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Pharmacophore mapping of Angiotensin Converting Enzymes (ACEs): Insight to Binding Site of ACE1 and ACE2

Navista Sri Octa Ujiantari*, Cintya Nurul Apsari
Laboratory of Medicinal Chemistry, Departement of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada
Sekip Utara Yogyakarta, 55281, Indonesia.

Corresponding author*
navista.sri.o@ugm.ac.id

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Abstract

Angiotensin Converting Enzymes (ACEs) are carboxypeptidase enzymes involved in the renin-angiotensin system (RAS), which catalyze angiotensin I by cleavage of the peptide bond. ACE1 has been known as a target for antihypertensive drugs. Another homolog of ACE1, ACE2 has been popular since 2020 because this enzyme is responsible for the SARS-COV2 infection in the human body. Interestingly, it was found that ACE1 inhibitors did not inhibit ACE2. Hence, this study aims to elucidate the pharmacophore of ACE1 and ACE2 in order to understand the mechanism of these different inhibitions. Pharmacophore mapping was carried out using a pharmacophore query editor in the Molecular Operating Environment (MOE). The 3D structures of both enzymes bound to respective inhibitors were prepared and their pharmacophore features were extracted. Besides that, the similarity of both enzymes was analyzed by comparing their amino acid sequences using Align in Uniprot. In addition to pharmacophore mapping, the surfaces of both binding sites were analyzed to obtain a comprehensive evaluation. Results showed that both ACE1 and ACE2 contain nine and eight pharmacophore features, respectively. The amino acid residues of both enzymes were quite similar, especially in the active site. However, both ACE1 and ACE2 inhibitors showed different interactions even though both were well aligned. It was found because the functional groups of both inhibitors were slightly different as well as the active site size of both enzymes. Thus, this might result in different ability of ACE1 inhibitors to occupy the binding site of ACE2. These findings could provide useful information in the design of new selective ACE1 compounds as well as ACE2 compounds.

Keywords: pharmacophore mapping; ACE1; ACE2; active site; homolog protein.

Abbreviations: ACE: angiotensin-converting enzyme; MOE: molecular operating environment; RAS: renin-angiotensin system; ACEI: ACE inhibitor; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

INTRODUCTION

The renin-angiotensin system (RAS) plays a role in blood regulation as well as glucose metabolism, electrolyte balance, and homeostasis in the body. The components of this system include angiotensinogen, angiotensin I, angiotensin II, angiotensin-converting enzyme (ACE) (Ahmad et al., 2023; Alvarez-Zaballos & Martinez-Selles, 2023). ACE1 is a carboxypeptidase that catalyzes angiotensin I, a decapeptide by cleavage of the peptide bond into an octapeptide, angiotensin II (Ames et al., 2019; Ahmad et al., 2023). This enzyme has been known as antihypertensive drugs and the synthetic drugs inhibit this enzyme namely ACE inhibitor (ACEI) (Messerli et al, 2018). Those inhibitors are orally administered and they usually contain the suffix "-pril" in their name, for instance captopril, lisinopril, fosinopril, enalapril, and ramipril (Ahmad et al., 2023).

Pharmacophore modeling has been applied in drug design and discovery to identify compounds with specific biological activities and to understand their interaction with protein or mapping interaction (Seidel et al., 2018). The functional groups or parts of molecules responsible for biological activities are called pharmacophores (Güner dan Bowen, 2014). The pharmacophore features include hydrogen bond acceptors and donors (HBA dan HBD), ionized groups, hydrophobic (HY), aromatic rings (RA), metal coordination (M), and halogen bond (Schaller et al., 2020). Pharmacophore modeling can be approached in two ways: ligand-based and structure-based (Vuorinen & Schuster, 2015). Ligand-based pharmacophore modeling relies on known compound activities, whereas structure-based pharmacophore modeling is derived from 3D experimental protein structures (Seidel et al., 2018). The 3D structures provide the shape of active site as well as the important amino acid residues and the type interaction (Vuorinen & Schuster, 2015).

