In Silico, Molecular Docking and In Vitro angiotensin-converting enzyme-inhibitory activity of PGYALQR peptide derived from fish waste hydrolysate

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Abstract. In recent decades, Angiotensin-converting enzyme (ACE) inhibitory peptides derived from various proteins have become crucial sources of health-enhancing components for clinical use. Abundant proteins in fish waste entrails can be used to produce ACE inhibitory peptides. Catfish, Tilapia, and Mackerel entrails were digested by pepsin and passed through the 3kDa cutoff column. The protein hydrolysate passed through the 3kDa cutoff column and C18 column were analyzed for an ACE inhibitory activities and sequenced using LC-MS/MS. Five candidate peptides from De novo sequencing was chemically synthesized and tested for ACE inhibitory activity. The ACE inhibitory activity result revealed that PGYALQR peptide contains ACE inhibitory activity as captopril did. This study aims to predict the conformation and orientation of the PGYALQR peptide into the binding site of ACE. Molecular docking analysis using AutoDock Vina was performed to elucidate the mechanisms underlying the ACE-inhibitory activity of the PGYALQR peptide. Computational analysis revealed that the peptide binds to the ACE active site with −11.2 kcal/mol, forming hydrogen bonds with Glu162, Gln281, His353, Ala354, Lys511, His513, and Tyr523. In comparision, captopril interacted with Gln281, His353, Lys511, His513, Tyr520, and Tyr523 with a binding energy of −5.9 kcal/mol. Additionally, the peptide interacts with the Zn (II) ion in the ACE active site, coordinating with the residues Glu411, His383, and His387, which is crucial for enhancing its inhibitory activity of ACE. It may contort the tetrahedral coordination of the Zn (II), resulting in loss of ACE activity.

1. Introduction

High blood pressure (Hypertension) is a condition in which the blood pressure in the arteries is greater than 140/90 millimeters of mercury, exerting pressure on the walls of the blood vessels while the heart contracts to pump blood to various parts of the body. Hypertension can be classified into two types based on its causes: the type with no clear cause (Essential hypertension), which is found in approximately 90-95% of cases, and the type with known causes (Secondary hypertension), which accounts for about 5-10% of cases [1]. Therefore, most patients with hypertension have the type with no clear cause, which is believed to result from a combination of multiple factors. A significant factor is the influence of an enzyme called renin and the hormone angiotensin on the kidneys. These two substances work together with the adrenal glands and the pituitary gland to regulate water, sodium minerals, and the contraction of blood vessels throughout the body to control blood pressure. This process is referred to as the renin-angiotensin system (RAS) [2].

The use of ACE inhibitors is one option for treating high blood pressure. Most of these drugs are synthesized, with the first drug developed being captopril, which is taken orally. Examples of synthetic drugs include captopril and enalapril, both of which can inhibit ACE activity by interacting with the enzyme's active site. Enalapril is a prodrug, meaning it is inactive on its own but becomes active after undergoing metabolism in the body. The drug is converted through a process called de-esterification. Captopril, on the other hand, has a structure containing a sulfhydryl (SH) group, allowing it to bind well with ACE at the active site and competitively inhibit angiotensin I. Captopril is rapidly absorbed with a bioavailability of around 70%, although food can reduce its absorption by 30-40%. The drug is metabolized in the liver into a disulfide compound and excreted through the kidneys [3].

Research has begun to explore natural substances with ACE-inhibitory effects, revealing that peptides from various foods are a source of ACE inhibitors. Typically, these peptides are inactive in their natural form as part of food proteins, but they can be broken down by protease enzymes from the digestive system. Once absorbed into the body, they exhibit ACE-inhibitory properties. Studies on peptides from food sources have shown their potential in lowering blood pressure. Food sources of bioactive peptides with ACE-inhibitory properties include dairy products, fish protein, meat, and plants. A key factor influencing the effectiveness of peptides as ACE inhibitors is their type and size. The ideal peptide size for ACE inhibition is between 2 to 12 molecules. The active site of angiotensin-converting enzyme (ACE) cannot effectively bind to large peptides, but it interacts well with tripeptides containing hydrophobic amino acids at the carboxyl terminus. Amino acids that are effective as ACE inhibitors include tyrosine (Y), tryptophan (W), phenylalanine (F), proline (P), leucine (L), isoleucine (I), valine (V), lysine (K), and alanine (A) [4-6].

