Avens Publishing Group
J Proteomics Computational Biol
October 2022 Volume:5, Issue:1

Open Access Research Article

Journal of Proteomics & Computational Biology

Peptides Digested from Some *Allium Sativum* and *Solanum lycopersicum* Proteins Serve as Antihypertensive Agents: Computational Analysis

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Submission: 21 September, 2022
Accepted: 25 October, 2022
Published: 29 October, 2022

Keywords: Hypertension; Anti-hypertensive peptide; Peptide-protein docking; *Allium sativum*; *Solanum lycopersicum*

Abstract

Hypertension (HTN) still constitutes a worldwide problem to the healthcare section and individual's life. The current angiotensin converting enzyme (ACE) inhibitors are first-choice option but pose deleterious side effects upon prolonged administration. The goal of the present *in silico* study is to evaluate the *Allium sativum* and *Solanum lycopersicum* proteins as source for anti-hypertensive natural peptides. The corresponding protein sequences were obtained from UniProt database and then inputted to antihypertensive peptides predictor online tool. Using the same tool, 3 digestive enzymes (pepsin, trypsin and elastase 1) were chosen for digestion of proteins into small peptides and subsequently assess their hypotensive activity. Some biochemical characteristics of positive bioactive peptides were calculated via Pepstats web interface while HAPPENN tool examined hemotoxicity. Afterwards, the secondary structure of positive bioactive peptides was de novo predicted through PEPstrMOD server prior to docking against human ACE (PDB ID: 1o86) using HPEPDOCK and ClusPro platforms. *A.sativum* peptides are more potent than *S.lycopersicum* ones in terms of IC50 as well as docking score albeit neither of which showed hemotoxicity. The present *in silico* work suggests the hypotensive activity of *A.sativum* and *S.lycopersicum* as natural treatment option of HTN.

Introduction

Hypertension (HTN) continues to be the second main risk factor of death cases from cardio- and cerebrovascular accidents as well as end-organs damage globally [1]. It is estimated in the United States (US) that one in every three persons suffers from HTN [2]. The recent redefinition of HTN is blood pressure (BP) levels ≥ 130/80 mm Hg or having HTN medicines. Accordingly, the percentage of prevalence of HTN in US rises to 50% [3]. Moreover, about half of COVID-19-hospitalized patients have HTN which increases the risk of mortality even more [4].

Various risk factors are traced to drive BP up that can be categorized into modifiable and non-modifiable factors (Figure 1). Modifiable factors include, but not limited to, diet (particularly Na-rich fast foods), sedentary lifestyle and obese people. Non-modifiable factors involve aging and the presence of chronic diseases besides such as type 2 diabetes and kidney diseases [5,6]. In addition, genetic inheritance and, more recently, epigenetics programming especially in prenatal stage increases the incidence of HTN later in adulthood [7].

Once patient's BP got elevated, therapeutic intervention should be introduced to restore the levels of BP to the accepted values. One of the mainstay pathways regulating BP is renin-angiotensin-aldosterone system (RAAS) (Figure 2). As the name implies, there are 3 cooperating hormones to control BP, renin (also known as angiotensinogenase), angiotensin and aldosterone. Driven by the HTN, juxtaglomerular cells of kidney sense this rise in BP and respond by secreting renin to the bloodstream. Therein, it proteolytically

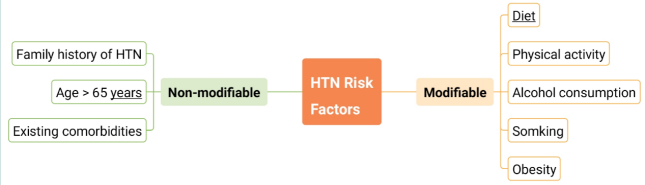

Figure 1: Most common risk factors of developing HTN.

