308.52	-1372.67	1491.86

Distances between the active site amino acids and groups of inhibitors

We musured distances between inhibitors groups of side chains responsible for interaction of amino glycosides (Table 2).

Interaction energy

In order to establish correlations between the interaction energies and activities, and to consider what kind of forces by the nature of interactions DPP-4-substrate are governed, we often refer to a decomposition of the total potential energy of the molecule in different terms (electrostatic energy, energy of VDW, ...). The energy of interaction between substrates studied and DPP-4 are obtained using the following relationship:

E interaction = (E total potential complex DPP-4-substrate) - (E total potential DPP-4 only + E total potential substrate)

We must also take account of Van der Waals interactions since these are the interactions between atoms that

stabilize the unbound enzyme-inhibitor complex (Table 3). VDW energy of the DPP-4 only = 489.827 Kcal/mol Steric energy of DPP-4 only = 328.761 Kcal / mol. The first ranking is based on the total interaction energy. The second ranking is based on the interaction energy of van der Waals. In light of the results, it appears that the introduction of bulky groups causes a conformational rearrangement within the active site pocket which probably will increase complementarity and therefore activity. We measured the distances between the inhibitors and amino acids that constitute the active site.
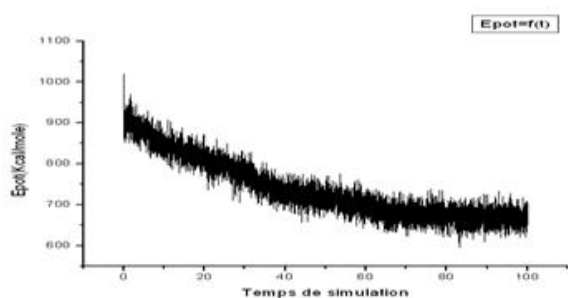
The measured distances vary between 2.71 Å and 12.45 Å. Interactions between 2.5 Å and 3.1 Å are considered high and those between 3.1 Å and 3.55 Å are supposed to be average. Interactions greater than 3.55 Å are weak (Table 2).[13] Based on the total interaction energy, we notice that the inhibitor 2 (1491.86 kcal / mol) is more active than the inhibitor 1 (6623.34). For the interaction energy of Van der Waals (generally used to explain the interactions between atoms that stabilize the unbound complex enzyme-substrate).[14]
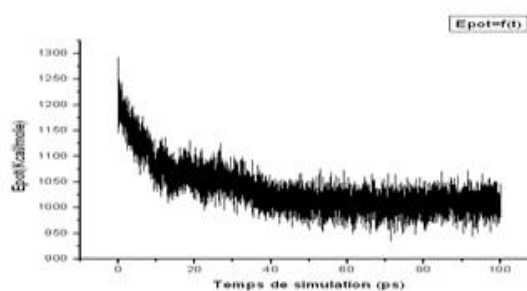

Figure a: Variation of the energy function of time complex 1

Figure b: Variation of the energy function of time complex 2

Figures 4: Variation of the energy potential function of time complexes

The inhibitor2 (-1372.67 kcal / mol) is more stable than inhibitor1 (-1281.41 kcal / mol) (Table 3).
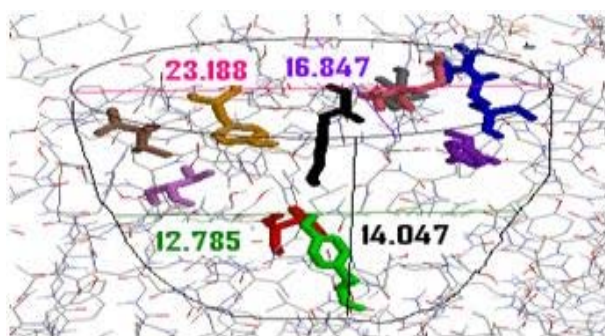

Figure 5: Size of the enzyme cavity

Note that we can discuss complementarity increasing or decreasing in the range of dimensions of the active site pocket, in our case with a deep geometry 14.04 Å, 23.18 Å an opening and 16.84 Å, this pocket shrunk up a width 12.78 Å [15] (Figure 5). Taking into account different geometrical constraints, the approach considered inhibitors may influence the complementarity and later activity.

CONCLUSION

The results obtained from this work, into which the inhibition of Dipeptidyl peptidase-4 (DPP-4) by molecular modeling methods was elucidated, allow us to conclude that inhibitor 2 presents a more optimised inhibition of Dipeptidyl peptidase-4(DPP-4).

REFERENCES

1. Grimaldi A, Tabuteau D, Bourdillon F, Pierru F, Olivier LC, Manifeste pour une santé égalitaire et solidaire, Odile Jacob, 2011, P200.

2. Grimaldi A, Diabète de type 2, Elsevier, 2004, P504.

3. Grimaldi A, Sachon C, Le diabète de type 2, Guide à l'usage des patients et de leur entourage, Broché, 2004, P199.

4. Grimaldi A, La santé écartelée - Entre santé publique et business, Dialogues, 2013, P220.

5. Grimaldi A, Cosserat J, Breton C, Cathébras P, Les pathologies dites fonctionnelles, Elsevier, 2004, P162.

6. Miller FP, Vandome AF, Brewster JM, Dipeptidyl Peptidase-4: Cluster of Differentiation, Protein, Gene, Development of Dipeptidyl Peptidase-4, Broché, 2010, P80.

7. Sheikhpour R, Incretins, dipeptidyl peptidase-4 inhibitors and diabetes: New medicines for diabetes, Broché, 2012, P112.

8. Geuns JMC "Stevia: six month beyond authorisation", EUPRINT, 2012, P226.

9. Geuns JMC "Estevia y glicósides de esteviol", EUPRINT, 2010, P307.

10. Sirshendu D, Mondal S, Banerjee S, Stevioside: Technology, Applications and Health, John Wiley & Sons Inc, 2012, P248.

11. Ewan RG, xiong Y, Melanie J, Andrea L, Regan L, Design of stable α-Helical arrays from an Idealized TPR Motif, Elsevier science Ltd, 11, 2003, P497-508.

12. HyperChem (Molecular Modeling System) Hypercube, Inc., 1115 NW 4th Street, Gainesville, FL32601, USA, 2005.

13. Ritchie D, Macromolecular Docking Using Spherical Polar Fourier Correlations, Department of Computing Science, University of Aberdeen, copyright © 1996-2005.

14. Société de chimie physique, Journal de chimie physique et de physico-chimie biologique, Edition Société de chimie physique, 95(4 à), 1998, 6.

15. Soufi W, Merad M, Boukli F, Ghalem S, The Complementarity Effect for Cdc25 Phosphatase Inhibitors, Advances in Molecular Imaging, 2011, P17-23.