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Study of Interaction between enzymes (maltase-glucoamylase (2QMJ) and glycogen synthase kinase-3 (3F7Z)) and Products Extracted from the Stevia Plant by Molecular Modeling

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ABSTRACT

Molecular modeling techniques are widely used in chemical, pharmaceutical and agrochemical industries. Most current drugs target enzymes. This theoretical approach enables to predict the mode of interaction of a ligand with its receptor. The inhibition of enzymes maltase - glucoamylase (2QMJ) and glycogen synthase kinase-3 (3F7Z) is an important approach in the treatment of disease type 2 diabetes. Extraction of the stevia plant has given us an insight to develop new types of antidiabetic medication. Rebaudioside A and Stevioside are two major components of the stevia plant used for inhibiting 2QMJ et3F7Z. Our work is the study of molecular interaction between the enzymes (maltase - glucoamylase and Glycogen synthase kinase-3) and the substrates (Stevioside and Rebaudioside A). Various tools of molecular modeling are used to carry out this work (molecular mechanics, molecular dynamics and molecular docking) (MOE). Our research objective is to study the inhibition of maltase - glucoamylase and Glycogen synthase kinase-3 by molecular modeling methods. The introduction of bulky groups causes a conformational rearrangement in the active site pocket, which will probably be reinforced and thus complement its activity. The results obtained from this work, in which the inhibitions of maltase - glucoamylase and Glycogen synthase kinase-3 by molecular modeling methods have been demonstrated. In conclusion, taking into account the results obtained in this study, inhibition of maltase - glucoamylase and Glycogen synthase kinase-3 by molecular modeling methods has been elucidated, which allow us to conclude that Rebaudioside A and Stevioside have a better inhibition of maltase - glucoamylase and Glycogen synthase kinase-3.

Keywords: maltase - glucoamylase (2QMJ) Glycogen synthase kinase-3, Rebaudioside A, Stevioside, molecular modeling (molecular mechanics, molecular dynamics and dockingmolecular), MOE (Molecular Operating Environment).

INTRODUCTION

The discovery of type 2 diabetes is common in ambulatory medicine, the increasingly growing epidemic is linked to an reduced life expectancy, obesity, lack of physical activity, and due to an unbalanced diet particularly rich in fats and refined sugars. [1] This disease has a significant genetic and frequently associated predisposition to obesity, hypertension and dyslipidemia[2].

Type 2 diabetes is a metabolic disease characterized by chronic hyperglycaemia with pathophysiological factors including increased resistance in peripheral tissues (liver, muscle) to the action of insulin, insufficient insulin secretion from β-cells in pancreas, inappropriate glucagon secretion, and a decrease in the effect of incretins, gut hormones stimulating postprandial insulin secretion [3].

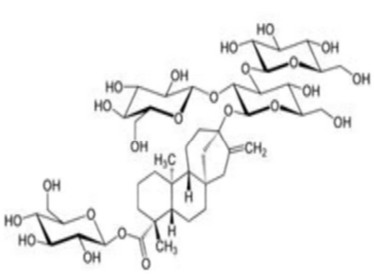
MATERIALS AND METHODS

Maltase-Glucoamylase:
Maltase - glucoamylase is digestive enzyme saccharides. There are mainly in the mucosa and its function is to hydrolyse maltose, beta-limit dextrins and certain cyclodextrins [4]. Expressed in kidney, small intestine and granulocytes, Maltase-glucoamylase (MGA) is one of the enzymes of the brush border membrance which plays a role in the final digestion of starch [5].

Glycogen Synthase Kinase-3 :
Glycogen synthase kinase-3 (GSK-3) is an important regulator of different signal transduction pathways. Regulation processes such as synthesis of glycogen, insulin-dependent, microtubule function, cell polarity, cell adhesion, cell survival [6], protein synthesis and the input of some transcription factors function. Poor signaling GSK-3 has been associated with diseases such as Alzheimer's, bipolar disorder and type II diabetes, making it an attractive target [7].
We have chosen for inhibiting these two enzymes: Stevioside and Rebaudioside A (stevia extract from the plant).