In 2019, pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in

Wuhan, China, became a global pandemic (Ni et al., 2020; Ahmad et al., 2021). The virus can penetrate the human body through a receptor called ACE2 (Ahmad et al., 2021; Pagliaro et al., 2022). This enzyme catalyzes angiotensin II into angiotensin 1-7 and is expressed in several organs such as blood vessels, the brain, kidneys, lungs, and the digestive system (Pagliaro et al., 2022; Dutta, 2022). Although ACE2 is a homologous protein with ACE1, ACE1 inhibitors do not inhibit ACE2 activity (Danser et al., 2020; Kai & Kai, 2020). Therefore, this research aims to examine the differences in the active sites of ACE1 and ACE2 using structure-based pharmacophore modeling by mapping interactions in both enzymes. The results of this study can be used as valuable information, especially in designing selective ACE2 inhibitors.

MATERIALS AND METHODS

Pharmacophore mapping was conducted using the pharmacophore query editor in the Molecular Operating Environment (MOE, 2022). The 3D structures of ACE1 with PDB ID: 1O86 (Natesh et al., 2003); 1UZF & 1UZE (Natesh et al., 2004), and ACE2 with PDB ID 1R4L (Towler et al., 2004) were obtained from the Protein Data Bank (Burley et al., 2017). All of those enzymes are bound to respective inhibitors. The enzymes were prepared, and their pharmacophore features were extracted using the pharmacophore query editor. Furthermore, the similarity between the two enzymes was analyzed by comparing their amino acid sequences using the Align tool in Uniprot (Boutet et al., 2016). Both 2D and 3D interactions between the inhibitor and ACEs were visualized to identify the types of interactions formed. Additionally, the active site surfaces of both enzymes were analyzed to obtain a comprehensive evaluation.

RESULTS AND DISCUSSION

Result

Based on the analysis of amino acid sequence similarity using Align, the results indicated that ACE1 and ACE2 share 36.61% similarity (per*cent identity). Homolog proteins share 30-40% sequence identity (Cavasotto & Phatak, 2009; Pearson, 2013).

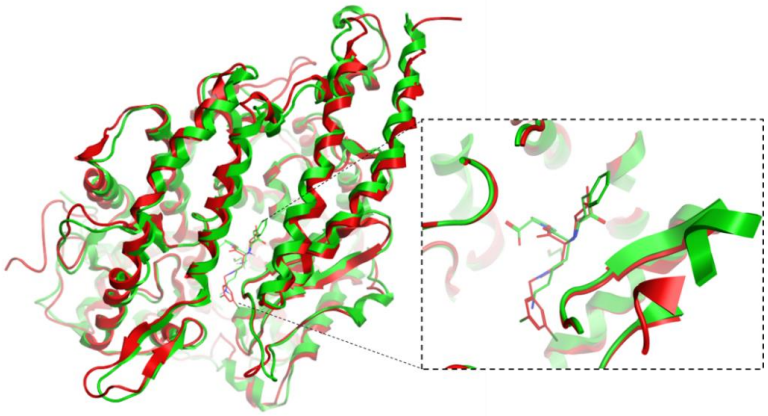

Figure 1. The alignment of ACE1 (green) and ACE2 (red) along with their inhibitors, namely lisinopril (green) and MLN-4760 (red), at the active site of the enzyme.

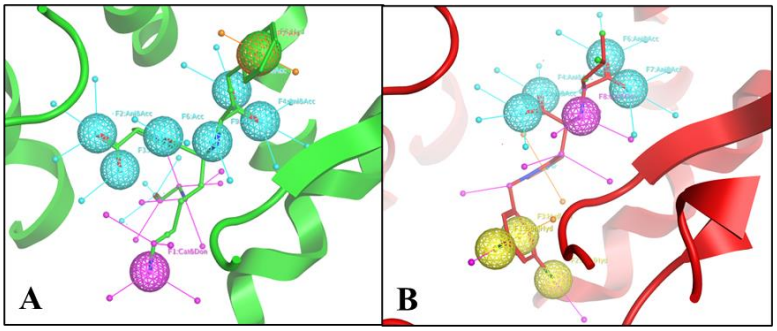

Figure 2. Pharmacophore features obtained from 3D structures of ACE1 (A) and ACE2 (B).