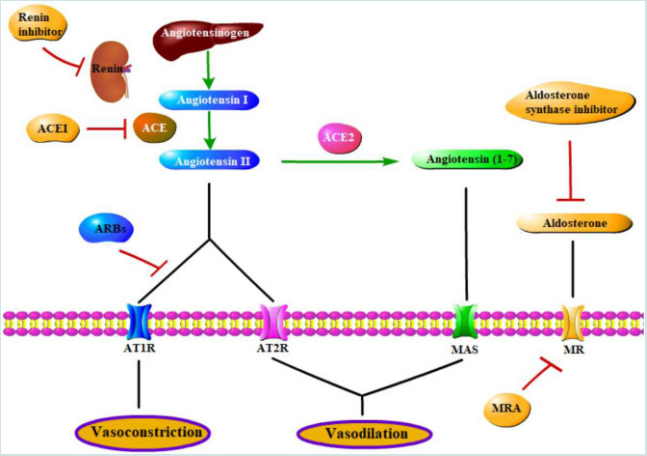

Figure 2: Renin-angiotensin-aldosterone system (RAAS) with the corresponding druggable targets, Reprinted from with permission from Elsevier [8].

ISSN: 2572-8679

activates angiotensinogen produced from liver forming angiotensin I and the latter catalyzed by the enzyme angiotensin-converting enzyme 1 (ACE1) is cleaved further to angiotensin II. The produced angiotensin II acts as powerful vasoconstrictor by activating its corresponding receptors ATR (angiotensin II receptors) on smooth muscle cells. With the assistance of ATR binding by angiotensin II, aldosterone is secreted from adrenal gland to enhance Na reabsorption contributing further to the establishment of HTN [8]. Therefore, interrupting ACE1 action cancels the vasoconstriction as well as Na reabsorption actions reducing BP to the normal set. ACE1 inhibitors are considered first-choice treatment of HTN [9].

Not only small organic molecules can achieve ACE1 inhibition. Many food proteins upon enzymic digestion in the gut releases bioactive peptides exhibiting inhibitory action toward different targets of RAAS including ACE1 [10,11]. So, we purposed to explore the anti-hypertensive activity of peptides digested from some *A.sativum* proteins *in silico*. The present study provides a probable means to nutraceutically treat HTN through the food taken on a regular daily routine.

Materials & Methodology

Workflow

The Repeated workflow strategy employed in this study is illustrated in Figure 3. However, the details of each step are explained below.

Source protein sequences retrieval

The primary structure (amino acid sequence) of the examined proteins was retrieved from UniProt database (https://www.uniprot.org/) [12]. Three proteins were chosen randomly: Alliin lyase 1 (Uniprot ID#Q01594), mannose-specific lectin (Uniprot ID#P83886) and peroxidase (Uniprot ID#H2CLW6) limiting the organism to Allium sativum only. Similarly, three proteins from Solanumlycopersicum were selected for further analysis (Uniprot ID#P27260, G1JUH1 & C1K5M2).

Proteins digestion and prediction of antihypertensive activity

The 3 mentioned proteins were subjected to three digestive enzymes (randomly chosen) namely: Pepsin, Trypsin and Elastase 1 in combination. Afterwards, the anti-hypertensive activity of the generated peptides were evaluated using anti-hypertensive peptide predictor (AHPP) server [13]. This server enables enzymatic digestion and activity assessment simultaneously. Only the peptide showed positive anti-hypertensive activity and having ≥ 7 AA were selected for further analysis.

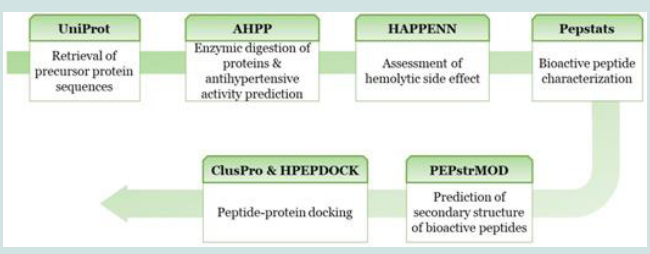

Figure 3: Flowchart summarizing the workflow of the current in silico study.

Examining hemolytic activity of the bioactive peptides

Despite of posing biological activity, some peptides have a coinciding side effects including hemotoxicity and many neglect such aspect [14]. HAPPENN web server was utilized to examine the hemotxicity of the positive peptides [15]. Results are expressed as probability score between 0 (non-hemolytic) to 1 (highly hemolytic).

Biochemical features of the bioactive peptides

The peptides succeeded in achieving positive antihypertensive activity and had at least seven AA were uploaded to pepstats web server (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/) for detecting some characteristics such as molecular weight, isoelectric point and charge, among others. The obtained results were collected and tubulated.

