JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2014, Vol. 2, No. 6, pp: 675-679
Available at www.jcrs010.com

ORIGINAL ARTICLE

DOCKING STUDIES OF INHIBITORY EFFECT OF HERBAL COMPOUNDS ON HUMAN PANCREATIC ALPHA-AMYLASE

Fatemeh Taktaz
[1]*Department of Biology, Faculty of Basic Sciences, Hakim Sabzevari University.*
f.taktaz@gmail.com

ABSTRACT: Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen, is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity, as well as, dental caries and periodontal diseases. Plants are an important source of chemical constituents with potential for inhibition of α-amylase and can be used as therapeutic or functional food sources. In this study the inhibitory effects of flavonoid and Tannin Compounds on enzyme activity with docking server were investigated and the parameters for inhibition effect and Inhibition Constant (Ki) were determined. The high inhibitory capacity is observed in flavones groups. The main inhibitory effects of the tannins is related with its the ability to strongly bind to carbohydrates and proteins.
Key words: Human pancreatic alpha-amylase, Inhibitory effects, Plant source.

INTRODUCTION

The α–amylase (α -1,4-glucan-4-glucanohydrolases; E.C. 3.2.1.1) is one of the major secretory products of the pancreas (about 5–6%) (16) and salivary glands, playing a role in digestion of starch and glycogen and can be found in microorganisms, plants and higher organisms[1]. α-Amylase catalyze the hydrolysis of starch via a double displacement mechanism involving the formation and hydrolysis of a covalent *β*- glycosyl enzyme intermediate by using active site carboxylic acids for it[2]. The residues, in particular, Asp197, Glu233, and Asp300 were described to function as catalytic residues [3]. You can see human pancreatic alpha-amylase enzyme structure in Figure 1. Methanol extracts of 41 plants, used in traditional Mongolian medicine have been tested for α-amylase inhibitory properties and significant inhibition of the enzyme was shown [4]. Phenolic compounds are a large group of structurally diverse naturally occurring compounds that possess at least a phenolic moiety in their structures. Flavonoids are abundant class of natural phenolic compounds with several biological activities. flavonoids and human α- amylase in order to understand the molecular requirement for enzyme inhibition [5]. Tannins are another heterogenous polyphenol group widely distributed in the plant kingdom that are often present in unripe fruits, but can disappear during ripening. They have a relatively high molecular weight and can be classified into two major classes: hydrolysable tannins and condensed tannins [6]. The main inhibitory effects of the tannins is related with its the ability to strongly bind to carbohydrates and proteins [7].

MATERIALS AND METHODS

In the first step, amino acid sequences human pancreatic alpha-amylase enzyme with the number of 1XGZ was taken from Protein Data Bank website. Molecular docking web server is used for docking study (http://www.dockingserver.com/web).
flavonoid and tannin Compounds in plants obtained from chemispider web site (Table 2) and their structure shown in Figure 2. Docking calculations were carried out using Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations.

RESULTS

The alpha-amylase consists of 496 amino acids, and its molecular weight is 55 KD. Enzyme catalytic residues are Asp197-Glu233-Asp236-Asp300 (Table 1**)**. Chemical Information of. docking calculations were carried out using Docking Server that interaction parameters for different inhibitors and their structure shown in Table 3 and Figure 3 respectively. The docking energy values were calculated as the sum of the electrostatic, Van der Waals energies and the flexibility of the ligand itself. Low docking energy indicates high binding ability. Based on the results obtained, Tannic acid with Ki = 1.33 uM showed the highest inhibitory. The main inhibitory effects of the tannins is related with its the ability to strongly bind to carbohydrates and proteins. From the present kinetic data, we conclude that the tannin inhibitor can bind to the active site of the free enzyme to give rise to EI complexes. Since tannins play such varied biological roles, and because of the enormous structural variation among tannins, it is difficult to develop models that allow an accurate prediction of tannin effects in enzyme systems.

Table 1. Amino acid sequence analysis of human pancreatic alpha-amylase (www.uniprot.org).

No	Feature key	Position(s)	Length	Description
1	Chain	16-511	496	Pancreatic alpha- amylase
2	Signal peptide	1-15	15	
4	Active site	248		Proton acceptor
5	Metal binding	182-173 216-115	1	Calcium
6	Glycosylation	476	1	N-linked (GlcNAc...)
7	Modified residue	16	1	Pyrrolidone carboxylic acid
8	Binding site	210-313 352	1	Chloride

Table 2. Chemical Information of flavonoid and tannin Compounds in plants (www.chemispider.com).