This research took the waste materials to use as a source for producing hydrolyzed fish protein, which consists of short-chain peptides, to study and analyze its properties in inhibiting the action of the angiotensin-converting enzyme (ACE) by using modeling methods

based on computer simulations, an enzyme that plays a crucial role in reducing high blood pressure. This research might lead to the development of health products and/or medications in the future.

2. Materials and Methods

2.1 Materials

For the in silico molecular docking study, the receptor and ligands were prepared as follows: The three-dimensional (3D) structure of Angiotensin Converting Enzyme (ACE) (PDB ID: 1O86) was obtained from the RCSB Protein Data Bank (https://www.rcsb.org/). The molecular structure of the ligand captopril (Compound CID: 44093) was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The structure of the PGYALQR peptide was constructed and optimized using the polypeptide builder function of the PepBuild web server.

2.2 Peptides preparation

The crude entrails of fish entrails of catfish, tilapia and mackerel were dissolved in 50mM sodium acetate buffer, pH 3.7 (1:5 w/v) and digested by pepsin at 37 oC for 16 hours. The supernatant from the hydrolysate was subjected to 3kDa cutoff column and the went through Nano/Capillary LC (Ultimate3000 LC System, Thermo Scientific, UK) for peptide separation in C18 column (75 µm X 15 cm) (Acclaim PepMap RSLC, Thermo Scientific). This instrument was designed to operate with Hybrid quadrupole Q-ToF impact II™ (Bruker Daltonics), and Nano-captive spray ion source. The elution was performed in linear gradient mode from 0.1% formic acid to 80% acetonitrile containing o.1% formic acid for 30 mins at a flow rate of 0.30 µL/min. The peptide sequencing was identified in the fraction that revealed remarkable ACE-inhibitory activity by matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF-TOF MS). The peptide sequencing was analyzed using DeCyder MS differential analysis 2.0 software (DeCyderMS, GE Healthcare) and Mascot software (Matrix Science). The peptides were matched to the published sequence of the relative protein data in Scombridae database (NCBInr databank). Amino acid sequence of peptides with potential ACE-inhibitory activity was acquired from some literatures [7-11]. The synthesis peptides peptide sequences were chemically synthesized by GenScript Biotech (NJ, USA). The purity (all above 94%) and sequence of those peptides were verified by HPLC.

2.3 ACE-inhibitory activity assay

The chemically synthesized peptides were determined ACE-inhibitory activity was measured according to the modified procedure [12]. The substrate, N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine, FAPGG, was used as a substrate and captopril, an angiotensin-converting enzyme (ACE) inhibitor, was used as a reaction of control. The reactions were incubated in 37oC heat box for 5 min. Decreasing of an absorbance at 334 nm was monitored every 1 min for a period of 20 min. Each activity value was determined from an average of three replicates.

2.4 In silico molecular docking analysis

Docking simulations to investigate the binding modes of each ligand in the substrate-binding sites of ACE were performed using AutoDock Vina (Molecular Graphics Laboratory, La Jolla, CA, USA), which predicts binding poses and energies. Polar hydrogens were added, and Gasteiger partial charges were assigned. The search grid for the key site of all receptors was set to x: 37.26, y: 35.35, and z: 46.21. The thoroughness parameter in AutoDock Vina was adjusted to ensure a comprehensive search for the lowest affinity energy. After the docking simulations, receptor-ligand interactions were visualized using BIOVIA Discovery Studio Visualizer v20.1.0.0 (Accelrys, San Diego, CA, USA).

