Angiotensin-Converting Enzyme 2 (ACE2) of marine biota: a preliminary study of potential therapy for SARS-CoV-2 infection

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Abstract. A new coronavirus, SARS-CoV-2, is responsible for the current pandemic causing severe respiratory disease. It has been known that the receptor for SARS-CoV-2 entry into the host cell is the angiotensin-converting enzyme 2 (ACE2). This receptor is expressed in a wide array of organs, for example, the kidneys and gastrointestinal tract, but rarely present in the circulation. The soluble form of ACE2 proposed as a potential therapy for SARS-CoV-2 infection. This research aimed to explore angiotensin-converting enzyme 2 (ACE2) from marine biota as the source of ACE2, which is potential for the therapy of SARS-CoV-2 infection. This explorative study was conducted by retrieving the angiotensin-converting enzyme 2 (ACE2) from the database of protein (UniProt). The samples of the study were ACE2 of marine vertebrate, namely Delphinapterus leucas and ACE2 of marine invertebrate, namely Protunus trituberculatus. 3-D structures of ACE2 proteins unavailable in the protein database were modeled in Swiss Model. Molecular docking was conducted by using ClusPro.2.2. The data were analyzed descriptively. The molecular docking results revealed that the binding energy of spike glycoprotein of SARS-CoV-2 and human ACE2 was -988.5 kcal/mol. The binding energy of spike glycoprotein of SARS-CoV-2 and Delphinapterus leucas (Beluga whale) ACE2 was -946.4 kcal/mol. Meanwhile, the binding energy of spike glycoprotein of SARS-CoV-2 and Protunus trituberculatus (swimming crabs) ACE2 was -778.4 kcal/mol. The binding energy of spike glycoprotein of SARS-CoV-2 and Delphinapterus leucas (Beluga whale) ACE2 was close to the binding energy of spike glycoprotein of SARS-CoV-2 and human ACE2. Hence, ACE2 of Delphinapterus leucas has the potential to be used as a therapeutic candidate from marine biota to suppress the SARS-CoV-2 transmission.

1. Introduction
The coronavirus disease 2019 (COVID-19) pandemic is caused by widespread transmission and infection of severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) [1]. SARS CoV-2 spread to various countries around the world, including Indonesia. WHO reports that as of 25 May 2020, this pandemic has reached 5,304,772 cases and caused 342,029 deaths and in Indonesia, there have been 22271 positive cases and 1372 deaths [2].

SARS CoV-2 caused a widespread outbreak [3]. This virus also undergoes mutations along with its spread [4]. Experts have also conveyed the dangers of human-to-human transmission and interspecies transmission [1] [5] [6]. Various attempts to inhibit viral transmission have been made, including
vaccine development, use of antiviral drugs, and regulation (binding) of angiotensin-converting enzyme 2 (ACE2) [7].

ACE2 was identified as the SARS-CoV-2 receptor [8] [9][10][11]. SARS CoV-2 enters the host cell by binding to the ACE2 receptor, namely by binding to spike glycoproteins [12] [13]. ACE2 is a receptor that plays a role in the hormonal system renin-angiotensin (RAS) and widely expressed in the endothelial tissue of various organs, such as the kidney and digestive tract, but is rarely found in the circulatory system [14] [15] [7]. The presence of ACE2 expression in these other organs contributes to the incidence of multi-organ dysfunction. Dissolved ACE2 has been proposed as a potential therapy for SARS-CoV-2 infection [15].

Human recombinant soluble angiotensin-converting enzyme 2 (hrsACE2) had previously developed [16], which has shown that the soluble ACE2 receptor is a target protein that binds easily to SARS-CoV-2. The virus that binds to ACE2 on the cell surface will decrease so that the probability of virus to infecting cells are reduced. Blocking of spike glycoprotein domains in circulating SARS-CoV-2 by dissolved ACE2 will block its binding with ACE2 which is localized in the tissue thus inhibiting the entry of SARS-CoV-2 into cells [16].