Inhibitor	ChemSpider ID	Molecular Formula	Average mass
Daidzein	4445025	$C_{15}H_{10}O_4$	254.237503Da
Myricetin	4444991	$C_{15}H_{10}O_8$	318.235107Da
Tannic acid	17286569	$C_{76}H_{52}O_{46}$	1701.198486Da
Quercetin	4444051	$C_{15}H_{10}O_7$	302.235687Da

Table 3. Interaction parameters for different inhibitors.

Inhibitors	Free Energy of Binding	Inhibition Constant, (Ki)	Electrostatic Energy	Interact Surface.	Frequency
Daidzein	-5.42 kcal/mol	106.57 uM	-0.14 kcal/mol	585.754	80%
Quercetin	-5.21 kcal/mol	152.60 uM	+0.00 kcal/mol	597.449	70%
Myricetin	-5.77 kcal/mol	58.92 uM	+0.05 kcal/mol	604.061	50%
Tannic acid	-3.92 kcal/mol	1.33 mM	+0.00 kcal/mol	764.202	10%

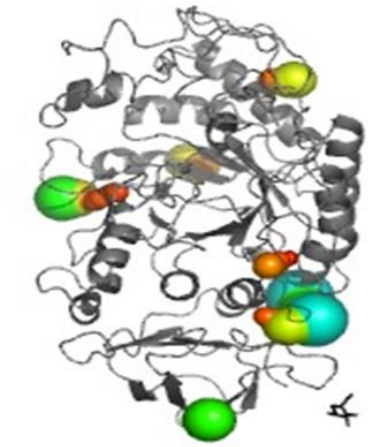
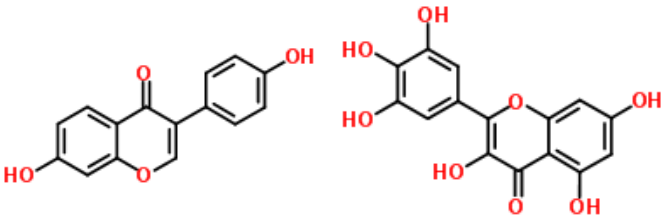
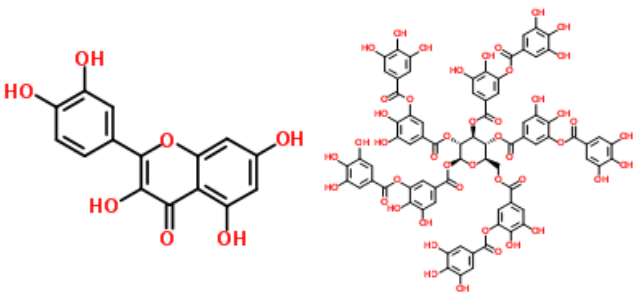