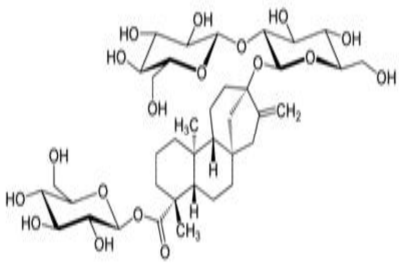
Rebaudioside A (Inhibitor 1) :
Rebaudioside A is a natural intense sweetener. It is obtained by extracting leaves of Steviarebaudiana (Bertoni). It is used to impart a sweet taste to foods or used in table sweeteners [8].

Stevioside (Inhibitor 2):
Stevioside is a natural sweetener extracted from the leaves of Steviarebaudiana (Bertoni). It is a known glycoside for its intense sweetness, and therefore used as a sweetener [9].



Rebaudioside A - Reb A

Stevia



Stevioside

Preparation of enzymes:
- Download of maltase - glucoamylase data was made from the database (Bookhaven Protein Data Bank) its code is (2QMJ).
- Download of Glycogen synthase kinase-3 data was made from the database (BookhavenProtein Data Bank) its code is (3F7Z).
The three-dimensional structure of the two enzymes was obtained by X-ray diffraction with aresolution (1.90 Å) and for 2QMJ (2.40 Å) for 3F7Z [10].

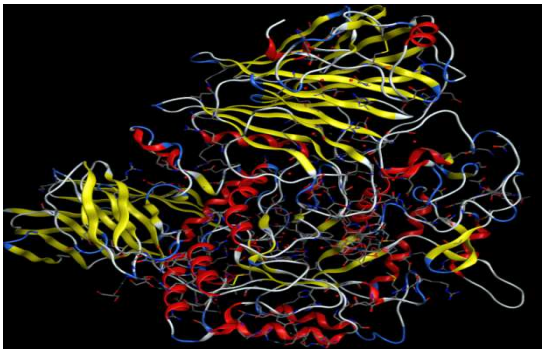


Figure 1: maltase - glucoamylase (2QMJ)

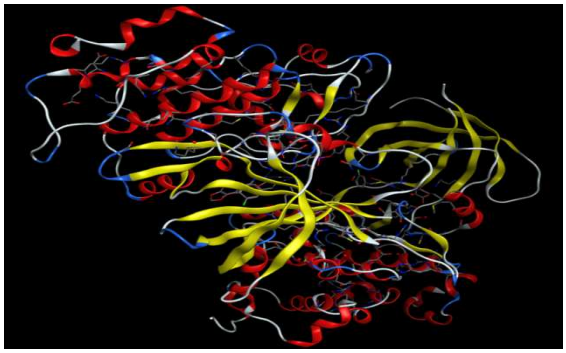


Figure 2: Glycogen synthase kinase-3 (3F7Z)

Optimization enzymeset inhibitors:
- Optimising the geometry of both enzymes was performed using the field strengths in the MMFF94x implanted software MOE (Molecular Operating Environment) [11].

The main chain has been maintained rigid while the side chains are flexible. This allows approximation of the protein side chains to more easily find the position in which the interactions are the most favorable.

- The construction and optimization of ligands were made by the MOE (Molecular Operating Environment) [11] software.

Table 1: Minimization of energies of molecules 4 (Kcal / mol)

molecules	2QMJ	3F7Z	Rebaudioside A	Stevioside
Energies	-9.03757e+002	-8.11887e+002	1.32534e+002	1.13532e+002

Molecular dynamics of enzymes and ligands only one:

We performed molecular dynamics calculation of single enzymes and ligands only to find the most stable conformation using the MOE software.

At 300 K there is an equilibration: The speeds are adjusted to maintain the temperature constant (there is an exchange between the kinetic energy and potential energy). Then, there is generation of conformations. The time of the molecular dynamics simulation is 100 ps.