In addition, the alignment results of ACE1 and ACE2 (Figure 1) showed that ACE1 and ACE2 share similar protein backbone or tertiary structure. Figure 1 also depicted that both inhibitors were well aligned in the active site situated in the centre of both enzymes. Lisinopril, an ACE1 inhibitor, also closely shared a similar orientation with MLN-4760, an ACE2 inhibitor. Pharmacophore mapping was conducted to identify potential interactions between the inhibitors and both enzymes (Figure 2). In Figure 2, ACE1 exhibited nine pharmacophore features, including 6 HBA, 1 HY, 1 RA, and 1 HBD, while eight pharmacophore features were extracted from ACE2 consisting of 4 HBA, 3 HY, and 1 HBD.

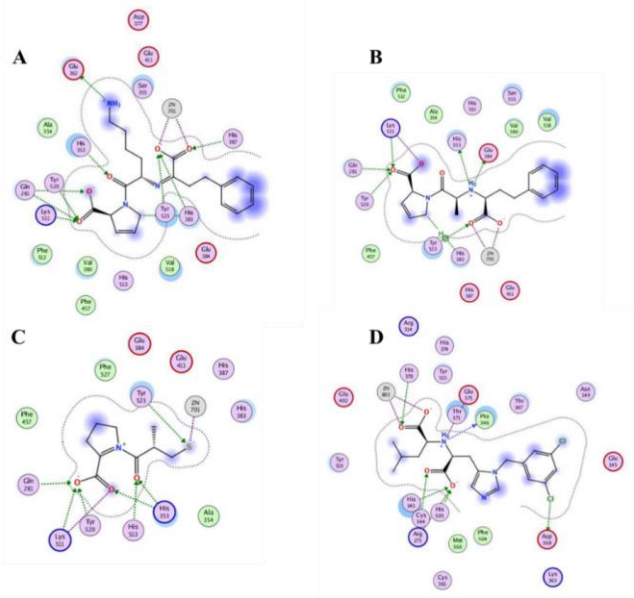

Figure 3. 2D interaction of ACE1 with lisinopril (A), enalaprilat (B), captopril (C), and ACE2 with MLN-4760 (D).

Table 1. The amino acid residues in the active site of both enzymes and the type of interactions.

ACE1	ACE2	Type of interactions
His353	**His345**	Hydrogen bond
Ala354	Pro346	
Ser355	Thr347	
Asp377	**Asp368**	
His383	**His374**	metal coordination/complex with Zn and inhibitor
Glu384	**Glu375**	
His387	**His378**	Hydrogen bond
Glu411	**Glu402**	metal coordination/complex with Zn and inhibitor
Phe512	**Phe504**	
His513	**His505**	
Val518	Tyr510	
Tyr523	**Tyr515**	

Note: bold line means same amino acid residue

The 2D visualization between the inhibitor and the enzyme was performed to identify the types of interactions. At the active sites of both enzymes, there are similarities in several amino acid residues, and these residues also interact with the inhibitors (table 1). Figure 3 illustrates the interactions between the inhibitors and the amino acid residues present at the enzyme's active site. In the ACE1 inhibitor, captopril, enalaprilat, and lisinopril had similar interactions, where the bound amino acid residues include Glu162, Gln281, His353, His383, Glu384, His387, Lys511, His513, Tyr520, and Tyr 523. Additionally, metal coordination bonds are formed between the carboxylate groups of the inhibitors and Zn, an ACE cofactor. Some interaction patterns observed in ACE1 are also present in ACE2, such as the carboxylate group of MLN-4760 binding to Zn. Furthermore, MLN-4760 also bound to several amino acid residues such as Arg273, Cys344, His345, Pro346, Asp368, Thr371, Glu375, His378, and His505. Figure 4 depicted the 3D visualization for both enzymes, which was used to identify the types of interactions between the inhibitors and the amino acid residues. Hydrogen bonds and metal coordination with Zn were formed in both ACE1 and ACE2. Additionally, an analysis of the active site surface was conducted to determine the shape of the active site and the hydrophobicity of the enzyme's active site. The ACE2 active site is narrower than ACE1, especially in the central area and it is dominated by hydrophobicity.