Docking analysis

The secondary structure of the best 3 peptides were predicted de novo via PEPstrMOD online tool [16] (https://webs.iiitd.edu.in/raghava/pepstrmod/) in provision to be docked against human ACE1(PDB ID: 1O86) after removal of water molecules, chloride and zinc ions and the inhibitor lisinopril by UCSF Chimera v1.16 [17]. Then, peptides and receptor PDB files to uploaded to and HPEPDOCK protein-protein docking platform [18].

Results

Biochemical features and anti-hypertensive peptide prediction

Table 1 Obtained biochemical features of the fragmented peptides from *A.sativum* proteins along with hemotoxicity values.

The 3 examined proteins of *A.sativum*, only 8 peptides found positive (antihypertensive). As we chose 3 digestive enzymes (pepsin, trypsin and elastase 1) the residues were short (<10 AA). Most of the fragmented peptides had neutral charge and, hence, pI around 5. Furthermore, the digested peptides posed high probability of being expressed in inclusion bodies which facilitates the large-scale production and isolation of the examined peptides. Moreover, none of the peptides showed hemolytic activity as reflected by values near zero (indicative of non-hemolytic) (Table 1). Overall, the digested peptides from *A.sativum* are both anti-hypertensive as well as non-hemolytic peptides particularly 3rd peptide (FTGHSGSR). This outcome is good for provision to molecular docking against human ACE1 to further confirm the findings.

Only 6 positive peptides were obtained from precursor proteins of *Solanum lycopersicum*. The fragmented peptides were significantly potent in terms of anti-hypertensive activity as mirrored by the IC_{50} values. The digested peptides are composed of a combination of neutral, acidic and basic amino acids with net charge between -2 to 1 and pI in the range 3.55 to 8.9. In addition, none of the obtained peptides exhibited hemolytic activity (Table 2).

Docking analysis

Table 3 Docking output of A.sativum peptides to ACE1 using HPEPDOCK and ClusPro servers

Apparently from table 3, the fragmented peptides from allium sativum exhibited good inhibition profile toward ACE1, confirming thus their anti-hypertensive activity predicted via AHPP server. The

ISSN: 2572-8679

Table 1: Obtained biochemical features of the fragmented peptides from *A. Sativum* proteins along with hemotoxicity values.

Peptide	IC_{50} (μM)	MW	Residues	Charge	pI	Expression probability	Hemotoxicity
FFNPVSN	61.76	823.9	7	0	5.55	0.693	0.023
FIDQTETA	71.83	923.98	8	-2	3.55	-0.66	0.001
FTGHSGSR	25.43	847.89	8	1.5	10.0208	0.975	0.033
INTQNGVG	61.76	801.85	8	0	5.55	0.879	0.057
NINCSEHGR	61.76	1029.1	9	0.5	6.9681	0.958	0.001
PPSPSYA	46.09	717.78	7	0	5.5494	0.036	0.019
VNNNNMVQA	61.76	1003.1	9	0	5.55	0.958	0.021
YVNVSNPEQ	61.76	1049.11	9	-1	3.8497	0.726	0.002

MW: molecular weight, pI: isoelectric point.

Table 2: Obtained biochemical and hemotoxicity findings of generated peptides from *S. lycopersicum* proteins.

Peptide	IC_{50} (μM)	MW	Residues	Charge	pI	Expression probability	Hemotoxicity
CNPGPQSS	29.68	788.83	8	0	5.5289	-0.187	0.03
KSSSDVKS	13.43	836.9	8	1	8.9961	0.975	0.002
TYPKCDL	38.02	838.97	7	0	5.7681	0.596	0.002
WVSENVMDA	59.83	1050.15	9	-2	3.55	0.787	0.049
YIDKDGDV	74	923.98	8	-2	3.826	0.172	0.001
YNCTNQR	49.76	897.96	7	1	7.9908	0.653	0.004

Table 3: Docking output of *A.sativum* peptides to ACE1 using HPEPDOCK and ClusPro servers.

Peptide	HPEPDOCK	ClusPro
	Docking score	Docking score
FFNPVSN	-230.048	-805.1
FIDQTETA	-161.654	-725.8
FTGHSGSR	-240.190	-805.1
INTQNGVG	-185.367	-561.3
NINCSEHGR	-230.454	-718.4
PPSPSYA	-211.221	-704.6
VNNNNMVQA	-197.001	-597.4
YVNVSNPEQ	-200.368	-621.7

Table 4: The docking result of *S.lycopersicum* peptides to ACE1.