Table 2: Dynamics of four molecules (Kcal / mol)

Molécules	2QMJ	3F7Z	Rebaudioside A	Stevioside
Energies	-2911.9216	-552.8135	242.6503	268.8654

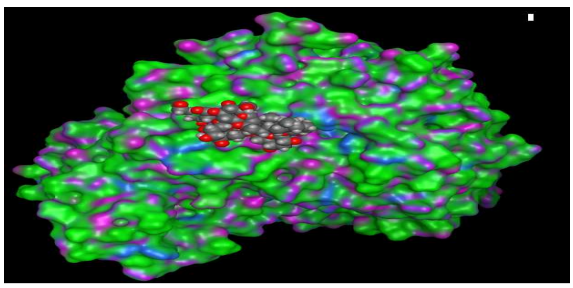
Docking of substrates and building complexes:
The next step, after the construction of ligands, is the positioning of these molecules in the active site of enzymes (2QMJ and 3F7Z). For this, we used the Dock module (Molecular Docking) using the MOE (Molecular Operating Environment) software [11].

Once the ligand-receptor complex is formed, it will adapt to the most stable conformation, i.e. with the lowest energy level.

The purpose of the Dock application is looking at favorable conformational binding between medium size ligands and a not so soft macromolecular target, which is usually a protein[12]. For each ligand, a number of conformations called poseswere generated (for the first complex 28 conformations were generated, 29 for the second, and 30 conformations for the 3rd and 4th) to identify favorable binding modes [11].

The search for binding modes is generally constrained to a small specific region of the receptor called the active site.

Figures 3: Docking of complexes 4



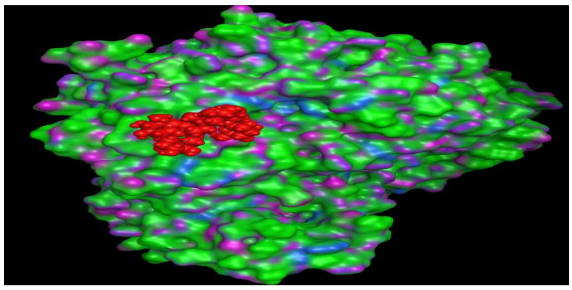
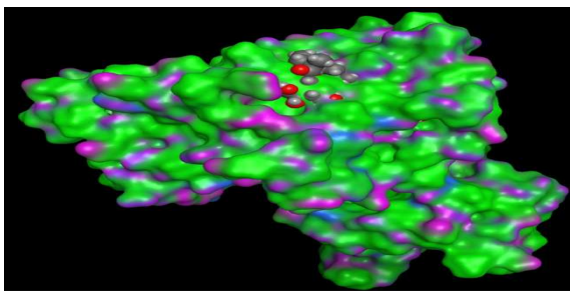


Figure a: Complex-1 (Rebaudioside A 2QMJ) Figure b: Complex-2 (Stevioside 2QMJ)



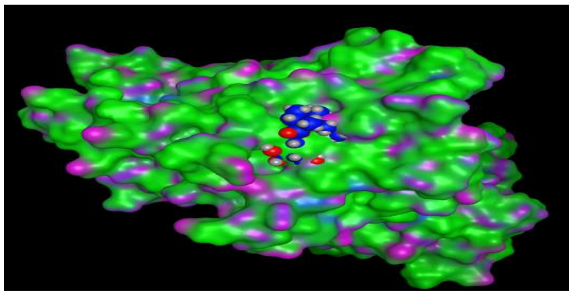


Figure c: Complex-3 (Rebaudioside A 3F7Z) Figure d: Complex-4 (Stevioside 3F7Z)

The energy balance (lowest energy) complex four is presented in the following table:

Table 3: Energy Balance of 4 complexes (Kcal / mol)

Molecules	S	Rmsd	Rmsd_Refine	E_Conf	E_Place	E_Scor1	E_Refine	E_Scor2	Nbre de poses
Complex-1	-4.5423	17.0148	5.9517	-322.7267	-29.8450	-9.2586	-13.4819	-4.5423	28
Complex-2	-4.7515	8.3521	2.9395	-367.7993	-28.0340	-13.5570	-16.8141	-4.7515	29
Complex-3	-7.1649	11.6607	2.9100	266.4092	-53.7913	-9.7746	-28.0415	-7.1649	30
Complex-4	-6.3901	10.8544	2.3868	295.6576	-95.1085	-13.5589	-23.4983	-6.3901	30

S: the final score; is the score of the last step, rmsd_refine: the mean square deviation between the laying before refinement and after refinement pose, E_conf: energy conformer, E_place: score of the placement phase, E_scor1: score the first step of notation, E_refine: score refinement step and number of conformations generated by ligand E_scor2: score the first step notation, number of poses: Number of conformations [13].