3. Results and Discussion

3.1 ACE inhibitory peptide sequences from fish entrail hydrolysates

After, investigating by using the method of Nano/Capillary LC-MALDI-TOF-MS/MS The results of De novo sequencing (picture 1) showed the amino acid sequence containing a key effect on ACE inhibitory activity. The positively charged amino acids (L and R) have been associated with the stronger efficiency of ACE inhibitory peptides [13, 14]. LEKFVR, KFAGPSVLAPPPL, ASNLHGV, LFKDLR, and PGYALQR peptides were chemically synthesized as potent peptide sequences. Except LFKFVR, other peptide sequences presented an ACE inhibitory activity (Table 1). Compared to those synthesized ACE inhibitory peptides with captopril, PGYALQR obtained higher ACE inhibitory activity than captopril.

3.2 In silico molecular docking analysis

To date, there is limited information available on the molecular mechanisms underlying the interaction between ACE and inhibitory peptides. Automated docking is an effective method for examining the structure-activity correlations between bioactive peptides and ACE. In this study, the synthesized PGYALQR peptide demonstrated ACE-inhibitory activity similar to captopril, an established ACE-inhibitory compound. This peptide was then analyzed through in silico methods to predict its interaction with human ACE using computational modeling. Molecular docking simulations of PGYALQR peptide and captopril with the active site of ACE were performed to evaluate their binding energies. The results showed that

PGYALQR exhibited a lower score with a binding energy of −11.2 kcal/mol, whereas captopril had a binding energy of −5.9 kcal/mol (Table 2). This study examined the residues located in the S1, S2, and S1 pockets as well as the zinc ion (Zn (II)) binding motif because these areas are crucial for the formation of an ACE-inhibitor complex. According to Pina and Roque (2009) [15], the three active pockets in ACE are S1, S2, and S1'. Ala354, Glu384, and Tyr523 were present in S1, Gln281, His353, Lys511, His513, and Tyr520 were present in S2, and the residue Glu162 was present in S1. Additionally, Zn (II) in the active site of ACE coordinates with Glu411, His383, and His387.

The molecular docking results revealed that the PGYALQR peptide and captopril could form 19 and 7 interactions, respectively, with ACE residues. As shown in Table 2, the PGYALQR peptide establishes 19 interactions with key amino acids in all 3 active pockets of ACE (Glu162, Gln281, His353, Ala354, Lys511, His513, and Tyr523), comprising 11 hydrogen bonds and 8 other types of interactions. In contrast, captopril interacts with 7 key amino acids in only 2 of the active pockets (Gln281, His353, Lys511, His513, Tyr520, and Tyr523), including 6 hydrogen bonds and one other type of interaction. Hydrogen bonds are the primary interactions responsible for maintaining the stability of the ligand inhibitor-ACE complex [16]. However, other interactions, such as electrostatic and hydrophobic bonds, also contribute to the stability of the ligand inhibitor-ACE complex. Additionally, a crucial factor promoting the inhibition of ACE activity is the direct coordination between the ligand and Zn (II) [15]. Fig. 2 displays the 3D interactions between the captopril docking poses and the PGYALQR peptide. Moreover, the close ACE active pockets 13 and 2 amino acids may interact with the PGYALQR peptide and captopril, respectively (Figs. 2A and 2B). Captopril, on the other hand, was shown to directly coordinate with the Zn (II) atom directly, whereas the PGYALQR peptides did not; instead, they were able to share bonds with three important residues (His383, His387, and Glu411) (Fig. 2A and 2B). Jimsheena and Gowda (2010)[17] reported similar findings, showing that arachin ACE-inhibitory peptides did not directly coordinate with the Zn(II) atom. Instead, their interaction with Glu411, His383, and His387 was crucial for enhancing their inhibitory activity. These results suggest that the PGYALQR peptide could serve as an effective ACE inhibitor. Additionally, the PGYALQR peptide exhibits superior binding to ACE compared to captopril, which is reflected in the binding energy values (Table 2). This finding is consistent with the ACE inhibition data presented in Table 1, indicating that the PGYALQR peptide inhibits ACE more effectively than captopril.