Peptide	HPEPDOCK	ClusPro
	Docking score	Docking score
CNPGPQSS	-181.314	-608.7
KSSSDVKS	-155.069	-416.8
TYPKCDL	-183.970	-658.2
WVSENVMDA	-211.474	-862.8
YIDKDGDV	-137.870	-673.3
YNCTNQR	-225.358	-711.2

docking scores in HPEPDOCK ranged from -185 to -240 kcal/mole. Similarly, from -561 up to -805 kcal/mole predicted against ACE1 via ClusPro platform. The ranking of peptides in the two servers are almost the same which confirm the results of each other with the 3rd peptide were the best in the two servers.

To further reveal the detailed peptide-protein interactions between 3rd peptide and ACE1, the 3D surface view and in-depthrepresentation have been performed (Figure 4). It has been figured out that peptides lie well within the active center of ACE1 as shown in surface view (Figure 4A).

The docking profile of the digested peptides from *S.lycopersicum* showed slightly weaker binding energy to ACE1 explored by HPEPDOCK server in comparison to *A.sativum* peptides. While the

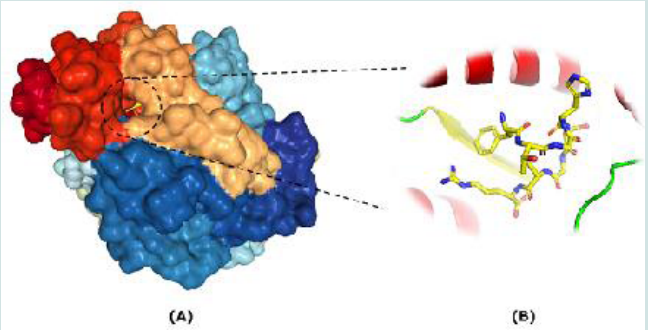

Figure 4: Peptide-protein interactions between 3rd peptide and ACE1, the 3D surface view.

highest docking score of S.*lycopersicum* peptides were peptide 6 which gave inhibitory value of -225 kcal/mole, 3rd peptide of *A.sativum* had a docking score of -240 kcal/mole, suggesting better potency. Nonetheless, peptide 4 of *S.lycopersicum* showed best docking energy compared to the 6th peptide in HPEPDOCK (-862 vs -711 kcal/mole) (Table 4). This docking energy is even stronger than best docking energy of 3rd peptide (best one) of *A. sativum* peptides.

Discussion

Developing HTN worsen individual's health life since it is accompanied by complications progression such as cardiovascular diseases, stroke and dementia. Beyond 1970s, BP levels have been declined in high-income nations whilst raised substantially in Middle-east and Southeast regions [19]. In 2016, about third of adult population worldwide are diagnosed with HTN [20]. Men on common have age-standardized systolic BP higher than women counterparts in several countries [21]. This data necessitates should urge to find more potent therapeutics with minimized side effects because the first-choice treatments (ACE1 inhibitors) exhibit deleterious side effects involving headache, visual disturbances, cough, dizziness and insomnia on prolonged use [22]. In recent years, the direction of HTN

ISSN: 2572-8679

treatment has been transformed into food peptides including milk, egg, meat and marine meats. The hypotensive peptides are released upon hydrolysis through gastrointestinal tract enzymes [23]. Jogi et al. reviewed long list of hypotensive peptides derived from food sources [24]. However, garlic and tomato-hypotensive peptides were not mentioned. The present *in silico* study was designed to simulate the in vitro and in vivo works for assessment of hypotensive peptides generated from food sources and predict their ACE1 inhibition. The results of the current study suggest the hypotensive activity of both garlic *(A.sativum)* and tomato *(S.lycopersicum)* as evidenced by IC_{50} values and validating molecular docking against ACE1 target albeit garlic peptides are more potent. Moreover, the fragmented peptides are non-hemolytic and had a high probability of being expressed in inclusion bodies for large-scale production. Overall, the present theoretical exploration provided a notable suggestion which should be validated by in-vitro and in-vivo experiments

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