As an essential part of the RAS hormone system, ACE2 is not only produced by human cells, but by other organisms. The data contained in the UniProt protein database show that some marine life also produces ACE2 and ACE2-like structures. Thus, the ACE2 and ACE2-like structure of this marine biota can be tested for binding to the SARS CoV-2 spike protein. The administration of excess dissolved ACE2 could block the binding of SARS CoV-2 to the host cell [15]. This study was aimed to analyze the potential of angiotensin-converting enzyme 2 of marine biota for the therapy of SARS-CoV-2 infection.

2. Method
This research was a descriptive exploratory study using the in-silico test approach. The in-silico test method used was molecular docking. The samples were ACE2 of marine vertebrates, namely Delphinapterus leucas (Beluga whale) and ACE2 of marine invertebrates, namely Portunus trituberculatus (swimming crabs), which were collected from Uniprot. In addition, the ACE2 of human collected from UniProt was also tested as control.

Procedure of molecular docking of ACE2 D. leucas and P. trituberculatus with SARS CoV-2 spike virus included: the collection of ACE2 molecules from Uniprot; visualization of ACE 2 3D proteins from D. leucas and P. trituberculatus using the Swiss Model; Glycoprotein spike collection from Protein Data Base; molecular docking using the ClusPro.2.2 web server; visualization of docking results. A macromolecule collection of the ACE2 receptor protein was performed from the Uniprot database (https://www.uniprot.org/). Protein visualization was carried out using the Swiss model website at https://swissmodel.expasy.org/. Through modelling, the fastened protein sequences were pasted on the modelling template of Swiss model. Thus, the results are 3D models displayed in PyMol software. SARS CoV-2 Glycoprotein Spikes were obtained from the Protein Data Base web at https://www.rcsb.org.

Before doing molecular docking, the molecules were first sterilized using Autodoc software. Furthermore, molecular interaction simulation between ACE2 and Spike Glycoprotein docking was conducted using Cluspro (https://cluspro.bu.edu/). The docking results could be seen in the program display. The docking model was downloaded as a PDB file. The coefficient could be changed to balanced, Electrostatic-favored (electrostatic), Hydrophobic-favored (hydrophobic), or VdW + elec (Van der waals + electrostatic). The docking model results and scores will follow the choice of coefficients.

Parameters used were lowest binding energy of the two proteins and the binding of the protein structure of ACE2 with the glycoprotein spike. The data obtained were the binding affinity score and the 3D structure of the ACE2 protein binding with glycoprotein spikes.

3. Results and Discussion
An essential part of the RAS hormone system, angiotensin-converting enzyme 2 (ACE2) is not only produced by human cells, but also by many other organisms. The data in the UniProt protein database shows that a number of marine organisms also express ACE2 and ACE2-like structures. The ACE2 used
for this study was ACE2 of *Portunus trituberculatus* retrieved UniProt, the entry code was A0A5B7IQP6; length was 85; mass was 9.769 Da. ACE2 of *Delphinapterus leucas* was retrieved UniProt, the entry code was A0A2Y9M9H3; length was 804; mass was 92,478 Da.

The process of molecular docking proteins and interaction simulation using Cluspro was used to determine the binding energy formed between spike glycoprotein (ligand) and ACE2 receptor (marine biota and human suspect) when the SARS-CoV-2 attachment occurs in the host cell. The molecular docking results revealed that the binding of ACE2 of *Delphinapterus leucas* to the SARS CoV-2 glycoprotein spike was similar to the binding of ACE2 of human to the SARS CoV-2 glycoprotein spike. The binding energy of ACE2 of human to the SARS CoV-2 glycoprotein spike was -988.5 kcal/mol, while the binding energy of ACE2 of *D. leucas* to the SARS CoV-2 glycoprotein spike was -946.4 kcal/mol (Table 1). The binding energy of ACE2 of *Protunus trituberculatus* to the SARS CoV-2 glycoprotein spike was much higher, at -778.4 kcal/mol. The complex of molecular docking displayed using PyMol with representative structures and colour selections to identify between receptors (ACE2) and ligands (SARS-CoV-2 spike glycoprotein) (Fig. 1).