4. Conclusions

This study demonstrated that anti-ACE peptides can be successfully synthesized chemically. The synthesized PGYALQR peptide showed ACE-inhibitory activity comparable to captopril. Molecular docking studies revealed that both the PGYALQR peptide and captopril effectively bind to the ACE active pockets and interact with Zn(II) coordinating residues, with binding energies of −11.2 kcal/mol and −5.9 kcal/mol, respectively. This study suggests that both PGYALQR peptide and captopril can inhibit ACE activity. Compared with synthetic drugs, using natural peptides as drug gives low systemic toxicity, because most of these peptides were derived directly or indirectly naturally, and after degradation only amino acids left. Therefore, the PGYALQR peptide holds promise as a candidate for future industrial production of functional foods or peptide drugs aimed at hypertension treatment.

Acknowledgements: This research was funded by King Mongkut's University of Technology North Bangkok. Contract no. KMUTNB-61-GOV-B-32. The authors would like to acknowledge Mr. Yodying Yingchutrakul and Mr. Sucheewin Krobthong for mass spectrometry analysis. Finally, we would like to give special thanks to National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA) for any facility using in peptide sequencing.

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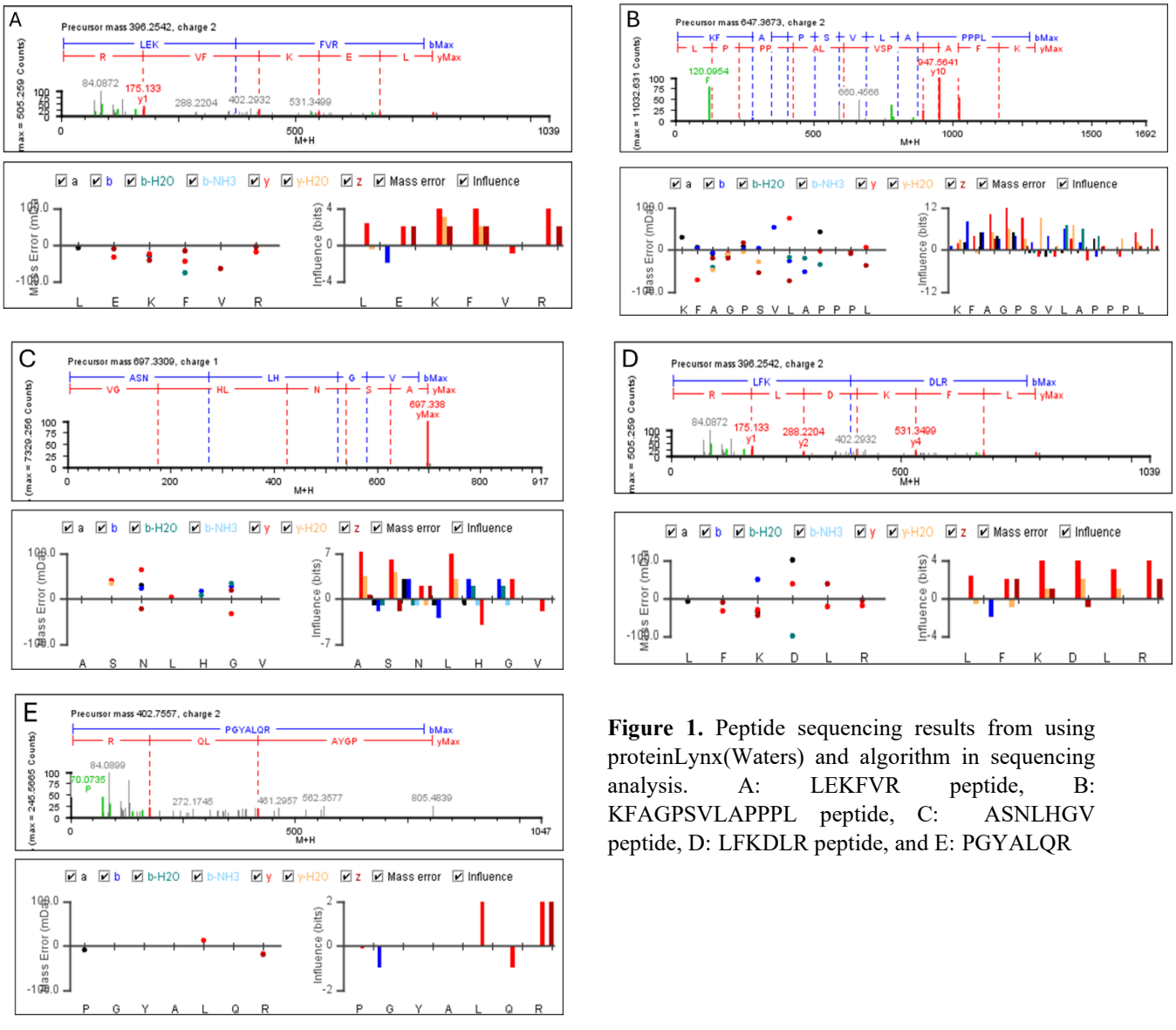