Figure 1. Human pancreatic alpha-amylase enzyme structure

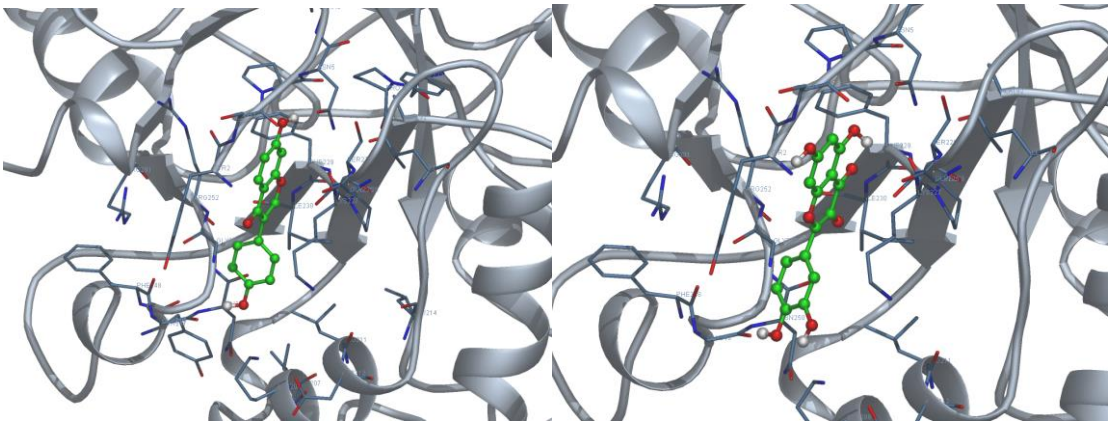


Daidzein Myricetin

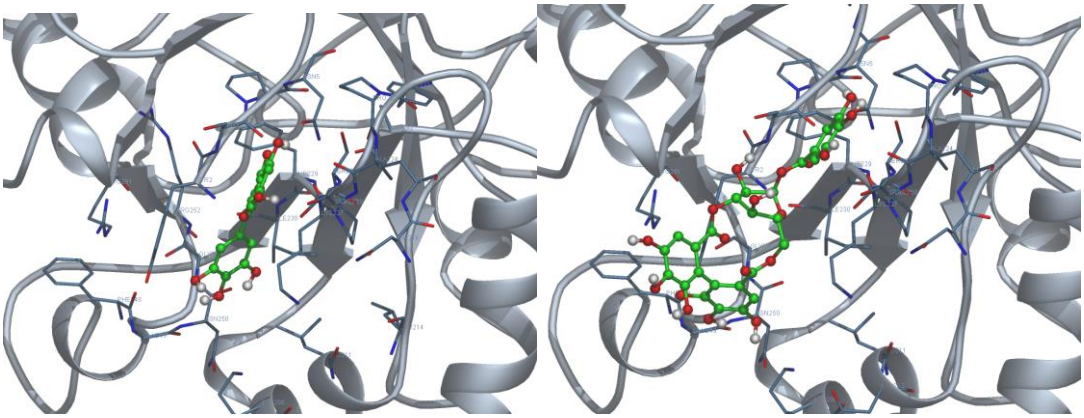


Quercetin Tannic acid

Figure 2. Flavonoids and Tannin presenting α-amylase inhibition activity.



Daidzein Quercetin



Myricetin Tannic acid

Figure 3. Docking plant compounds in enzyme structure.

DISCUSSION

Pancreatic α-Amylase enzyme plays an important role in early breakdown of complex carbohydrates into simple molecules. Modulation of α-amylase activity affects the utilization of carbohydrates as an energy source and stronger is this modulation; more significant is the reduction is the breakdown of complex carbohydrates. Majority of studies have focused on the anti amylase phenolic compounds**.** The action mechanism proposed for inhibitory capacity of flavonoids correlated the potency of inhibition of these compounds with the number of hydroxyl groups on the B ring of the flavonoid skeleton with the formation of hydrogen bounds between the hydroxyl groups of the polyphenol ligands and the catalytic residues of the binding site of the enzyme. The high inhibitory capacity is observed in flavonols and flavones groups. Also, there is need for novel agents, therapeutic strategies or designing functional foods that could act on the physiological regulation of sugar uptake, blood sugar levels, and prevention of oral diseases.

REFERENCES

Kandra L. α-Amylases of medical and industrial importance . Mol Struct 2003: 666-667 487-498.

Brayer GD, Sidhu G, Maurus R, Rydberg EH, Braun C, Wang Y, Nguyen NT, Overall CM, Withers SG. Subsite mapping of the human pancreatic alpha-amylase active site through structural, kinetic and mutagenesis techniques. Biochemistry 2000:US 39: 4778-4791.

Rydberg EH, Li C, Maurus R, Overall CM, Brayer GD, Withers SG. Mechanistic analyses of catalysis in human pancreatic α-amylase: Detailed kinetic and structural studies of mutants of three conserved carboxylic acids. Biochemistry 2002: 41: 4492-4502.

Kobayashi K, Baba E, Fushiya S, Takano F, Batkhuu J, Dash T, Sanchir C, Yoshizaki F. Screening of Mongolian Plants for

Influence on Amylase Activity in Mouse Plasma and Gastrointestinal Tube. Biol. Pharm. Bull 2003: 26: 1045-1048.

Lo Piparo E, Scheib H, Frei N, Williamson G, Grigorov M, Chou CJ. Flavonoids for Controlling Starch Digestion: Structural Requirements for Inhibiting Human α-Amylase. J. MedChem 2008:51: 3555-3561.

Kandra L, Gyémánt G, Zajácz A, Battab G. Inhibitory effects of tannin on human salivary aamylase. Biochem Biophys Res Commun 2004: 319: 1265-1271.

Quideau S, Jourdes M, Saucier C, Glories Y, Pardon P, Baudry C. DNA topoisomerase inhibitor acutissimin A and other flavano-ellagitannins in red wine. Chem 2003:42: 6012- 6014.