![Figure 1. 3D structure of the ACE2 protein binding with glycoprotein spikes of SARS-CoV-2: A: ACE2 of human; B: ACE2 of *Portunus trituberculatus*; C: ACE2 of *Delphinapterus leucas*. Information: ACE2 (red) molecular complex; SARS-CoV-2 spike glycoprotein (blue).](image)

In this study, the results showed that the molecular complex arrangement the lowest binding energy to achieve stability -946.4 until -811.5 kcal/mol from *Delphinapterus leucas* (Table 1), in the glycoprotein ACE2 binding formation. This finding indicated that *Delphinapterus leucas* has the potential to be used as a therapeutic candidate from marine biota. The lowest binding energy of spike glycoprotein of SARS-CoV-2 was closed to the binding energy of spike glycoprotein of SARS-CoV-2 and human. Hence, the lower binding energy values can trigger the formation of stable molecular complexes between ACE2 and spike glycoprotein [17],[18]. This stability can trigger the activation of biological responses to target proteins such as mediated endocytosis during the SARS-CoV-2 viral entry to the host cell [19].
Table 1. Binding energy of ACE-2 with SARS-CoV-2 spike glycoprotein

| Organisms                          | Code ACC Uniprot | Molecular complex      | Cluster | Lowest Energy (kcal/mol) |
|-----------------------------------|------------------|------------------------|---------|--------------------------|
| *Delphinapterus leucas* (Beluga whale) | A0A2Y9M9H3       | ACE-2_Spike Glycoprotein | 0       | -946.4                   |
|                                   |                  |                        | 1       | -900.1                   |
|                                   |                  |                        | 2       | -891.9                   |
|                                   |                  |                        | 3       | -811.5                   |
|                                   |                  |                        | 0       | -988.5                   |
|                                 |                  |                        |         |                          |
| *Homo sapiens* (Human)            | Q9BYF1           | ACE-2_Spike Glycoprotein | 1       | -924.4                   |
|                                   |                  |                        | 2       | -823.8                   |
|                                   |                  |                        | 3       | -821.5                   |
|                                   |                  |                        | 0       | -778.4                   |
|                                |                  |                        |         |                          |
| *(Portunus trituberculatus* (Swimming crab) | A0A5B7IQP6     | ACE-2_Spike Glycoprotein | 1       | -770.5                   |
|                                   |                  |                        | 2       | -816.6                   |
|                                   |                  |                        | 3       | -826.0                   |

Angiotensin-converting enzyme 2 (ACE2) was identified as the SARS-CoV-2 receptor [8] [9] [10] [11]. SARS CoV-2 enters the host cell by binding to the ACE2 receptor, namely by binding to spike glycoproteins [12] [13]. ACE2 is a receptor that plays a role in the hormonal system renin-angiotensin (RAS) and is widely expressed in the endothelial tissue of various organs, such as the kidney and digestive tract, but is rarely found in the circulatory system [14] [15] [7]. The presence of ACE2 expression in various other organs contributes to the incidence of multi-organ dysfunction often found in COVID-19 patients. Dissolved ACE2 has been proposed as a potential therapy for SARS-CoV-2 infection [15].

Human recombinant soluble angiotensin-converting enzyme 2 (hrsACE2) had previously developed as one of potential therapy for SARS-Cov-2 infection [16]. The results of these studies indicate that the soluble ACE2 receptor is the target protein that easily binds to SARS-CoV-2. The virus that binds to ACE2 on the cell surface will decrease so that the infected cells are reduced. This is evident from the results of his research on Vero E6 cells showing hrsACE2 exposure can inhibit the entry of SARS-CoV-2 into cells. Blocking of spike glycoprotein domains in circulating SARS-CoV-2 by dissolved ACE2 will block its binding with ACE2 localized in the tissue, thus inhibiting the entry of SARS-CoV-2 into cells [16].

4. Conclusion
It can be concluded that ACE2 of *Delphinapterus leucas* has the potential to be used as a therapeutic candidate from marine biota to suppress the SARS-CoV-2 transmission. Further studies are needed to explore more ACE2 from other marine biota as well as to synthesize the potential ACE2.

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