Figure 1. Peptide sequencing results from using proteinLynx(Waters) and algorithm in sequencing analysis. A: LEKFVR peptide, B: KFAGPSVLAPPPL peptide, C: ASNLHGV peptide, D: LFKDLR peptide, and E: PGYALQR

Table 1. ACE inhibitory activity of chemically synthesized peptides. The graph slope of FAPGG degradation was used to determine the enzyme activity)

Samples	A graph slope of FAPGG hydrolysis over time
ACE + FAPGG	-2.2711
ACE + FAPGG + captopril	1.067
ACE + FAPGG + LEKFVR	-2.9979
ACE + FAPGG + KFAGPSVLAPPPL	-1.2329
ACE + FAPGG + ASNLHGV	0.8901
ACE + FAPGG + LFKDLR	0.3963
ACE + FAPGG + PGYALQR	2.2313

Table 2. The Docking score, binding sites, bonding types and bond length of the PGYALQR peptide and Captopril

Subsites	Interaction of ACE binding sites	Types of interactions and their corresponding distance (Å)					
		PGYALQR peptide			Captopril		
		Hydrogen	Hydrophobic	Electrostatic	Hydrogen	Hydrophobic	Electrostatic
S1	Ala354	2.2 Å 2.3 Å	4.0 Å 4.0 Å	-	-	-	-
	Glu384	-	-	-	-	-	-
	Tyr523	2.2 Å	3.9 Å	-	3.4 Å	4.4 Å	-
S2	Gln281	2.9 Å 2.1 Å	-	-	3.1 Å	-	-
	His353	2.7 Å 1.9 Å 3.7 Å	4.1 Å 5.2 Å	4.5 Å	3.4 Å	-	-
	Lys511	2.6 Å	-	-	2.7 Å	-	-
	His513	1.9 Å	-	-	3.3 Å	-	-
	Tyr520	-	-	-	2.6 Å	-	-
S1'	Glu162	2.8 Å	-	4.1 Å 3.5 Å	-	-	-
Total of interactions		19			7		
Zn^{2+}		√			√		
Binding energy (kcal/mol)		-11.2			-5.9		