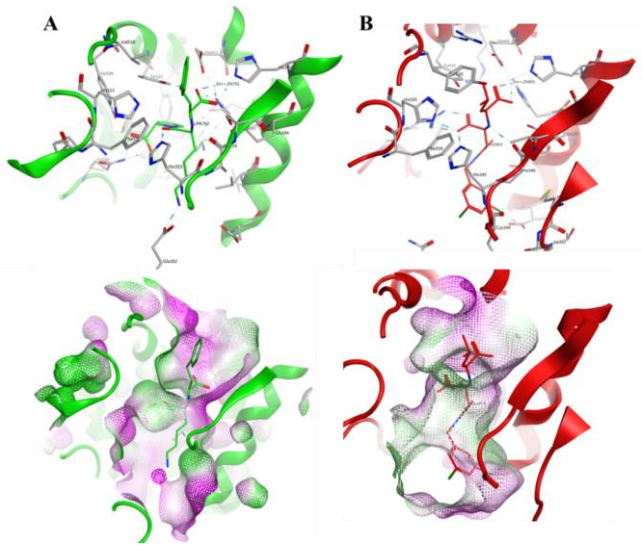

Figure 4. 3D visualization of lisinopril with ACE 1 (A) and MLN-4760 with ACE2. The 3D interaction of the inhibitors at the active site with amino acid residues (above); the surfaces of active site in both enzymes with purple for hidrophilicity/polar and green for hydrophibicity (below).

Discussion

In this in silico studies, pharmacophore mapping was conducted to analyze the differences in the active sites of ACE1 and ACE2, which are homologous proteins based on their backbone similarity or tertiary structures. Three

3D structures of ACE1 were used with their corresponding inhibitors: captopril, enalaprilat, and lisinopril, while ACE2 was bound to an inhibitor, MLN-4760. The active site of both enzymes is located at the centre of their respective structures. It can be observed that both share similarities in some amino acid residues thus ACE1 inhibitors and ACE2 inhibitors have similar type of interactions. However, it was found that there is a subtle difference in their interaction due to the functional groups of both inhibitors. In addition, the size of ACE2 active site is slightly narrower than ACE1 active site. It has been known that ACE1 inhibitors are mostly bulky compounds and therefore they could not occupy the ACE2 active site. Furthermore, the ACE2 active site is also dominated by hydrophobicity, making polar groups, especially in the central area of the compound, more likely to bind to ACE2. Consequently, these differences cause ACE1 inhibitors to be unable to occupy the ACE2 active site. In conclusion, these preliminary findings could provide useful information in designing compounds that are selective towards both ACE1 and ACE2.

CONCLUSIONS

ACE1 and ACE2 are homologous proteins based on their tertiary structure (backbone) and their sequence identity. ACE1 inhibitors, which are bulky compounds, could not occupy the active site of ACE2 due to its narrower shape. In addition, the active site of ACE2 tends to be more polar or dominated by hydrophobicity.

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Authors' Contributions: Navista Sri Octa Ujiantari designed the study, performed the pharmacophore modelling, analyzed the data, and wrote the manuscript. Cintya Nurul Apsari carried out the data collection and protein alignment. All authors read and approved the final version of the manuscript

Competing Interests: Authors declare that there are no competing interests.

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