The various interactions of complex 4:

2D molecular method of the screen has been attributed to the MOE (Molecular Operating Environment) software, which is designed to visualize the active sites of the complex (protein-ligand). The ligand is prepared and made with an improved 2D depiction layout algorithm, and protein residues version are arranged around it to indicate links spatial proximity [14].

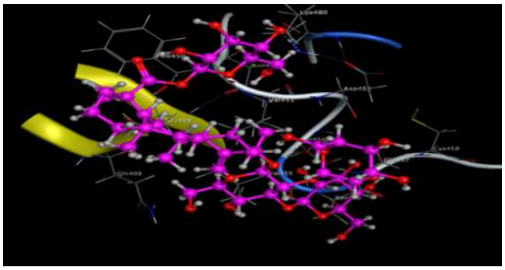
Residues are marked with their amino acid code of 3 letters, and job classification. If there are multiple channels in the system, the positions are prefixed by the letters of the alphabet.

There are 4 default colors for residues. Hydrophobic residues are colored with green inside, while polar residues are colored light purple. Basic residues are still marked by a blue inner ring and the acid residues with a red ring [15]. Hydrogen bonding interactions between the receptor and ligand are shown with an arrow to indicate the direction of the hydrogen bond (i.e. the donor is at the base of the arrow, and the acceptor at the head). In cases where there may be a hydrogen bond in both directions, we use double-headed arrow.

When the hydrogen bond is formed with the side chain residues, the arrow is drawn in green. The hydrogen bonds to the backbone residues are drawn in blue, with an additional point shot at the attachment point of residues [16].

Interactions between 2.5 Å and 3.1 Å are considered high and those between 3.1Å and 3.55Å are average. Greater than 3.55Å interactions are weak [17].

Figures 4: Diagrams of Interactions (protein-ligand) 4 complexes



3D image

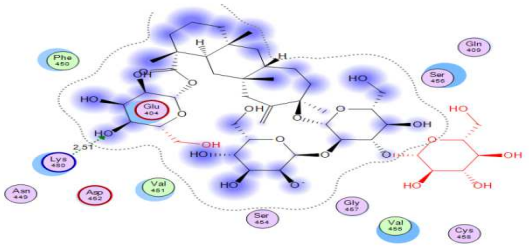


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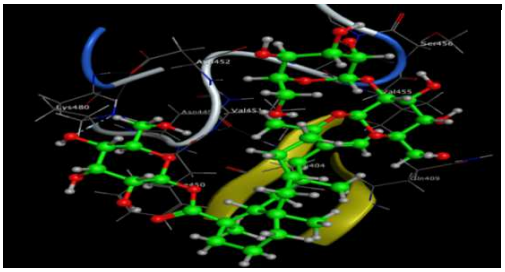
Figure (e): Diagram of interaction of complex-1 (2QMJ + Rebaudioside A)



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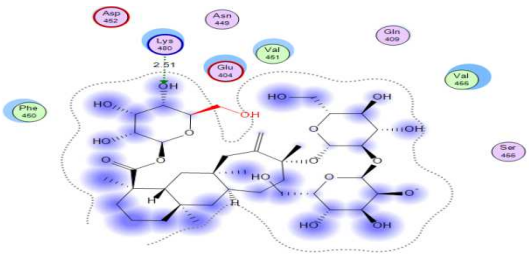