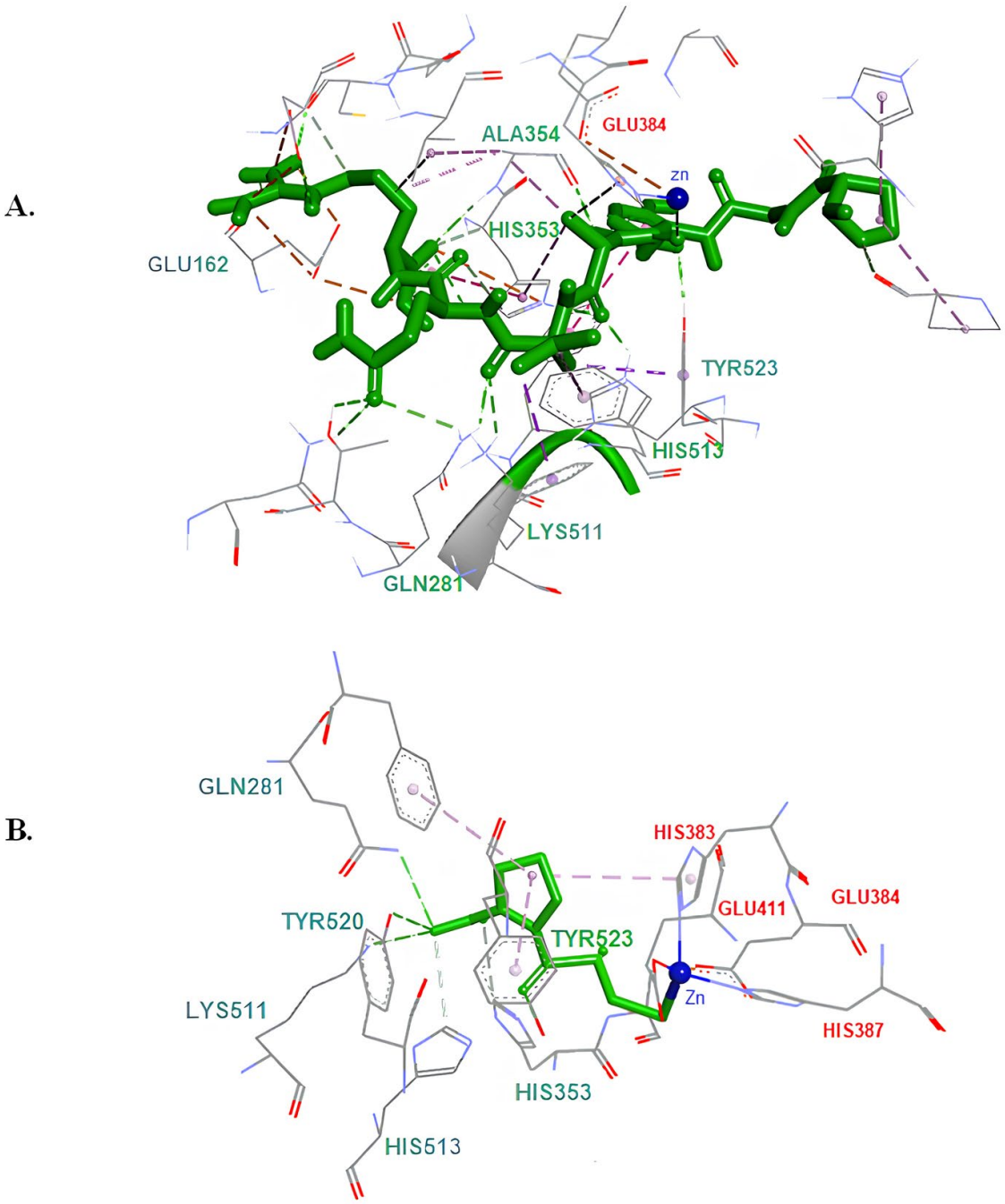

Figure 2. Predicted interaction for ACE (1O86) with PGYALQR peptide (A) and Captopril (B). Zn (II) atom and coordination bonds are shown with a circle and blue bold line, respectively. ACE binding key residues are represented in green and Zn (II)-coordinating residues in red.