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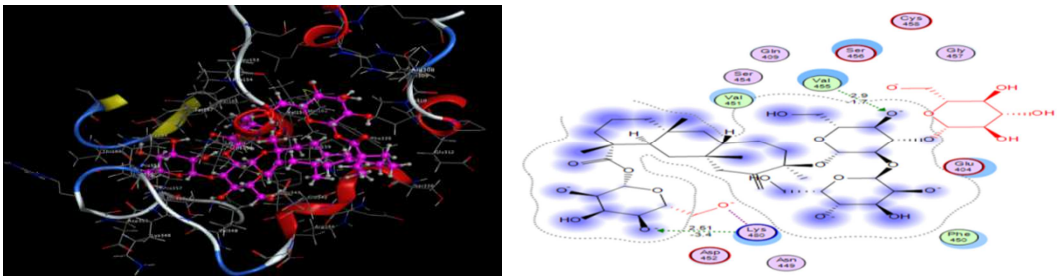
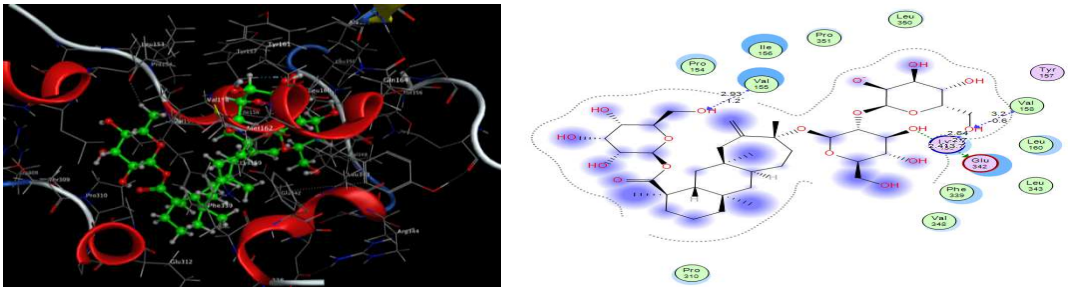
Figure (f): diagram of interaction of the complex-2 (+ 2QMJ Stevioside)



3D image **2D image**

Figure (g): diagram of interaction of the complex-3 (+ 3F7Z Rebaudioside A)



3D image **2D image**

Figure (h): diagram of interaction of the complex-4 (+ 3F7Z Stevioside)

RESULTS AND DISCUSSION

In light of these results, it appears that the introduction of bulky groups causes a conformational rearrangement within the active site pocket, which will probably be the fold in the complementarity and hence the activity [18].

The energy balance of the four complexes is shown in Table 3. It is noted that both inhibitors interact with both proteins.

These results show that the complex 3 has the lowest energy (-7.1649Kcal/mol) and is more active than complex 4 (-6.3901 Kcal/mol) which is more active than complex 2 (-4.7515 Kcal / mol) which in turn is more active than complex 1 (-4.5423 Kcal / mol) [19].

The results revealed that:
For complex 1: Rebaudioside-A interacts with the amino acid Lys 480 at a distance of 2.51 Å (strong interaction), suggesting that Rebaudioside-A can inhibit Maltase - glucoamylase (2QMJ) and interfere with Lys 480 [20].

For complex 2: Stevioside has an interaction with the amino acid Lys 480at a distance of 2.51 Å (strong interaction), suggesting that stevioside can inhibit Maltase - glucoamylase (2QMJ) and interfere with Lys 480 [20].

For complex 3: Rebaudioside A interacts with amino acid 455 Val at a distance of 2.9 Å (strong interaction), suggesting that Rebaudioside-A can inhibit Glycogen synthase kinase-3 (3F7Z) and interfere with Val 455 [20].

For complex 4: Stevioside interacts with the amino acids Glu 342, Val 158, Lys 159, Val 155 at distances of 2.39, 3.36, 2.94, 3.02 Å, respectively (for the 1st, 3rd and 4th strong interactions and for the 2nd average interaction), which suggests that stevioside can inhibit Glycogen synthase kinase-3 (3F7Z) interfere with Glu 342, Val 158, Lys 159, Val 155 [20].

Our present results showed that both inhibitors have interactions with residues of the two enzymes; we can say that there is an affinity between the two inhibitors and the two enzymes [21].

CONCLUSION

Comparing the four complexes; we can see that the two inhibitors (Rebaudioside A and stevioside) have a better

inhibition for the enzyme glycogen synthase kinase-3 (3F7Z) that the enzyme maltase - glucoamylase (2QMJ). We found that stevioside and Rebaudioside A have demonstrated growth inhibitory activity for both enzymes, suggesting that the two inhibitors can be used for the development of antidiabetic medication.

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